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Original Research

Isolation and identification of two extremely halophilic archaea from sebkhas in the Algerian Sahara

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Abstract: In Algeria, many salt lakes are to be found spread from southern Tunisia up to the Atlas Mountains in northern Algeria. Oum Eraneb and Ain El beida sebkhas (salt lakes), are located in the Algerian Sahara. The aim of this study was to explore the diversity of the halobacteria in this type of habitats. The physico-chemical properties of these shallow saline environments were examined and compared with other hypersaline and marine ecosystems. Both sites were relatively alkaline with a pH around 8.57- 8.74 and rich in salt at 13% and 16% (w/v) salinity for Oum Eraneb and Ain El beida, respectively, with dominant ions of sodium and chloride. The microbial approach revealed the presence of two halophilic archaea, strains JCM13561 and A33^T in both explored sebkhas. Growth occurred between 10 and 25% (w/v) NaCl and the isolates grow optimally at 20% (w/v) NaCl. The pH range for growth was 6 to 9.5 with an optimum at pH 7.5 for the first strain and 7 to 9.5 with an optimum pH at 8.5-9 for the second strain. On the basis of 16S rRNA gene sequence analysis, strains JCM13561 and A33^T were most closely related to *Halorubrum litoreum* and *Natronorubrum bangense* (99% and 96% similarity, respectively).

Key words: Sebkha; Hypersaline environments; Extremely halophilic; Archaea.

Introduction

The greatest part of the biosphere is saline. The oceans that cover most of the earth's surface contain 35 g of dissolved salts per liter. Higher concentrations of salt, up to saturation of NaCl and beyond, exist (1). Hypersaline environments are typical extreme habitats where high salt concentration is not the only environmental factor that can limit their biodiversity. They have low oxygen concentrations, depending on the geographical area, extreme temperature (high or low) and are sometimes very alkaline. These specific environments are represented by aquatic (thalassohaline) and terrestrial (athalassohaline) systems, as well as salty products, foods, or sea salt etc. (2,3).

Considered sterile in the past, these sites have revealed a number of interesting microorganisms (4) in each of the three domains of life perfectly adapted to grow in saline environments. But as the concentration of salt increases, the overall diversity of physiological groups decreases (5). These exceptional organisms that defy the laws of biology and create their own life where man could not dare imagine it, are called extremophiles (6).

The extremely halophilic archaea belonging to the family *Halobacteriaceae*, established by Gibbons (7), are highly successful inhabitants and dominant heterotrophic organisms in hypersaline environments where salt concentrations exceed 250-350 gl^{-1} (8). Most microorganisms of this fascinating group are red pigmented, exhibit optimal growth at 200- >260g NaCl and require at least 100g NaCl to develop (9).

An awareness of the haloarchaea has existed for a long time, with published descriptions of the "red waters" associated with salt mining, and the "redness" of salted fish (10). This distinct red color caused by the carotenoid pigments that protect the cells from the harmful effect of ultraviolet light, was reported in the Great Salt Lake Utah, the Dead Sea and the extremely alkaline brines of Lake Magadi and other soda lakes in Kenya (11,12).

In many athalassohaline environments, life at extremely high salt concentrations is combined with the need to thrive at alkaline pH and high temperatures, and the organisms that grow there do so at the physico-chemical limit of life (9).

The study of Algerian ecosystems' diversity such as the hypersaline chotts (Sebkhas) revealed the existence of new specific microbial groups: Hacene et al. (13), Kharroub et al. (14) and Boutaiba et al. (15).

Currently, no microbial research study has been done on the sebkhas Oum Eraneb and Ain El beida, in the Sahara desert located in Ouargla, 800 km, south west of Algiers.

The objective of this study was to elucidate halobacterial environments in search for interesting microor-

ganisms inhabiting particular ecosystems.

To achieve this objective, physicochemical analysis of the water and sediment samples were carried out, followed by the isolation, in pure culture, of halobacteria from these previous samples. We also characterized their morphology, physiology, biochemical characteristics, and 16S rRNA gene sequences.

Materials and Methods

Geographical location

Ouargla is located in the south-west of the country at 800 km from the capital, Algiers. It is bordered in the north by El Oued, Djelfa and Biskra, in the south by Illizi and Tamanrasset. In the east by the Tunisian border and in the west by the city of Ghardaia "Figure1A".

Sebkha Oum Eraneb is a wetland located about 7 km northeast of the city of Ouargla. It is oblong and extends in a north-south direction. The site is a lake of 1400 ha, surrounded by sand dunes. The surface of the salt lake is 7155 ha with a depth less than 2m.

The chott of Ain El beida is located 6 km east of the main town Ouargla. It is a salt depression which flooded part is constituted by the sebkha situated in the middle of a palm grove in the basin of Ouargla. Oblong, extending from north-west to south-east over 5.3 km, the surface area of the salty lake is 6853 ha with water covering 800ha (16).

Sample collection

At the level of each sebkha, Oum.Eraneb and A.El beida "Figure 1B", water and sediments were sampled. According to accessibility, the sebkhas have been divided into 4 sites. Sampling campaigns were organized in spring 2013, water samples of 200ml were collected a few centimeters below the surface. Sediment samples were also picked at the surface and several steps in each site, labelled and transported to the laboratory.

Sediment and water samples analysis

The chemical and physical properties of the water samples were analyzed in the laboratories of the ANRH Ouargla according to the methods described by Rodier (17). The chemical properties included compositional estimations of Mg^{2+} by a complexometric method using ethylene diaminetetra acetic acid and EDTA. HCO_3^- and CO_3^{--} by colorimetry at 497nm. Nitrates by colorimetry at 520nm; SO_4^- by colorimetry at 495nm, and Cl^- at 497nm, multi-ray spectrophotometry (DR2000). Na^+, Ca^{++} and K^+ by spectrophotometry with flame ionization DR 2000. Temperature and pH were measured in situ using portable instruments.

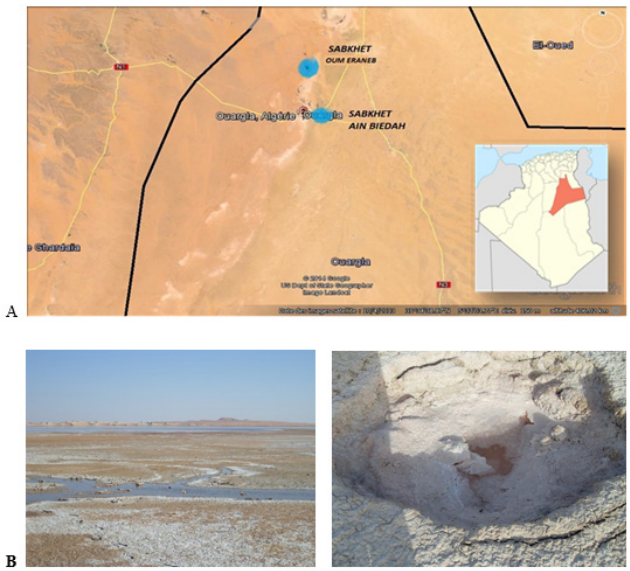

Figure 1. (A) Oum Eraneb and Ain El beida location map. (B) Representative images of the two sampling sites that show the high salt deposit.

Chemical and physical properties of the sediment samples were analyzed at the laboratories of the International Center for Environmental Technologies of Tunis CITET-Tunisia, according to the norms quoted below.

The pH was measured by the electrochemistry method (NF T 90-008 (2001), conductivity and salinity by electrochemistry (NF EN 27 - 888 (1994). Ca^{++} and Mg^{2+} by mineralization. Na^+ and K^+ by atomic emission-ICP (ISO 11885 (2007). Sulfate, Chlorure and Nitrate were estimated by extraction according to the 1/5 method (10g in 50ml of water) Ion chromatography (ISO 10304-1 (2007). Carbonate was measured according to the Bernard calcimeter method. All these characteristics are listed in Table 1.

Enrichment and isolation

Enrichment cultures and isolation procedures to collect aerobic, moderately or extremely halophilic microorganisms were conducted in halophilic medium.

The water samples were subcultured in SG medium (Sehgal and Gibbons) (18) containing (per liter): 250g of NaCl, 5g of Na_2CO_3 $10H_2O$, 3g of tri sodic citrate,7.5g casamino acid (Sigma Aldrish), 10g of yeast extract (Difco), 1g $MgSO_4$, 3g of KCl, adjusted to pH 7 with HCl.

The sediment samples were also subcultured in a medium containing gl^{-1}: 250g of NaCl (Sigma Aldrish), 13g of $MgCl_2$ $6H_2O$, 20g of $MgSO_4 7H_2O$ (Sigma Aldrish), 4g of KCl, 1g of $CaCl_2 2H_2O$, 0.5g NaBr, 0.2g $NaHCO_3$, 5g yeast extract (Difco), 8g of tryptone, 1g glucose, pH adjusted to 7,2 with NaOH.

Primary cultures were grown in 100ml of each medium in 250ml Erlenmeyer flasks in a rotary shaker at 37°C with stirring at 150 rpm. Aliquots (100 µl) of dilutions 10^{-1} to 10^{-4} were deposited on the same agar media solidified with 20 g of Agar. After 7 to 10 days of incubation at 37°C, the pigmented colonies were picked and restricted several times to obtain a pure culture.

Physiological and biochemical morphological characterization of isolates

The framework for studying aerobic halobacteria as suggested by Oren et al. (19), should contain a data set as complete as possible, including phenetic, chemical and molecular properties.

The isolates were examined for colony and cell morphology. The colonial morphologies were described using standard microbiological criteria, with particular emphasis on pigmentation, diameter, colonial elevation and opacity.

Gram staining was performed using samples fixed with acetic acid as described by Dussault (20). The routine culture was carried out at 37°C, pH 7,5. Cellular morphology was examined by differential interference

contrast (Normarski interference contrast DIC) of liquid exponentially growing cultures at room temperature using a ZEISS Axio Observer equipped with a target Immersion in oil 40x, 1.4 NA.

In order to classify our isolates according to their behavior with respect to salt, optimal growth conditions included the salinity tolerance tested in a solid medium containing 0, 5, 10, 15, 20, 25 and 30% (w/v) NaCl.

Growth at pH 5 to 9.5 (at intervals of 0.5) was examined using a solid medium. Growth was also tested at different temperatures 5, 10, 20, 25, 30, 37, 40 and 45 by incubating cultures on agar plates at optimal pH and salt concentrations.

Catalase and oxidase activities were tested using standard procedures. The capacity to grow anaerobically includes the use of an alternative electron acceptor as nitrate. Thus, nitrate reduction was detected using liquid media supplemented with 0,1% KNO_3 (w/v) (21).

Indole formation was performed as described by Takashima et al. (22), after 7 days of incubation at 40°C in a liquid medium containing tryptone. The production of H_2S was tested as indicated by Rodriguez et al. (23), using Kligler-Hajna medium supplemented with 25% (w/v) NaCl, incubating at 40°C for 7 days.

Since it seemed to be interesting to look for the ability of strains to produce exoenzymes, therefore the tests focused on hydrolysis of casein which was determined using halophilic media supplemented with 1g of yeast extract, 0,1% of casein, incubation at 40°C for 7 days.

The degradation of gelatin was also considered and tested on agar plates prepared by adding 12% of gelatin and 1g yeast extract, incubating at 40°C for 14 days (24). And hydrolysis of the 80 was performed following the protocols of Noris and Ribbons (25); in a medium containing 1 gl^{-1} $CaCl_2$ $2H_2O$ and 1 ml of tween 80. Incubation at 40°C for four weeks.

Aerobic halobacteria typically lead an heterotrophic life style. However, in spite of their common requirement for high salt concentrations for growth, their nutritional demands and metabolic pathways are quite different (26). So, the use of sugars as single carbon sources by our isolates was tested in solid medium supplemented with 1% (w/v) of the carbon source under test (19). Other phenotypic characteristics were determined using API 20^E (Kit Biomérieux).

As the biochemistry of Archaea is very different from that of other microorganisms, we can expect a difference in sensitivity to antibiotics. The reaction to chemotherapeutic agents was examined by propagating bacterial suspensions on culture plates and applying antibiotic discs (Bacitracin, 10U; streptomycin, 10µg; chloramphenicol, 30µg; erythromycin, 5µg; penicillin G, 10U; novobiocin, 30µg; ampicillin, 10µg; tetracycline, 30U; naldixic acid, 30µg; vancomycin, 30µg; gentamycin, 10µg; ciprofloxacin, 5µg; kanamycin, 30µg) (19). The plates were incubated for 2 weeks at 37°C.

16rRNA PCR Amplification

DNA from isolated strains (cultures) was extracted using NucleoSpin Tissue from Machery-Nagel according to the manufacturer's recommendations.

The 16rRNA gene of each strain was amplified by a standard PCR reaction using the High-Fidelity Q5 DNA polymerase from NEB in its buffer optimized for a high

Table 1. Chemical and physical properties of Oum Eraneb and Ain El beida salt lakes of the Algerian Sahara compared to other hypersaline and marine ecosystems[a].

Ecosystem	Chemical and physical properties								
	pH	**Na^{2+}**	**K^+**	**Mg^{2+}**	**Ca^{2+}**	**Cl^-**	**So_4^{2-}**	**$Hco3^-$**	**Salinity**
Hyper saline Solarsaltern	Nd	65.4	2.5	20.1	0.2	144	1.9	nd	254
Great Salt Lake (USA)	7.7	105	6.7	11.1	0.3	181	27	0.72	333
Lake Assal (Djibouti)	n.d	77.8	5.4	8	14.6	164	2.3	n.d	277
Dead Sea	7.8	40.1	7.6	44	78.2	225	0.44	0.26	340
WadiNatrun(Egypt)	11	142	2.3	UD	UD	155	22.6	67	394
Sambhar Salt Lake SSL (India)	9	37.5	0.50	0.00	0.00	21.46	6.00	1.94	/
Solar salternSfax (Tunisia)	7.55	87.5	8.5	0.29	0.043	261.09	43.2	nd	376.6
El Golea Salt Lake (Algeria)	9	107	Nd	0.3	0.4	198	nd	nd	296
SidiAmeur site (Algeria) water	7.4	67.1	0.17	3	0.51	111	2.1	0.19	200
SidiAmeur site (Algeria) sediment	7.15	94.5	0.23	29	1.7	170	1.1	nd	nd
Himalatt site (Algeria) water	7.2	24.5	0.12	1.6	0.22	63.8	3.1	nd	117
Oum Eraneb site (Algeria) water	**8.57**	**37.33**	**1.71**	**4.04**	**5.63**	**64.68**	**41.22**	**0.43**	**128**
Oum Eraneb site (Algeria) sediment	**8.32**	**27.6**	**2.52**	**22.27**	**24.32**	**39.15**	**30.32**	**70.42**	**17.125**
Ain El beida site (Algeria) water	**8.74**	**71.77**	**1.91**	**10.02**	**7.35**	**112.31**	**51.03**	**0.43**	**165**
Ain El beida site (Algeria) sediment	**8.65**	**50.4**	**3.98**	**26.85**	**11.37**	**55.2**	**69.92**	**106.9**	**22.67**
Marine	**Chemical and physical properties**								
	pH	**Na^{2+}**	**K+**	**Mg^{2+}**	**Ca^{2+}**	**Cl^-**	**So_4^{2-}**	**Hco3**	**Salinity**
Aral Sea	8.2	2.2	0.08	0.55	0.51	3.47	3.2	0.07	10.2
Caspienne Sea	8.3	3.18	0.09	0.73	0.34	5.33	3.0	0.4	12.8
Atlantic Ocean	8.5	10.6	0.38	1.29	0.42	19.2	2.68	0.14	34.85

[a]Salinity and ions are represented as g per litre . n.d., not determined. U.D., undetectable. pH of the water samples were 7.47 – 9.58 and 7.41 -9.9 for O.Eraneb and A.El beida respectively. References for abiotic features of other hypersaline and marine habitats were as follows: Boutaiba et al. (15), Upasani et al. (27) and Trigui et al. (28).

Table 2. Characteristics that differentiate *Halorubrum litoreum JCM 13561^T* from other closely related species of the genus.

Caracteristic	*JCM 13561^T*	1	2	3	4	5	6	7	8	9	10	11	12	13
Gram strain	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mobility	+	+	+	+	+	ND	+	-	-	-	+	+	+	ND
Colonies pigmentation	RD	O-RD	RD	RD	RD	RD	RD	RD	RD	RD	P	RD	RO	RD
NaCl range for growth (M)	1.7-5.1	2.5-5.2	1.8-5.2	2.-5.2	2.5-4.3	2.0-5.1	2.2-5.2	2.5	2.5-5	2.5-5.0	2.5-5	1.7-4.8	2.5-5.1	2.5-5.1
Optimum NaCl	3.4	4.2	3.9-4.3	3.1-3.4	3.4	4.2	3.4-3.9	3.4	3.4	3.4	3.5-4 .5	2.6	4.2	4.2
Optimum Temperature (°C)	40	37-45	38	40	37-40	37	42	37	35-40	37	37	45-48	37	35-40
pH range for growth	6.0-9.5	5.0-9.0	8.0-10.5	6.0-10	6.0-9.5	7.0-8.5	6.0-8.5	6.0-9.0	6.0-9.0	6.0-10	6.0-8.5	7.0-9.0	6.0-9.0	7-9
Optimum pH	7.5	7.5	9-10	7-7.5	7-7.5	7.4	7.5	7.5	7.5	7.5	7.3	7.5	7.4	8
Catalase	+	+	+	+	-	+	-	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	-	+	-	-	+	+	+	-	+
Indole	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Nitrate reduction to nitrite	+	-	+	-	+	+/-	+	+	+	+	+	+	+	+
H_2S	-	-	+	+	ND	ND	+	-	-	-	-	-	-	-
Hydrolyse of :														
Casein	-	-	-	ND	-	-	-	-	-	-	+	-	-	-
Gelatin	-	-	-	ND	-	-	-	-	-	-	-	+	ND	-
Tween 80	+	+	-	-	-	-	-	ND	+	+	-	+	ND	+
Utilisation of														
Glucose	+	-	+	+	+	+	+	-	+	-	+	+	+	+
Galactose	+	+	-	-	-	+	-	-	-	-	+	+	-	+
Fructose	-	-	+	+	-	-	-	-	+	-	-	+	ND	-
Sucrose	+	-	-	+	+	-	-	ND	+	ND	-	+	-	-
Lactose	+	ND	-	-	-	-	-	-	+	-	+	-	ND	+
Maltose	-	-	+	+	+	-	+	-	+	-	-	+	+	-
Mannitol	-	-	-	-	-	ND	-	-	-	-	-	-	ND	-
G+C%	64.9[a]	64.4	62.1	68.0	61.9	65.5	65.7	69.4	62.1	64.0	69.4	65.9	65.1	64.6

a G+C% : Cui et al. (29).Taxa:1, *Hrr. terrestre* VKM B-1739^T; 2, *Hrr. alkaliphilum* JCM 12358^T; 3, *Hrr.xinjiangense* BD-1^T; 4, *Hrr. ezzemoulense* 5.1^T; 5, *Hrr. choaviator* Halo-G*^{T} ; 6, *Hrr. arcis* AJ201^T; 7, *Hrr. kocurii* BG-1^T; 8, *Hrr. aquaticum* EN-2^T; 9, *Hrr. ejinorense*; 10, *Hrr. californiens* SFT-213^T ;11, *Hrr. lipolyticum* 9-3T ; 12, *Hrr. safexense* ETD6 ; 13, *Hrr. halophilum* B8^T. Unless indicated, data for reference strains were taken from Presenti et al. (30), Mancinelli et al. (31), Trigui et al. (28), Gutierréz et al. (32) and Yim et al. (33). +, Positive; -, négative ; + /-, doubtful variale; ND, no data available ; RD, red ; O, orange

GC content. Universal primers for archaea 21F (5'-TTCCGGTTGATCCYGCCGGA-3') and 1404R (5'-GGGTGTGTGCAAGGRGC-3') were used. Specific PCR products were obtained when the primer hybridization was performed at 55°C. The PCR products were then purified and sequenced. The nearly complete 16rRNA gene sequence of each strain was compared via the BLAST microbial nucleotide (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=MicrobialGenomes), using representative genomes only.

Results

Physicochemical properties

In this study, we aimed at isolating and characterizing the halophilic microorganisms present in the saline depressions of Oum Eraneb and Ain El beida. To fulfill this objective, we sampled water and sediment from both sites. Besides, we investigated the mineralization, the overall salinity and the individual ionic composition of these samples. The results are summarized in Table 1 and compared to other hypersaline and marine ecosystems as cited above.

Microbial properties

The detailed phenotypic and physiological properties of the two strains *Halorubrum litoreum* and *Natronorubrum bengense*, isolated and characterized from sediment and water samples of both sebkhas, are given in the description of the species and the differential characteristics listed in Tables 2 and 3.

The two isolated halobacteria were sensitive to bacitracin and novobiocin, but insensitive to common antimicrobial agents tested above.

16S rRNA analysis

Fragments ranging from 0.5 bp to 1.5 bp of the 16S rDNA sequences of the isolates.

Discussion

Situated in the Algerian Sahara, the hypersalinity of Oum Eraneb and Ain El Beida sebkhas, is derived from the dissolution of salts of continental origin. These waters can be considered as athalassohaline (36).

The athalassohaline composition reflects the composition of the surrounding geology, topography and climatic conditions, often particularly affected by the dissolution of mineral deposits; thus the composition of such waters varies widely (37).

At the time of sampling, the temperature of the water varied between 17°C in Oum Eraneb and 18°C in Ain El beida. All water and sediment samples were alkaline, with pH values ranging from 8.57 to 8.74 (Table 1). Such pH is closer to the ones observed in the lake El Golea, Sambhar Salt Lake SSL (India), the Caspian Sea, the Atlantic Ocean and the Aral Sea. But contrasts with the Algerian salt lakes previously described; Sidi Ameur, Himalatt and Wadi Natrun (Egypt) (15,27) .

The hypersaline alkaline lakes show another ionic composition with predominance of carbonate and chloride anions and Na^+ cations (38). Water and sediment samples are dominated by Na^{2+} and Cl^- ions that correspond with other studies as shown in Table 1. The proportions of the various salts of these waters are dis-

Table 3. Differential characteristics of ***Natronorubrum bangense A33^T*** and other species of *Natronorubrum.*

		A33^T	**1**	**2**	**3**	**4**
	Gram strain	-	-	-	-	-
	Mobility	-	-	+	ND	-
	Colonies pigmentation	O-RD	RD	RD	ND	P
	NaCl range for growth (M)	1.7-4.3	2-5.1	2.4-3	2.1-4.8	2.5-5
	Optimum NaCl	3.4	3.4	2.6-3.1	3.1	3.4
	Optimum Temperature °C)	45	45	45	44-47	37
	pH range for growth	7-9.5	8.5-11	6.5-9.5	8-10	8-11
	Optimum pH	8.5-9	9	8	7.8-9.2	9
	Catalase	+	+	-	-	+
	Oxidase	+	+	+	+	+
	Indole	+	+	+	+	-
	Nitrate reduction to nitrite	-	-	+	+	+
	H_2S	-	-	-	+	-
Hydrolyse of :	Casein	-	-	-	-	-
	Gelatin	-	+	-	-	-
	Tween 80	-	-	-	-	+
Utilisation of	Glucose	+	+	+	+	+
	Galactose	-	-	+	-	-
	Fructose	+	+	-	-	+
	Succrose	+	+	+	+	ND
	Mannitol	-	-	-	-	-
	G+C %	59.9[b]	60.1	61.2	60.9	62.5

(b) : Xu et al. (34). Taxa : 1, *Nrr. tibetense* GA33^T; 2, *Nrr. aibiense* 7-3^T ; 3, *Nrr. sulfidifaciens* AD2^T, 4, *Nrr. sediminis* CG-6^T. Data were taken from Xu et al. (34) and Gutierréz et al. (35). +, Positive; -, négative; ND, no data available. RD, red; O-RD, orange-red; P, pink.

tinctly different from those of sea-water. Compared to the Atlantic Ocean, the concentrations of sodium and chloride were 6 times higher in Ain El beida and 3 times higher in Oum Eraneb.

Bicarbonate ions constitute a significant part of the anion sum in such lakes, in addition to chloride and sulfate (1). The concentration of bicarbonate ions appeared very abundant in both sediment samples. In contrast, bicarbonate ions concentration in water samples is significantly lower.

Sulfate ions SO_4^{2-} are detected at high levels in both water and sediment samples, and their concentration is significantly higher than in most described hypersaline ecosystems (even compared to the high concentrations reported in the Great Salt Lake, USA and Wadi Naturn, Egypt). Similar values are reported from Oum Eraneb and the solar saltern of Sfax (Tunisia).

Calcium concentrations in the samples of Ain Elbeida and Oum Eraneb are higher than in most hypersaline ecosystems studied previously, except for the Assal Lake of Djibouti and the Dead Sea where these concentrations are even higher.

Altogether, sediment and water samples collected in both sites have a different chemical composition compared to the other Algerian site, Sidi Ameur, also located in the Sahara desert.

Large pure red colonies were isolated from the sediments of Oum Eraneb and Ain El Beida, and grew to a population density of $5.2x10^4$UFC $g^{-1}$and$7.5x10^4$UFCg^{-1} respectively. Aerobic growth was observed at 20 to 45°C (higher temperature not tested) with the most advantageous at 40°C. A pH range of 6 to 9,5 was tested at 37°C with an optimum pH of 7,5. A minimum concentration of 10% NaCl was required for development, up to 30% on the plate, with an optimum observed at 20% (3.4M). No growth observed at 0% and 5%.

Cells are mobile and Gram negative. Microscopic observation revealed some pleiomorphism, with rod-shaped and bacilli-like shaped cells "Figure 2A".

This isolate appeared catalase and oxidase-positive. Reduction of nitrate to nitrite was observed, but no indole was produced. Tween 80 was hydrolyzed, but casein and gelatin were not. Growth was observed only with glucose, galactose, malate, sucrose, lactose, glycerol, acetate, pyruvate, inositol, rhamnose, sucrose, melibiose, amylose and arabinose. No ortho-Nitro-Phenyl-Galactosidase, Argenine Deshydrogenase, Lysine Decarboylase and Urease activities were observed.

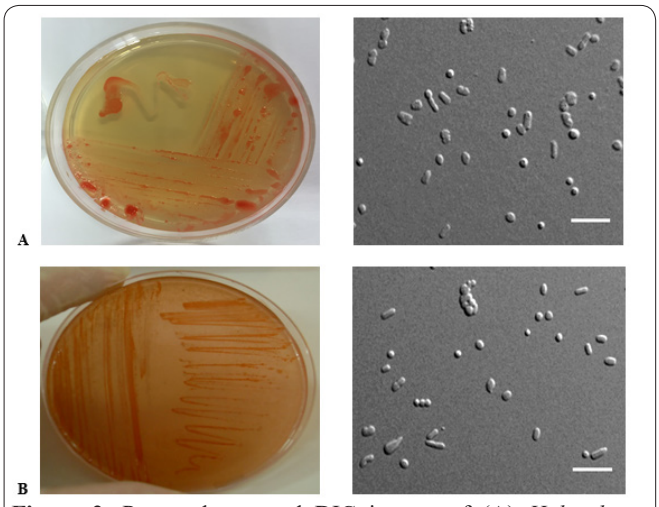

Figure 2. Pure cultures and DIC images of (A) *Halorubrum litoreum* and (B) *Natronorubrum bangense* cells isolated from the two sebkhas. Scale bar 10µm.

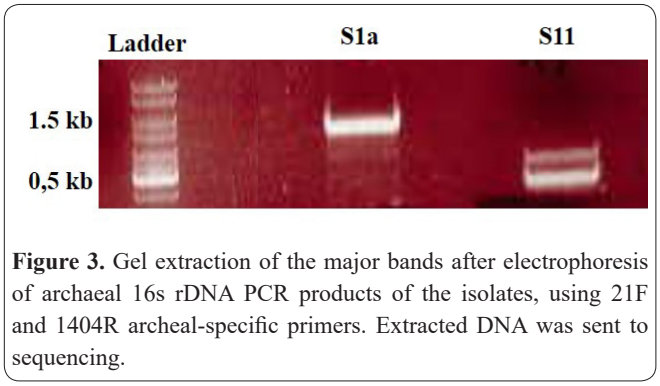

Figure 3. Gel extraction of the major bands after electrophoresis of archaeal 16s rDNA PCR products of the isolates, using 21F and 1404R archeal-specific primers. Extracted DNA was sent to sequencing.

DNA was isolated and 16S rRNA was amplified and sequenced. Blast analysis of the almost complete 16S rRNA gene showed a 99% identity to the 16S full-length16rRNA gene of the *Halorubrum litoreum* strain JCM13561 isolated from a solar marine saltern in Fujian, China (29).

Pure red-orange colonies isolated from Oum Eraneb and Ain Elbeida water samples appeared on halophilic medium with $5.1x10^3$UFC $ml^{-1}$and $9x10^3$UFC ml^{-1}respectively. Aerobic growth was observed at temperatures from 20 to 45°C, pH 7 to 9,5 and NaCl concentrations ranging from 10 to 25% with an optimum pH of 8,5-9 and 20% NaCl (3.4 M).

Cells are mobile and Gram negative. As already mentioned, microscopic observation revealed a pleomorphism in the form of a stem, bacilli, and triangle "Figure 2B". The selected strain showed to be catalase and oxidase-positive, indole formed from tryptone. Nitrate was not reduced to nitrite and no H_2S formation was observed. The casein was not hydrolyzed and the gelatin was not liquefied. Growth occurs on glucose, fructose, sucrose, succinate, manose, inositol, sorbitol, rhamnose, sucrose, melibiose, amylose and arabinose. No Ortho Nitrophenyl-Galactosidase, Arginine Deshydrogenase or Lysine Decarboxylase activity was observed.

Comparative 16S rRNA gene sequence analysis showed that the strain was the most closely related (96%) with a full-length gene from *Natronorubrum bangense* isolated from a soda lake in Tibet (34). The genus *Halorubrum* was proposed formally in 1995 by Mc Genity and Grant (39). The genus *Natronorubrum* was estabilished by Xu et al (34).

Because of the absence of murein in Archaea, these last are not susceptible to most antibiotics which inhibit the synthesis of the cell wall. So they are not affected by high concentrations of penicillin, vancomycin and cephalosporin which are all cell wall biosynthesis inhibitors.

The usual antibiotics such as chloramphenicol and streptomycin do not affect the protein synthesis of archaea, with the exception of neomycin which, at high concentrations, is an inhibitor. In addition, tetracycline is not very efficient as an inhibitor unless it inhibits the protein synthesis of bacteria and eukaryotes. As reported by Tortora et al., Perry and Staley (40,41), these results reveal a very different structure of the archaea ribosomes from that of bacteria and eukaryotes.

Aerobic halophilic Archaea thrive in environments with salt concentrations approaching saturation. Indeed, the salinity range of our isolates shows that they may grow in media containing up to 25 and even 30% (w/v) NaCl with an optimum of growth observed at 20% (w/v) NaCl. This profile corresponds to that of extremely halophilic Archaea, most species described grow optimally above a concentration of 150gL^{-1} salt and lyse at concentrations bellow 100g L^{-1} (26).

The examinated temperature range goes from 5 to 50°C and the isolates showed tolerance to variations between 20 and 50°C,with an optimum of growth at 40°C to 50°C, thus, these strains are thermotolerant, what may be explainable by the presence of the examined sites in a dryland area.

The pH of our isolates growth ranges from 6 to 9.5, with an optimum allowing to classify them as neutrophilic (pH 7.5) and alkaliphilic (pH 9). This result confirms those of Minegishi et al. (42), who emphasize "that most representatives of aerobic halophilic Archaea are neutrophilic, many are alkaliphilic, and a moderately acidophilic species, *Halarchaeum acidiphilum* does not grow above pH 6.0.

Glucose is the most assimilated monosaccharide by all strains presented. Studies on glucose and fructose degradation pathways in *Halococcus saccharolyticus* showed that glucose is entirely degraded via an Entner–Doudoroff (ED) type pathway, whereas fructose is almost completely degraded (96%) via an Embden–Meyerhof type (43).

Halobacterium does not grow on sugars, but its growth is stimulated by the addition of carbohydrates to the medium (44).

According to the results presented in Tables 2 and 3, our strains have more than one advantage to live in such environments.

The microbial community of hypersaline environments is dominated by well-adapted halophilic microorganisms which in many cases are polyextremophiles with the ability to grow optimally not only at high salt concentrations but also at high or low pH values and temperatures (9,45). Only a very few of the extreme halophiles are able to grow optimally under triply extreme conditions. *Natronorubrum bangense*, rises to this rank and appears with some poly-extremophily.

Microbial diversity of Algerian salt lakes has been revealed by some works. Among them, Boutaiba et al. (15) molecular studies revealed the presence of *Haloferax*, *Halorubrum*, *Halalkalicoccus*, *Haladaptatus*, *Halobacterium* and *Halosarcina,* in two Algerian saharian sebkhas.

Several years later, Imadalou-Idres et al. (46) reported the presence of cultivable halophilic archaea isolated from three Algerian environments (salterns at Ichekaben, Bejaia and sebkhas at Ouargla and Oran) belonging to three genera of *Halobacteriaceae*. *Haloarcula* was isolated from all sites, however *Halorubrum* and *Natrinema* were found only at Ouargla and Ichekaben.

Actually, Baati et al. (47) notified the predominance of *Haloferax* and *Halorubrum* in the isolates of halophilic archaea cultures studies.

Several species of *Halorubrum* have been reported to be present in Algerian salt lakes as underlined by Imadalou-Idres et al. (46) *Hrr. ezzemoulense* seems the best example being present both in lakes at the North of the country (14) and in Saharian areas. Already identified in China, our study remains the first report of the presence of *Halorubrum litoreum* and *Natronorubrum bangense* in salt depressions in the Algerian Sahara.

A physicochemical approach combined with cultural and molecular methods has been adapted to study two sebkhas unexplored from this point of view: Oum Eraneb and Ain El Beida, located in Ouargla, in the Algerian desert.

The physico-chemistry of the water and sediments of these sites made it possible, for the first time, to compare them with other terrestrial and aquatic saline environments of the world, highlighting their alkalinity and abundance in salt.

The microbial exploration of these saline environments was successful and revealed the presence of extremely halophilic alkaline-thermotolerant strains belonging to the Archaea domain, Halobacterials order, *Halorubrum* and *Natronorubrum* genera, previously unidentified in such ecosystems of the Algerian desert.

The microbial diversity of saline ecosystems and alkaline waters is not only essential to better understand the limits of life at high salt concentration or extreme pH, but also to our search for new useful biomolecules as well.

Microorganisms living in extreme habitats are a good source for such (extreme) enzymes which allow biotransformation reactions under unconventional conditions where many proteins are completely denatured.

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