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Diversity of hydrolytic enzymes in haloarchaea isolated from Algerian sabkhas

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Algeria has numerous natural hypersaline environments (sabkha and chott) located in the north and south of the country. In the course of screening microorganisms form these environments, we isolated and characterized 44 haloarchaeal strains. According to their phenotypic characteristics and comparative 16S rRNA sequence analysis, all the isolates belonged to the family *Halobacteriaceae* including members related to species of the genera *Halorubrum*, *Haloterrigena*, *Halogeometricum*, *Halobacterium*, *Haloferax*, *Halomicrobium* and *Haloarcula*. Our finding reveals that *Halorubrum* is the most prevalent genus retrieved. The production of hydrolase was qualitatively studied on these isolates. Several strains were able to produce amylase, gelatinase and lipase. None was able to produce DNase activity. Combined hydrolytic activity was also detected in many strains.

Key words: Haloarchaea, sabkha, screening, hydrolytic enzyme.

INTRODUCTION

Halophilic microorganisms have been isolated from a range of environments: the Dead Sea, the Great Salt Lake (Utah, USA), alkaline brines of Wadi Natrun (Egypt), lake Magadi (Kenya), the hypersaline lakes of Inner Mongolia, and saline soils (Arahal et al., 1996; Grant et al., 2011; Ma and Gong, 2013). In addition, halophiles have also been isolated from highly salty foods, and the human intestinal mucosa (Abriouel et al., 2011; Lee, 2013).

The studies of these environments have shown that the diversity of microbial populations is low (Benlloch et al., 2002; Burns et al., 2004) and that, in general, microbial diversity decreases with increased salinity (Benlloch et al., 1995). The low total diversity of hypersaline environments, makes them to be taken as an ideal model system for ecological studies (Burns and Dyall-Smith, 2006). Halophilic archaea constitute the family *Halobacteriaceae* within the order *Halobacteriales*. They represent a considerable fraction of the prokaryotic world in hypersaline environments in term of number, biomass and genetic heterogeneity (Antón et al., 1999; Maturrano et al., 2006). Their adaptations to grow at high salinity concentrations make them interesting for fundamental

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research and the exploration for biotechnological process (Oren, 2010). Halophilic enzymes are unusually more stable than their normal counterparts. It has been proved that many haloenzymes are polyextremophilic. These properties made them attractive for various biotechnological applications (Kanekar et al., 2012).

Algeria has many salt lakes, located generally in semi-arid and arid regions from which salt is extracted for human consumption. Our knowledge of the diversity of halophilic microorganisms in these environments is, however, still limited; even if some investigations were performed to survey the microbial diversity of the hypersaline lakes in the Algerian Sahara (Hacène et al., 2004; Boutaiba et al., 2011). Thus, in this study, we present the taxonomic affiliation of haloarchaeal isolates recovered from three hypersaline lakes of Algeria. In addition, the production of extracellular amylase, gelatinase and lipase was investigated.

MATERIALS AND METHODS

Sampling sites

Samples used for this study were taken from three Algerian sabkhas (Ezzemoul, Melghir and Bethioua) in 2004 and 2008. These sabkhas belong to the naturally-occurring salt lakes in Algeria receiving their water supply from high ground water levels, precipitation or run-off from adjacent areas. Variations in water levels and brine concentrations occurred cyclically, starting to change in spring and the lakes completely evaporated during June-July period. Total salt concentrations and pH values were measured with refractometer (Leica) and pH-meter (Hanna), respectively.

Strain isolation and culture conditions

To perform the screening of extreme halophiles producing hydrolytic activities, samples from different sabkhas were inoculated onto two saline media. The HM contained (per litre of distilled water): 5 g proteose-peptone (Difco), 5 g yeast extract (Difco), 1 g glucose with a final total salt concentration of ca. 25% (w/v). The stock of total salts at 30% (w/v) was prepared as described by Subov (1931): 234 g NaCl, 42 g $MgCl_2.6H_2O$, 60 g $MgSO_4.7H_2O$, 1 g $CaCl_2.2H_2O$, 6 g KCl, 0.2 g $NaHCO_3$, 0.7 g NaBr and 0.005 g $FeCl_3$ and 1000 mL distilled water. Halophilic medium modified from the formulation of Oren (1983) contained (per litre distilled water): 125 g NaCl, 100 g $MgCl_2.6H_2O$, 5 g K_2SO_4, 0.1 g $CaCl_2.2H_2O$, 1 g yeast extract, 1 g casamino-acids and 2 g soluble starch. For solidification, 20 g agar L^{-1} was added.

Each medium was adjusted to pH 7.0-7.2. Portions of 0.1 mL of water samples were directly plated on solid media. Incubation was carried out in sealed plastic containers at 37°C. Colonies arising on the plates were selected for isolation based on gross morphological differential characteristics (size, pigmentation and shape). They were transferred to fresh media and pure cultures were obtained.

Phenotypic characterization

Gram staining was performed using acetic-acid-fixed samples (Dussault, 1955). NaCl tolerance was determined in growth medium supplemented with 0, 3, 5, 7.5, 10, 15, 20, 25 or 30% (w/v) NaCl. The pH dependence of growth was tested in the pH range 5.0-10.0. The temperature range for growth was determined by using incubation temperature ranging from 10 to 55°C. Catalase production was detected with 10 % (w/v) H_2O_2. The oxidase reaction was performed on filter paper moistened with a 1% (w/v) aqueous solution of N, N, N', N'-tertraméthyl-p-phenylenediamine. Tests for formation of indole and hydrolysis of starch and aesculin were performed following Gonzalez et al. (1978). The urea hydrolysis was tested according to the procedure of Larpent and Larpent-Gourgaud (1985). Reduction of nitrate was tested using the sulfanilic acid and α-naphtylamine reagent (Smibert and Krieg, 1981).

Screening for extracellular hydrolytic activities

All the isolates were tested qualitatively for the production of extracellular enzymes on plates containing 20% (w/v) total salts. The production of amylase was tested by flooding cultures on solid medium containing 1% (w/v) starch with lugol's iodine (Barrow and Feltham, 1993). Hydrolysis of gelatine and Tween 80 were tested as outlined by Gutiérrez and Gonzalez (1972). The presence of DNase activity was determined on DNase test agar. After incubation, the plates were flooded with 1 N HCl solution. Clear halos around the colonies indicated the DNase activity (Jeffries et al., 1957).

DNA extraction, 16S rRNA gene amplification and sequencing

Genomic DNA was extracted and purified from cells in the mid-logarithmic growth phase by using the method of Lind and Ursing (1986).

The 16S rRNA gene sequences were amplified by specific forward primer D30 (5'– ATTCCGGTTGATCCTGC- 3') and reverse primer D56 (5'– GYTACCTTGTTACGACTT- 3') (Arahal et al., 1996). PCR amplification was carried out as follows: denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and elongation at 72°C for 1.5 min with additional 5 s added for each cycle for a total of 30 cycles, followed by a final elongation step at 72°C for 15 min. The resulting PCR products of the expected size were purified using Microcon-100 concentrator (Amicon) and sequenced using primers given in Table 1.

Molecular identification and phylogenetic analysis

The sequences obtained were identified by a similarity based search using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). Phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis (MEGA) version 4.1 (Tamura et al. 2007). Multiple alignment analyses were performed with Clustal W 1.8 software (Thompson et al., 1994) selecting related sequences from the NCBI Taxonomy Homepage (Tax-Browser) database. All alignments gaps were treated as missing data. The phylogenetic trees were determined by neighbour-joining method (Saitou and Nei, 1987) and a phylogenetic consensus tree was reconstructed at random by selecting 1,000 replicates. The sequence of the type strain of *Methanospirillum hungatei* DSM 864^T was used as outgroup.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences reported in this paper have been submitted to Genbank/NCBI databases under accession numbers DQ118426; DQ120725; DQ149846; EF488827; EU409597; GQ181207-GQ181213; GQ225084-GQ225095; GQ250584; GU166402; FJ794071; FJ794073; FJ897725; GU361123-GU361125; GU361130 and GU361133-GU381143.

Table 1. Oligonucleotide primers used for PCR amplification and sequencing of bacterial and archaeal 16S rRNA genes.

Primer designation	Sequence (5'-3')	Type	Orientation
D30	ATTCCGGTTGATCCTGC	PCR, sequencing	Forward
D99	GTGTTACCGCGGCTGCTG	Sequencing	Reverse
B36	GGACTACCAGGGTATCTA	Sequencing	Reverse
D34	GGTCTCGCTCGTTGCCTG	Sequencing	Reverse
X10	ACGGGCGGTGTGTRC	PCR	Reverse
D56	GYTACCTTGTTACGACTT	PCR, sequencing	Reverse

Table 2. Geographical location and physicochemical properties of water samples.

Parameter	Hypesalines lakes		
	Ezzemoul	Melghir	Bethioua
Localisation	35°53.137´N, 6°30.200´E	34°00´ 6°07,30´	35°41´33"N, 0°18´0"O
pH	7.9	7.1	6.9
Salinity (%)	30	36	32

RESULTS

Sampling sites

Brines samples were collected from a wide geographical area. To our knowledge, this is the first microbiological study on extremely halophilic archaea from Ezzemoul, Bethioua and Melghir sabkhas. Generally, the pH is quite close to neutrality. The salinity ranged from 30 to 36% (w/v). The most saline habitat was located south (sabkha Melghir) (Table 2).

Phenotypic characterization of isolates

The assessment of the archaeal diversity of three sabkhas has been carried out through the application of cultivation methods. The isolates formed colonies ranged in color from pale-pink to red and were 1-2 mm in diameter after one week of incubation. These colonies were smooth, circular and entire. The cells were extremely pleomorphic, rod or pleomorphic-rod and stained Gram-negative. Physiological tests were performed for the isolates. All of them were extreme halophilic, had a salt concentration for growth at least 10% (w/v) NaCl, and could tolerate salt concentration up to 25-30% (w/v) NaCl. The isolates grew best between 37 and 40°C, pH of 6.5 to 7.5 and were catalase, and oxidase positive. Among the strains tested for indole production and urea hydrolysis, the majority were negative and nitrate reduction was observed within 23 isolates (Table 3).

Hydrolytic activity of isolates

The extremely halophilic strains isolated from the sabkhas were tested for their capacity to hydrolyse using extracellular enzymes substrates such as starch, gelatine, DNA and Tween 80.

Extracellular lipase activities were detected in four isolates. In contrast to the few extracellular gelatinase producers, twenty-two isolates showed extracellular amylase activities, fourteen strains from Ezzemoul sabkha. None of the isolates exhibited DNase activities. Combined hydrolytic activities were also detected in many strains. The results are summarized in Table 3 indicating that starch hydrolytic activity was predominant.

Molecular identification and phylogenetic analysis

Genomic DNA was extracted from isolates and amplified using the archaeal 16S rRNA primers. The phylogenetic analysis was done with obtained sequences and related ones obtained from the Genbank database. The 16S rRNA gene sequence similarity percentages and phylogenetic relationship are shown in Table 4 and most isolates shared more than 97% identity with their closest phylogenetic relative.

The tree constructed by neighbor-joining method depicting the phylogenetic relationships of isolates and their closest relatives is shown in Figure 1. They are placed within the family *Halobacteriaceae* (Gibbons, 1974) belonging to *Halobacteriales* order (Grant et al., 2001). The genus *Halorubrum* accounted for the majority of the isolates (59%). Among strains assigned to genus *Halorubrum*, a cluster of 20 isolates was closely related to the type strain of *Halorubrum chaoviator* Halo-G^T (Mancinelli et al., 2011). The remaining isolates were phylogenetically related to the following genera: *Halobacterium* (4 isolates), *Halogeometricum* (1 isolate),

Table 3. Phenotypic features of halophilic archaeal isolates from Algeria.

Characteristic	Strains								
	5.1	**S1**	**4**	**S7**	**K-1**	**Ez59**	**L52**	**Ez26**	**Ez228**
Pigmentation	Red	Orange	Salmon pink	Red	Orange	Pink	Pink	Orange	Red
Cell shape	Pleomorphic	Pleomorphic	Pleomorphic	Rods	Short-rods	Rods	Rods	Rods	Rods
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
NaCl range (%)	15-25	10-30	10-30	15-30	20-30	15-30	15-25	15-25	15-25
pH range	6.5-9.0	5.5-9.0	6.0-9.0	6.5-9.0	6.5-10	6.0-9.0	6.5-9.0	5.0-9.9	6.0-9.0
Temperature range (°C)	22-50	30-55	30-50	30-50	30-50	22-50	22-55	30-50	22-50
Urease	-	+	-	-	-	-	-	-	-
Indol production	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	-	+	+	-	-	+
Hydrolysis of:									
Aesculin	-	+	+	-	-	-	-	+	-
DNA	-	-	-	-	-	-	-	-	-
Gelatin	-	+	-	-	-	-	-	-	-
Starch	-	+	+	-	+	+	-	-	-
Tween 80	-	-	-	-	+	-	-	-	-

+, Positive; -, negative.

Table 3. Contd.

Characteristic	Strains								
	L56	**Eza4**	**Ez21**	**Ez1.2**	**Ez5-1**	**Ez5-2**	**Ez5RB**	**EzB1**	**EzB3**
Pigmentation	Pale-pink	Red	Red	Orange-red	Red	Red	Red	Red	Pink
Cell shape	Rods	Short rods	Rods	Pleomorphic rods	Rods	Rods	Pleomorphic rods	Short rods	Short rods
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
NaCl range (%)	15-25	15-25	15-30	15-25	15-30	15-25	10-30	10-30	10-30
pH range	6.5-9.0	6.0-9.0	5.5-8.5	7.0-9.0	6.5-9.0	6.5-9.0	6.5-9.0	7.0-9.0	6.5-9.0
Temperature range (°C)	30-55	22-50	30-55	30-55	22-50	22-50	30-50	30-50	30-50
Urease	-	-	+	-	-	-	-	-	-
Indol production	-	+	-	-	-	-	-	-	-
Nitrate reduction	+	-	+	+	-	-	-	-	+
Hydrolysis of:									
Aesculin	-	+	-	+	+	+	-	-	-
DNA	-	-	-	-	-	-	-	-	-
Gelatin	+	-	+	+	-	-	-	-	-
Starch	-	+	-	+	+	+	+	+	+
Tween 80	-	-	-	-	-	-	-	-	-

Haloterrigena (3 isolates), *Haloferax* (4 isolates), *Halomicrobium* (4 isolates) and *Haloarcula* (4 isolates).

DISCUSSION

Sabkhas and chotts are examples of high salty environments inhabited by halophilic microorganism. Our study is the first attempt to investigate halophilic archaea in the sabkhas of Ezzemoul, Bethioua and chott Melghir.

Throughout the course of this work, we further characterized 44 isolates. All were contained within the family *Halobacteriaceae*, a typical and dominant group in

Table 3. Contd.

Characteristic	Strains								
	EzS2	**EzS6**	**Ez522**	**Ez526**	**Ez24**	**EzA1**	**Sm**	**A**	**Beja5**
Pigmentation	Red	Red	Red	Pink	Orange	Red	Pale-pink	Red	Pale-pink
Cell shape	Rods	Rods	Short rods	Rods	Pleomorphic	Rods	plleomorphic	Rods	Pleomorphic
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
NaCl range (%)	15-30	15-30	12.5-30	15-30	10-30	15-30	10-30	20-30	12.5-30
pH range	6.5-9.0	6.5-9.0	6.0-8.5	6.5-9.0	6.5-8.5	6.0-8.5	6.5-8.5	6.5-9.0	6.0-9.0
Temperature range (°C)	30-50	30-50	22-30	22-50	30-50	22-50	22-50	30-50	22-50
Urease	-	-	-	-	-	-	-	-	-
Indol production	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	+	-	-	-	+	+	-
Hydrolysis of:									
Aesculin	-	-	+	+	-	-	-	-	+
DNA	-	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-
Starch	+	-	+	-	-	+	-	-	-
Tween 80	-	-	-	+	+	-	-	-	-

Table 3. Contd.

Characteristic	Strains								
	bejS3	**bej51**	**KL**	**MGG2**	**MGG3**	**MG23**	**MG25**	**MG215**	**MG525**
Pigmentation	Beige-orange	Pink	Orange	Red-orange	Orange	Pink	Pink-red	Orange	Red
Cell shape	Rods	Pleomorphic	Pleomorphic	Pleomorphic	Rods	Short rods	Rods	Pleomorphic rods	Short rods
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
NaCl range (%)	10-25	10-25	10-25	10-30	10-30	10-30	10-25	15-30	10-30
pH range	6.0-8.5	6.5-9.0	6.0-9.0	5.5-9.0	6.0-9.0	6.5-9.0	5.5-8.5	6.5-9.0	5.5-8.5
Temperature range (°C)	22-50	30-50	30-55	22-50	22-50	30-50	22-50	30-50	30-55
Urease	-	-	-	+	+	-	-	-	-
Indol production	+	-	-	-	-	+	+	+	-
Nitrate reduction	+	+	+	+	+	-	-	-	+
Hydrolysis of:									
Aesculin	-	-	-	+	+	-	-	-	+
DNA	-	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-
Starch	+	+	-	+	+	-	-	-	-
Tween 80	-	+	-	-	-	-	-	-	-

hypersaline environments by cultivation methods. We discovered seven genera: *Halorubrum*, *Halobacterium*, *Haloferax*, *Halomicrobium*, *Haloarcula*, *Haloterrigena*, *Halogeomtricum* and two novel species (Kharroub et al., 2006, 2008). The haloarchaea were characterized by their obligate halophilic lifestyle and their aerobic heterotrophic metabolism. The sequence similarities of isolates ranged between 95.8 and 99.8% to closely related species. The majority of the strains from this study were more closely related to *H. chaoviator* Halo-G^T isolated from sea salt in Baja California, Mexico, Western Australia and the Greek island of Naxos (Mancinelli et al.,

Table 3. Contd.

Characteristic	Strains							
	MG526	Set21	KM	Bet 58	Bet25	Bet213	Bet217	Bet512
Pigmentation	Pink-red	Red	Red-orange	Red-orange	Pale-pink	Pink	Orange	Orange
Cell shape	Short rods	Short rods	Rods	Rods	Pleomorphic	Short rods	Rods	Rods
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
NaCl range (%)	15-30	10-25	15-30	15-30	15-30	10-30	10-30	10-30
pH range	6.0-9.0	6.0-8.5	6.0-9.0	6.0-9.0	6.0-8.5	6.0-9.0	6.5-8.5	6.0-8.5
Temperature range (°C)	22-50	30-55	30-55	22-50	30-55	30-55	30-50	22-55
Urease	-	-	-	-	-	-	-	+
Indol production	+	+	-	-	+	-	-	-
Nitrate reduction	-	+	+	+	-	-	-	+
Hydrolysis of:								
Aesculin	+	-	+	+	+	+	+	-
DNA	-	-	-	-	-	-	-	-
Gelatin	-	+	-	-	+	-	+	-
Starch	-	+	-	+	-	+	-	+
Tween 80	-	-	-	-	-	-	-	-

Table 4. Affiliations of the haloarchaeal 16S rRNA gene sequences of the basis pairwise comparison by the Ez Taxon server 2.1.

Strain	Isolation site	Accession no.	Identity (%)	Taxon (type strain)
5.1[a]	S1	DQ118426	100	*Halorubrum ezzemoulense* (DQ118426)
S1	S1	DQ120725	96.5	*Haloarcula salaria* HST01-2R^T (FJ429317)
4	S1	DQ149846	95.8	*Haloferax prahovense* TL6^T (AB258305)
S7	S1	EF488827	99.4	*Halorubrum chaoviator* Halo-G^T (AM048786)
K-1[a]	S1	EU409597	100	*Halomicrobium katesii* Al-5^T ((EF533994)
Ez59	S1	GQ181207	99	*Halorubrum chaoviator* Halo-G^T (AM048786)
L52	S1	GQ181208	99.7	*Haloterrigena thermotolerans* PR-5^T (AF115478)
Ez26	S1	GQ181209	99.5	*Halorubrum chaoviator* Halo-G^T (AM048786)
Ez228	S1	GQ181210	99.5	*Halorubrum chaoviator* Halo-G^T (AM048786)
L56	S1	GQ181211	99	*Halorubrum chaoviator* Halo-G^T (AM048786)
Eza4	S1	GQ181212	99.2	*Halorubrum californiense* SF3 213^T(EF139654)
Ez21	S1	GQ181213	98.2	*Halobacteriumjilantaiense* NG4^T (DQ256409)
Ez1.2	S1	GQ225084	98.9	*Haloarcula quadrata* 801030^T (AB010964)
Ez5-1	S1	GQ225086	98.9	*Halorubrum chaoviator* Halo-G^T (AM048786)
Ez5-2	S1	GQ225087	99.1	*Halorubrum chaoviator* Halo-G^T (AM048786)
Ez5RB	S1	GQ225088	99.7	*Halorubrum chaoviator* Halo-G^T (AM048786)
EzB1	S1	GQ225089	99.8	*Halorubrum chaoviator* Halo-G^T (AM048786)
EzB3	S1	GQ225090	97.5	*Haloterrigena thermotolerans* PR-5^T (AF115478)
EzS2	S1	GQ225091	98.8	*Halorubrum chaoviator* Halo-G^T (AM048786)
EzS6	S1	GQ225092	99.8	*Halorubrum chaoviator* Halo-G^T (AM048786)
Ez526	S1	GQ225094	99	*Halorubrum californiense* SF3 213^T(EF139654)
Ez24	S1	GQ225095	99.4	*Halorubrum chaoviator* Halo-G^T (AM048786)
EzA1	S1	GQ250584	99.1	*Halorubrum chaoviator* Halo-G^T (AM048786)
EzSm	S1	GU166402	99.1	*Haloterrigena thermotolerans* PR-5^T (AF115478)
EzA	S1	GQ225085	99.7	*Halobacterium salimarum* NRC-1 (AE004437)
beja5	S2	GU361123	98.5	*Halorubrum chaoviator* Halo-G^T (AM048786)
bejS3	S2	GU361124	98.9	*Haloferax lucentense* JCM 9276^T (AB081732)
bej51	S3	GU361125	97.8	*Haloferax prahovense* TL6^T (AB258305)

[a]Strain described as new species.

Table 4. Contd.

Strain	Isolation site	Accession no.	Identity (%)	Taxon (type strain)
KL	S2	FJ794071	98.4	*Halogeometricum rufum* R01-4^T (EU887286)
MGG2	S3	GU361137	98	*Haloarcula marismortui* ATCC43049^T (AY596298)
MGG3	S3	GU361138	98	*Haloarcula vallismortis* CGMCC1.2048 rrnB (EF645688)
MG23	S3	GU361139	99.8	*Halorubrum chaoviator* Halo-G^T (AM048786)
MG25	S3	GU361140	98.6	*Halorubrum chaoviator* Halo-G^T (AM048786)
MG215	S3	GU361141	99.6	*Halorubrum chaoviator* Halo-G^T (AM048786)
MG525	S3	GU361142	98.3	*Halorubrum chaoviator* Halo-G^T (AM048786)
MG526	S3	GU361143	99.2	*Halorubrum californiense* SF3 213^T(EF139654)
Set21	S3	GU361130	99.5	*Haloferax lucentense* JCM 9276^T (AB081732)
KM	S2	FJ794073	96.6	*Halomicrobium katesii* Al-5^T (EF533994)
Bet 58	S2	FJ897725	97.9	*Halomicrobium katesii* Al-5^T (EF533994)
Bet25	S2	GU361133	98.8	*Halorubrum chaoviator* Halo-G^T (AM048786)
Bet213	S2	GU361134	96.8	*Halomicrobium katesii* Al-5^T (EF533994)
Bet217	S2	GU361135	99.1	*Halorubrum californiense* SF3 213^T(EF139654)
Bet512	S2	GU361136	99.5	*Halorubrum chaoviator* Halo-G^T (AM048786)

S1, Ezzemoul sabkha; S2, Bethioua sabkha; S3, Chott Melghir.

2011). Such dominance of the genus *Halorubrum* is in agreement with previous cultivation-based study on halophilic archaeal communities inhabiting hypersaline environments (Burns et al., 2004; Xu et al., 2007; Trigui et al., 2011; Chen et al., 2013). The genus, *Halorubrum* contains the largest number of species (currently 27 species) and was among the most frequently revealed and probably ubiquitous archaeon in different hypersaline lakes that were confirmed by several diversity studies (Pašić et al., 2007; Manikandan et al., 2009). The predominant population tends to be made up of strains belonging to the genera *Natrinema* and *Haloferax* found in Rambla Salada (Spain) (Luque et al., 2012); *Halorubrum* and *Haloferax* in solar salterns of Tamil Nadu (India) (Manikandan et al., 2009); *Halobacterium* in Ocnei hypersaline lake (Romania) (Baricz et al., 2014). Compared to several lakes, at genus level, almost the same rate (6 and 7 genera) is observed (Pašić et al., 2007; Tsiamis et al., 2008).

The culture media applied in this study had varying degrees of selectivity on the detected species. Some of the isolates were able to reduce nitrate to nitrite, which suggests that they might be involved in the nitrogen cycle within these environments. A survey of the literature on the taxa of haloarchaea showed that some strains have been reported to possess the urease activity (Mizuki et al., 2005). In the present work, only four strains affiliated to genus *Haloarcula* were shown to be urease producers.

Most research studies performed on hypersaline environments have focused on the microbial diversity and on ecology. However, the studies based on the diversity of halophilic microorganisms showing hydrolytic activities in hypersaline habitats remain unexplored. It is interesting to emphasize that multiple hydrolytic activity was detected in the isolates of this study supporting previous studies in other hypersaline environments. Many species of the family *Halobacteriaceae* produce hydrolases such as proteases, lipases, amylase and amyloglucosidases that function at high salinity (Pérez-Pomares et al., 2003; Oczan et al., 2009; Siroosi et al., 2014). The most recurrent hydrolytic activity detected in our study was amylase (50% of total isolates), in agreement with those found by several authors (Moreno et al., 2009; Makhdoumi Kakhki et al., 2011). Several halophilic enzymes also function at high temperatures (Vidyasagar et al., 2006; Moshfegh et al., 2013). Some archaeal enzymes are of potential interest, such as amylase of *Haloarcula* sp. (Fukushima et al., 2005) and β-galactosidase of *Halorubrum lacusprofundi* (Karan et al., 2013). Thus, the hypersaline environments represent a valuable source of extracellular hydrolytic enzymes with potential in different economical fields (DasSarma et al., 2010; Delgado-Garcia et al., 2012; Schreck and Grunden, 2014).

A general conclusion that emerged from this study is that the diversity of halophilic archaea described above is undoubtedly a small fraction of the true diversity of halophilic archaea in Algerian hypersaline environments. These environments could be used as a starting point, for more cultivation attempts. Also, their localization in semi-arid and arid regions where solar heating, especially in the chott Melghir, results in temperature up to 50°C in summer. These conditions make these habitats a good potential for halothermophiles prokaryotes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

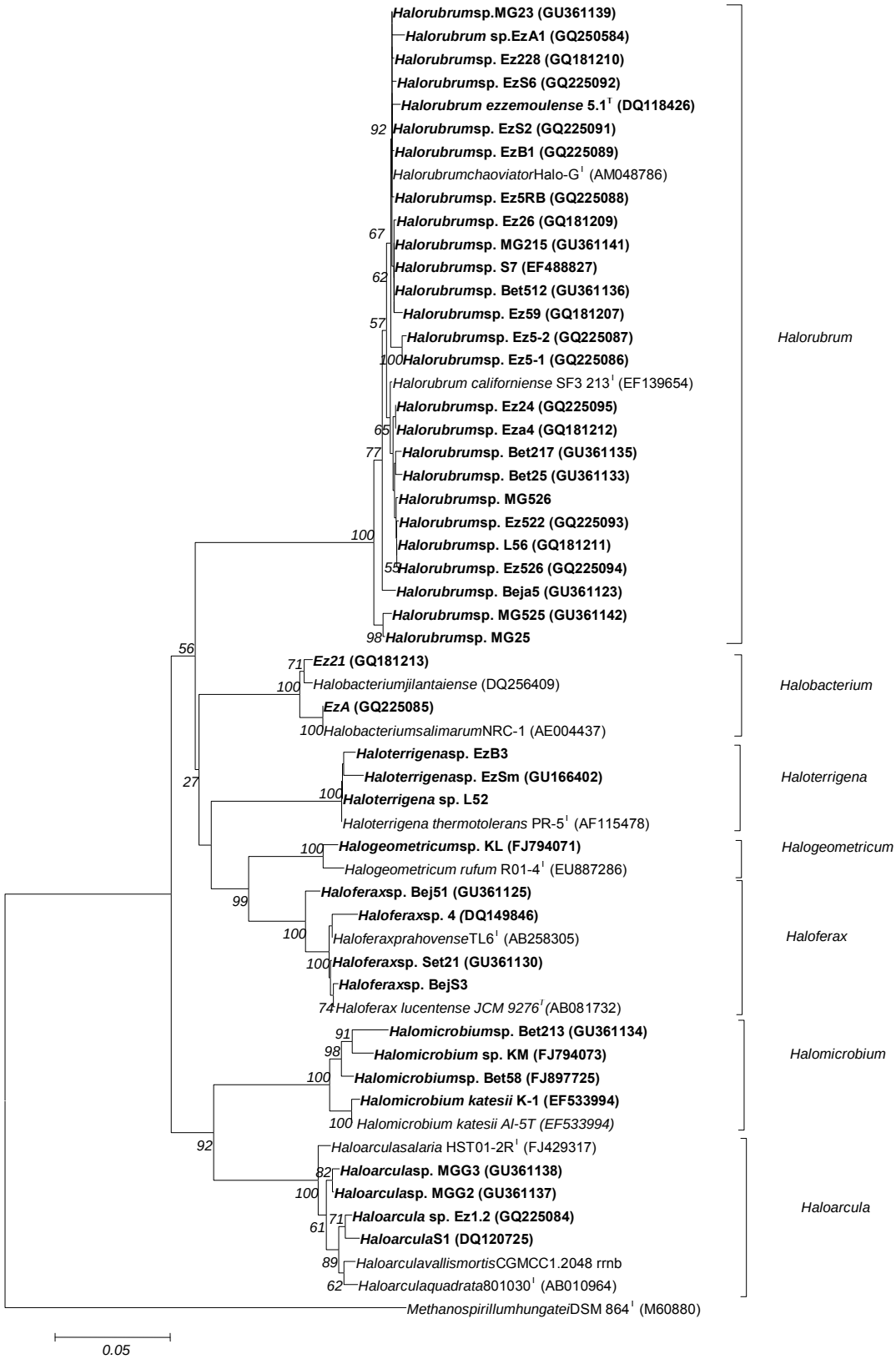

Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strains with respect to other species of the family *Halobacteriaceae*. The 16S rRNA gene sequence of *Methanospirillum hungatei* DSM 864^T was used as outgroup. Numbers at branch points indicate the level of bootstraps support, based on 1,000 resamplings.

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