

EXTREMELY HALOPHILIC ARCHAEA FROM ALGERIAN SALT LAKES: ISOLATION, PHYLOGENETIC IDENTIFICATION AND BIOPROSPECTION OF HYDROLYTIC ENZYMES

KHELFAOUI, M. E. A.¹ – LENCHI, N.^{2,3*} – KEBBOUCHE-GANA, S.³ – GANA, M. L.⁴ – MEDHKOUR, H.⁵
– GRINE, G.⁵ – KHEMILI-TALBI, S.³ – AKMOUSSI-TOUMI, S.³

¹ *Laboratoire de recherche Conservation et Valorisation des Ressources Biologiques, Faculté des Sciences, Université M'Hamed Bougara de Boumerdes, Independence Avenue, Boumerdes 35000, Algeria*

² *Department of Natural and Life Sciences, Faculty of Sciences, University Algiers 1 BenYoucef Benkhedda, Algiers, Algeria*

³ *Bioinformatics, Applied Microbiology and Biomolecules Laboratory, Faculty of Sciences, University M'Hamed Bougara of Boumerdès, Boumerdès, Algeria*

⁴ *Research and Development Center, SONATRACH, Independence Avenue, Boumerdes 35000, Algeria*

⁵ *Aix Marseille University, MEPHI, IHU Fondation Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France*

**Corresponding author*

*e-mail: n.lenchi@univ-alger.dz ; phone: +213-555-975-420
m.khelfaoui@univ-boumerdes.dz*

(Received 25th Jan 2022; accepted 2nd May 2022)

Abstract. Hyper-saline aquatic ecosystems are among the most inhospitable habitats where life can be found; Chotts and sebkhas constitute a model of extreme environments where lives a halophilic microflora adapted to these conditions. These habitats are not sufficiently studied in Algeria and this research has been carried out on four distant and different sebkhas where the salt reaches more than 300 g/L. The physical-chemical analysis showed that these waters contain numerous minerals, of which the chlorides are the most dominants. The study of the microflora revealed the presence of an important morphologic, physiologic, metabolic and phylogenetic diversity, including archaeal species. Twelve strictly halophilic archaea (XA1, XC1, XC2, XE1, XE2, WA1, WC1, YA1, YA2, YF2, ZC1 and ZD1) were selected for further characterization. The growth occurred between 15 and 30% (w/v) NaCl with an optimum at 20% (w/v) NaCl. 16S rRNA gene sequencing revealed that the strains belonged to *Halorubrum sp*, *Natribaculum sp*, *Haloarcula sp*, *Natrinema sp*, *Halostagnicola sp*, *Haloferax sp* and *Haloterrigena sp* genera. Noting that these halophilic *Archeae* are capable of producing different enzymes at high salt concentrations (20% NaCl). The production yields obtained are very promising for applications in the biotechnology and industrial microbiology.

Keywords: *extreme environments, halophiles archaea, phylogenetic diversity, enzymes*

Introduction

A certain number of particular ecosystems have been formed, following climatic and geophysical changes, in the course of the evolution of the earth (Henriet et al., 2014). These so-called “extreme” ecosystems are special habitats whose physicochemical conditions are, not very favorable to the development of life, characterized by the absence of several life forms and by a distinct microbial diversity (McGenity and Grant, 1995). The organisms found there must have particular properties to be able to

proliferate under these hostile conditions (Kebbouche-Gana et al., 2009). Among the extreme environments, ecosystems with high or low pH values, high or low temperatures or high content of salt can be found in many parts of the world (Antranikian and Egorova, 2007). This is the case of the hyper-saline aquatic environments that contain in their solutions mineral salts that can reach saturation; usually referred to as sebkha or over-salted mediums.

Arid and Saharan habitats (North-Western Algerian Sahara), characterized by a saline and gypsum-saline substrate, occupy small areas. This type of habitat corresponds to depressions known as Chott or Sebkh. Geologically and topographically, Sebkh are depression in the form of basins, periodically flooded, in which an accumulation of salt occurs. They function as evaporation tanks, which after drying, reveal a layer of salt whose concentration is maximum in the center and decreases towards the periphery, hence a composition of the vegetation according to the degree of salinity. These media have a dissolved salt content higher than that of seawater (Baliga et al., 2004). When one exceeds 100 g/l in salt, the media becomes extreme and inhibits the growth of a large majority of microorganisms (Antranikian and Egorova, 2007).

In hyper-saline environments, *Halobacteria* (members of the halophilic *Archaea*) are the dominant organisms. The halophilic *Archaea* are associated with prokaryotes (Antranikian and Egorova, 2007). They are cells without nucleus, very diverse in morphology as well as in physiology (Menasria et al., 2018). In addition to their gene sequences coding for ribosomal RNAs, they are distinguished from the other two kingdoms by many points concerning the structure and chemistry of the wall, the structure of membrane lipids and certain metabolic pathways (Castillo et al., 2006).

In Algeria, the hyper-saline Chotts and Sebkh aquatic ecosystems have been few studied; they are still rich in divers microorganisms (Kharroub et al., 2014; Quadri et al., 2016; Lenchi et al., 2020). Genera that have been retrieved are: *Halorubrum*, *Halobacterium* and *Haloterrigena* isolated by Menasria et al. (2018); the genera *Haloarcula* and *Halovivax* isolated by Kebbouche-Gana et al. (2009); the genera *Haloarcula*, *Halorubrum*, *Natrialba*, *Halovivax* and *Haloferax* are isolated by Quadri et al. (2016) and Imadalou-Idres et al. (2013) and the species *Haloferax mediterranei* isolated by Akmoussi-Toumi et al. (2018). These halophilic microorganisms have different biotechnological applications such as, pigments for food coloring, extracellular polysaccharides (EPS), poly-hydroxyalkanoate used in the production of biodegradable plastic, the production of fermented foods, compatible solutes and hydrolytic enzymes (Bajpai et al., 2015). The reports on the diversity of halophilic microorganisms producing hydrolytic enzymes in hypersaline habitats, such as solar salt marshes, salt lakes, salt deserts and salt deposits are sparse (Makhdoumi-Kakhki et al., 2011).

Due to its biotechnological potential and industrial applications, the detection and isolation of hydrolytic enzymes produced by extreme halophiles is an interesting research field. These enzymes, including amylase, lipase, protease, gelatinase and cellulase, are the most used in the industrial field such as the process of biosynthesis, environmental bioremediation and food processing (Makhdoumi-Kakhki et al., 2011; Delgado-García et al., 2012). They tend to be very stable in organic solvents that allow their use in green chemistry (Oztetik and Cakir, 2014). These microorganisms considered non-pathogenic are found in hyper-saline lakes, the Dead Sea (Jordan), Salt Marshes (Spain) and in large, extremely saline alkaline lakes such as Oued Natron in Egypt, Lake Magadi in Kenya, ponds in solar distillation and Salt Lake City (USA) (Menasria et al., 2018).

To our knowledge, except the sebkha of Tinsilt in Oum el Bouaghi, there is no study on the diversity of the Archean communities living in the Sebkhas of El Outaya in Biskra, Ain Beida and Oum Reneb in Ouargla in the Algerian Sahara Desert. The main purpose of this research was to isolated halophilic archaeal strains from different salt lakes of Algerian desert and characterize them with respect to some phenotypic and phylogenetic characteristic, with a view to screening for enzymes of an industrial and biotechnological interest.

Materials and methods

Geographical location

The sampling study was carried out in Sebkhha El Outaya (Biskra), Tinsilt (Oum el Bouaghi), Ain Beida and Oum Reneb (Ouargla) in January 2020. Biskra location is in the north-east of the Algerian Sahara. This sebkha is characterized by a depth of 150 cm and an area of 50 m²; it is located 28 km north of the city of Biskra (35°01' North, 5°44' East and Altitude 282 m). The location of Oum El Bouaghi is in northeast Algeria. This sebkha is characterized by a depth of 50 cm and an area of 2.15 ha; it is located in the region of Ain M'Lila 5 km from the Commune of Souk-Naâmane and 17 km south of the city of Aïn-M'lila (35°53'14" Nord, 06°28'44" East and Altitude 792 m). Ouargla's location is in south-eastern Algeria. the sebkha of Ain Beida is characterized by a depth of 60 cm and an area of 6.85 hectares; it is located east of the wilaya of Ouargla (6 km) and near the town of Ain El Beida (31°59'2" North, 5°21'52" East and Altitude 139 m) and the sebkha of Um Reneb is characterized by a depth of 70 cm and an area of 7.15 hectares; it is located to the north of the wilaya of Ouargla (10 km) and in the municipality of Sidi Khouiled (32°01'31" North, 5°21'51" East and Altitude 150 m) (see *Fig. 1*). The geographical position of the study areas, the Aurès Mountains in the north, and the Sahara in the south, gives it an unstable arid climate with frankly Saharan tendencies. Climate is influenced relatively by the cold weather especially in winter and by the high temperatures due to the winds blowing from the south in summer and the sirocco. Therefore, the vegetation is spontaneous and governed by both climatic conditions and degradations from anthropogenic causes. Apart from the irrigated areas, the reliefs are practically devoid of vegetation.

Sample collection

Water samples are obtained from three different points in the shape of a triangle; on the surface and at a depth of 20 cm. The three samples were mixed in order to obtain a representative sample of 200 ml (American Public Health Association, 2005). Water was collected in sterile jerry cans, completely filled after three overflows and sealed directly with screw caps to avoid contamination. The samples were immediately transported at ambient temperature to the laboratory and stored at 4 °C until analyses. Samples were treated within 24 h after collection. Temperature, pH, electrical conductivity (Cs) and salinity were measured *in situ* using a multi-parameter probe (Symphony, VWR).

Water samples analysis

The physico-chemical analyzes of water samples are carried out at the Research and Development Center (CRD) according to standard methods 4500-S⁻² F (American

Public Health Association, 2005). The chemical and physical properties included estimates of the composition of Na^+ , Ca^{2+} and K^+ by spectrophotometry with DR 2000 flame ionization. Mg^{2+} by a complexometry method using ethylene diaminetetraacetic acid and EDTA. Cl^- at 497 nm, multi-ray spectrophotometry (DR2000). CO_3^- and HCO_3^- by colorimetry at 497 nm. Nitrates by colorimetry at 520 nm. SO_4^- by colorimetry at 495 nm and salinity by electrochemistry (NF EN 27-888 (1994)). The different characteristics are given in *Table 1*.



Figure 1. Location of sampling sites

Enrichment and halophilic archaea isolation

Cultivation of the halophilic species requires a stage of reactivation and enrichment (Oren, 2008). A specific medium for aerobic, neutrophilic and alkalophilic halophilic microorganisms given by Oren (2008), has been used, it is used both for enrichment, isolation, and also for physiological identification tests, containing (per liter); 250 g of NaCl, 6 g of KCl, 29 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 19.5 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.1 g of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.8 g of NaBr, 0.2 g of NaHCO_3 and 5 g of yeast extract, adjusted to pH 7 with HCl. The sodium chloride and sodium bicarbonate are sterilized separately in 250 ml bottles.

They are added extemporaneously to the other components of the medium (in order to avoid precipitation of the salt). Enrichments are made in 250 ml Erlen Meyer autoclaved at 120 °C for 20 min, filled with 100 ml of culture medium then inoculated 1/10 with the water sample and the various dilutions prepared. Incubation of the cultures takes place in a shaker incubator at 40 °C with a stirring speed of 120 rpm, this temperature promotes the growth of most *Halobacteriaceae* (Oren, 2008). So, and dilutions of 10^{-1} to 10^{-4} were made on the same agar medium solidified with 20 g of agar. After 7-10 days of incubation at 40 °C, the pigmented colonies were picked and sub-cultured several times to obtain a pure culture.

Physiological and biochemical morphological characterization of isolates

The identification of *Halobacteria* is based on the study of phenotypic, physiological, biochemical and phylogenetic characteristics according to several recommendations (Amoozegar, 2017; Joint, 2010; Oren, 2008). The macroscopic study of colonies is considered the first step that guides the progress of strain identification. Macroscopic examination of the colonies obtained on the solid medium based on morphological characters observed with the naked eye such as colony shape, pigmentation, diameter, elevation and opacity. The study of micro-morphological characters allows to have an observation of the isolates in the fresh state in the absence of any fixation or staining in a saline solution (20%). The modified Gram stain applied to the halophilic microflora was performed by samples fixed with acetic acid as described by Kebbouche-Gana et al. (2009). To study certain classification criteria, liquid enrichment media have undergone modifications by varying the concentration of NaCl (%) (5, 10, 15, 20, 25, 30, and 35) (p/v), pH (4.0, 6.0, 7.0, 8.0 and 9.0) and temperature (10, 25, 32, 37, 42, 50 and 60 °C). This study is carried out by varying one of the parameters while the other two are kept constant, the latter being inoculated with archaeal cultures aged 72 H in 100 ml Erlenmeyer flasks containing 20 ml each of medium or it is isolated, in the presence of controls (tubes containing the unseeded sterile culture medium) (Kebbouche-Gana et al., 2009). The growth in anaerobiosis is affected by the use of another acceptor of electrons such as nitrate. The enzymatic activities of oxidase and catalase were tested using standard procedures (Oren and Ventosa, 2000). The reduction of nitrates was tested using the liquid enrichment medium supplemented with 0.1% KNO₃ (w/v) (Oren, 2008). The TSI medium confirms the fermentation of glucose and the attack of lactose and sucrose, the production of H₂S and the gas, using Kligler-Hajna medium supplemented with 25% (w/v) NaCl, in incubating at 40 °C for 7 days. The indole formation was tested after 7 days of incubation at 40 °C in a liquid medium in the presence of tryptone (Oren, 2008). Some strains have the ability to use different sources of carbon to grow; this source is sometimes a sugar (glucose, sucrose, lactose, arabinose and rhamnose), an alcohol (inositol, sorbitol and mannitol) or an organic acid (citrate). To study this capacity, we performed the various tests given by Oren (2008). The other phenotypic characteristics were tested using API 20E (Biomérieux Kit), Modified by adding sterile NaCl. The study of antibiotic susceptibility is a criterion for the classification of strains. The test consists of determining the resistance or sensitivity of isolated archaeal strains to certain antibiotics: Nalidixic acid, 30 µg; Ofloxacin, 5 µg; Penicillin G, 6 µg; Ampicillin and derivatives, 10 IU; Amoxicillin + clavulamic acid, 20/10 µg; Erythromycin, 15 µg; Pristinamycin, 15 µg; Trimethoprim, 1.25/23.75 µg; Furans, 30 µg; Gentamicin, 30 µg; Chloramphenicol/Thiamphenicol, 5 µg; Tetracycline, 30 µg; Oxacillin, 5 µg;

Cefotaxime, 30 µg; Cefalexin, 30 µg; Novobiocin, 30 µg; Streptomycin, 300 µg; Bacitracin, 200 µg. The test is performed on solid medium, inoculated with young archaeal cultures and cultured with the presence of antibiotic discs, then incubated at 40 °C for one week (Kebbouche-Gana et al., 2009).

16S rRNA gene sequencing of the isolates

The extraction of the DNA from each strain tested is carried out with the PCI method (phenol/chloroform/isoamyl alcohol) (Baliga, 2004; Castillo, 2007; Quadri, 2016). The gene coding for the 16S RNA is amplified by PCR from the previously extracted genomic DNAs. The partial 16S rRNA gene was amplified using broad-spectrum *Archaea* primers SDArch0333aS15 (5'-TCCAGGCCCTACGGGG-3') and SDArch0958aA19 (5' YCCGGCGTTGAMGCCGATTCCAATT-3') (Tamura, 2013). In order to verify the presence of DNA and the size of the fragments of the PCR products, agarose gel electrophoresis is performed (Tamura, 2013). The amplicon was sequenced using the Sanger method at the laboratory Microbes, Evolution, Phylogeny and Infection (MEPHI) (Marseille-France). The alignment of the sequences for the homology search with the closest sequences set to the database was carried out using the program: the Basic Local Alignment Search Tool (BLAST) on the NCBI (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov). The sequences are then deposited in GenBank data base and an accession number is obtained for each strain. Phylogenetic tree construction based on strains sequences and database sequences was constructed using the Neighbor-Joining algorithm implemented in MEGA 7.0 (Tamura, 2013).

Screening of extracellular hydrolytic enzymes

The search for halophilic enzymes is such an important criterion. The search for enzymatic activity was carried out on agar plates using a drop technique after incubation at 40 °C for 7 days. The cultures used for the enzymatic screening were obtained by cultivating the halophilic microorganisms in 10 ml of enrichment media supplemented with 20% NaCl while stirring at 120 revolutions per minute for approximately one week. A sample of (10 µl) of liquid culture from each test culture was identified on an appropriate medium. The subsequent experimentation was carried out in duplicate according to the standard protocols described below (Akmoussi-Toumi et al., 2018; Menasria et al., 2018).

Amylase activity

The detection of the amylase is carried out by cultivating the strains to be tested on a solid culture medium supplemented with 0.1% of the starch; the presence of amylase activity was confirmed by the appearance of a clear halo zone around the colonies after staining with Gram's iodine solution (Abd-Elhalem et al., 2015; Akmoussi-Toumi et al., 2018; Menasria et al., 2018).

Cellulase activity

The presence of cellulase is examined on enrichment medium containing 0.5% (w/v) Carboxy-Methyl Cellulose (CMC) (Saxena et al., 2007). The results were expressed by the appearance of clarification zones around the archeal colony (Latorre et al., 2016).

Lipolytic activity

The search for lipase is carried out on a medium supplemented with 0.1% Tween 80 indicator. An opaque halo around the colonies indicated the positive lipolytic activity (Ghanem, 2007).

Gelatine hydrolysis

The detection of gelatinase is carried out on a solid medium supplemented with 0.1% of gelatin indicator, the appearance of a clear zone around the colony indicated gelatinase positive (Delgado-García et al., 2012).

Caseine hydrolysis

The presence of caseinase is achieved by adding to the double-agar isolation medium the same volume of milk containing no fat. The positive reaction results in zones of clarification around the spots (Delgado-García et al., 2012).

Results and discussion

Water analysis

In the purpose of isolating and characterizing of the halophilic *Archaea* present in the studied Sebkhas, the physicochemical analysis of water samples was firstly performed. Besides studying its salinity and ionic composition, *Table 1* presents the results of the salt analyzes in the sample compared to other hyper-saline ecosystems. The hyper-salinity of the studied sebkhas, located in the North-East of the Algerian Sahara, comes from the dissolution of salts of continental origin; these waters are classified as athalassohaline. This is the case of the Pink Salt Lake in Senegal and most sebkhas located in semi-arid and arid zones (Atanasova et al., 2013; Liu et al., 2015; Roussel et al., 2008). These environments have a saline ionic composition different from that of seawater (Litchfield and Dalmat, 2009). The day of the sampling; the water temperature was 10 °C. This average temperature is explained by a sampling carried out in winter (January). The waters are alkaline with a pH value of 8.07. We noted that these pH values are close to that of the Great Salt Lakes in the USA (Grant et al., 2011) and higher than that of the Dead Sea (5.9 - 6.3) but remains well below that of Wadi Natrun of Egypt (pH 11) (Quadri et al., 2011). The waters analyzed are highly mineralized, in which the salinity equal 374 g per liter and Na⁺ and Cl⁻ ions are dominant, such as those found in the large salt lakes (USA) and Lake Natrun in Egypt and the Dead Sea in Jordan (Madigan and Martinko, 2006). The concentration of Na⁺ ion is much higher than that of the Dead Sea. On the other hand, the high chloride content is also noted in the Dead Sea in Jordan (Madigan and Martinko, 2006). As for sulfate ions (SO₄⁻), it is also observed that they are present in relatively high concentration. Sulphate is the most oxidized form of sulfur. It plays an essential role in the life cycle, thus promoting the development of sulphate-reducing bacteria (Gana et al., 2010). In addition, the total alkalimetric titre (HCO₃⁻) of the two samples is low, close to that of the Dead Sea (0.2 g/l). These ions are used as a carbon source by the halophilic autotrophic (Grant et al., 2006). These waters have a considerable content of Mg⁺⁺ ion and a mean concentration of Ca⁺⁺ and K⁺ ions. In fact, these trace elements act as cofactors or enzymatic activators (Amoozegar et al., 2017; Madigan and Martinko, 2006; Oren,

2008). In particular, Mg⁺⁺ ions, which promote the proliferation of extreme halophilic flora, Ca⁺⁺ ions are necessary for the bacterial metabolism (Grant et al., 2011; Madigan and Martinko, 2006; Oren, 2008).

Table 1. Chemical properties of the studied salt lakes of the North Algerian Sahara compared to other hyper-saline and marine ecosystems

Ecosystems	Chemical properties								
	pH	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	Salinity
Chott of Biskra (Algeria)	8.7	190	7.41	2.16	0.5	207	69	0.21	374
Chott of Oum Bouaghi (Algeria)	7.9	157	1	6.45	0.7	180	45	0.95	324
Chott of Ain Beida (Algeria)	8.07	120	0.21	2.74	0.1	124	66	0.16	224
Chott of Oum Reneb (Algeria)	8.10	117	0.19	2.62	0.13	184	66	0.15	332
Caspienne Sea ¹	8.3	3.18	0.09	0.73	0.34	5.33	3.0	0.4	12.8
Aral Sea ¹	8.2	2.2	0.08	0.55	0.51	3.47	3.2	0.07	10.2
Great Salt Lake (USA) ¹	7.7	105	6.7	11.1	0.3	181	27	0.72	333
Lake Natrun (Egypt) ¹	11	142	2.3	UD	UD	155	22.6	67	394
Lake Magadi (Kenya) ¹	11.5	46	0.06	0.8	0.62	14	nd	34.9	277
Salt Lake El Goléa (Algeria) ²	9	107	nd	0.3	0.4	198	nd	nd	296
Chott of Ouargla (Algeria) ²	8.57	37.33	1.71	4.04	5.63	64.68	41.22	0.43	128

Salinity and ions are represented as g per liter. nd., not determined. UD., undetectable. References for abiotic features of other hypersaline and marine habitats were as follows:

¹Grant et al. (2011)

²Boutaiba et al. (2011)

Isolation and identification of the microorganisms

Among the 43 strains isolated from the water of these Sebkhass, twelve distinct strictly halophilic strains: (XA1, XC1, XC2, XE1 and XE2) from Biskra, (WA1 and WC1) from Oum El Bouaghi, (YA2, YA3 and YF2) from Ain Beida and (ZC1 and ZC2) from Oum Reneb were chosen for further characterization. These twelve isolates were unable to grow in the absence of NaCl and required at least 10% NaCl to grow, which showed their strict halophilic character (Oren et al., 2008). The isolates were morphologically, biochemically, and physiologically characterized (Table 2). Pure orange and red colonies (Fig. 2) were isolated from Sebkhass waters with a population density of 6.1×10^4 CFU g⁻¹. Growth under aerobic conditions was noticed between 25 and 50 °C, with an optimum temperature of 40 °C. A pH range of 6 to 9 was tested at 40 °C, with an optimal pH of 8. No growth noted at 0% and 5% NaCl, with slight growth to 10% NaCl, go up to 30% and a growth optimum of 25%. The isolated strains are immobile and Gram negative. Microscopic observation shows pleomorphic or cocci forms. Glucose is assimilated by all the strains, only the XA1 and WA1 strains use lactose, others use mannitol (XA2 and ZC1) and two others use xylose (NE1 and ZD1). Sucrose, fructose, arabinose, rhamnose, ribose and sorbitol are not assimilated by all the strains isolated. All the strains studied are sensitive to Novobiocin and Bacitracin, this is a major criterion of halophilic *Archaea* (Atanasova et al., 2013). The majority of strains are resistant to antibiotics generally active on bacteria (Penicillin, Ampicillin, Amoxicillin, Erythromycin, Pristinamycin and Streptomycin) which is an important feature for the description of halophilic *Archaea*. Resistance to penicillin G, ampicillin, amoxicillin, clavulamic acid, oxacillin, cefotaxime, cefalexin, cefixime and fosfomycin that inhibit cell wall synthesis can be explained by a very different biochemical composition of the *Archaea* wall (Atanasova et al., 2013; Imadalou-Idres, 2013). Likewise, *Archaea*-dependent DNA RNA polymerase is insensitive to rifampicin, which inhibits the bacterial enzyme at very low concentrations. In addition, the protein synthesis of *Archaea* is not affected by the usual antibiotics, chloramphenicol and

streptomycin; tetracycline is also a weak inhibitor although it inhibits the protein synthesis of Bacteria and eukaryotes (Klingl, 2014). The pH ranges investigated varies from 6 to 10, the pH above 11 and below 6 are inhibitory for all strains. On the other hand, all strains can grow relatively well at pH 7 and 8, they are therefore neutro-alkalophilic strains. So, Castillo et al. (2006), report that 55% of the known halophilic *Archaea* species have a pH optimum of 7-8, the rest of the halophilic *Archaea* species are alkalophilic (optimal pH 9). In this study, the temperature range explored varies from 25 to 50 °C. The temperature above 50 °C and below 25 °C inhibited the isolates obtained but grew optimally at 35 and 40 °C. Castillo et al. (2006), report that 63% of the known halophilic *Archaea* species have a temperature optimum of 38-40 °C. Furthermore, the rest of the *Archaea* halophilic species are either thermo-tolerant (optimal temperature equal 50 °C) or mesophilic (28 and 35 °C). Oren (2008), report that several obligate alkalophilic *Archaea* species have been isolated from Magadi Lake and Rift Valley, Kenya, *Natronobacterium gregoryi*, *Halorubrum vacuatum*, *Natrialba magadii*, *Natronomonas pharaonis* and *Natronococcus amylocticus*; each of these microorganisms has a pH optimum of 9.0 or higher. Of all the known extreme halophilic *Archaea*, only two species are extremely halophilic, necessarily alkalophilic and thermophilic and called poly-extremophiles, *Natrialba hulunbeirensis*, which has an optimum (Na^+) of 3.4 M, an optimum pH of 9.0, and an optimum temperature equal 50 °C. So, *Natronolimnobius aegytiacus* has an optimum of 4.5 M (Na^+), a pH optimum of 9.5 and an optimum temperature of 55 °C (Liu and al., 2015; Oren and Ventosa, 2000). It should also be noted that with the exception of glucose, with an acid production for all strains, our isolates have shown a low assimilable fermentative power of most sweet compounds tested. Although there are species using carbohydrates, such as *Haloferax mediterranei*, *Haloarcula mamarismortui* and *Halococcus sacharolyticus*, which catabolize hexoses (glucose, fructose), pentoses (arabinose, xylose), sucrose and lactose, other halophilic *Archaea* such as *H. salinarum* are not able to degrade them (Oren, 2008). Species that do not use carbohydrates degrade amino acids and compounds typical of hypersaline habitats. *Haloferax volcanii*, for example, is able to grow on glycerol and organic acids excreted by halophilic algae *Dunaliella salina* and *Microcoleus chthonoplasts*, respectively (Akmoussi-Toumi et al., 2018; Cuadros-Orellana et al., 2012; Henriot et al., 2014; McGenity and Grant, 1995).

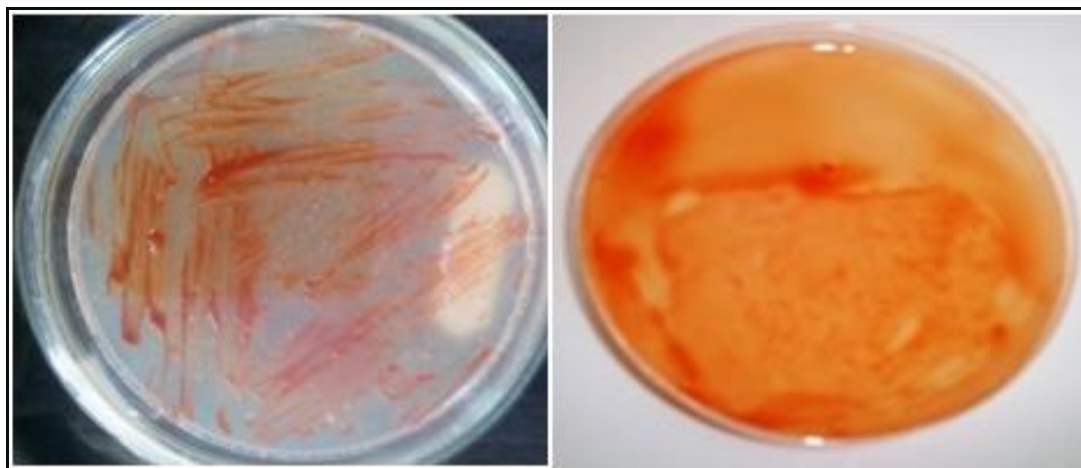


Figure 2. Colony of strain XA2 obtained on enrichment agar medium, after 7-10 days of incubation at 40°C.

Table 2. Phenotypic, biochemical and physiological characters of the isolates

Characteristics	Isolated strains											
	XA1	XA2	XC1	XC2	XE1	WA1	WC1	YA2	YA3	YF2	ZC1	ZD1
Pigmentation	Brick red	Orange red	Red	Orange	Light red	Red	Dark red	Light red	Orange red	Orange	Orange	Orange red
Cells shape	P	P	C	C	P	P	C	P	C	C	C	C
Gram staining	-	-	-	-	-	-	-	-	-	-	-	-
NaCl range (%)	10- 30	20- 30	20- 30	15- 30	15- 30	20-30	15-30	20-30	15-30	15-30	20-30	15-30
pH range	7- 9	6- 9	7- 8	7- 9	7- 9	6-9	7-9	7-8	7-9	7-9	6-9	6-9
T° range (°C)	30- 50	25- 50	30- 50	30- 50	30- 50	30-60	30-50	30-60	30-50	30-50	30-50	30-50
Acid production from												
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	-	-	-	+	-	-	-	-	-	-
Mannitol	-	+	-	-	-	-	-	-	-	-	+	-
Saccharose	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	+	-	-	-	-	-	-	+
Fructose	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-

P, Pleomorph, C, Cocci, +, Positive; -, Negative

Phylogenetic studies based on the partial 16S rRNA gene are the main tool for delineating hierarchical relationships between different organisms (Amoozegar et al., 2017; Grant et al., 2011). The phylogenetic tree based on the gene encoding the partial 16S rRNA of the isolated identified strains is shown in *Figure 3*. The phylogenetic characteristics of isolated halophilic *Archaea* are given in *Table 3*. After DNA isolation, amplification and sequencing of 16S rRNA, partial sequence analysis from the *Archaeal* strain library indicated that the isolated strains were related to the order *Halobacteriales*. Partial sequencing showed a high degree of similarity with the closest species, with significant phylogenetic diversity (*Tables 3* and *4*).

The sequencing allowed us to identify strains isolated from seven different *Archean* genera belonging to *Halobacteriaceae*: for Biskra sebkha; *Halorubrum sp.* strain XA1, *Natribaculum sp.* strain XC1, *Haloarcula sp.* strains XC2 and XE1 and *Natrinema sp.* strain XE2. Their partial 16S rDNA gene sequences have been deposited in GenBank under the accession numbers: MN393054, MN393056, MN393057, MN393058 and MN393086, respectively (*Fig. 3*). The partial 16S rDNA sequence of the isolated strain XA1 (559 bp) gave 94.29% match with *Halorubrum terrestre* (KY129965), *Halorubrum litoreum* (LN649886), *Halorubrum chaoviator* (LN649850) and *Halorubrum distributum* (NR_113475). The partial 16S rDNA sequence of the XC1 strain (567 bp) showed a correspondence of 99.47% with *Natribaculum longum* (KF739019) and 99.12% with *Natribaculum breve* (KF739011). However, the phylogenic position of this strain places it in the genus *Natribaculum*. The partial 16S rDNA sequence of strain XC2 (547 bp) has 93.20% sequence identity with *Haloarcula hispanica* (NR112194). The partial 16S rDNA sequence of the halophilic isolate XE1 (560 bp) gave a match of 99.64% with *Haloarcula amylyolytica* (KY411772), *Haloarcula argentinensis* (LC198790), *Haloarcula janonica* (LC085249), *Haloarcula hispanica* (LN649974), *Haloarcula tradensis* (KF962648) and *Haloarcula salaria* (KF962645). Finally, the partial 16S rDNA sequence of the halophilic isolate XE2

(571 bp) gave a match of 99.88% with *Natrinema altunense* (MK490895), *Natrinema pallidum* (LC331311), *Natrinema salaciae* (AB935413), *Natrinema gari* (JF802154) and *Natrinema ajinwuensis* (AY570917).

Table 3. Phylogenetic characterization of the isolated strictly halophilic *Archaea* strains (Biskra and Oum Bouaghi sebkhas)

Strain ID	Strain name (accession number)	Sequence length	Closely related validly published taxa	Similarity of 16S rRNA gene sequence (%)
XA1	<i>Halorubrum sp.</i> (MN393054)	559 bp	<i>Halorubrum terrestre</i> (KY129965)	94.29
			<i>Halorubrum litoreum</i> (LN649886)	94.29
			<i>Halorubrum chaoviator</i> (LN649850)	94.29
			<i>Halorubrum distributum</i> (NR_113475)	94.29
XC1	<i>Natribaculum sp.</i> (MN393056)	567 bp	<i>Natribaculum longum</i> (KF739019)	99.47
			<i>Natribaculum breve</i> (KF739011)	99.12
XC2	<i>Haloarcula sp.</i> (MN393057)	547 bp	<i>Haloarcula hispanica</i> (NR112194)	93.20
XE1	<i>Haloarcula sp.</i> (MN393058)	560 bp	<i>Haloarcula amylyolytica</i> (KY411772)	99.64
			<i>Haloarcula argentinensis</i> (LC198790)	99.64
			<i>Haloarcula janonica</i> (LC085249)	99.64
			<i>Haloarcula hispanica</i> (LN649974)	99.64
			<i>Haloarcula tradensis</i> (KF962648)	99.64
			<i>Haloarcula salaria</i> (KF962645)	99.64
XE2	<i>Natrinema sp.</i> (MN393086)	571 bp	<i>Natrinema altunense</i> (MK490895)	94.88
			<i>Natrinema pallidum</i> (LC331311)	94.88
			<i>Natrinema salaciae</i> (AB935413)	94.88
			<i>Natrinema gari</i> (JF802154)	94.88
			<i>Natrinema ajinwuensis</i> (AY570917)	94.88
WA1	<i>Halorubrum sp.</i> (MN393054)	590 bp	<i>Halorubrum xinjiangense</i> (JF261094)	99.83
			<i>Halorubrum trapanicum</i> (NR_112869)	99.83
			<i>Halorubrum terrestre</i> (KY129965)	99.66
			<i>Halorubrumchaoviator</i> (LN649850)	99.66
			<i>Halorubrum litoreum</i> (HM748596)	99.66
WC1	<i>Haloarcula sp.</i> (MN392906)	569 bp	<i>Haloarcula amylyolytica</i> (KJ499812)	93.10
			<i>Haloarcula marismortui</i> (AY994193)	93.10
			<i>Haloarcula vallismortis</i> (LC198794)	92.92
			<i>Haloarcula salaria</i> (NR_116666)	92.74
			<i>Haloarcula hispanica</i> (DQ089682)	92.74

For Oum El Bouaghi sebkha; *Halorubrum sp.* strain WA1, *Haloarcula sp.* strain WC1. Their partial 16S rDNA gene sequences have been deposited in GenBank under the accession numbers: MN393054 and MN392906, respectively (Fig. 3). The partial 16S rDNA sequence of the isolated strain WA1 (590 bp) gave 99.83% match with *Halorubrum xinjiangense* (JF261094), *Halorubrum trapanicum* (NR_112869), 99.66% match with *Halorubrum terrestre* (KY129965), *Halorubrum chaoviator* (LN649850) and *Halorubrum litoreum* (HM748596). The partial 16S rDNA sequence of the isolated strain WC1 (569 bp) gave 93.10% match with *Haloarcula amylyolytica* (KJ499812) and *Haloarcula marismortui* (AY994193), 92.92% match with *Haloarcula vallismortis* (LC198794) and 92, 74% match with *Haloarcula salaria* (NR_116666) and *Haloarcula hispanica* (DQ089682).

For Ain Beida Sebkha; *Halostagnicola sp.* strain YA2, *Haloferax sp.* strain YA3 and *Halorubrum sp.* strain YF2. Their partial sequences of the 16S rDNA gene have been deposited in GenBank under the accession numbers: MN393084, MN393084 and

MN393087 respectively (Fig. 3). The partial 16S rDNA sequence of the isolated strain YA2 (568 bp) gave 99.82% match with *Halostagnicola larsenii* (NR_028169), 98.40% match with *Halostagnicola kamekurae* (KF650665), 98.23% match with *Halostagnicola alkaliphila* (MN713677) and 97.88% match with *Halostagnicola bangensis* (NR_134744). The partial 16S rDNA sequence of the isolated strain YA3 (576 bp) gave 99.82% match with *Haloferax lucentense* (MH062946), 99.66% match with *Haloferax sulfurifontis* (MG213726), 99.65% match with *Haloferax volcanii* (JX646768) and *Haloferax alexandrinus* (JX646752) and 99.30% match with *Haloferax prahovense* (NR_028165). The partial 16S rDNA sequence of the isolated strain YF2 (565 bp) gave 99.82% match with *Halorubrum xinjiangense* (NR_113491) and *Halorubrum trapanicum* (NR_112869) and 99.29% match with *Halorubrum terrestre* (KY129965), *Halorubrum litoreum* (LN649886) and *Halorubrum chaoviator* (LN649850).

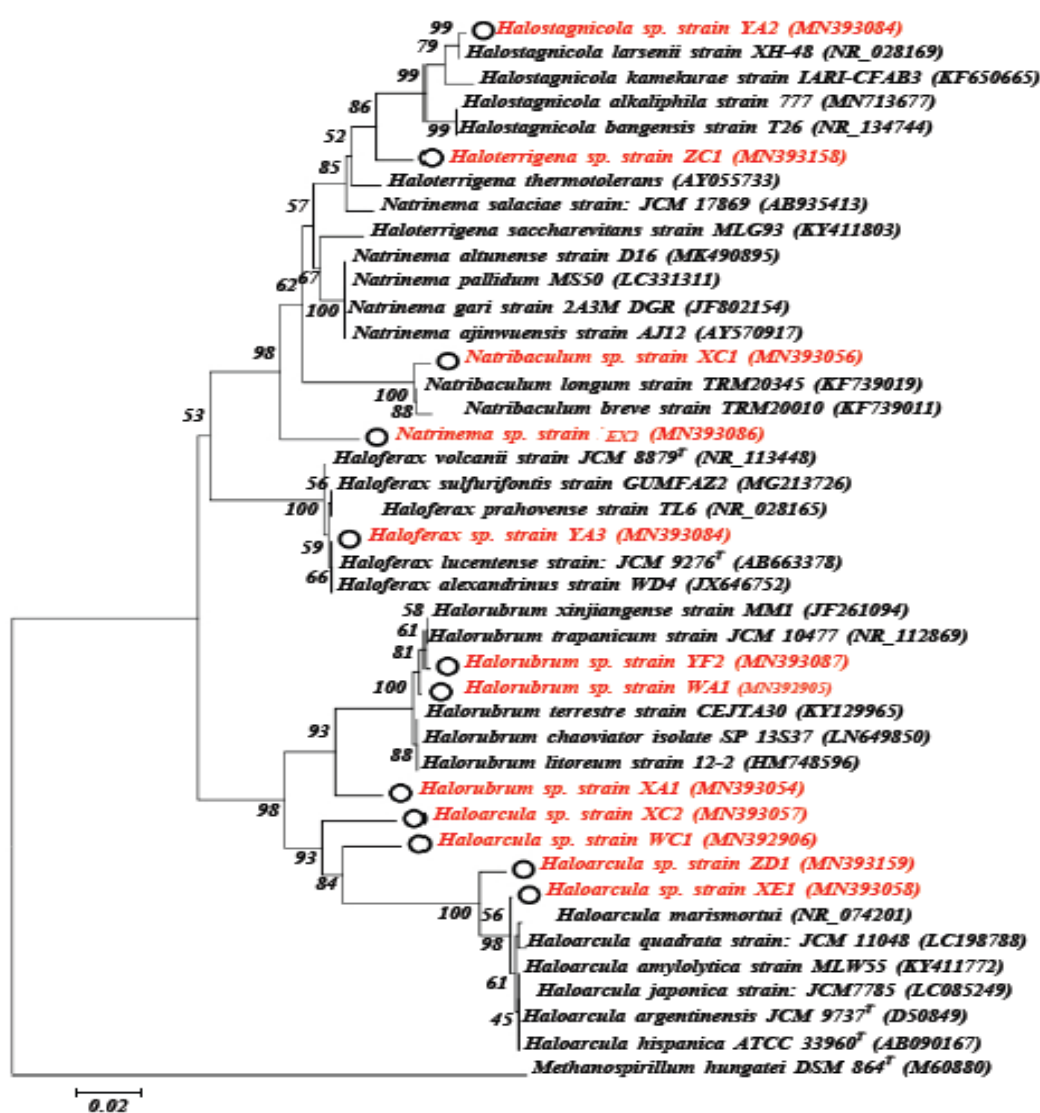


Figure 3. Phylogenetic tree based on the gene encoding 16S rRNA showing the result of the phylogenetic identification of identified and isolated strains of studied sebkhas. The tree is obtained using the software MEGA.7. Rooting was performed using the ribosomal sequence of Archaea *Methanospirillum hungatei*

Table 4. Phylogenetic characterization of the isolated halophilic *Archaea* strains (Ain Beida and Oum Reneb sebkhas)

Strain ID	Strain name (Accession number)	Sequence length	Closely related validly published taxa	Similarity of 16S rRNA gene sequence (%)
YA2	<i>Halostagnicola sp.</i> (MN393084)	568 bp	<i>Halostagnicola larsenii</i> (NR_028169)	99.82
			<i>Halostagnicola kamekurae</i> (KF650665)	98.40
			<i>Halostagnicola alkaliphila</i> (MN713677)	98.23
			<i>Halostagnicola bangensis</i> (NR_134744)	97.88
YA3	<i>Haloferax sp.</i> (MN393084)	576 bp	<i>Haloferax lucentense</i> (MH062946)	99.82
			<i>Haloferax sulfurfontis</i> (MG213726)	99.66
			<i>Haloferax volcanii</i> (JX646768)	99.65
			<i>Haloferax alexandrinus</i> (JX646752)	99.65
			<i>Haloferax prahovense</i> (NR_028165)	99.30
YF2	<i>Halorubrum sp.</i> (MN393087)	565 bp	<i>Halorubrum xinjiangense</i> (NR_113491)	99.82
			<i>Halorubrum trapanicum</i> (NR_112869)	99.82
			<i>Halorubrum terrestre</i> (KY129965)	99.29
			<i>Halorubrum litoreum</i> (LN649886)	99.29
			<i>Halorubrum chaoviator</i> (LN649850)	99.29
ZC1	<i>Haloterrigena sp.</i> (MN393158)	582 bp	<i>Haloterrigena thermotolerans</i> (AY055733)	96.20
			<i>Halostagnicola alkaliphila</i> (MN713677)	95.68
			<i>Haloterrigena saccharevitans</i> (KY411803)	95.68
ZD1	<i>Haloarcula sp.</i> (MN393159)	588 bp	<i>Haloarcula vallismortis</i> (KR866136)	98.62
			<i>Haloarcula marismortui</i> (NR_074201)	98.62
			<i>Haloarcula californiae</i> (LC198792)	98.45
			<i>Haloarcula amylytica</i> (KY411772)	98.28
			<i>Haloarcula argentinensis</i> (LC198790)	98.28
			<i>Haloarcula quadrata</i> (LC198788)	98.28
			<i>Haloarcula japonica</i> (LC085249)	98.28
			<i>Haloarcula tradensis</i> (KJ875316)	98.28

For Oum Reneb sebkha; *Haloterrigena sp.* strain ZC1 and *Haloarcula sp.* strain ZD1. Their partial sequences of the 16S rDNA gene have been deposited in GenBank under the accession numbers: MN393158 and MN393159 respectively (Fig. 3). The partial 16S rDNA sequence of the isolated strain ZC1 (582 bp) gave 96.20% match with *Haloterrigena thermotolerans* (AY055733), 95.68% match with *Halostagnicola alkaliphila* (MN713677) and *Haloterrigena saccharevitans* (KY411803). The partial 16S rDNA sequence of the isolated strain ZD1 (588 bp) gave 98.62% match with *Haloarcula vallismortis* (KR866136) and *Haloarcula marismortui* (NR_074201), 98.45% match with *Haloarcula californiae* (LC198792), 98.28% match with *Haloarcula amylytica* (KY411772), *Haloarcula argentinensis* (LC198790), *Haloarcula quadrata* (LC198788), *Haloarcula japonica* (LC085249) and *Haloarcula tradensis* (KJ875316). Obviously, more extensive analysis, as well as sequencing of the total 16S rDNA genes and DNA/DNA hybridation, are required in order to determine the correct phylogenetic position of the halophilic isolated strains. So, the genus *Halorubrum* was proposed in 1995 by Mc Genity and Grant (1995), the genus *Haloarcula* was created by Torreblanca et al. cited by Baliga et al. (2004). The genus *Natribaculum* was created by Liu et al. (2015) and the genera cited by Oren and Ventosa (2000). Finally, identification of a part of a DNA sequence, usually then related to the full-length sequence by alignment. The determinations of the phylogenetic relationships of some of these obligatorily inter and intracellular organisms are essential and a DNA-

DNA hybridization study is needed to confirm. The complete 16S rRNA gene sequence analysis can better identify poorly described, rarely isolated, or phenotypically aberrant strains, can be used for identification of halobacteria. So far, only a few reports have been produced on isolation, the characterization of *Archaea* halophiles isolated from the Algerian saline ecosystem. These studies focused mainly on saline lakes (Akmoussi-Toumi et al., 2018; Boutaiba et al., 2011; Imadalou-Idres et al., 2013; Kebbouche-Gana et al., 2009; Quadri et al., 2016; Makhdoumi-Kakhki et al., 2011; Menasria et al., 2018). Kharroub et al. (2014) reported the isolation of ten strains of Oum Bouaghi sebkha. These strains are related to four genera of halophilic *Archaea* (*Halorubrum*, *Halobacterium*, *Haloarcula*, *Haloferax* and *Haloterrigena*). Quadri et al. (2016) isolated eighteen strains from the Ouargla sebkha belonging to genera (*Natrialba*, *Halovivax* and *Haloferax*). Imadalou-Idres (2013) isolated six strains of *Archaea* halophilic from the sebkha of Oran description of four strains of *Haloarcula* and two strains of *Halorubrum*. Moreover, to the best of our knowledge, our study remains the first report of the presence of *Natribaculum* in the salt lakes of the Algerian Sahara.

Screening of enzyme producing strains

Halophile enzymes, useful in biotechnology, represent the main industrial interest, because they can function at salinity within the limits of life. The low level of contamination in their culture media due to the high salt concentration is another advantage for the industrial application of halophilic enzymes (Antranikian and Egorova, 2007; Bajpai et al., 2015; Delgado-García et al., 2012; Menasria et al., 2018). The extreme adaptation of halophilic *Archaea* has attracted the attention of scientific researchers because of their capacity to produce active enzymes and pigments with important biotechnological applications (Antranikian and Egorova, 2007; Bajpai et al., 2015; Delgado-García et al., 2012; Klingl, 2014; Menasria et al., 2018).

In the present study, the screening of twelve isolates of *Archaea* for active extracellular halophilic enzymes showed that our isolates produced at least one hydrolytic enzyme apart from the YA3 *Haloferax* sp strain (Tables 5 and 6; Fig. 4). The halophilic *Archaea* strains tested exhibit significant enzymatic potential, reflected by their ability to hydrolyze gelatin and casein (*Natribaculum* sp “XC1”, *Haloarcula* sp “XC2, XE1, ZD1 and WC1”, *Halorubrum* sp “WA1” and *Haloterrigena* sp “ZC1”), to produce lipase (*Haloarcula* sp “XE1”, *Halostagnicola* sp “YA2” and *Haloterrigena* sp “ZC1”) and degrades cellulose (*Haloterrigena* sp “ZC1” and *Haloarcula* sp “ZD1”). However, the isolates (*Halorubrum* sp “XA1 and YF2”, *Haloarcula* sp “XC2 and XE1” and *Haloterrigena* sp “ZC1”) showed extracellular amylase activity. It is important to note that all these enzymatic activities occurred at high salt concentration (20% NaCl). Furthermore, Kharroub et al. (2014), reported that the most degraded substrate by the strains tested, is starch, while several strains had gelatinase, amylase and lipase. The diversity of extreme halophilic archaea producing different hydrolases isolated from salt lakes, concluding that *Halorubrum* and *Haloarcula* are the dominant haloarchaeon genera with high-throughput enzyme production, including amylase and lipase (Makhdoumi-Kakhki et al., 2011). The diversity of hydrolytic enzymes in halophilic *Archaea* strains isolated from Salt Lake in Iran has shown that the genera *Halorubrum*, *Haloarcula* and *Natrinema* contain the largest number of strains with enzymatic activity (Delgado-García et al., 2012). *Archaea* are an important source of enzymes, including proteases, for applied research as well as basic enzymology. Protease activities of the *Haloferax* and *Natrialba*

species have been the subject of previous studies (Grant et al., 2012). Hyper-saline environments represent a source of potentially interesting hydrolytic enzymes, whose haloarchaeon strains isolated from these environments could generate a number of viable economic applications (Antranikian and Egorova, 2007; Henri et al., 2014; Oztetik and Cakir, 2014). The catalytic properties of these halophilic enzymes have applications in different fields such as the food industry and biotechnological processes as additives in detergents (Antranikian and Egorova, 2007; Amoozegar et al., 2017; Henri et al., 2014; McGenity and Grant, 1995).

Table 5. Screening result of enzyme producing strains

Hydrolysis of	Isolated strains						
	XA1	XC1	XC2	XE1	XE2	WA1	WC1
	<i>Halorubrum sp</i>	<i>Natribaculum sp</i>	<i>Haloarcula sp</i>	<i>Haloarcula sp</i>	<i>Natrinema sp</i>	<i>Halorubrum sp</i>	<i>Haloarcula sp</i>
Starch	+	-	+	+	+	-	-
Gelatin	-	+	+	+	-	+	+
Casein	-	+	+	+	-	+	+
Tween 80	-	-	-	+	-	-	-
Cellulose	-	-	-	-	-	-	-

+, positive; -, negative

Table 6. Screening result of enzyme producing strains (continued)

Hydrolysis of	Isolated strains				
	YA2	YA3	YF2	ZC1	ZD1
	<i>Halostagnicola sp</i>	<i>Haloferax sp</i>	<i>Halorubrum sp</i>	<i>Haloterrigena sp</i>	<i>Haloarcula sp.</i>
Starch	-	-	+	-	+
Gelatin	-	-	-	+	+
Casein	-	-	-	+	+
Tween 80	+	-	-	+	-
Cellulose	-	-	-	+	+

+, positive; -, negative

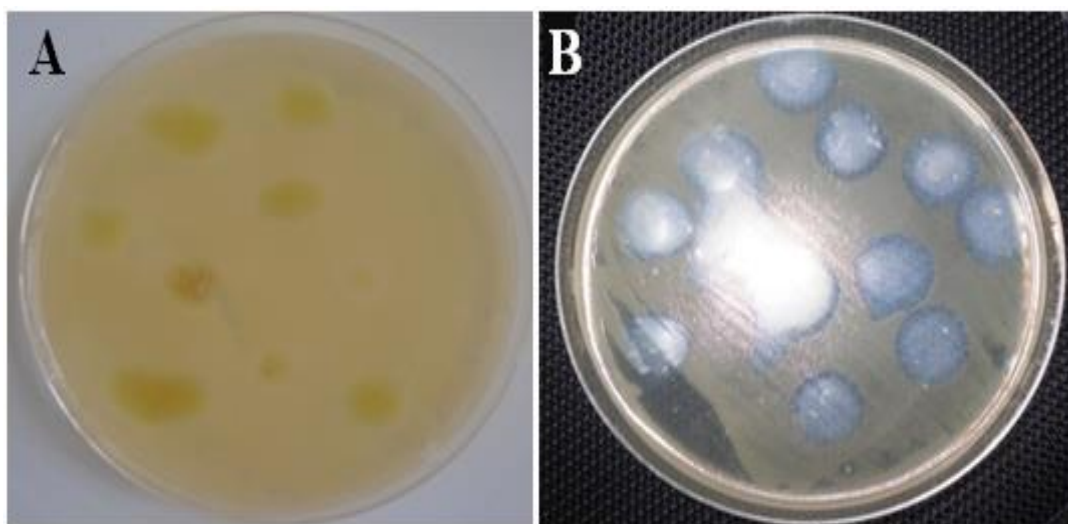


Figure 4. Screening of halophilic active enzymes (XE1 strain) using a drop spot technique. (A) Amylase; (B) Lipase

Conclusion

The physicochemical analysis study of the waters of the sebkhas studied located in the north-east of the Algerian Sahara, and their comparison with other aquatic super-saline ecosystems of the world, in particular their alkalinity and salinity, was studied for the first time. The microbiological study showed a remarkable morphological, physiological and metabolic diversity of the isolated strains. We have new access to an archaeal diversity including extremely halophilic alkalo-thermotolerant strains belonging to the order *Halobacteriales* : *Halorubrum sp*, *Natribaculum sp*, *Haloarcula sp*, *Natrinema sp*, *Halostagnicola sp*, *Haloferax sp* and *Haloterrigena sp*, until now isolated for the first time in the hyper-saline environments of northern Algerian Sahara. The hyper-saline environments represent a source of potentially interesting bioactive molecules, whose haloarchaeal strains isolated from these environments could generate a number of viable economic applications. As most of the currently used enzymes are known mainly for their instability and fragility under extreme conditions, the search for new, more stable enzymes has therefore become a property. In the present study, many isolates showed several combined hydrolytic activities mainly the ZD1 strain affiliated with *Haloarcula*. The *Haloterrigena* affiliated ZC1 strain also displays interesting hydrolytic activities. Complementary work on these two strains is planned for the study of the other enzymes of biotechnological interest and secondary metabolites with novel therapeutic activities (e.g. anti-cancer effect).

Acknowledgments. We thank Pr Idir BITAM and Pr Michel DRANCOURT Michel, the members of MEPHI (Aix-Marseille France) for their technical assistance, and the access to the laboratories. Also, to DGRSDT for the support provided to the research.

Disclosure statement. No potential conflict of interests was reported by the authors.

REFERENCES

- [1] Abd-Elhalem, B. T., El-Sawy, M., Gamal, R. F., Abou-Taleb, K. A. (2015): Production of amylases from *Bacillus amyloliquefaciens* under submerged fermentation using some agro-industrial by-products. – *Ann AgricSci* 60: 193-202.
- [2] Akmoussi-Toumi, S., Khemili-Talbi, S., Kebbouche-Gana, S., Ferrioune, I. (2018): Purification and characterization of an organic solvent-tolerant and detergent stable lipase from *Haloferax mediterranei* CNCMM 50101. – *International Journal of Biological Macromolecules* 116: 817-830.
- [3] American Public Health Association. (2005): *Standard Methods for the Examination of Water and Wastewater*. 21st Ed. – American Water works Association and Water Pollution Control Federation, Baltimore, MD.
- [4] Amoozegar, M. A., Siroosi, M., Atashgahi, S., Smidt, H., Ventosa, A. (2017): Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. – *Microbiology* 163: 623-645.
- [5] Antranikian, G., Egorova, K. (2007): Extremophiles a Unique Resource of Biocatalysts for Industrial Biotechnology. – In: Gerday, C., Glansdorff, N. (eds.) *Physiology and Biochemistry of Extremophile*. Wiley, Hoboken, NJ.
- [6] Atanasova, N. S., Pietila, M. K., Oksanen, H. M. (2013): Diverse antimicrobial interactions of halophilic archaea and bacteria extend over geographical distances and cross the domain barrier. – *Microbiol* 2(5): 811-825.

- [7] Bajpai, B., Chaudhary, M., Saxena, J. (2015): Production and characterization of alpha-amylase from an extremely halophilic archaeon, *Haloferax* sp. HA10. – *Food Technology and Biotechnology* 53: 11-17.
- [8] Baliga, N. S., Bonneau, R., Facciotti, M. T., Pan, M., Glusman, G. et al. (2004): Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. – *Genome Research* 14: 2221-2234.
- [9] Boutaiba, S., Hacène, H., Bidle, K. A., Maupin-Furlow, J. A. (2011): Microbial diversity of the hypersaline SidiAmeur and Himalatt Salt Lakes of the Algerian Sahara. – *Arid Environments* 75(10): 909-916. <https://doi.org/10.1016/j.jaridenv.2011.04.010>.
- [10] Castillo, A. M., Gutiérrez, M. C., Kamekura, M., Ma, Y., Cowan, D. A. et al. (2006): *Halovivax asiaticus* gen. nov., sp. nov., a novel extremely halophilic archaeon isolated from Inner Mongolia, China. – *International Journal of Systematic and Evolutionary Microbiology* 56: 765-770.
- [11] Cuadros-Orellana, S., Pohlschroder, M., Grossman, M. J., Durrant, L. R. (2012): Biodegradation of aromatic compounds by a halophilic archaeon isolated from the Dead Sea. – *Chemical Engineering Transactions* 27: 3-18.
- [12] Delgado-García, M., Valdivia-Urdiales, B., Aguilar-González, C. N., Contreras-Esquivel, J. C., Rodríguez-Herrera, R. (2012): Halophilic hydrolases as a new tool for the biotechnological industries. – *J. Sci. Food Agric* 92: 2575-2580.
- [13] Gana, M. L., Kebbouche-Gana, S., Touzi, A., Pauss, A., Lounici, H. et al. (2010): Antagonistic activity of *Bacillus* sp. obtained from an Algerian oilfield and chemical biocide THPS against Sulfate-reducing bacteria consortium inducing corrosion in the oil industry. – *Journal of Industrial Microbiology and Biotechnology* 38(3): 391-404.
- [14] Ghanem, A. (2007): Trends in lipase-catalyzed asymmetric access to antiomerically pure/enriched compounds. – *Tetrahedron* 63(8): 1721-1754.
- [15] Grant, W. D., Pagaling, E., Marquez, M. C., Gutiérrez, M. C., Cowan, D. A. et al. (2011): The Hypersaline Lakes of Inner Mongolia. – In: Ventosa, A., Oren, A., Yanhe, M. (eds.) *Halophiles and Hypersaline Environments*. Springer-Verlag, Berlin, pp. 65-107.
- [16] Henriot, O., Fourmentin, J., Delincé, B., Mahillon, J. (2014): Exploring the diversity of extremely halophilic archaea in food-grade salts. – *International Journal of Food Microbiology* 191: 36-44.
- [17] Imadalou-Idres, N., Carré-Mlouka, A., Vandervennet, M., Yahiaoui, H., Peduzzi, J. et al. (2013): Diversity and antimicrobial activity of cultivable halophilic archaea from three Algerian sites. – *Journal of Life Sciences* 7: 1057-1069.
- [18] Joint, I., Mühling, M., Querellou, J. (2010): Culturing marine bacteria an essential prerequisite for biodiscovery. – *Microb. Biotechnol* 3: 564-575.
- [19] Kebbouche-Gana, S., Gana, M. L., Khemili, S., Fazouane-Naimi, F., Bouanane, N. A. et al. (2009): Isolation and characterization of halophilic Archaea able to produce biosurfactants. – *Journal of Industrial Microbiology & Biotechnology* 36(5): 727-738.
- [20] Kharroub, K., Gomri, M. A., Aguilera, M., Monteoliva-Sanchez, M. (2014): Diversity of hydrolytic enzymes in haloarchaea isolated from Algerian sabkhas. – *African Journal of Microbiology Research* 56: 1583-1588.
- [21] Klingl, A. (2014): S-layer and cytoplasmic membrane – exceptions from the typical archaeal cell wall with a focus on double membranes. – *Frontiers in Microbiology* 5: 624-2. DOI: 10.3389/fmicb.2014.00624.
- [22] Latorre, J. D., Hernandez-Velasco, X., Wolfenden, R. E., Vicente, J. L., Wolfenden, A. D., et al. (2016): Evaluation and selection of *Bacillus* species based on enzyme production, antimicrobial activity, and bio-film. – *Front Vet Sci*. DOI: 10.3389/fvets.2016.00095.
- [23] Lenchi, N., Kebbouche, S., Khelifaoui, M. L., Laddada, B., Khemili, S., Gana, M. L., Akmoussi, S., Ferioune, I. (2020): Phylogenetic characterization and screening of halophilic bacteria from Algerian salt lake for the production of biosurfactant and

- enzymes. – World Journal of Biology and Biotechnology 5(2).
<https://doi.org/10.33865/wjb.005.02.0294>.
- [24] Litchfield, B., Dalmat, S. (2009): Microbial diversity in hypersaline environments. – Proceedings of the 2nd International Conference on the Ecological Importance of Solar Saltworks (CEISSA2009). Merida, Yucatan, Mexico, pp. 26-29.
- [25] Liu, Q., Ren, M., Zhang, L. L. (2015): *Natribaculum* breve gen. nov., sp. nov. and *Natribaculumlongum* sp. nov., halophilic archaea isolated from saline soil. – Int J Syst Evol Microbiol 65(Pt 2): 604-608.
- [26] Madigan, M. T., Martinko, J. M. (2006): Brock Biology of Microorganisms. 11th Ed. – Pearson Prentice Hall, Upper Saddle River, NJ.
- [27] Makhdoumi-Kakhki, A., Amoozegar, M. A., Mahmodi-Khaledi, E. (2011): Diversity of hydrolytic enzymes in haloarchaeal strains isolated from Salt Lake. – Int. J. Environ. Sci. Technol 8: 705-714.
- [28] McGenity, T. J., Grant, W. D. (1995): Transfer of *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium trapanicum* NRC 34021 and *Halobacterium lacusprofundito* the genus *Halorubrum* gen. nov., as *Halorubrum saccharovorum* comb. nov., *Halorubrum sodomense* comb. nov., *Halorubrum trapanicum* comb. nov., and *Halorubrum lacusprofundi* comb. nov. – Syst Appl Microbiol 18: 237-243.
- [29] Menasria, T., Aguilera, M., Hocine, H., Benammar, L., Ayachi, A. et al. (2018): Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes. – Microbiological Research 207: 289-298.
- [30] Oren, A. (2008): Microbial life at high salt concentrations: phylogenetic and metabolic diversity. – Saline Systems 4: 2.
- [31] Oren, A., Ventosa, A. (2000): International Committee on Systematic Bacteriology Subcommittee on the taxonomy of Halobacteriaceae. Minutes of the meetings, 16 August 1999, Sydney, Australia. – Int. J. Syst. Evol. Microbiol 50: 1405-1407.
- [32] Oztetik, E., Cakir, A. (2014): New Food for an old mouth: new enzyme for an ancient Archaea. – Enzyme and Microbial Technology 55: 58-64.
- [33] Quadri, I., Hassani, I., Chalopin, M., Hacene, H., Jebbar, M. (2016): Characterization and antimicrobial potential of extremely halophilic archaea isolated from hypersaline environments of the Algerian Sahara. – Microbiological Research 186-187: 119-3.
- [34] Roussel, E. G., Cambon Bonavita, M. A., Querellou, J., Cragg, B. A., Webster, G., et al. (2008): Extending the subsea-floor biosphere. Science 320(5879): 1046. *Salinicoccus iranensis* sp. nov., a novel moderate halophile. – Int J Syst Evol Microbiol 58: 178-183.
- [35] Saxena, K. R., Dutt, K., Agarwal, L., Nayyar, P. (2007): A highly and thermostable alkaline amylase from a *Bacillus* sp. PN5. – Bio-resource Techno 198: 260-265.
- [36] Tamura, K., Nei, M., Kumar, S. (2004): Prospects for inferring very large phylogenies by using the neighbor-joining method. – Proc Natl AcadSci USA 101: 11030-11035.