

Characterization by polyphasic approach of some indigenous halophilic archaea of Djelfa's rock salt "Hadjr el Meelh", Algeria

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ABSTRACT

Background and Objectives: Hadjr El Melh of Djelfa is an example of hypersaline ecosystems, which can harbor a wide variety of microorganisms under hostile physicochemical conditions. Given the importance of the study of halophilic microorganisms present there in terms of fundamental and applied microbiology, the purpose of this study was to characterize some halophilic archaea isolated from the brines of this environment.

Materials and Methods: Eight water samples were chosen randomly and collected for physicochemical and microbiological analyses. Isolation of halophilic archaea was carried out by membrane filter technique. Ten strains were identified by polyphasic approach and tested for enzymes production.

Results: Water samples of Djelfa's rock salt were slightly acidic to neutral in pH (6.55-7.36) with salinity ranging from 258.68 g/l to 493.91 g/l. Phenotypic, biochemical, taxonomic and phylogenetic characteristics indicated that all strains were classified within the family of *Halobacteriaceae*. Based on the comparison of DNA sequences encoded 16S rRNA, it was determined that seven strains were affiliated to the genus *Haloarcula*, two strains were related to the genus *Halobacterium* and one strain within the genus *Haloferax*. Production of different enzymes such as protease, amylase, esterase, lipase, lecithinase, gelatinase and cellulase on solid medium indicated that one strain (S2-2) produced amylase, esterase, lecithinase and protease. However, no strains showed cellulolytic or lipolytic activity. Gelatinase was found in all tested strains.

Conclusion: This report constitutes the first preliminary study of culturable halophilic archaea recovered from the brines of Djelfa's rock salt with a promising enzymatic potential in various fields of biotechnology.

Keywords: *Halobacteriaceae*; Isolation and purification; Salinity; Enzyme; Archaea; Phylogeny; Algeria

INTRODUCTION

Hypersaline ecosystems are widespread through Algeria. They can be found at sea level, in arid and semiarid regions. Among the natural ones, sebkhas

and chotts are a good example, but are only occasionally studied. Their microbial diversity was poorly investigated and limited to some works carried out by Hacene et al., Boutaiba et al., and Khellaf et al. (1-3).

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Hadjr El Melh of Djelfa, the second rock salt in Algeria is an example of original extreme environments which is exposed like other hypersaline ecosystems to extreme physicochemical conditions including high radiation (UV), significant temperature and dryness changes that render it an important target of study for microbiologists (4). The brines of permanent and temporary streams of this site that are also rich in divalent ions such as Calcium and Magnesium result from evaporite concentration and crystallization of leaching water from rock by evaporation (5). This type of environment is colonized by halotolerants and halophilic microorganisms belonging to the three domains of life: Archaea, Bacteria and Eukarya (6). Species of the order *Halobacteriales* within the family of *Halobacteriaceae*, that are the best known extreme halophiles, require at least 1.5 M NaCl for growth (7). According to their salt requirement, Kushner differentiated between extreme halophiles that grow well at 2.5-5.2 M NaCl, borderline extreme halophiles with optimum growth at 1.5-2.5 M NaCl and moderate halophiles who prefer NaCl concentration ranging from 0.5 to 2.5 M (8). Colonization of such environments by these heterotrophic microorganisms was achieved by diverse adaptation mechanisms tolerating periodic desiccation and rewetting (4). Since there were not any reported studies on the biodiversity of Hadjr El Melh, a preliminary study was undertaken to get an overview on the culturable halophilic archaea species that exist in hypersaline waters of the rock salt by using both biochemical tests and molecular methods. This study was also performed to test the ability of isolated strains to produce enzymes of industrial interests. A physicochemical analysis was also conducted to determine the major ions in the brines and mimic the environment conditions in the isolation culture medium.

MATERIALS AND METHODS

Sampling. The salt water samples were taken from the surface of eight streams of the salt rock chosen randomly and collected in sterile 500 ml bottles and sent to the laboratory in coolers. Each sample was taken in duplicate; one for physicochemical analyses, the other for the isolation of the halophilic strains.

Description of the sampling site (Hadjr El Melh).

The rock salt named Hadjr El Melh is located on the road to Algiers 25 Km North of the city of Djelfa, county of Ain Maâbed (latitude: 34° 77 '39.04" North and longitude: 3° 17 '37.89 "East, altitude of 1083 m) in the basin Zahrez El Gharbi (Fig. 1) (Google earth.com). It is a triassic outcrop which culminate 110m in height. It is composed mainly of rock salt, salt mud and gypsum. The salt rock is devoid of vegetation, except for a few tufts of Alfa, some shrubs of Tamarisk. In the whole salt rock, and especially in the region of the big steep cliffs, there are several valleys containing permanent or temporary streams which reappear after a long circulation of rainwater (Fig. 2). There are also elongated potholes at the bottoms of which are sections of the Oued El Malah River, which runs along this site from east to west.

Physico-chemical analysis of the water. The pH and conductivity of water samples were determined using a Hach pH meter and conductivity meter successively and values were recorded. The Chlorinity was measured by titration with AgNO_3 (9). Salinity was calculated by the following formula: $S\text{‰} = 1.80655 \cdot \text{Cl}\text{‰}$ (9). Na^+ concentration was determined with PFP7 flame Photometer. Ca^{+2} and Mg^{+2} were also measured by a titrimetric method using ethylene-diaminetetraacetic acid (EDTA) (9).

Isolation of halophilic strains. Due to the low microbial concentration in the hypersaline water, halophiles were isolated by membrane filter technique (0.2 μm) (10). Membranes of each sample were deposited on the modified medium "MSH" of the following composition NaCl 150.0/200.0/250.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 50.0 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 32.0 g; KCl 5.0 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.8 g; NaBr 0.6 g; NaHCO_3 0.16 g; Yeast extract 5.0 g; Agarose 15.0 g; water 1000ml, pH 7.2 (6). Petri dishes were incubated at a temperature of 40°C for an incubation period of 5 to 15 days in hermetically sealed plastic bags (10). The pure culture collection strains were maintained on slopes of the same medium at 4°C.

Screening of obligate halophilic strains. In order to select the obligate halophilic strains of the other halotolerant strains, a Na-free-MSH liquid culture at 40°C with shaking (130 rpm) was prepared for each strain. Growth monitoring was done by measuring the optical density at 600 nm after six incubation days.

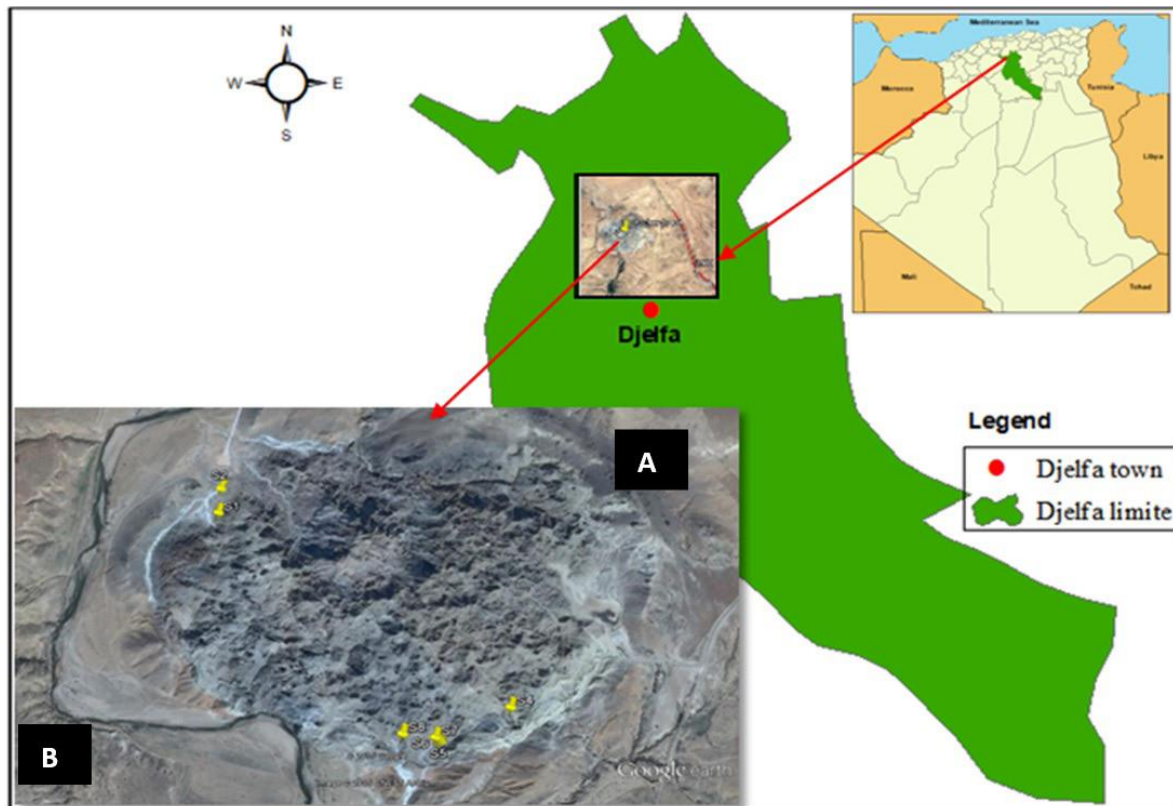


Fig. 1. Location of the study area in Algeria. A) Satellite image of the study area “Hadjr El Melh” located in the north of Djelfa City. B) Satellite image of the sampling sites of the rock salt “Hadjr El Melh” (Source: Google Earth.com).

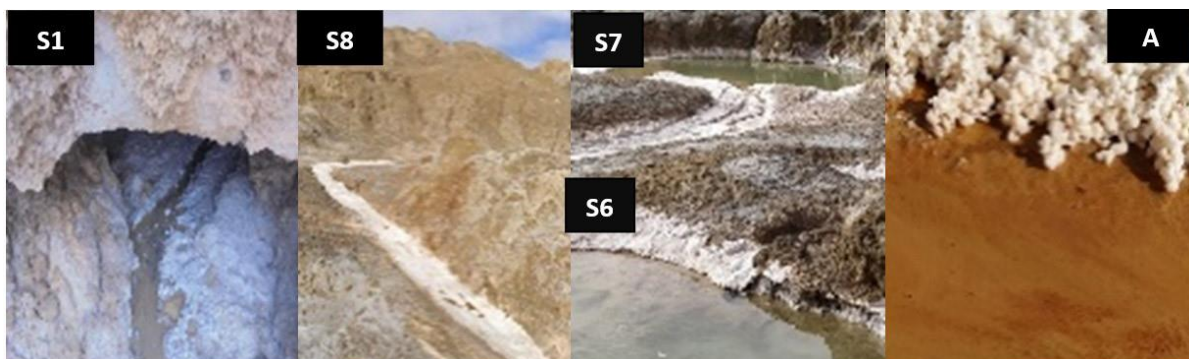


Fig. 2. An overview of some sampling sites in the rock salt “Hadjr El Melh” Djelfa. S1 (34, 83571° N, 3, 08809°E). S6, S7 and S8 (34, 83038°N, 3, 09513°E); A) Sampling sites are saturated with salt which forms white crust and the availability of water is reduced under solar evaporation and dryness conditions.

Preliminary characterization of obligate halophilic isolates. Phenotypic characterization of isolates was carried out by following the description of the *Halobacteriaceae* family (11). The isolates were Gram stained using sample fixed with acetic acid and examined by a light microscope (3). Cell morphology and motility were determined on fresh liquid culture by phase contrast microscope (Zeiss). Scanning elec-

tron microscope was used to visualize the external appearance of isolated strains which pre-fixed by 3% glutaraldehyde for 1.5 h, washed with 0.1 M cacodylate buffer, post-fixed by 1% OsO_4 for 2 h at 4°C and dehydrated by a series of increasing gradient (50%, 70%, 80%, 90%, 95%, and 99.8%) of acetone for 10 minutes each and then in 100% acetone for 30 minutes twice. After air-drying, samples were mounted

on a stub of metal with adhesive, coated with 40-60 nm of Gold and then examining with JEOL JSM-6610 scanning electron microscope (12). Oxidase reaction was carried out following the Kovacs technique (13), Catalase was determined by crushing a small inoculum of each strain in a drop of 10 volumes of H_2O_2 . The affiliation of the isolated strains to the domain archaea was ascertained as shown in the protocol of Kamekura et al. (14), by their sensitivity to the bile acid Na-deoxycholate (0.04%) or their resistance to chloramphenicol (30 μ g). In order to confirm whether the isolated strains belong to extreme non-cocoid haloarchaea, cell lysis in distilled water was tested for each strain by transferring thick inoculums in 100 μ l distilled water. A very viscous suspension indicates cell lysis. Furthermore, examining cell lysis was done by re-streaking of the viscous suspension on solid MSH medium. The salt tolerance of the isolates was studied by growing in MSH liquid medium with different NaCl concentrations ranging from 0.3 M to 4.28 M. Anaerobic growth of the isolates by fermentation of L-Arginine was tested. For the nitrate reduction test, the MSH liquid medium supplemented with 1 g/l of KNO_3 instead 0.5% of $NaNO_3$ was used (10). The ability to use a single source of carbon and energy was tested according to the protocol of Montalvo-Rodriguez and his collaborators with slight modifications; a liquid yeast extract-free MSH medium (pH 7.2) supplemented with 10 g/l of tested sugar (Glucose, arabinose, starch, mannose, lactose, fructose, galactose, maltose, saccharose) or 1 g/l of tested alcohol (mannitol, glycerol), 0.005 g/l of KH_2PO_4 and 0.5 g/l NH_4Cl was used (10). The growth was estimated by measuring of the A_{600nm} after 6 days of incubation at 40°C with orbital agitation (130 rpm). The sensitivity to different antibiotics (ampicillin, chloramphenicol, novobiocin, tetracycline, bacitracin, trimethoprim, ceftazidime, nalidixic acid, neomycin and gentamycin) was assessed by agar disk diffusing method (10) with slight modifications regarding the test medium. The solid MSH medium was used instead of the Sehgal-Gibbons (SG) medium.

Polar lipids analysis was performed by thin layer chromatography on silica gel G plate after their extraction following the technique describing by Litchfield and Oren (15).

Genomic DNA extraction amplification of 16S rDNA and phylogenetic analysis. According to the manufacturer's instructions, genomic DNA was ex-

tracted from the cultured cells using the DNeasy® blood & tissue kit (Qiagen). The 16S rRNA genes were amplified by PCR, using the archaeal specific forward and reverse primers (5'-TCCGGTTGATCCTGCCG-3') and (5'-GGAGGTGATCCAGCCG-3'), respectively (16). All reactions were carried out in 50 μ l reaction mixture containing 25 μ l of GoTaq® Hot Start Green Master Mix (2 \times), 10 μ M of each primer, 1 μ l of DNA template (<250ng) and Nuclease free water with the following thermocycling program: 2 minutes denaturation at 95°C, followed by 35 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 55°C, 1 minute 30 seconds extension at 72°C, and a final extension step of 5 minutes at 72°C. In the presence of a molecular weight-size marker (1Kb DNA leader, New England Biolabs), RedSafe™ stained amplicons were electrophoresed on 1% (w/v) agarose gel, viewed and recorded under UV- trans illumination, The 1.5Kb PCR products obtained were purified using a PureLink® PCR purification Kit (invitrogen) and sent for sequencing to CeMIA SA (Cellular and Molecular Immunological applications, Larissa Greece) by using an ABI 3730 XL sequencer. The nucleotide sequences received were compared using the EzBioCloud database server (<http://Eztaxon-e.ezbiocloud.net>). MEGA X software was used to construct the phylogenetic tree, which was inferred using the Neighbor-joining method (17, 18).

Qualitative detection of extracellular hydrolytic enzymes. Extracellular hydrolytic enzyme production was tested on modified solid MSH medium by the reduction of the amount of yeast extract to 0.3 g/l instead of 5 g/l, at the optimum NaCl concentration supplemented with the suitable substrate for each enzyme. Hydrolysis of starch (amylase activity), tween 80 (esterase activity), olive oil (lipase activity), skim milk (protease activity), gelatin (gelatinase activity), lecithin (lecithinase) and carboxymethyl cellulose (cellulase activity) was tested by adding to the modified solid MSH medium the following substrates (1% soluble starch, 1% tween 80; 2.5% olive oil; 10% skim milk; 15% gelatin; 2 ml of egg yolk solution and 0.5% carboxymethyl cellulose, respectively) (19).

RESULTS

Physicochemical analyses. The physicochemical characteristics of the water rock salt samples were

reported in Table 1. Hypersaline ecosystems differ greatly in ionic composition, total salt concentration, and pH. Rock salt water with salinity varying from 258.68 g/l to 493.91 g/l was slightly acidic to neutral with pH values of 6.55 to 7.36.

The values of conductivity, ranging from 194.03 ms/cm to 562.5 ms/cm, showed an average mineralization. To determine the nature of elements contributing to the salinity, chemical analysis of the rock salt water indicated that Cl⁻ concentrations ranged from 181.08 g/l to 273.4 g/l, where equivalent concentrations of Mg²⁺ (0.4147 g/l to 0.5597 g/l) and Ca²⁺ (1.4188 g/l to 1.7034 g/l) were recorded. Sulphate and bicarbonate ions concentration was ranging from 1.79525 g/l to 3.551175 g/l and 0.061 g/l to 0.1403 g/l, respectively.

Isolation of halophilic strains. As a first step, Sehgal and Gibbon's medium with the following composition in g/l (Yeast extract: 10 g; Casamino acid: 7.5 g; Trisodic Citrate: 3 g; MgSO₄ · 7H₂O: 20 g; KCl: 2 g; FeCl₂ · 4H₂O: 36 mg; MnCl₂ · 4H₂O: 0.36 mg; Agar: 20 g) containing different NaCl concentrations (15%, 20% and 25%) (w/v) was used to isolate halophilic microorganisms by membrane filtration technique (0.2 µm). In spite of its high contents in proteins, vitamin B and amino acids, no colony was obtained on this culture medium. Furthermore, comparing with magnesium content of water samples, Sehgal and Gibbon's medium exhibits a low magnesium concentration, which could make it unsuitable isolation medium in our case. Another culture medium (MSH) with elevated level of magnesium was used later. After 7 to 18 days of incubation at 40°C, large number of white, pink, orange-red colonies was obtained. Only, pink

and orange-red colonies were picked and streaked for purification. However, many colonies failed to grow during repeated purification streaking steps. This growth failure may be due to the fact that the isolates were not sufficiently provided with culture conditions mimicking the in situ environmental conditions. In addition, accidental loss of some pure isolated strains after a number of streaking may be due the cell damage. This characteristic was actually assigned to the members of the Archaeal domain. It was noted that the growth rate on solid MSH medium was much slower than the growth on liquid MSH medium. The dry environmental conditions of the agar plates and the high salt concentration actually constitute a limitation factor of the microbial growth.

Phenotypic characterization of the isolates. Ten strains were chosen randomly to polyphasic identification. All isolates formed tiny, pink or red-orange-pigmented, round, smooth, opaque and convex colonies (Fig. 3).

Homogeneous cells suspensions in MSH liquid medium were difficult to prepare and require vigorous shaking. The red pigmentation characterized the most members of the family *Halobacteriaceae* was due to the richness of their membrane with C-50 carotenoid pigments (K-bacterioruberin and derivatives) associated in some cases by the bacteriorhodopsin (20). At 30°C, no pigmentation was observed for all strains. Cells of all strains were Gram negative and motile. Only the two strains 6.OF.3 and 5RS8 were rod-shaped while the rest were pleomorphics with unusual shapes (triangle and trapezoid cell shapes) which is widely distributed among haloarchaea, and especially the genus *Haloarcula* (Fig. 3) (21).

Table 1. Physicochemical characteristics of the water Rock Salt samples

Samples	pH	Conductivity ms/cm	Cl (g/l)	S (g/l)	Na ⁺ (g/l)	Ca ⁺² (g/l)	Mg ⁺² (g/l)	HCO ⁻³ (g/l)	SO ₄ ⁻² (g/l)
S1	7.07	233.33	202.36	356.57	n.d	1.524	0.4382	0.0732	3.5511
S2	6.87	233.67	231.97	409.06	n.d	1.554	0.4147	0.061	2.8312
S3	6.77	235	273.4	493.91	n.d	n.d	n.d	n.d	00
S5	6.55	n.d	194.11	350.67	n.d	1.733	0.4519	n.d	2.7163
S6	6.59	194.03	143.19	258.68	110	1.703	0.4483	0.1098	1.7952
S7	7.05	557.5	247.37	446.88	112.5	1.621	0.4851	0.1403	3.1796
S8	7.36	234.67	181.08	327.13	107.5	1.418	0.5151	0.0915	3.3915
S9	6.75	562.5	190.55	344.24	n.d	1.425	0.5597	n.d	3.525

n.d: not determined.

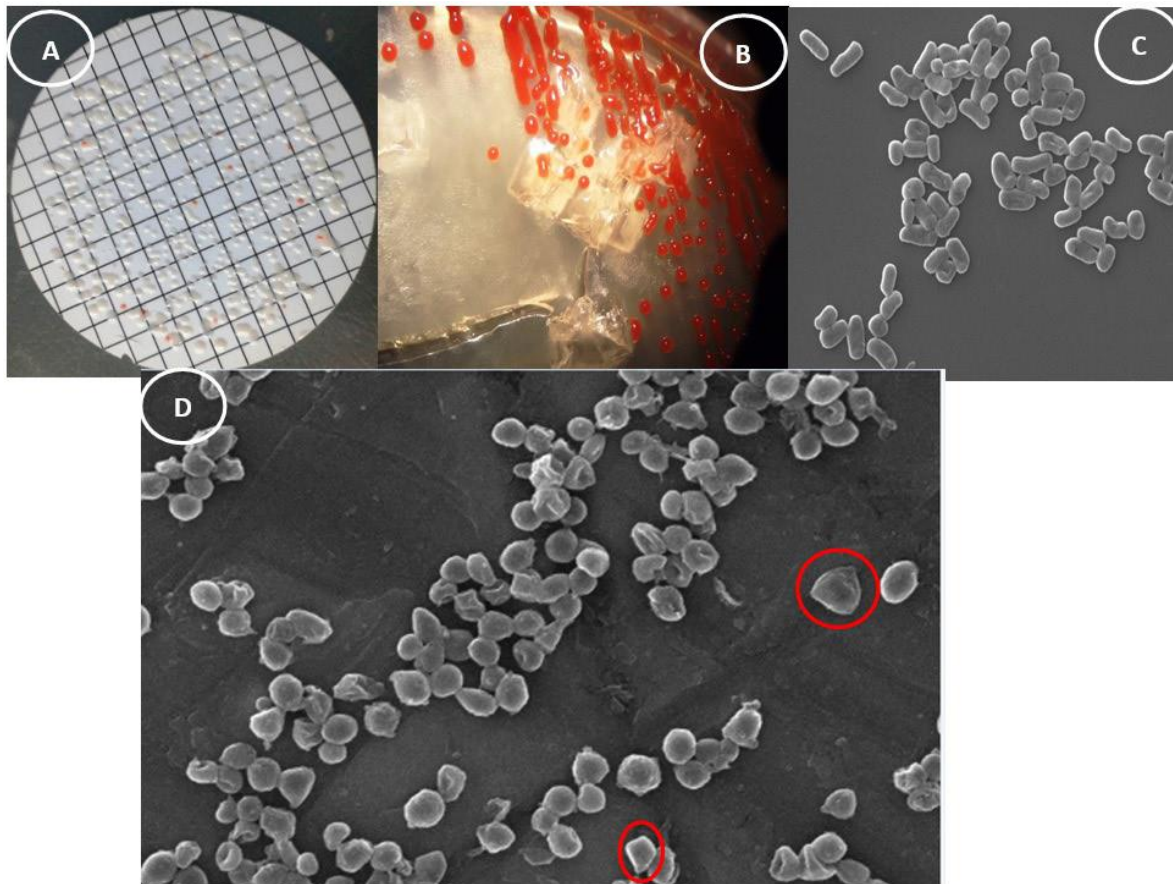


Fig. 3. Isolated colonies on 15% MSH and colony morphology. A. Isolated colonies obtained from S6 by membrane filter technique; white and red pigmented colonies are observed on membrane filter. B. Colonies of S2-2 on 20% MSH. C. Rod shaped cells of the isolated strain 5RS8. D. Pleomorphic cells (S1-1) with triangle and trapezoid shape.

All strains which were catalase and oxydase positive required high concentration of magnesium in the MSH culture medium (0.34 M). No growth occurred in the absence of one of the two magnesium salts sources. All isolates were found to be resistant to ampicillin, gentamycin, chloramphenicol, nalidixic acid and ceftazidin and sensitive to bacitracin and novobiocin. Sensitivity to tetracyclin, trimethoprim and neomycin was different among strains. Cell lysis of the isolated strains by Na-deoxycholate proved to be of a non-cocoid haloarcheal origin. All archaeal strains grew on MSH culture medium with NaCl concentration higher than 10% (w/v) with optimum of growth ranging from 15%-25% (w/v). However, Non-growth was observed in MSH culture medium with NaCl concentration lower than 10% (w/v). In addition, all strains lysed in distilled water. Eight isolates (8.OF.1, 1.OC.2, S2-2, S1-3, 6.OF.3, 9.OF.1, 9.OC.2 and S1-1) did not grow anaerobically on L-Arginine. Whereas, among ten surveyed strains, seven (8.OF.1,

S2-2, S1-3, 9.OF.1, 9.OC.2, S1-1 and 6.OF.1) were able to grow anaerobically with either nitrate or nitrite. Out of these seven strains, four (S1-3, 9.OF.1, 9.OC.2 and S1-1) carried out a complete denitrification with reduction of nitrate (NO_3^-) to dinitrogen (N_2) via (NO_2^-). The ability to use a single source of carbon test revealed that the lactose was not utilized by any of the strains.

Only two strains (S2-2 and 6.OF.3) were able to use arabinose as a single source of carbon. Saccharose and glucose were the most utilized sugars by most of the strains. However, glucose did not have a significant effect on growth compared with saccharose. While the strain S2-2 used most of the tested sugars, 5RS8 was the strain that uses less sugar. The restricted use of sugars as a single carbon source by 5RS8 revealed its belonging to the genus *Halobacterium*. 8.OF.1, 1.OC.2 and S2-2 were able to use starch, mannose, maltose, galactose, mannose and glycerol as sole carbon source.

Taxonomic characterization of the isolates. The polar lipids of halophilic archaea which represent the acetone insoluble fraction of the total extracted lipids are important taxonomic markers to determine the genus level of the isolates (22). The lipid extract was separated by TLC, and the principal polar lipids were determined by comparing the R_f values of isolated strains with R_f from the literature review. According to the results, the presence of the methylated phosphatidylglycerophosphate (Me-PGP) in all strains proved their belonging to the family *Halobacteriaceae* (15). The phospholipid (PGS) found in all strains indicated their placement among members of one of the two genera *Halobacterium* or *Haloarcula* (21). Both strains 6.OF.3 and 5RS8 had the same lipid profile that characterized the genus *Halobacterium*; they were both characterized by the presence of tetraglycosylarchaeosulphate (S-TeGD) and sulfated triglycosyldiphytanylglycerol (S-TDG-1) (23). S2-2, 1.OC.2, S1-3 and 8.OF.1 showed the presence of TGD-2. This glycolipid characterized the genus *Haloarcula* (21).

Phylogenetic analysis. Genomic DNA of each isolated strain was extracted and purified. 16S rRNA genes were amplified by using archeal forward and reverse primer (20F and 1540R). All strains showed an amplicon of 1500 bp. The Genbank accession numbers of the resulting 16S rRNA gene sequences were from OM089650 to OM089659.

Comparison of 16S rRNA gene sequences with reference sequences of 16S rRNA gene of representative members of the *Halobacteriaceae* family available in EzBioCloud server database showed that all strains belong to the family *Halobacteriaceae*. Seven isolated strains (S2-2, S1-3, 8.OF.1, 1.OC.2, 9.OF.1, 9.OC.2 and S1-1) were placed in the genus *Haloarcula*, two strains (5RS8 and 6.OF.3) belonged to the genus *Halobacterium* and one isolate (6.OF.1) was related to the genus *Haloferax*. Thus, the Eztaxon result of the 16S rDNA sequences extracted from different tested strains indicated that S2-2 and 8.OF.1 exhibited 99.90% and 99.47% similarity with *Haloarcula argentinensis* JCM 9737 strain, respectively. However, 9.OC.2, S1-1 and 9.OF.1 were close to *Haloarcula hispanica* ATCC 33960 strain with similarity of about 99%. S1-3 was also affiliated to *Haloarcula hispanica* ATCC 33960 with 98.53% of similarity. 1.OC.2 was highly related to *Haloarcula salaria* HST01-2R with 99.30% of sequence similarity. 6.OF.1 showed high

similarity (99.90%) with *Haloferax lucentense* DSM 14919. Two strains (5RS8 and 6.OF.3) were homologous to *Halobacterium salinarum* NRC-1 strain with 99.86% and 98.84% similarity, respectively (Fig. 4). The same Neighbor-joining tree (Fig. 4) obtained by using different substitution models showed that 8.OF.1 and *Haloarcula salaria* HST01-2R were closely related (99.47% sequence similarity). Furthermore, the isolated strains S2-2 shared with *Haloarcula hispanica* ATCC 33960 99.50% sequence similarity. As for the sequence of the 16S rRNA gene corresponding to 6.OF.1, it was clustered within the clade that included *Haloferax lucentense* DSM 14919 and *Haloferax alexandrinus* TM (99.80% sequence similarity). However, the isolated strain 1.OC.2 which was not strongly related to other members of the genus *Haloarcula* may belong to a novel species which requires another molecular markers such as DNA-dependent RNA polymerase subunit B' gene (*rpoB'*), DNA-DNA hybridization and DNA G+C content.

Extracellular hydrolytic enzymes. All tested strains were able to degrade gelatine. Only one strain (S2-2) revealed a positive esterase, lecithinase and protease activity. No strain was able to produce lipase or cellulase enzyme. Three strains (S2-2, 9.OF.1 and 9.OC.2) were characterized as amylase-producing archaea.

DISCUSSION

This study was based on the isolation and polyphasic characterization of ten different archaeal strains from the rock salt brines of Djelfa located in semi-arid regions of Algeria. The high salinity, the richness in Cl^- and the pH which was slightly acidic to neutral indicated that the brines of the rock salt were convenient habitats for halophilic archaea which require at least 1.5 M NaCl for growth with optimal growth at 3.5 M to 4.5 M NaCl and pH 7.0 to 7.5 (23). However, because of the high concentrations of Mg^{2+} and Ca^{2+} , which were ten times and fifty times higher than the Dead Sea water (0.0461 g/l; 0.0237 g/l), respectively (24), the brines of Djelfa's rock salt were even more hostile thalassohalin environment for microbial life. Mg^{2+} has a chaotropic influence on proteins and other cellular components. Lunescu et al indicated that the Dead Sea is an extreme environment where most species cannot tolerate the increased salinity and the

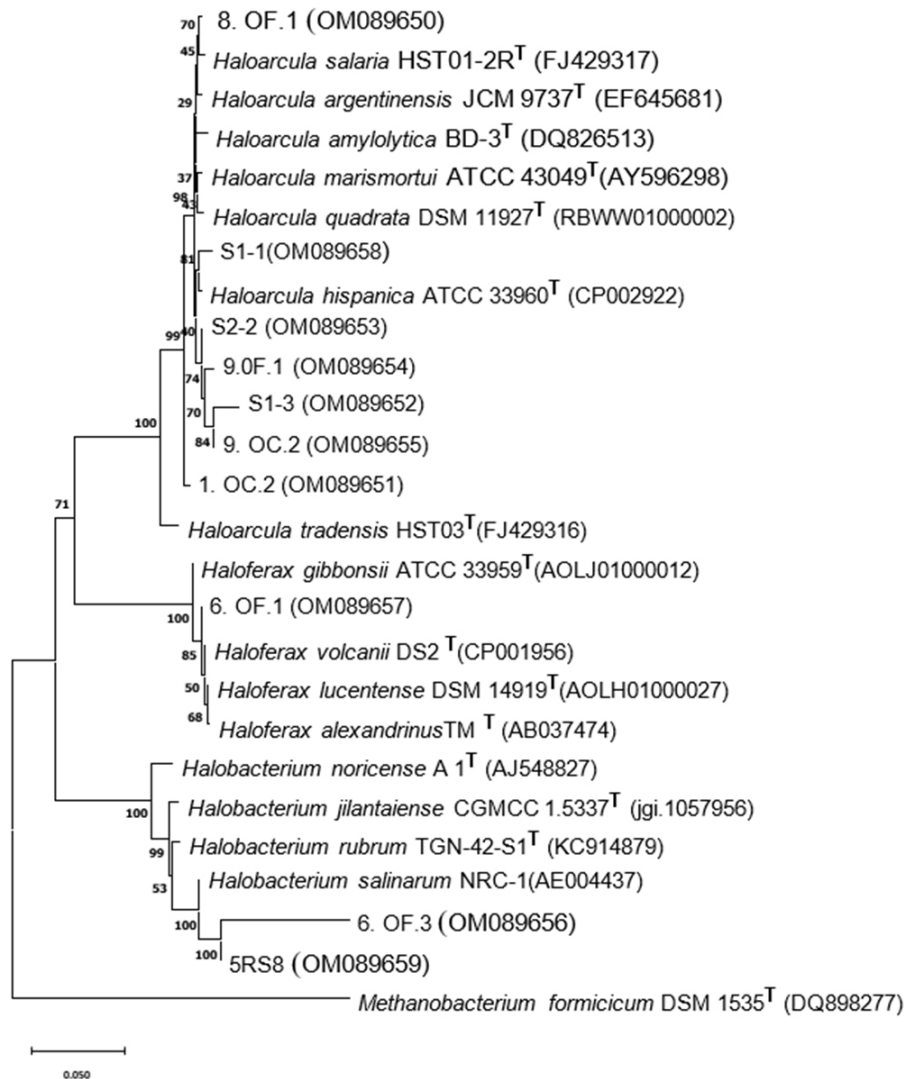


Fig. 4. Molecular phylogenetic analysis of the haloarchaeal isolated strains based on the 16S rRNA gene sequences. The Neighbor-joining tree was inferred using the Kimura 2-parameter method and expressed in terms of the number of base per site. All ambiguous positions were deleted (pairwise deletion option). The final dataset contained 1495 positions in total. MEGA X was used to perform the evolutionary analysis.

high concentration of Mg^{2+} ions. This reflects the low abundance of microorganisms in the Dead Sea (25). In spite of the extreme physicochemical conditions, Hadjr El melh of Djelfa hosts a variety of extremely halophilic archaea represented mainly by the genus *Haloarcula*, *Haloferax* and *Halobacterium*. These results were confirmed by the sequential analysis of the 16S rRNA gene. *Haloarcula*, *Halorubrum* and *Natrinema* are the main genera isolated from Meyghan Lake in Iran (26). However, the phylogenetic analysis carried out by Menasria et al. on 68 halophilic archaeal strains isolated from saline sediments

in Algeria showed that among seven identified genera (*Haloferax*, *Haloarcula*, *Halococcus*, *Halogeometricum*, *Haloterrigena*, *Natrialba* and *Natrinema*) within the class *Halobacteria*, *Haloferax* is the dominated genus followed by *Halococcus* (27). In another Algerian study, Boutaiba et al reported six different haloarchaeal genera, including *Haloferax*, *Halobacterium*, *Haladaptatus*, *Halalkalicoccus*, *Halorubrum* and *Halosarcina* (2). Phylogenetic analysis of 16S rRNA genes indicated that each hypersaline ecosystem has its own species richness where its members distribution depend mainly on total salt concentra-

tion, ionic composition, nutrient availability and some physical conditions. The qualitative test of extracellular hydrolytic enzymes production (amylase, protease, gelatinase, esterase, lipase, lecithinase and cellulase) by the isolated archaeal strains revealed that amylase and gelatinase was the most frequently encountered hydrolytic activities. Only one strains (S2-2) related to *Haloarcula hispanica* ATCC 33960 exhibited five hydrolytic activities (gelatinae, protease, esterase, licithinase and amylase). This strain could be useful in biotechnological applications. Two strains (9.OF.1 and 9.OC.2) demonstrated two hydrolytic activities (amylase and gelatinase). Nevertheless, cellulase and lipase activities were absent within all isolated strains. Finding in this study agree with a previous study carried out on Algerian arid and semi-arid wetland ecosystems where halophilic archaea display a large spectrum of hydrolytic enzyme ability (27). In contrast to the current study, Robert et al. established that the hydrolytic activity of the acrchaeal strains is limited in the production of amylase and cellulase (19).

CONCLUSION

In conclusion, the obtained results that showed the presence of members of the *Halobacteriaceae* family could help with archaeal taxonomy, in particular the halophilic archaeal species from the rock salt brines of Djelfa. Furthermore, the attempt to assess their ability of enzyme production by qualitative test could make the rock salt of Djelfa a good source for isolating halophilic archaea and bacteria that produce industrial enzymes.

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REFERENCES

- Hacene H, Rafa F, Chebhouni N, Boutaiba S, Bhatnagar T, Baratti JC, et al. Biodiversity of prokaryotic microflora in El Golea salt lake, Algerian Sahara. *J Arid Environ* 2004; 58: 273-284.
- Boutaiba S, Hacene H, Bidle KA, Maupin-Furlow JA. Microbial diversity of the hypersaline Sidi Ameur and Himalatt salt lakes of the Algerian Sahara. *J Arid Environ* 2011; 75: 909-916.
- Khallef S, Lestini R, Myllykallio H, Houali K. Isolation and identification of two extremely halophilic archaea from sebkhas in the Algerian Sahara. *Cell Mol Biol (Noisy-le-grand)* 2018; 64: 83-91.
- Mckew BA, Taylor JD, Mcgenity TJ, Underwood GJC. Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. *ISME J* 2011; 5: 30-41.
- Oren A. The microbiology of red brines. *Adv Appl Microbiol* 2020; 113: 57-110.
- Schneegurt MA (2012). Media and conditions for the growth of halophilic and halotolerant Bacteria and Archaea. In: *Advances in Understanding the Biology of Halophilic Microorganisms*. Ed, Russell H. Vreeland. Springer, pp. 35-58.
- Yildiz E, Ozcan B, Caliskan M. Isolation, characterization and phylogenetic analysis of halophilic Archaea from a salt mine in central Anatolia (Turkey). *Pol J Microbiol* 2012; 61: 111-117.
- Ventosa A, Marquez C, Sanchez-Porro C, de la Haba R R (2012). Taxonomy of Halophilic Archaea and Bacteria. In: *Advances in Understanding the Biology of Halophilic Microorganisms*. Ed, Russell H. Vreeland. Springer, pp. 59-80.
- Grasshoff K, Kremling K, Ehrhardt M (2007). *Methods of Seawater analysis*. 3rd, completely revised and extended ed. Wiley.
- Montalvo-Rodriguez R, Lopez-Garriga J, Vreeland RH, Oren A, Ventosa A, Kamekura M. *Haloterrigena thermotolerans* sp. nov., a halophilic archaeon from Puerto Rico. *Int J Syst Evol Microbiol* 2000; 50: 1065-1071.
- Oren A (2014). The family *Halobacteriaceae*. In: *The prokaryotes*. Ed, Rosenberg E., deLong E.F., Lory S., Stackebrandt E., Thompson F. Springer, Berlin, Heidelberg, pp. 41-121.
- Fischer ER, Hansen BT, Nair V, Hoyt FH, Dorward DW. Scanning electron microscopy. *Curr Protoc Microbiol* 2012; 25:2B.2.1-2B.2.47.
- Ruginescu R, Purcăria C, Dorador C, Lavin P, Cojoc R, Neagu S, et al. Exploring the hydrolytic potential of cultured halophilic bacteria isolated from the Atacama Desert. *FEMS Microbiol Lett* 2019; 366: fnz224.
- Kamekura M, Oesterhelt D, Wallace R, Anderson P, Kushner DJ. Lysis of halobacteria in bacto-peptone by bile acids. *Appl Environ Microbiol* 1988; 54: 990-995.
- Litchfield CD, Oren A (2001). Polar lipids and pigments

- as biomarkers for the study of the microbial community structure of solar salterns. In: *Saline Lakes*. Ed, J.M. Melack, R. Jellison & D.B. Herbst. Kluwer Academic Publishers, Netherlands, pp. 81-89.
16. Enache M, Itoh T, Kamekura M, Teodosiu G, Dumitru L. *Haloferax prahovense* sp. nov., an extremely halophilic archaeon isolated from a Romanian salt lake. *Int J Syst Evol Microbiol* 2007; 57: 393-397.
 17. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 2018; 35: 1547-1549.
 18. Gascuel O, Steel M. Neighbor-joining revealed. *Mol Biol Evol* 2006; 23: 1997-2000.
 19. Ruginescu R, Gomoiu I, Popescu O, Cojoc R, Neagu S, Lucaci I, et al. Bioprospecting for novel halophilic and halotolerant sources of hydrolytic enzymes in brackish, saline and hypersaline lakes of Romania. *Microorganisms* 2020; 8: 1903.
 20. Oren A. Molecular ecology of extremely halophilic Archaea and Bacteria. *FEMS Microbiol Ecol* 2002; 39: 1-7.
 21. Oren A, Arahal DR, Ventosa A. Emended descriptions of genera of the family *Halobacteriaceae*. *Int J Syst Evol Microbiol* 2009; 59: 637-642.
 22. Lobasso S, Lopalco P, Mascolo G, Corcelli A. Lipids of the ultra-thin square halophilic archaeon *Haloquadratum walsbyi*. *Archaea* 2008; 2: 177-183.
 23. Oren A, Rodrigues-Valera F. The contribution of halophilic Bacteria to the red coloration of saltern crystallizer ponds. *FEMS Microbiol Ecol* 2001; 36: 123-130.
 24. Jacob HJ, Hussein EI, Shakhathreh MAK, Cornelison CT. Microbial community analysis of the hypersaline water of the Dead Sea using high-throughput amplicon sequencing. *Microbiologyopen* 2017; 6(5): e00500.
 25. Ionescu D, Siebert C, Polerecky L, Munwes YY, Lott C, Häusler S, et al. Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. *PLoS One* 2012; 7(6): e38319.
 26. Naghoni A, Emtiazi G, Amoozegar MA, Cretoiu MA, Stal LJ, Etimadifar Z, et al. Microbial diversity in the hypersaline Lake Meyghan, Iran. *Sci Rep* 2017; 7: 11522.
 27. Menasria T, Aguilera M, Hocine H, Benammar L, Ayachi Ammar, Si Bachir A, et al. Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes. *Microbiol Res* 2018; 207: 289-298.