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Microbial Diversity of the Hypersaline Sidi Ameer and Himalatt Salt Lakes of the Algerian Sahara

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Abstract

Microbial populations within hypersaline lakes often exhibit high activities of photosynthesis, dissimilatory sulphate reduction and other processes and, thus, can have profound impacts on biogeochemical cycles of carbon, nitrogen, sulphur and other important elements within arid lands. To further understand these types of ecosystems, the physicochemical and biological properties of Sidi Ameer and Himalatt Salt Lakes in the Algerian Sahara were examined and compared. Both lakes were relatively neutral in pH (7.2 to 7.4) and high in salt, at 12% and 20 % (w/v) salinity for Himalatt and Sidi Ameer Lakes, respectively, with dominant ions of sodium and chloride. The community compositions of microbes from all three domains (*Bacteria*, *Archaea* and *Eukarya*) were surveyed through the use of 16S and 18S ribosomal gene amplification and clone library clustering using amplified ribosomal DNA restriction analysis (ARDRA) in conjunction with DNA sequencing and analysis. A high level of microbial diversity, particularly among the bacteria of the Himalatt Salt Lake and archaea of Sidi Ameer Lake, was found within these environments. Representatives from all known halophilic bacterial phyla as well as 6 different genera of halophilic archaea were identified. Moreover, several apparently novel phylotypes among both archaea and bacteria were revealed.

Keywords

molecular biology; microbial diversity; halophiles; haloarchaea; hypersaline

INTRODUCTION

The use of culture-independent molecular methodology has become an essential and reliable tool for surveying the diversity of microbial life in any given habitat. Extreme environments, namely those subjected to conditions uninhabitable by most living organisms, offer unique opportunities to assess the types of microbes able to withstand the harsh conditions within a

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given ecosystem and further our understanding of the types of growth parameters and/or conditions under which these organisms are able to survive.

One group of extreme environments is that of hypersaline, or high-salt lakes. These habitats are dominated by halophilic archaea, or haloarchaea, which require a minimum of 9% (w/v) NaCl for growth with most displaying optimal growth at 20 to 26% (w/v) NaCl and some growing well in saturated salt conditions of > 30% (w/v) NaCl (Oren, 2000). Over the last decade, the diversity of haloarchaea in various hypersaline environments has been examined and more fully characterized, aided largely through the use of culture-independent molecular methodology (Rodríguez-Valera *et al.*, 1999). Included among these studies are naturally occurring salt lakes (Arahal *et al.*, 1996; Maturrano *et al.*, 2006; Mutlu *et al.*, 2008), hypersaline microbial mats (Sorensen *et al.*, 2005) and man-made solar salterns (Benlloch *et al.*, 1996; Benlloch *et al.*, 2001; Bidle *et al.*, 2005; Ochsenreiter *et al.*, 2002).

Until recently, it was thought that haloarchaea were the sole colonizers of extreme hypersaline habitats. However, a survey performed by Antón *et al.* (2000) revealed the surprising presence of extremely halophilic bacteria in a saltern crystallizer pond in Alicante, Spain. This new halobacterial genus and species, *Salinibacter ruber* has since been found ubiquitously in numerous hypersaline environments (Antón *et al.*, 2008). In addition to the extreme halophile *S. ruber*, moderate halophilic bacteria within phyla such as *Cyanobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* are also found in hypersaline habitats (Oren, 2008). However, haloarchaea, uniquely adapted for life in high salt, appear to remain the dominant members of these types of habitats.

Bou Saâda (meaning “place of happiness”) is located in north-central Algeria approximately at 280 km south of the coast of Algeria between the el-Hodna Depression and the mountains of the Saharan Atlas (Fig. 1). This oasis town at the edge of the Algerian Sahara lies in stark contrast to the surrounding Saharan desert, nearby barren Ouled Naïl Mountains and adjacent hypersaline lakes including Sidi Ameur and Himalatt Salt Lakes. The biology of these two hypersaline lakes, which are related yet separated by 80 km, is of interest not only in furthering our understanding of the microbial diversity of extreme environments but also in assessing the impact of human activity on these types of water ecosystems in arid environments. Hypersalinity and pollution are likely to be two of the major parameters selecting for the microbial biodiversity of these Algerian lakes and, thus, may facilitate the identification of new genera and/or species.

In this paper, culture-independent 16S rDNA and physicochemical analyses were performed to survey the microbial diversity and further our understanding of the hypersaline Sidi Ameur and Himalatt Salt Lakes in the Algerian Sahara. Ribosomal gene amplification combined with amplified ribosomal DNA restriction analysis (ARDRA) was performed and revealed the diversity of both bacterial and archaeal DNA sequences within these environments was widespread, dependent on the properties of the site (*e.g.*, salinity, pollution) and, in several cases, novel.

MATERIALS AND METHODS

Sampling site description

Sidi Ameur and Himalatt Lakes are shallow salt lakes, or sebkhas, 55 km to the northwest and 25 km to the southeast of Bou Saâda station, respectively (Fig. 1). The waters are considered athalassohaline because their salinity derives from the dissolution of salts of continental origin. Both lakes are influenced by human activity. Sidi Ameur Lake, in particular, is closest to the city of Sidi Ameur and is bordered on one side by an open field oriented towards the city and enclosed by sand dunes on the other side. It is filled with the

drainage water of local palm groves and some rainwater (198 mm/year). A high evaporation rate (1827 mm/year) during the dry season increases the salt concentrations of this lake. The average annual temperature of the Bou Saâda region, where both lakes are located, is 30 °C with minimum and maximum recorded temperatures of 20 °C and 40 °C, respectively (National Office of Meteorology, Algeria).

Sample collection

Sidi Ameur and Himalatt Salt Lakes are about 4 km in length, with width and depth fluctuating between 800 m to 1.5 km and 0 to 30 cm, respectively. In order to have homogenous and representative samples, the areas of the Sidi Ameur and Himalatt Lakes were divided into 16 and 10 sub-sampling sites, respectively. Water samples (200 ml) were collected at 10 cm depth from the water surface with 250 ml sterile flasks. Sediment cores of 10 cm (length) by 8 cm (diameter) were also collected. Samples were gathered during the summer season (March for Himalatt Lake, July for Sidi Ameur Lake). A total of 52 water and sediment samples were collected from the two lakes, dispatched on the day of collection in ice boxes to the laboratory and stored at -4 °C prior to DNA isolation.

Sediment and water sample analysis

Chemical and physical properties of the samples were analyzed at the laboratories of Laghouat and University of Djelfa according to the methods described by Rodier (1996). Chemical properties included compositional estimations of: Ca^{2+} and Mg^{2+} by a complexometric method using ethylene-diaminetetraacetic acid (EDTA), HCO_3^- and Cl^- by a titrimetric method, SO_4^{2-} by UV/Vis-spectrophotometry (Beckman/DU 520) and Na^+ and K^+ by spectrophotometry with flame ionization (Jenway PEP7). Physical parameters included pH and temperature measured in situ using a portable instrument (Hanna Hi 8915). In addition, the colour and odour of the lake water and sediments were noted. The conductivity was monitored using a Hach conductivity meter. The biological oxygen demand (BOD) was determined by standard methods (Eaton *et al.*, 1995) with a dissolved oxygen meter (Hach Lange OXI 315i).

DNA isolation and ARDRA (amplified ribosomal DNA restriction analysis)

DNA was extracted from 250 mg of sediment and 100 ml of water samples using the PowerSoil and UltraClean Water DNA kits (MO BIO Laboratories, Carlsbad, CA). The amount of DNA extracted from each sample was approximately 10 to 25 µg for sediment and 50 to 100 µg for water samples. Amplified ribosomal DNA restriction analysis (ARDRA) of the isolated DNA was performed using the following primer pairs: bacterial 16S rDNA specific primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3'); archaeal 16S rDNA specific primers 21F (5'-TTCCGGTTGATCCYGCCGGA-3') and 1404R (5'-GGGTGTGTGCAAGGRGC-3'); and eukaryal 18S rDNA specific primers 18sF (5'-ACCTGGTTGATCCTGCCAG-3') and 18sR (5'-TGATCCTTCYGCAGGTTTCAC-3') (Lane, 1991; Moon-van der Staay *et al.*, 2001). PCR reactions (50 µl) contained: 5 to 100 ng isolated DNA sample as template, 60 mM Tris-HCl (pH 9.2), 2 mM MgSO_4 , 30 mM NaCl, 200 µM each dNTP, AccuPrime proteins and enhancers, 0.2 µM each primer, 1.8 U AccuPrime GC-Rich DNA Polymerase (Invitrogen) and 0.2 U Vent DNA polymerase (New England BioLabs). PCR was performed with an iCycler (BioRad Laboratories, Hercules, CA). PCR cycles were as follows: 1 cycle at 95 °C for 5 min; 30 cycles at 94 °C for 1 min, 55 to 60.2 °C for 1 min and 72 °C for 2 min; 72 °C for 10 min. PCR products were analyzed on 0.8% (w/v) agarose gels (SeaKem GTG Agarose) (Cambrex Bio Science, Rockland, ME) in 40 mM Tris-acetate with 1 mM EDTA (TAE) buffer at pH 8.0. PCR products of appropriate size (1.3 to 1.7 kb) were purified by gel extraction as needed (Qiagen, Valencia, CA) and cloned into the HincII site of pUC19 using *E. coli* DH5α as the host. Plasmid DNA was purified from isolated colonies using the

QIAprep Spin Miniprep Kit (Qiagen) and used as template for PCR as described above. PCR products (5 µl) were analyzed by restriction digest with *AluI*, *HaeII* and *RsaI* in 10 µl reactions as recommended by supplier (NE BioLabs, Beverly, MA). Restriction fragments were separated on 3.5% Metaphor agarose gels (Cambrex Bio Science) in TAE buffer. Gels were photographed after staining with ethidium bromide at 0.5 µg ml⁻¹ with a Mini visionary imaging system (FOTODYNE, Hartland, WI). Sizes of the fragments were estimated using the Hi-Lo DNA molecular weight marker (Minnesota Molecular, Minneapolis, MN). Final grouping of sequences was performed by visual and computer-aided alignments of restriction patterns. The number of PCR cycles used, the stringency of the reaction conditions and the harshness of the DNA extraction method employed were optimized to minimize the likelihood of PCR chimera formation.

DNA sequence analysis and phylogenetic tree construction

Representative plasmids from each restriction group were sequenced by Sanger automated DNA sequencing using an Applied Biosystems Model 3130 Genetic analyzer (ICBR Genomics Division, University of Florida). Sequencing primers were as described for ARDRA. Each 16S rDNA sequence generated in these studies was searched for its closest relative in the GenBank database using BLASTN (Altschul et al., 1997). In general, only one representative of a group of closely related sequences was chosen for phylogenetic tree construction. Multiple alignments of sequences were performed using the CLUSTALX program (Thompson et al., 1997) and the trees were constructed using NJPlot (Perrière & Gouy, 1996).

Sequences of the cultivated species and environmental clones used for the phylogenetic analyses are as follows: archaeal analysis - *Haloferax volcanii*, *Halorubrum sodomense*, *Haladaptatus paucihalophilus*, *Halalkalicoccus* str. C15, *Halobacterium noricense*, *Halorubrum* str. CGSA-42, *Halorubrum* str. HALO-G*, *Halosarcina pallida*, haloarchaeon clone ARCH05, clone TX4CA_26, clone TX4CA_59, clone MSP41, clone YA66; bacterial analysis - *Psychroflexus* str. YIM C238, halophilic eubacterium EHB-5, *Salinibacter ruber*, *Euhalothece* str. MPI 95AH13, *Halomonas* str. GTW, *Bacillus macyae* str. JMM-4, *Desulfotomaculum* str. Lac2, *Haloanaerobium acetethylicum*, *Nitrobacter* str. PBAB10, *Rhodobacteraceae* bacterium BF A20(17), *Loktanella vestfoldensis*, *Roseobacter* str. TM1038, *Rhodovibrio salinarum* str. JA137, *Acidovorax* str. R-24667, *Desulfobacterium corrodens*, *Thioalcalovibrio denitrificans*, *Haloanaerobacter chitinovorans* and uncultured clones *Flavobacteriaceae* LA1-B21N, 3-45B, 3-55B, E4bC07, Chun-s-2, E4aB08, E6aH12, PS-B19, LL8B, HMMVPog-18, K-4b6, ctg_NISA101, MAT-CR-M4-D08, MAT-CR-P6-E04, MAT-CR-M3-B09, 419, *Bacteroidetes* clone 1G94, δ proteobacterium JS_SRB100Hy, *Haloanaerobiaceae* clone 2P90, *Desulfocapsa* clone SB1_88, α proteobacterium clone C5, NP25, *Haloanaerobiaceae* clone 2P67, EV818SWSAP65. *S. ruber* and *Hfx. volcanii* 16S rDNA sequences served as the outgroups for archaeal and bacterial tree construction, respectively.

Nucleotide sequence accession numbers

The sequences of 9 archaeal and 49 bacterial 16S rDNA genes used to generate the phylogenetic trees of this study were submitted to GenBank and have the following accession numbers: EU869365 to EU869374 for the archaeal clones and EU869375 to EU869426 for the bacterial clones. BAC and ARC designate bacterial and archaeal 16S rDNA sequences, terminal S and H represent Sidi Ameur and Himalatt Salt Lakes and subterminal S and W designate sediment and water samples, respectively.

RESULTS AND DISCUSSION

Physicochemical properties

To gain a deeper understanding of two Algerian lakes, Sidi Ameur and Himalatt Salt Lakes which are in close proximity, physicochemical properties of water and sediment samples were determined including pH, conductivity and ion composition (Table 1). All of the water and sediment samples from these lakes were relatively neutral with pH values ranging from 7.15 to 7.4 (Table 1), similar to the Dead Sea and the Great Salt Lake and in contrast to the more basic lakes such as Lake Wadi Natrun (pH 11) and El Golea Salt Lake (pH 9). The water and sediment of Sidi Ameur Lake appeared highly mineralized based on the extreme conductivity of the samples, with conductivity values greater than 5000 ms/cm (Table 1).

To further investigate this mineralization, the overall salinity and individual ion composition of water and sediment samples from Sidi Ameur Lake were determined and compared to that of the neighbouring Himalatt Lake and other previously characterized hypersaline and marine ecosystems (Table 1). Consistent with their high conductivity, the overall salinity of the Sidi Ameur Lake water was 20 % (w/v), within the range previously reported which includes values up to 30% (w/v) for this lake (National Office of Meteorology, Algeria). This range of salinity (20 - 30%) for Sidi Ameur Lake is comparable to a number of well characterized hypersaline ecosystems including both natural and man-made habitats, such as the Great Salt Lake and solar salterns of Puerto Rico (Table 1). In contrast, the salinity of the water from Himalatt Lake at 11.7% (w/v), was nearly 2-fold less than Sidi Ameur Lake yet several-fold higher than the salinity of seawater considered to be 3-3.5% (Table 1). Thus, both of these Algerian lakes are hypersaline environments, *i.e.* environments with salt concentrations well above that of seawater.

Like most hypersaline ecosystems, with some exceptions such as the Dead Sea, sodium and chlorine ions dominated the Sidi Ameur and Himalatt Lake samples. The major ions detected for the two Algerian lakes were sodium and chloride at 67 to 170 g per liter with magnesium and sulphate ions less abundant at 1.1 to 3 g per liter (Table 1). Low levels of calcium, potassium and bicarbonate were also detected, often at less than 1 g per liter. The sodium and chloride concentrations detected for Sidi Ameur Lake were 6 times higher than those of the Atlantic Ocean. In contrast, the magnesium and calcium ion concentrations of Sidi Ameur and Himalatt Lake water were somewhat comparable to the Atlantic Ocean and much less abundant (15- to 150-fold) than the Dead Sea.

Although Sidi Ameur and Himalatt Lakes differ from other hypersaline environments in several aspects of their physicochemical characteristics, both can be considered hyperhalobe biotopes according to the classification of saline waters (Ricard, 1977) and represent a relatively untapped resource for the identification of salt tolerant microbes and enzymes with properties desirable for industrial application (*e.g.*, extreme tolerance to desiccation, salts and/or solvents).

Microbial activity

To gain insight into the microbial activity of the two Algerian salt lakes, the biological oxygen demand (BOD) of the water was measured. The BOD values for Sidi Ameur and Himalatt Salt Lakes were relatively robust for natural lake ecosystems at 39 mg·l⁻¹ and 73 mg·l⁻¹, respectively, yet consistent with their close proximity to human activity. Similar to what has been observed for other saline ecosystems such as the El Golea Salt Lake (Hacene *et al.* 2004), the BOD values were inversely proportional to the salinity of the site.

Culture-independent molecular analyses

Culture-independent molecular analyses were used to examine the microbial community structure of the water and sediment of Sidi Ameur and Himalate Salt Lakes. PCR-based retrieval of 16S/18S rDNA was performed using domain-specific primer pairs (*i.e.*, *Bacteria*, *Archaea* and *Eukarya*), cloning of the PCR-generated rDNA into plasmids, grouping of the cloned rDNA by restriction fragment polymorphisms and rDNA sequence analysis of representative members from each group. A total of 259 total clones were screened from the waters and sediments from these two different sampling sites (Table 2). Of these, 132 clones were screened from the sediments and water of Sidi Ameur Salt Lake and were de-replicated by ARDRA to 46 unique clones for sequencing analysis (10 archaeal, 32 bacterial and 4 eukaryotic). In the second site, the Himalatt Salt Lake, 127 clones were examined from sediment and water samples, and of these, a total of 62 unique isolates were sequenced (4 archaeal, 58 bacterial). Overall, 108 clones (14 archaeal, 90 bacterial and 4 eukaryotic) were sequenced from the original total of 259 clones, and, of these, a total of 49 bacterial and 9 archaeal clones were further analyzed in phylogenetic tree construction. While the phylotype groups indentified by identical ARDRA fingerprints may include microbes that are not conspecific, ARDRA-based de-replication did enable the enrichment of DNA from diverse microbes for sequencing.

Sequences from both cultivated species as well as uncultured representatives from clone libraries were retrieved from the GenBank database and used in multiple alignments for the construction of phylogenetic trees for both the archaeal and bacterial sequences determined in this study (Figs. 2 and 3, respectively). It is noteworthy that the majority of the uncultivated clones found in GenBank and used in the creation of the phylogenetic trees were retrieved from hypersaline environments such as hypersaline microbial mats, hypersaline lakes and alkaline salterns (*e.g.*, Grant *et al.*, 1999; Mutlu *et al.*, 2008; Sorensen *et al.*, 2005).

Archaeal community composition

Community analysis of 16S rDNA sequences from the sediment and water samples of Sidi Ameur and Himalatt Salt Lakes yielded a total of nine unique archaeal clones from the 14 ARDRA de-replicated 'unique' clones (Table 2; Fig. 2). Six different haloarchaeal genera were represented among these clones, including *Haloferax*, *Halobacterium*, *Haladaptatus*, *Halalkalicoccus*, *Halorubrum* and *Halosarcina*. In addition, three clones from the archaeal library, ARDARCWS1, ARDARCSS5 and ARDARCSS13, all from Sidi Ameur Lake, grouped independently of any known cultivated haloarchaeon. Only one uncultivated clone in GenBank, MSP41 isolated from an East African alkaline saltern (Grant *et al.*, 1999), showed any 16S rDNA sequence similarity to clone ARDARCSS13 (~87%). The remaining two clones, ARDARCWS1 and ARDARCSS5, revealed weak 16S rDNA sequence similarity (~87%) to isolates of the genus *Halorubrum*.

Of the two lakes, the DNA sequences generated from the archaeal clone library of the sediment samples from Sidi Ameur Lake were most diverse with all nine of the unique archaeal clone sequences represented among the 13 clones from this library. In contrast, the diversity of archaeal-specific DNA sequences obtained from the water samples of this lake as well as those from sediment and water samples of Himalatt Salt Lake were less diverse. Although ten clones specific for archaeal primer pairs were isolated from the water samples of Sidi Ameur Lake, all were related and were similar to DNA sequences from environment clones that have yet to be cultured. In both the water and sediment samples from Himalatt Salt Lake, the dominant genus of the archaeal DNA retrieved from the clone libraries was *Halobacterium* at 54% and 100% of the clones, respectively. Two other genera were identified in the water samples from this lake, *Halalkalicoccus* and *Haloferax*, representing

20 – 25% of the clone sequences. Although, the Himalatt Salt Lake archaeal clones were less diverse overall than those of Sidi Ameer, these last two genera were not identified in the Sidi Ameer Lake clone library.

Bacterial community composition

Bacterial community analysis of 16S rDNA sequences obtained from the combined sampling sites of the two Algerian lakes of this study provided a wide representation of halophilic microbial diversity (Fig. 3). Forty-nine of the 91 ARDRA de-replicated 'unique' clones were used to create a phylogenetic tree that depicted the diversity of this habitat. Of the 49 clones, 15 clones showed less than 90% identity to any known sequences in the database, cultivated or clonal. An additional 5 clones showed less than 90% identity to any known cultivated species in the database, although they were greater than 92% identical to other clones within the database. Of the remaining 29 clones, each clone grouped into one of four distinct phyla, the *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*, or *Proteobacteria* (α , β , γ , or δ) (Fig. 3), all four of which include well-characterized halophilic bacterial species (Oren, 2008).

In contrast to the haloarchaea, the vast majority of the bacterial diversity detected in the clone libraries was from Himalatt Salt Lake, most likely due to the lower salinity and higher pollution of this ecosystem compared to the Sidi Ameer Lake. Interestingly, the majority of the bacterial diversity of Himalatt Salt Lake that was detected was from water compared to sediment samples. Of the bacterial clones isolated from the water of this lake, nearly half clustered to the phylum *Proteobacteria* with over 40 % of these related to cultivated α -proteobacteria including *Nitrobacter* sp., *Roseobacter* sp., *Loktanella vestfoldensis* and *Rhodovibrio salinarum*. The remaining 60% of proteobacterial clones clustered evenly among cultivated species of the β class (*Acidovorax* strain B-24667), γ class (*Thioalkalivibrio denitrificans* and *Halomonas* sp.), δ class (*Desulfobacterium* and *Desulfocapsa* sp.) in addition to environmental clones from uncultivated proteobacteria such as clone EV818SWSAP65 isolated from the subsurface water of the Kalahari shield in south Africa (GenBank DQ337088). The second most represented phyla among the Himalatt Salt Lake water samples were clones of the phylum *Firmicutes* (~ 40% of the total) with the majority of these (70%) related to uncultivated environmental clones and the remaining clones equally clustering with cultivated firmicutes such as *Desulfotomaculum* sp., *Halanaerobium acetethylicum* and *Halanaerobacter chitinivorans*. Only a small percent (less than 3%) of the bacterial clones from the water of Himalatt Salt Lake were of the phyla *Cyanobacteria* and *Bacteroidetes*. The latter of which was also identified in the clonal library from sediment of this lake.

In contrast to Himalatt Lake, the bacterial community of Sidi Ameer Lake appeared less diverse based on ARDRA-16S rDNA sequence analysis. A significant number of non-identical clones ($n = 9$) related to *Salinibacter ruber* of the phylum *Bacteroidetes*, however, were identified in Sidi Ameer Salt Lake that were not detected in the Himalatt sebkha. Since the discovery of *S. ruber* in 1998, a wide diversity of representatives of this extremely halophilic bacterium or close relatives have been found in hypersaline environments across the world (Antón *et al.*, 2008). All of the clones isolated from the water samples of Sidi Ameer Lake were related to *Salinibacter* sp. suggesting this microbe may dominate the bacterial community of this hypersaline water system. In contrast to Himalatt Lake where the majority of bacterial diversity was detected in the water, most of the bacterial diversity of the Sidi Ameer Lake was detected in the sediment samples. Consistent with the water samples, however, many of the bacterial clones from the sediment (~40%) clustered to the phylum *Bacteroidetes* including 16S rDNA sequences related to *Salinibacter*, *Psychroflexus* and *Flavobacteria* sp. A smaller percentage of the clones from the Sidi Ameer sediment clustered to the phyla *Cyanobacteria* (*Eubacter* and *Cyanobacter* sp.) and *Firmicutes*

(*Halanaerobacter chitinivorans*) (10% for each group) with the remaining ~40% of the clones related only to DNA sequences from the environment.

Eukaryal community composition

In contrast to the archaea and bacteria, very little diversity in the eukaryotic community of the salt lakes surrounding Bou Saâda was identified by 18S rDNA sequence analysis. Of the 4 clones sequenced, each showed strong similarity (93-97%) to either *Dunaliella salina* or its related species *Dunaliella* sp. ABR11. These results are not surprising as this carotenoid-containing microalgae is a well-known and dominant halophilic eukaryotic inhabitant of a wide range of hypersaline habitats (Oren, 2005).

Discussion

Based on 16S and 18S rDNA sequencing of ARDRA de-replicated clonal libraries, the microbial communities of the two Algerian lakes, Sidi Ameur and Himalatt Salt Lake, were found to be largely dominated by obligatory halophilic microorganisms in both water and sediment. There were distinct differences, however, in the composition of the microbial community structure between the water and sediment as well as between the two lakes. Whereas the bacterial community of Sidi Ameur Lake water and sediment were largely dominated by *Salinibacter*, the bacterial communities of the Himalatt sebkha were relatively diverse with the majority of this diversity in the water compared to sediment. Interestingly, a large number of the Himalatt Lake clones were related to sulfate reducing bacteria isolated from salt- and sulfide-rich springs, *Haladaptatus paucihalophilus*, *Halosarcina pallida*, *Desulfotomaculum* sp. and *Desulfobacterium corrodens* (Savage *et al.*, 2007; Savage *et al.*, 2008; Dinh *et al.*, 2004; Campbell and Postgate, 1965), suggesting the presence of sulfide reducing activity in this lake. This finding is supported by the dark sediments with sulfide observed at the redox boundary of the water-sediment interface for the Himalatt Lake during sample collection.

Differences in the phylogenetic composition of the archaeal communities were also observed between the water and sediment as well as between the two lakes based on 16S rDNA sequencing of ARDRA de-replicated clones. Sidi Ameur Lake sediment included the nine unique sequences affiliated to four genera of haloarchaea (*Halobacterium*, *Haloadaptatus*, *Halorubrum* and *Halosarsina*), while the water samples from this lake contained only clones related to a single 16S rDNA sequence that clustered with environmental DNA sequences from haloarchaea that have yet to be cultivated. The overall diversity of archaea in the clone libraries from the Himalatt Salt Lake samples was even lower than Sidi Ameur Lake with three distinct genera of haloarchaea (*Halobacterium*, *Halalkalicoccus* and *Haloferax*) identified in the water and only *Halobacterium* identified in the sediment.

The eukaryotic microbes identified in this study are represented by the unique genus *Dunaliella*, halophilic microalgae common to hypersaline environments. While DNA clones of this genus were not detected in Himalatt Salt Lake, they were isolated from both the water and sediment of Sidi Ameur Lake. *Dunaliella* produce high amounts of glycerol and β -carotene, enabling them to survive the extreme osmotic pressure and intense light encountered in hypersaline environments. Thus, finding these organisms dominating the eukaryotic microbial community of Sidi Ameur Lake is not surprising. The inability to detect these microalgae in Himalatt Salt Lake may be due to a number of factors including: 1) the low temperature of 9 °C recorded during sample collection and 2) the anaerobic properties of this lake based on its relatively high BOD values, the presence of microbial communities rich in sulfide-reducing bacteria and the presence of dark sediments of sulfide.

Both low temperature and reduced oxygen availability would limit growth of aerobic mesophiles such as *Dunaliella* (optimal growth at 25 to 30 °C).

Based on these findings, we propose that the qualitative and quantitative distribution of the microorganisms in the water and the sediments of both Sidi Ameer and Himalatt Salt Lakes are governed by the following parameters: 1) the physicochemical conditions of the lake particularly temperature, salinity and pollution that exert a strong pressure of selection on the microflora and 2) the effect of redox status on microbial metabolism. In general, a pronounced difference in salinity was observed across the water-sediment with a precipitate on the lake surface of nearly pure salt, the salinity of the lake water at 11 to 20 ‰ and the presence of other minerals in addition to halite salt in the lake sediments. Furthermore, the anoxic environment of Himalatt Salt Lake appeared unfavorable for the growth of strongly aerobic microorganisms, such as *Dunaliella*.

Differences in the abundance and distribution of microbial communities between lake water and sediment samples of hypersaline ecosystems have been previously described. For example, Jiang *et al.* (2006) identified distinct differences in the microbial diversity in water and sediment of an athalassohaline lake in Northwestern China. Similar to our study, these distinct changes in the microbial assemblage across the water-sediment interface were most likely due to differences in the salinity and redox state of the lake.

Overall, our findings reveal a greater diversity of bacterial phylotypes as compared to the diversity of archaea (and eukaryotes) in samples extracted from the Algerian salt lakes. In contrast to the Himalatt Salt Lake that was lower in salinity and dominated by bacterial diversity, a relatively equal distribution of clonal representatives was detected among the haloarchaeal and bacterial phylogenetic clades in Sidi Ameer Salt Lake. However, the diversity of haloarchaeal clades remained relatively low. Our results are consistent with a recent survey of over 173 published 16S rDNA libraries which found archaeal libraries to be rarely as diverse as bacterial libraries from the same environments (Aller & Kemp, 2008). Although the biological reason for this remains to be determined, it has been suggested that haloarchaea are energetically taxed by their extreme habitat, thus, limiting their ability to diversify within hypersaline environments (Oren, 1999).

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Abbreviations

ARDRA	amplified ribosomal DNA restriction analysis
PCR	polymerase chain reaction

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Fig. 1. Sidi Ameur and Himalatt sites of the Algerian Sahara. **A.** The hypersaline Sidi Ameur and Himalatt sites are respectively located 55 km to the northwest and 25 km to the southeast of Bou Saâda, Algeria (latitude of $35^{\circ}13'$, longitude of $4^{\circ}11'$ and altitude of 663 m). **B.** Representative images of Sidi Ameur Lake depicting the barren landscape and high salt concentrations.

Fig. 2.

Neighbor-joining phylogenetic tree based on archaeal 16S rDNA sequences found in the sediments and waters of Sidi Ameer site and Himalatt site [ARDARC sequences with Sidi Ameer water (WS), and sediment (SS) and Himalatt water (WH) and sediment (SH) samples indicated]. Bootstrap values (expressed as percentages of 1000 replications) > 70% are shown at branch points. *S. ruber* was used as an outgroup. Bar, 0.02 substitutions per nucleotide position. See methods for details and accession numbers.

Fig. 3. Phylogenetic tree based on bacterial 16S rDNA sequences found in the sediments and waters of Sidi Ameer site and Himalatt site [ARDBAC sequences with Sidi Ameer water (WS), and sediment (SS) and Himalatt water (WH) and sediment (SH) samples indicated]. Tree was constructed by neighbor-joining with bootstrap values (expressed as percentages of 1000 replications) > 70% shown at branch points. *Hfx. volcanii* was used as an outgroup. Bar, 0.02 substitutions per nucleotide position. See methods for details and accession numbers.

Chemical and physical properties of the Sidi Ameur and Himalatt Salt Lakes of the Algerian Sahara compared to other hypersaline and marine ecosystems.

Table 1

Ecosystem	Chemical and physical properties ¹									
	pH	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻	HCO ₃	Salinity	
Hypersaline:										
Solar Saltern (Puerto Rico)	n.d.	65.4	5.2	20.1	0.2	144	1.9	n.d.		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	8.0	14.6	164	2.3	n.d.		277
Dead Sea	7.8	40.1	7.6	44.0	78.2	225	0.44	0.26		340
Wadi Natrun (Egypt)	11	142	2.3	u.d.	u.d.	155	22.6	67		394
El Golea Salt Lake (Algeria)	9.0	107	n.d.	0.3	0.4	198	n.d.	n.d.		296
Sidi Ameur site (Algeria) - water	7.4	67.1	0.17	3.0	0.51	111	2.1	0.19		200
Sidi Ameur site (Algeria) - sediment	7.15	94.5	0.23	2.9	1.7	170	1.1	n.d.		n.d.
Himalatt site (Algeria) - water	7.2	24.5	0.12	1.6	0.22	63.8	3.1	n.d.		117
Marine:										
Aral Sea	8.2	2.2	0.08	0.55	0.51	3.47	3.2	0.07		10.2
Caspian 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

¹ Salinity and ions are represented as g per liter. n.d., not determined. u.d., undetectable. For Sidi Ameur site: conductivity of the water and sediment samples was 5090 and 5337 ms/cm, respectively, turbidity was 23.3 NTU/FTU, and ash content was 26.3 and 31.6 g per liter for water and sediment samples, respectively. References for abiotic features of other hypersaline and marine habitats were as follows: (Copin-Montégut, 1996; Gavrieli, 1997; Hacene *et al.*, 2004; Imhoff *et al.*, 1979; Oren, 2002; Post, 1981).

Microbial community composition of Sidi Ameur and Himalatt Salt Lakes by analysis of clones isolated by PCR-based retrieval of 16S/18S rDNA.

Table 2

Domain	Sidi Ameur site				Himalatt site				Overall			
	sediment total ^a	sediment unique	water total	water unique	sediment total	sediment unique	water total	water unique	overall total	overall unique	total	unique
<i>Archaea</i>	13	9	10	1	11	1	24	3	58	14		
<i>Bacteria</i>	51	26	18	6	9	1	83	58	161	91		
<i>Eukarya</i>	30	2	10	2	u.d.	u.d.	u.d.	u.d.	40	4		
Total	94	37	38	9	20	1	107	61	259	108		

^a Number of total and ARDRA de-replicated 'unique' clones isolated by PCR-based retrieval of 16S/18S rDNA with domain-specific primer pairs.

^b u.d., undetectable.