


Prokaryotic diversity in a Tunisian hypersaline lake, Chott El Jerid

Manel Ben Abdallah^{1,2} · Fatma Karray^{1,3}  · Najla Mhiri^{1,3} · Nan Mei² ·
Marianne Quéméneur² · Jean-Luc Cayol² · Gaël Erauso^{2,3} · Jean-Luc Tholozan² ·
Didier Alazard^{1,2} · Sami Sayadi^{1,3}

Received: 20 August 2015 / Accepted: 6 December 2015 / Published online: 2 January 2016
© Springer Japan 2015

Abstract Prokaryotic diversity was investigated in a Tunisian salt lake, Chott El Jerid, by quantitative real-time PCR, denaturing gradient gel electrophoresis (DGGE) fingerprinting methods targeting the 16S rRNA gene and culture-dependent methods. Two different samples S1-10 and S2-10 were taken from under the salt crust of Chott El Jerid in the dry season. DGGE analysis revealed that bacterial sequences were related to *Firmicutes*, *Proteobacteria*, unclassified bacteria, and *Deinococcus-Thermus* phyla. Anaerobic fermentative and sulfate-reducing bacteria were also detected in this ecosystem. Within the domain archaea, all sequences were affiliated to *Euryarchaeota* phylum. Quantitative real-time PCR showed that 16S rRNA gene copy numbers of bacteria was 5×10^6 DNA copies g^{-1} whereas archaea varied between 5×10^5 and 10^6 DNA copies g^{-1} in these samples. Eight anaerobic halophilic fermentative bacterial strains were isolated and affiliated with the species *Halanaerobium alcaliphilum*, *Halanaerobium saccharolyticum*, and *Sporohalobacter salinus*. These data showed an abundant and diverse microbial community detected in the hypersaline thalassohaline environment of Chott El Jerid.

Keywords Archaea · Bacteria · Microbial diversity · Hypersaline lake · 16S rRNA · PCR-DGGE · Quantitative PCR · Anaerobic fermentative bacteria

Introduction

Hypersaline environments are abundant worldwide and are characterized by salt concentrations higher than that of seawater (Ventosa et al. 2008). The microbial community of hypersaline environments close to saturation has been studied by both culture (Emmerich et al. 2012) and molecular methods (Maturrano et al. 2006; Foti et al. 2008) regarding the potential applications of halophiles in biotechnology (Oren 2010). These environments are generally limited to arid and semi-arid areas. In North Africa, salt lakes are called *sebkhath* or *chott*. In Tunisia, shallow salt lakes extend from the south coast of Tunisia to the Atlas Mountains (Algeria). Chott el Jerid is the largest saline depression, located in the south-west of Tunisia. It covers an area of approximately 5000 km². During the dry season, salt-covered depression where salt crusts are precipitated covers most of this area. Sometimes in winter when a lot of rain falls, the area may be largely flooded and it acquires the aspect of lake during some months (Ori et al. 2009). Recent investigations have shown that solid salts in this lake come from the geological formations of the surrounding mountains. Differently to other continental *sebkhath*s and according to its physicochemical properties, this shallow lake contains similar ions to seawater with a salt concentration varying between 250 and 330 g L⁻¹, although it does not communicate with the Mediterranean Sea (Kbir-Arigoib et al. 2001). The major elements are sodium and chloride followed in abundance by SO₄²⁻ obtained in previous studies in this ecosystem (Kbir-Arigoib et al. 2001). Chott El

Communicated by A. Oren.

✉ Fatma Karray
fatma.karray@cbs.rnrt.tn

¹ Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, University of Sfax, Route Sidi Mansour, km 6, BP 1177, 3018 Sfax, Tunisia

² IRD, University of Aix-Marseille, University of Toulon, CNRS, MIO, UM 110, 13288 Marseille Cedex 09, France

³ Laboratoire Mixte International « Contaminants et Ecosystèmes Marins Sud Méditerranéens » (LMI COSYS-Med), Sfax, Tunisia

Jerid is a terrestrial evaporitic environment that has been the subject of sedimentological and hydrochemical studies but its microbial diversity has never been studied using molecular approaches. This is the first report describing the microbial populations present in the water and sediment of Chott El Jerid using molecular methods [denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR]. Few ecological studies of this environment based on the cultivation approach have been investigated (Ben Abdallah et al. 2015; Mezghani et al. 2012). The main purpose of this research is to obtain an overview of the abundance, the structure, and microbial diversity of both archaeal and bacterial communities in water and sediment samples of Chott El Jerid using culture-dependent and culture-independent techniques. It is also aimed at comparing the results obtained with other studied hypersaline ecosystems.

Materials and methods

Site description, samples collection

Sampling was done from a continental salt lake: Chott El Jerid located in the south of Tunisia. Two samples S1-10 (33°54'42.21"N and 8°31'7.98"E) and S2-10 (33°54'44.15"N and 8°31'9.01"E) were taken from Chott El Jerid in the dry season (October 2010). S1-10 and S2-10 samples, a mixture of saturated salt waters and sediments, were collected 10 cm below the salt crust surface in sterile bottles and kept aseptically at -80 and 4 °C until further analysis. The sediments of S1-10 and S2-10 samples have a black and sandy color, respectively. Temperature, salinity, and pH of water samples were determined. The water at the samples site was at 23 °C. The pH of the water samples S1-10 and S2-10 were 6.61 and 6.9, respectively. The total salt concentration in the water was 346 g L $^{-1}$.

Isolation of anaerobic halophilic fermentative bacteria

In an attempt to isolate anaerobic halophilic fermentative bacteria; the following medium was used: (per liter distilled water): NH_4Cl , 1.0 g; K_2HPO_4 , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g; NaCl , 200 g; tryptone, 0.5 g; KCl , 1 g; yeast extract, 0.5 g; cysteine hydrochloride, 0.5 g; 10 ml trace mineral element solution (Balch et al. 1979). The pH was adjusted to 6.8 with 10 M KOH. The medium was boiled under a stream of O_2 -free N_2 gas and cooled to room temperature. 5 mL aliquots or 25 mL were then dispensed into Hungate tubes or serum bottles, respectively, under a stream of N_2/CO_2 (80:20, v/v) gas and autoclaved for 20 min at 120 °C. Prior to inoculation of the medium in each bottle, 0.5 mL 2 % (w/v) $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$; 0.5 mL 10 %

(w/v) NaHCO_3 ; 0.5 mL (1 M) glucose or 0.625 mL (20 %) tryptone or 0.625 mL (20 %) yeast extract were injected from sterile stock solutions. The medium in each Hungate tube was similarly supplemented, using 0.1 mL of each stock solution. For enrichment cultures in serum bottles, 10 % of sample was inoculated into growth medium followed by incubation at 37 °C for 1 week. The culture was purified by repeated use of the Hungate roll tube method in medium with 2 % (w/v) agar added (Hungate 1969). Cellular morphology and purity of the cultures were assessed under an Optiphot (Nikon) phase-contrast microscope.

DNA extraction

DNA was extracted from 250 mg of sediment of S1-10 and S2-10 samples using Ultra Clean Soil DNA kit (MO BIO) according to the protocol of the manufacturer. Fermentative bacterial genomic DNA was extracted by Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's recommendations.

PCR amplification of bacterial and archaeal 16S rRNA genes

For DGGE analysis, PCR amplifications of the variable region V3–V5 of bacterial and archaeal 16S rRNA genes were performed by Nested PCR. Bacterial 16S rRNA genes were amplified from all the samples using the following primer sets (Table 1): FD1/RD1 tested in the first round to amplify the entire DNA. In the second round of amplification, PCR products obtained were amplified with a second pair of primers: 341F-GC/907R. Within the domain archaea, DNA was amplified with the primer pairs 21F/1492R in a first amplification. Amplified archaeal 16S rRNA gene fragments were used as the template for a nested PCR with archaeal specific primers 344F-GC/915R (Table 1). PCR reactions were performed using a thermocycler (Applied Biosystems).

PCR amplification was performed in a total volume of 50 μL mixtures containing $1 \times$ Taq buffer, 0.2 mM each dNTP, 0.2 μM of each primer, 0.1 mg mL $^{-1}$ BSA (Sigma), 50 ng DNA template and 1.25 U of Taq DNA polymerase (Fermentas). The first round of amplification program for bacteria was 94 °C for 2 min, and 30 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min 45 s. For the archaea, the following protocol was used for the first round of amplification: 94 °C for 4 min, and 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min 45 s. The final extension step was at 72 °C for 10 min for both bacteria and archaea. The nested PCR program was 94 °C for 4 min; followed by 30 cycles of 94 °C for 1 min, 63 °C for 1 min (for archaea) or 58 °C for 45 s (for bacteria), and 72 °C for 45 s; ending with 10 min at 72 °C. Negative

Table 1 Primers used in this study

Primer ^a	Target site ^b	Sequence ^c (5′–3′)	Targeted group	PCR	References
Primer for DGGE					
FD1	8–28	AGAGTTTGATCCTGGCTCAG	Bacteria	First step PCR	Weisburg et al. (1991)
RD1	1547–1531	AAGGAGGTGATCCAGCC			Weisburg et al. (1991)
341F-GC ^d	341–357	CCTACGGGAGGCAGCAG	Bacteria	Second step PCR	Muyzer et al. (1993)
907R	907–926	CCGTCAATTCMTTGTGAGTTT			Lane (1991)
21F	21–40	TTCCGGTTGATCCYGCCGGA	Archaea	First step PCR	DeLong (1992)
1492R	1492–1510	GGTTACCTTGTTACGACTT			Lane (1991)
344F-GC ^d	344–363	ACGGGGYGCAGCAGGCGCGA	Archaea	Second step PCR	Casamayor et al. (2002)
915R	915–934	GTGCTCCCCCGCCAATTCCT			Casamayor et al. (2002)
Primer for qPCR					
331F	331–349	TCCTACGGGAGGCAGCAGT	Bacteria	qPCR	Nadkarni et al. (2002)
797R	797–822	GGACTACCAGGTATCTAATCCTGTT			Nadkarni et al. (2002)
344F	344–363	ACGGGGYGCAGCAGGCGCGA	Archaea	qPCR	Casamayor et al. (2002)
519R	519–536	GWATTACCGCGGCKGCTG			Ruff-Roberts et al. (1994)
27F	9–27	AGAGTTTGATCMTGGCTCAG	Bacteria	Conventional PCR	Lane (1991)
907R	907–926	CCGTCAATTCMTTGTGAGTTT			Lane (1991)
109F	109–125	ACK GCT CAG TAA CAC GT	Archaea	Conventional PCR	Großkopf et al. (1998)
958R	958–976	YCCGGC GTT GAMTCCAATT			DeLong (1992)
Primer for sequencing ^e					
T7	2999–3	TAATACGACTCACTATAGGG			
SP6	139–158	TATTTAGGTGACACTATAG			

^a F (forward) and R (reverse) indicate the orientation of the primers in relation to the rRNA sequence

^b Based on *Escherichia coli* numbering (Brosius et al. 1981)

^c M = A or C; Y = C or T; W = A or T; K = G or T

^d GC-clamp is a 40-nucleotide GC-rich sequence attached to the 5′ end, 5′-CGCCCCGCGCGCCCCGCGCCCGTCCCCGCCGCCCGCCCGC-CCG-3′ as described (Muyzer et al. 1993)

^e T7/SP6 specific primers localized in *pGEM-T Easy* vector (Promega)

controls were included with no addition of template DNA. Molecular identification of fermentative bacterial isolates was performed. The 16S rRNA genes were amplified with specific bacterial primers FD1/RD1 (Table 1). The PCR condition was as follows: after initial denaturation (95 °C for 1 min), 30 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min 30 s were performed, followed by a final extension (10 min, 72 °C).

DGGE analysis of bacterial and archaeal diversity

For each sample, 300 ng of archaeal and bacterial amplicons were subjected to DGGE analysis. DGGE was performed as previously described by using the D-Code multiple system (BioRad, USA). PCR products obtained were electrophoresed on a 6 % (w/v) polyacrylamide gel (acrylamide: bis-acrylamide, 37.5:1) with 40–80 % (archaeal) and 30–50 % (bacterial) denaturing gradient (100 % of

denaturing agents were 7 M urea and 40 % (v/v) deionized formamide) in 1 × TAE buffer (40 mM tris, pH 8.5; 20 mM acetic acid; 1 mM EDTA) at 60 °C. Electrophoresis conditions were 150 V for 5 h for both domains. After 30 min of ethidium bromide staining, DGGE gels were photographed under UV light and photographed with a Gel doc XR Imaging system (BioRad). Selected bands of each sample (in terms of intensity and frequency of appearance) were cut from the gel, resuspended overnight in 35 µL of MilliQ water, reamplified (using the appropriate corresponding bacteria or archaea primers devoid of the GC clamps), cloned, and sequenced. PCR products were ligated into the vector *pGEM-T easy* vector (Promega Corporation, Madison, WI) following the manufacturer's recommendations. Recombinant plasmids were isolated from overnight cultures using EZ-10 Spin Column Plasmid DNA kit and screened by restriction analysis with *EcoRI*. Positive transformants were selected for sequencing.

Sequencing and phylogeny analysis

DGGE bands were sequenced using the Big Dye® Terminator cycle Sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The primers used for sequencing were T7 and SP6 presented in *pGEM-T easy* vector (Table 1). PCR products from each isolate were sequenced using FD1 and RD1 primers (Table 1). Sequence similarity searches were performed using the online sequence analysis resources “BLAST” (Basic Local Alignment Search Tool) (Altschul et al. 1997) and “Seq-match” (Ribosomal Database project II; Release 10) (Cole et al. 2009). Multiple alignments were generated by the MUSCLE program (Edgar 2004) and phylogenetic tree was constructed with MEGA program version 6 (Tamura et al. 2013) based on evolutionary distances that were calculated using the Neighbour-Joining method with Juke-Cantor distance model. We performed bootstrap resampling analysis for 1000 replicates to estimate degrees of confidence in tree topologies. Then, chimerical sequences were checked using chimera check on DECIPHER database (<http://decipher.cce.wisc.edu/FindChimeras.html>) (Wright et al. 2012).

Quantitative real-time PCR (qPCR)

To evaluate the abundance of archaea and bacteria in all samples, qPCR was used by targeting 16S rRNA genes. The archaeal and bacterial amplification reactions were performed in triplicate on a Bio-Rad CFX-96 real-time system (Bio-Rad). Archaeal and bacterial 16S rRNA genes were quantified using the primer sets 344F/519R and 331F/797R, respectively (Table 1). The same reaction components and protocol were used for the bacterial and archaeal real-time PCR. Each qPCR mixture contained the following components: 1× SsoAdvanced SYBR Green Supermix (Bio-Rad), 250 nM of each primer, 50 ng of DNA template (tenfold dilution series of standard PCR product or environmental DNA sample) or distilled water (negative control), and RNase/DNase-free water to a final volume of 20 µL. The qPCR reactions were carried out as follows: Initial denaturation at 98 °C for 2 min, followed by 39 cycles of a 2-step PCR protocol with a 5 s denaturation phase at 98 °C and a 30 s annealing/elongation phase at 56 °C for the archaeal real-time PCR.

The bacterial qPCR reactions had an initial denaturing step of 10 min at 95 °C, followed by 35 cycles of 30 s of denaturing at 95 °C, 30 s of annealing phase at 60 °C, and 30 s of primer extension at 72 °C. Fluorescence was measured at the end of each cycle. To assess the specificity of the primers, a post-PCR melting curve analysis was carried out, in which the temperature varied between 65 and 95 °C in 0.5 °C increments. qPCR standard curves were constructed from serial tenfold dilutions of DNA standards of

known concentration. For bacterial 16S rRNA gene qPCR, standard DNA fragments were amplified from *Halomonas alimentaria* YKJ-16^T using the primer sets 27F-907R (Table 1). For archaeal 16S rRNA gene qPCR, standard DNA fragments were amplified from *Halorubrum chaoviator* DSM 19316 using the primer sets 109F-958R (Table 1). Triplicate PCR products were pooled and purified with Wizard® SV and PCR Clean-up System (Promega), according to the manufacturer’s instructions. Purified PCR products were subsequently quantified using the *BioSpec-nano* Spectrophotometer (Shimadzu) and used as DNA standards. Measured concentrations of purified PCR products were then converted to copies per microliter as described and the concentration was adjusted to 1×10^9 copies µL⁻¹ prior to performing serial dilutions from 10⁸ to 10¹ copies per µL of DNA. For all standard curves, the coefficients of determination (R²) were higher than 99.0 %. Cell numbers per g sediment were calculated from the qPCR 16S rRNA gene copy numbers according to the average 16S rRNA copy number in bacterial (3.82) and archaeal genomes (1.62).

Nucleotide sequence accession numbers

The 16S rRNA gene sequences from bands DGGE determined in this study were deposited in the GenBank database under accession numbers KT363000 to KT363010 (bacteria) and KT363011 to KT363036 (archaea). The 16S rRNA gene sequences of the fermentative isolates have the accession numbers KU180220 to KU180227.

Results

DGGE profiles

The microbial community structure was investigated in Chott El Jerid through PCR-DGGE analysis. Bacterial and archaeal community profiles elucidated by DGGE are shown in Fig. 1. Similar DGGE profiles are obtained for the S1-10 and S2-10 samples from Chott El Jerid for bacteria or archaea. Numerous predominant bands are presented in all profiles (archaeal or bacterial) indicating diverse microbial community present in S1-10 and S2-10 samples. For bacteria, 19 bands were excised. Only seven of them (B1, B4, B6, B7, B9, B10, B18) were successfully reamplified, cloned and sequenced. For archaea, 19 excised DGGE bands (A1–A19) were reamplified, cloned, and sequenced. Nucleotide sequences were compared to the GenBank and RDP databases using BLASTN and Seq-Match, respectively. Sequencing results on bacterial and archaeal 16S rRNA gene sequences from DGGE bands are reported in Tables 2 and 3. Phylogenetic analyses based on

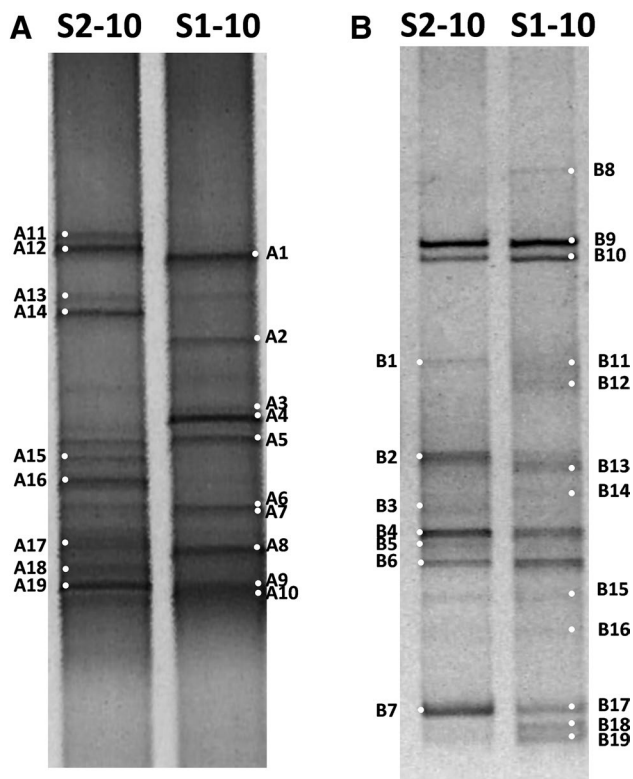


Fig. 1 Denaturing gradient gel electrophoresis profiles of PCR-amplified archaeal (a) and bacterial (b) 16S rRNA gene fragments obtained from samples S1-10 and S2-10

the bacterial and archaeal 16S rRNA gene sequences were performed (Figs. 2, 3).

Phylogenetic affiliations of bacterial community members

Phylogenetic groups within the bacterial domain were regrouped in the *Firmicutes*, *Proteobacteria* (*Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria*), *Deinococcus-Thermus* phyla and unclassified bacteria.

Within the *Proteobacteria* Phylum, the sequences were clustered with the *Rhodobacteraceae*, *Aeromonadaceae*, and *Desulfobacteraceae* family's bacteria belonging to *Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* classes, respectively. Band B1.C23 sequence was related to uncultured bacterium clone HSS104 (97 % similarity), detected in coastal saline soils in India and presented 86 % similarity with anaerobic halophilic sulfate-reducing *Deltaproteobacteria* belonging to *Desulfatibacillum* and *Desulfosalsimonas* genus. The sequences B4.C15 and B7.C13 were related to a facultative anaerobic bacteria strain *Aeromonas sharmana* DSM 17445, isolated from water collected from a warm spring in India. Band B10.C72 and B18.C17 sequences showed 97 % similarity with

Roseovarius pacificus, isolated from deep-sea sediment and 98 % similarity with *Oceanicola marinus*, isolated from seawater collected from the coast of Taiwan, respectively. Band B18.C26 sequence was affiliated to uncultured bacterium clone, which was isolated from urban stormwater sediments. This band showed 85 % similarity with sulfate-reducing bacterium *Desulfofrigus oceanense*, isolated in marine Arctic sediments.

The second major phylum was the *Firmicutes* and the sequences were grouped with members of *Clostridia* and *Bacilli* classes. Band B1.C1, detected only in the S2-10 sample, presented 99 % similarity with uncultured bacterium clone kasin-B2-F08 recovered from iron-rich salt lake sediments lake Kasin, southern Russia. Band B1.C1 sequence was also related to anaerobic halophilic fermentative genera *Halanaerobium* and *Halocella* species belonging to *Halanaerobiaceae* family. The sequence of B6.C6 showed 97 % similarity with denitrifying bacterium *Bacillus azotoformans* LMG 9581.

Within the *Deinococcus-Thermus* phylum, Band DGGE B10.C14 sequence, detected only on S1-10 DGGE profile, was closely related to *Truepera radiovictrix* DSM 17093 isolated from hot spring runoffs on the Island of São Miguel in the Azores. This species was extremely ionizing radiation resistant (60 % of the cells survive 5.0 kGy). Two sequences from band B9.C16 and B4.C18 were affiliated with uncultured clones of bacteria retrieved from a hypersaline lake in Iran.

Phylogenetic affiliations of archaeal community members

Within the domain archaea, all sequences were grouped with members of *Euryarchaeota* phylum including *Halo bacteriaceae*, *Natrialbaceae*, *Haloferacaceae*, *Methanosarcinaceae* and *Methanocaldococcaceae* families. Bands A2, A4, A13, A15, A17, A18 and A19 sequences were related to the genus *Halorubrum* spp. (*Haloferacaceae* family) which were widely found in diverse hypersaline environments such as solar salterns, salt lakes, coastal sabkhas, soda lakes. Band A3.C18 is related to *Halorubrum ejinorensis* isolated from lake Ejinor, Inner Mongolia, China. It also appears that band A5.C51 and A6.C63 showed 91 % of similarity with *Halococcus hamelinensis* and *Halococcus dombrowskii* isolated from stromatolites in Shark Bay, Australia and from a Permian alpine salt deposit, respectively. Band A7.C73 is affiliated to *Natrinema versiforme* (*Natrialbaceae* family) found in Aibi salt lake in China, whereas, band A12.C24 was closely related to uncultured bacterium clone described in hypersalin sediments of lake Kasin in Russia. Band A12.C12 sequence was related to species of *Natronomonas* (97 % of similarity), isolated from salt-saturated lakes, from a marine solar

Table 2 Blast results on bacterial 16S rRNA sequences derived from excised DGGE bands from the two samples collected in 2010

Band	Length (bp)	Phylogenetic affiliation	Best match/accession number	Percentage of similarity	Source	Closest cultured bacterium/accession number	Percentage of similarity
B1.C1	588	<i>Firmicutes</i> <i>Halanaerobiaceae</i>	Uncultured bacterium clone Kasin-B2-F08/ (HE604688)	99	Hypersaline Sediments of Lake Kasin, Southern Russia	<i>Halocella cellulosilytica</i> DSM 7362/(X89072)	90
B1.C23	578	<i>Deltaproteobacteria</i> <i>Desulfobacteraceae</i>	Uncultured bacterium clone HSS104/(HQ397460)	97	Coastal saline soils in India	<i>Desulfatibacillum aliphaticivorans</i> DSM 15576/(AY184360)	86
B4.C15	586	<i>Gammaproteobacteria</i> <i>Aeromonadaceae</i>	Uncultured bacterium clone S22 068/(KC872593)	98	Surface water of northern south china sea	<i>Aeromonas sharmana</i> DSM 17445/(DQ013306)	96
B4.C18	586	Unclassified bacteria	Uncultured bacterium clone RB130/(HQ425215)	99	Aran-bidgol salt Lake in Iran	<i>Sorangium cellulosum</i> DSM 14627/(EU240497)	79
B6.C6	586	<i>Firmicutes</i> <i>Bacillaceae</i>	Uncultured Firmicutes bacterium clone MFC-10/ (EU194836)	99	Marine sediments	<i>Bacillus azotoformans</i> LMG 9581/ (AB363732)	97
B7.C13	584	<i>Gammaproteobacteria</i> <i>Aeromonadaceae</i>	Uncultured bacterium clone S22 068/(KC872593)	97	Surface water of northern south china sea	<i>Aeromonas sharmana</i> DSM 17445/(DQ013306)	96
B9.C16	523	Unclassified bacteria	Uncultured bacterium clone RB130/(HQ425215)	97	Aran-bidgol salt Lake in Iran	<i>Haloferula rosea</i> MBIC08340/ (AB372853)	79
B10.C72	561	<i>Alphaproteobacteria</i> <i>Rhodobacteraceae</i>	<i>Roseovarius pacificus</i> LMG 24575/(DQ120726)	97	Deep-sea sediment		
B10.C14	556	<i>Deinococcus-Thermus</i> <i>Therapeaceae</i>	<i>Therapeera radiovictrix</i> DSM 17093/ (DQ022076)	91	hot spring runoffs on the Island of São Miguel in the Azores		
B18.C26	588	<i>Deltaproteobacteria</i> <i>Desulfobacteraceae</i>	Uncultured bacterium clone TFMacopo175/ (HE659258)	99	Urban stormwater sediments	<i>Desulfotribes oceanense</i> DSM 12341/ (AF099064)	85
B18.C17	560	<i>Alphaproteobacteria</i> <i>Rhodobacteraceae</i>	<i>Oceanicola marinus</i> LMG 23705/(DQ822569)	98	Seawater collected off Taiwan		

Table 3 Blast results on archaeal 16S rRNA sequences of selected DGGE bands from two samples collected in 2010

Band	Length (bp)	Phylogenetic affiliation	Best match/accession number	Percentage of similarity	Source	Closest cultured archaeon/ accession number	Percentage of similarity
A1.C10	553	<i>Euryarchaeota</i> <i>Methanocaldococcaceae</i>	Uncultured archaeon clone RA10/(HQ425177)	99	Aran-Bidgol Salt Lake in Iran	<i>Methanotorris igneus</i> DSM 5666/(M59125)	83
A2.C1	555	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured halobacterium sp. Clone SFG1F051/ (AM947501)	96	Solar saltern Mediterranean seawater Sfax in Tunisia	<i>Halorubrum kocurii</i> JCM 14978/(AM900832)	95
A3.C18	555	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone Kasin-A3-G10/(HE604632)	93	Hypersaline sediments of Lake Kasin, southern Russia	<i>Halorubrum ejinorensis</i> JCM14265/(AM491830)	91
A4.C20	556	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum chaoviator</i> DSM 19316/(AM048786)	99	Sea salt Beja California Mexico		
A5.C51	556	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone TX4CA 30/(EF690585)	93	Soil of former lake Texcoco in Mexico	<i>Halococcus hamelinensis</i> JCM 12892/(DQ017835)	91
A6.C63	555	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured haloarchaeon clone ARDARCWH2/ (EU869373)	94	Hypersaline Sidi Ameur and Himalatt salt lakes of Algerian Sahara	<i>Halococcus dombrowskii</i> DSM 14522/(AJ420376)	91
A7.C73	555	<i>Euryarchaeota</i> <i>Natrialbaceae</i>	Uncultured archaeon clone CK599/(HQ400567)	94	Petroleum contamination in saline-alkali soil	<i>Natriema versiforme</i> JCM10478/(AB023426)	92
A8.C86	555	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured euryarchaeo- te clone SFH1E051/ (FN391283)	99	Sediments of Tunisian multi- pond solar saltern	<i>Halapricum salinum</i> JCM 19729/(NR126307)	96
A9.C13	555	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone TX4CA 30/(EF690585)	96	Soil of former lake Texcoco in Mexico	<i>Halapricum salinum</i> JCM 19729/(NR126307)	94
A10.C14	554	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Haloarchaeon LDS1D3.3/ (GQ375041)	94	Australian saltern crystallizer ponds	<i>Halosimplex pelagicum</i> JCM 17263/(HMI159602)	93
A11.C6	543	<i>Euryarchaeota</i> <i>Methanosarcinaceae</i>	<i>Methanohalobium evestigatum</i> DSM 3721/(FR733675)	99	Mud from a salt lagoon in Crimea, Russia		
A11.C101	546	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone BA3/ (HQ425146)	99	Aran-Bidgol Salt Lake in Iran	<i>Halomicrobium zhouti</i> JCM 17095/(HQ215546)	94
A12.C24	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone Kasin-A1-C11/(HE604442)	98	Hypersaline sediments of Lake Kasin, southern Russia	<i>Salarchaeum japonicum</i> JCM 16327/(AB454051)	91
A12.C12	546	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	Uncultured archaeon clone GA12/(HQ425159)	97	Aran-Bidgol Salt Lake in Iran	<i>Natronomonas moolapensis</i> DSM 18674/(AY498645)	97
A13.C27	546	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum terrestre</i> JCM 10247/(AB090169)	99	Saline soils		
A13.C31	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	98	An Australian solar saltern		

Table 3 continued

Band	Length (bp)	Phylogenetic affiliation	Best match/accession number	Percentage of similarity	Source	Closest cultured archaeon/ accession number	Percentage of similarity
A14.C44	547	<i>Euryarchaeota</i> <i>Halobacteriaceae</i>	Uncultured <i>Halomicrobium</i> sp. clone L4-H11/(KF452248)	96	Hypersaline Ocnei Lake Romania	<i>Haloarcula marismortui</i> JCM 8966/(AB663354)	95
A14.C36	516	<i>Euryarchaeota</i> <i>Halobacteriaceae</i>	Uncultured <i>Halomicrobium</i> sp. clone L4-H11/(KF452248)	96	Hypersaline Ocnei Lake Romania	<i>Haloarcula marismortui</i> JCM 8966/(AB663354)	96
A15.C47	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum terrestre</i> JCM 10247/(AB090169)	99	Saline soils		
A15.C55	548	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	97	An Australian solar saltern		
A17.C126	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	99	An Australian solar saltern		
A17.C130	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	98	An Australian solar saltern		
A18.C148	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	98	An Australian solar saltern		
A18.C140	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum terrestre</i> JCM 10247/(AB090169)	99	Saline soils		
A19.C166	547	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum terrestre</i> JCM 10247/(AB090169)	99	Saline soils		
A19.C162	516	<i>Euryarchaeota</i> <i>Haloferacaceae</i>	<i>Halorubrum saccharovororum</i> JCM 8865/(U17364)	98	An Australian solar saltern		

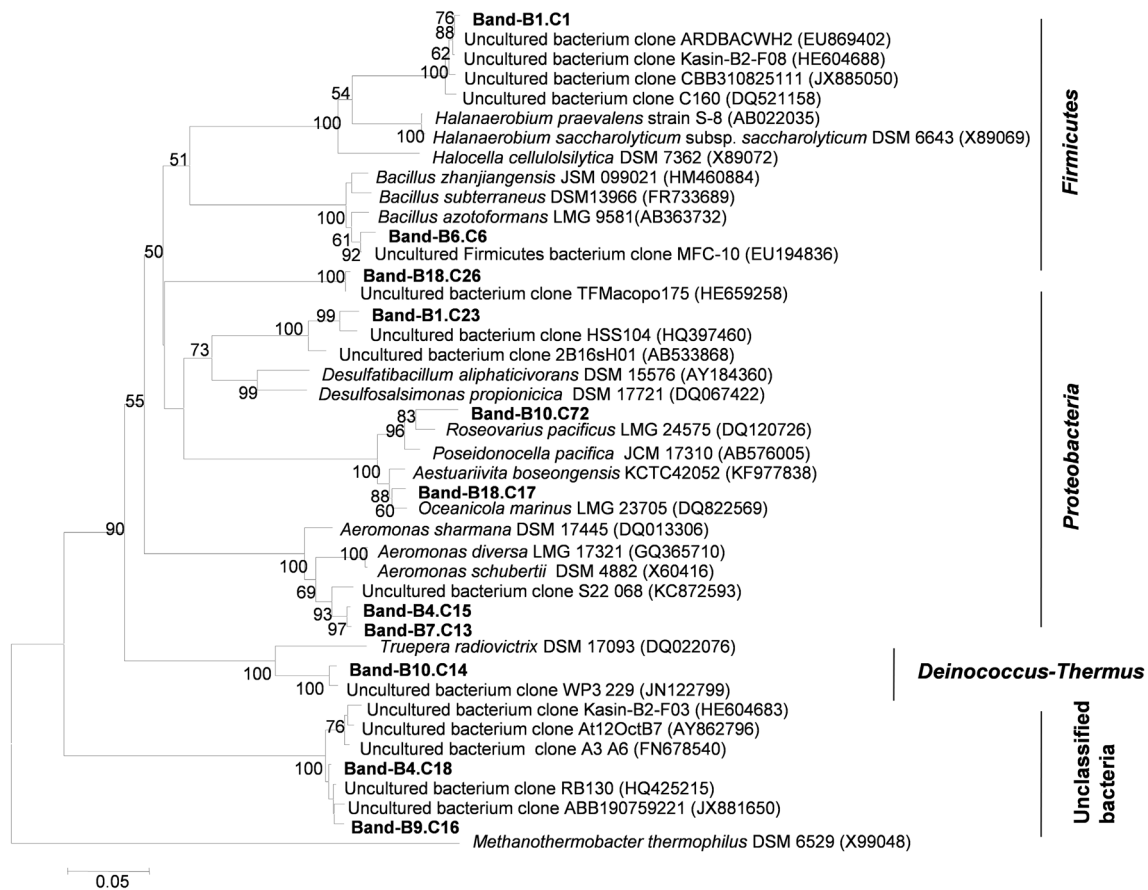


Fig. 2 Phylogenetic tree based on 16S rRNA gene of excised DGGE bands compared to representatives of the domain bacteria and environmental clones. The tree is based on Neighbour-Joining method.

The scale bar represents 5% estimated sequence divergence. The sequence of *Methanothermobacter thermophilus* was used as the out-group

saltern crystallizer, and from a solar saltern in Korea. Band A14.C36 and A14.C44 sequences were affiliated to *Haloarcula marismortui* isolated from Dead Sea. Band A10.C14 exhibited 93% of sequence similarity with *Halo simplex pelagicum* isolated from salted brown alga *Laminaria* China. The sequence of Band A8.C86 was related to an extremely halophilic archaeon *Halapricum salinum* (96% similarity) identified from non-purified solar salt. Furthermore, sequences from bands A11.C101 and A9.C13 showed high similarity to the uncultured archaeal clones previously described in hypersaline environments. Within the *Methanococci* class, bands A1.C10 and A11.C6 sequences were clearly affiliated to the family *Methanocaldococcaceae* and *Methanosarcinaceae*, respectively. Sequences of band A11.C6 and A1.C10 were related to *Methanohalobium vestigatum* isolated from a salt lagoon in Crimea, Russia and *Methanotorris igneus* isolated from a coastal hydrothermal environment in Iceland with sequence similarity of 99 and 83%, respectively.

Quantification of 16S rRNA genes

The abundance of bacterial and archaeal groups in all samples, determined by quantitative real-time PCR, was reported as DNA copy numbers of 16S rRNA genes per gram of wet sediment. The abundance of archaea was 5×10^5 to 10^6 , whereas bacteria abundance was 5×10^6 DNA copies g^{-1} .

Identification of fermentative bacteria

Eight fermentative anaerobic halophilic bacterial strains obtained in pure culture were selected based on their morphological characteristics of colonies and were characterized by 16S rRNA gene analysis. Cells of strictly anaerobic isolates were rods occurring singly or in pairs. All strains were motile. The isolates are designated by letters indicating first the origin of strains from Chott El Jerid (CEJ), Fermentative (F) followed by type of substrate Tryptone (T),

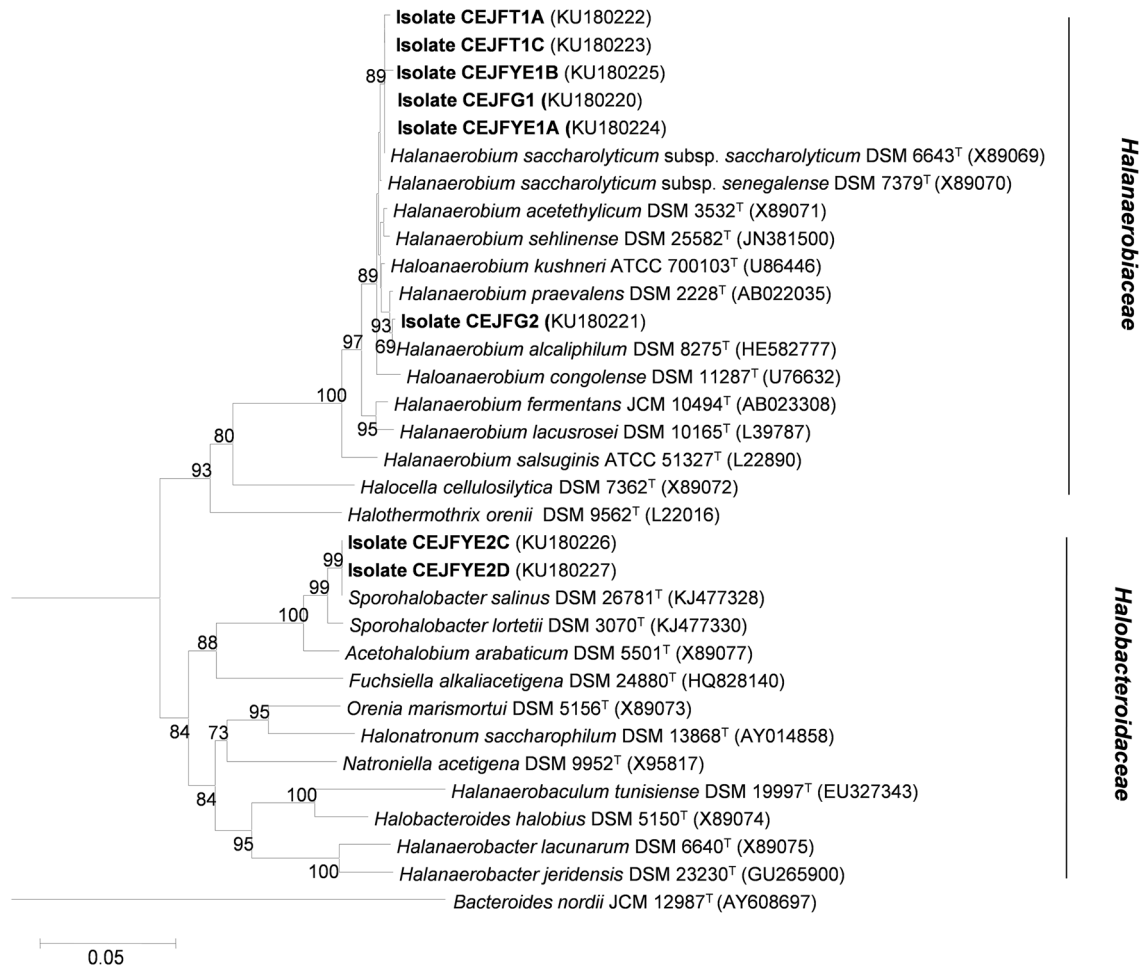


Fig. 4 Phylogenetic tree based on similarities of 16S rRNA sequences of isolates and its relatives. The tree is based on the Juke-Cantor model and the Neighbour-Joining method. The sequence of

Bacteroides nordii was used as the outgroup. Bootstrap values based on 1000 replicates are shown

Halanaerobiaceae family and two strains to *Halobacteroidaceae* family. CEJFT1A, CEJFT1C, CEJFYE1B, CEJFG1, CEJFYE1A were related (98–99 % of similarity) to species *H. saccharolyticum*. The strain CEJFG2 exhibited 99 % of sequence similarity with *Halanaerobium alcaliphilum*. Strains CEJFYE2C, CEJFYE2D were affiliated (98 % of similarity) to species *Sporohalobacter salinus*.

Discussion

In the present study, the microbial diversity present in the largest Tunisian salt lake, Chott El Jerid, was investigated for the first time by culture-independent methods. Denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR) were performed to assess the bacterial and archaeal communities' structure and to evaluate their abundance. A remarkable microbial diversity was

obtained in the extremely halophilic ecosystem, Chott El Jerid, among both the bacteria and archaea domains. Predominant phylogenetic groups were represented by *Proteobacteria* and *Firmicutes* of the bacteria domain. These taxonomic groups were also detected previously in hypersaline lakes (Demergasso et al. 2008; Makhdoumi-Kakhki et al. 2012; Emmerich et al. 2012), salterns, and hypersaline sediments (Mouné et al. 2003; Baati et al. 2010).

What is notable in our study is the absence of sequences representing the *Bacteroidetes* group. This group is reported to dominate with *Proteobacteria* in many different saline environments including hypersaline lakes such as the Tebenquiche in the Salar de Atacama (Demergasso et al. 2008), the largest hypersaline playa Aran-Bidgol in Iran (Makhdoumi-Kakhki et al. 2012), and Tunisian multipond solar saltern (Baati et al. 2008).

In this work, eight obligately halophilic anaerobic fermentative bacteria were isolated from the same samples

(S1-10) and (S2-10) used for DGGE analysis. Those strains were affiliated with three species *H. alcaliphilum*, *H. saccharolyticum* and *S. salinus*. The species *H. alcaliphilum* was a strictly anaerobic, moderately halophilic, Gram-negative bacterium and was isolated from the sediments of Great Salt Lake (Tsai et al. 1995). *H. saccharolyticum* was retrieved from sediments of hypersaline lakes (Cayol et al. 1994). The species *S. salinus* was recently isolated from Chott El Jerid (Ben Abdallah et al. 2015). These results are consistent with those obtained from DGGE. In fact, in our study, a number of DGGE band sequences were related to groups of anaerobic bacteria, principally fermentative bacteria. Previous studies were established by the isolation of anaerobic halophilic fermentative bacterial strains from surface sediments of Chott El Jerid (Hedi et al. 2009; Mezghani et al. 2012).

Moreover, a number of DGGE band sequences related to sulfate-reducing bacteria were detected in our study, but no sulfate-reducing strains have been isolated in pure cultures from this lake up to now.

Within the bacterial clone libraries, the most abundant *Deltaproteobacterial* representatives related to halophilic sulfate-reducing bacteria and members of the phylum *Firmicutes* related to the strictly anaerobic fermentative bacteria were found inhabiting the hypersaline ponds in Mediterranean salterns and in salt lakes (Mouné et al. 2003; Makhdoumi-Kakhki et al. 2012).

Interesting was the detection of *Deinococcus-Thermus* in Chott El Jerid. It is a small group of bacteria that are highly resistant to radiation. The presence of representatives of this group was scarce in hypersaline ecosystems studied using rRNA gene clones libraries. Members of *Deinococcus-Thermus* were also detected from the RNA-derived DGGE profiles in Cock Lake, a soda lake in Russia at low salinity 60 g L⁻¹ and this group was absent at high salinities in other investigated soda lakes (Foti et al. 2008).

With regard to the archaea population on the earth, halophilic archaea are the dominant microbial flora in hypersaline environments such as solar salterns, hypersaline lakes, hypersaline microbial mats, and the Dead Sea (Oren 2002). In this work, it could be noticed that all the 16S rRNA gene sequences obtained were affiliated with the orders *Halobacteriales* and *Haloferacales* positioned in the phylum *Euryarchaeota*, with most representatives belonging to the *Halobacteria* class, extreme aerobic halophiles growing at high salinity close to saturation.

In this study, no sequences affiliated with the *Crenarchaeota* group were detected. However, clones related to this phylum were recovered in anoxic sediments of hypersaline ponds in Salin-de-Giraud salterns from the station of lower salinity (150–200 g L⁻¹) (Mouné et al. 2003).

The same results were obtained in other studies assessing the diversity of Haloarchaea in salt lakes which they

have shown that most sequences were affiliated to *Halobacteria* (Makhdoumi-Kakhki et al. 2012). Here, interestingly, we detected via DGGE method two sequences, retrieved from each sample, related to members of the orders *Methanosarcinales* and *Methanococcales*. Methanogenic orders seem to be in low rates of all archaeal 16S rRNA gene sequences from clone's libraries from hypersaline lakes, and hypersaline ponds in Mediterranean salterns (Mouné et al. 2003). It is well known that in hypersaline environments, characterized by high concentrations of sulfate, sulfate-reducing bacteria outcompete methanogens for the substrate such as H₂, acetate and formate.

According to qPCR results, Chott El Jerid shelters an important number of bacterial and archaeal populations (10⁶ cells per g wet sediment) in comparison with previous studies performed in other hypersaline systems using quantitative PCR. In contrast, higher numbers of cells 10⁷ to 10⁹ per g wet sediment were detected in saline Qinghai Lake, China (Dong et al. 2006). Most studies demonstrated that the microbial communities in many hypersaline environments were dominated by halophilic archaea numbers. Spatial and temporal effects on the structure of microbial communities should be further investigated. Other samples will be taken in the rainy season in order to analyze the spatial and seasonal effects on the prokaryotic community in the Chott El Jerid.

Our results showed that Chott El Jerid presents an important diversity of bacterial and archaeal types found in thalassohaline hypersaline environments from different geographic regions (Baati et al. 2010; Makhdoumi-Kakhki et al. 2012).

In conclusion, culture-independent study reports on the microbial diversity, using 16S rRNA gene sequences analysis within two samples from Chott El Jerid. The diversity of bacteria and archaea found in Chott El Jerid was specified. Bacteria domain was represented exclusively by *Proteobacteria* and *Firmicutes*. *Archaea* domain was closely related to the *Euryarchaeota* phylum. More importantly, *Firmicutes* were affiliated with the anaerobic genera *Halanaerobium*, *Halocella* and the group *Proteobacteria* with anaerobic halophilic sulfur reducing *Deltaproteobacteria*. Additionally, the DGGE analysis confirmed the presence of microorganisms previously described through clone libraries in these ecosystems, such as methanogen archaea and *Deinococcus-Thermus* phylum. Our results suggest that microorganisms as well as uncultured clones detected in Chott El Jerid were found in hypersaline thalassohaline environments, despite the continental origin of our ecosystem. The cultivation approach led us to isolate anaerobic fermentative bacteria related to three species *H. alcaliphilum*, *H. saccharolyticum* and *S. salinus* grouped within the phylum *Firmicutes*. The culture-dependent and culture-independent are complementary methods and give a good description of the prokaryotic

diversity in hypersaline environments. However, using novel molecular strategies such as metagenomic approaches is an essential step to gaining a better understanding of microbial ecology in hypersaline environments.

Acknowledgments MBA was supported by the Tunisian Ministry of Higher Education, Scientific Research and Technology fellowship. This work was published with the support of AIRD (JEA HALOBI-OTEC project “Traitement anaérobie des effluents industriels salins et hypersalins par des bioréacteurs membranaires”).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. doi:10.1093/nar/25.17.3389
- Baati H, Guermazi S, Amdouni R, Gharsallah N, Sghir A, Ammar E (2008) Prokaryotic diversity of a Tunisian multipond solar saltern. *Extremophiles* 12:505–518. doi:10.1007/s00792-008-0154-x
- Baati H, Guermazi S, Gharsallah N, Sghir A, Ammar E (2010) Novel prokaryotic diversity in sediments of Tunisian multipond solar saltern. *Res Microbiol* 161:573–582. doi:10.1016/j.resmic.2010.05.009
- Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 43:260–296
- Ben Abdallah M, Karray F, Mhiri N, Cayol JL, Tholozan JL, Alazard D, Sayadi S (2015) Characterization of *Sporohalobacter salinus* sp. nov., an anaerobic, halophilic, fermentative bacterium isolated from a hypersaline lake. *Int J Syst Evol Microbiol* 65:543–548. doi:10.1099/ijs.0.066845-0
- Brosius J, Dull TJ, Sleeter DD, Noller HF (1981) Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 148:107–127. doi:10.1016/0022-2836(81)90508-8
- Casamayor EO, Massana R, Benlloch S, Øvreås L, Díez B, Goddard VJ, Gasol JM, Joint I, Rodríguez-Valera F, Pedrós-Alió C (2002) Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environ Microbiol* 4:338–348. doi:10.1046/j.1462-2920.2002.00297.x
- Cayol JL, Ollivier B, Lawson A, Soh ALS, Fardeau ML, Ageron E, Grimont PAD, Prensier G, Guezennec J, Magot M, Garcia JL (1994) *Haloicola saccharolytica* subsp. *senegalensis* subsp. nov., isolated from the sediments of a hypersaline lake, and emended description of *Haloicola saccharolytica*. *Int J Syst Bacteriol* 44:805–811. doi:10.1099/00207713-44-4-805
- Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 37:141–145. doi:10.1093/nar/gkn879
- DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* 89:5685–5689. doi:10.1073/pnas.89.12.5685
- Demergasso C, Escudero L, Casamayor EO, Chong G, Balagué V, Pedrós-Alió C (2008) Novelty and spatio-temporal heterogeneity in the bacterial diversity of hypersaline Lake Tebenquiche (Salar de Atacama). *Extremophiles* 12:491–504. doi:10.1007/s00792-008-0153-y
- Dong H, Zhang G, Jiang H, Yu B, Chapman LR, Lucas CR, Fields MW (2006) Microbial diversity in sediments of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. *Microb Ecol* 51:65–82. doi:10.1007/s00248-005-0228-6
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. doi:10.1093/nar/gkh340
- Emmerich M, Bhansali A, Lösekann-Behrens T, Schröder C, Kappler A, Behrens S (2012) Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, southern Russia. *Appl Environ Microbiol* 78:4386–4399. doi:10.1128/AEM.07637-11
- Foti MJ, Sorokin DY, Zacharova EE, Pimenov NV, Kuenen JG, Muyzer G (2008) Bacterial diversity and activity along a salinity gradient in soda lakes of the Kulunda Steppe (Altai, Russia). *Extremophiles* 12:133–145. doi:10.1007/s00792-007-0117-7
- Großkopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* 64:960–969
- Hedi A, Fardeau ML, Sadfi N, Boudabous A, Ollivier B, Cayol JL (2009) Characterization of *Halanaerobaculum tunisiense* gen. nov., sp. nov., a new halophilic fermentative, strictly anaerobic bacterium isolated from a hypersaline lake in Tunisia. *Extremophiles* 13:313–319. doi:10.1007/s00792-008-0218-y
- Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* 3B:117–132
- Kbir-Arighuib N, Ben Hassan Chehimi D, Zayani L (2001) Treatment of Tunisian salt lakes using solubility phase diagrams. *Pure Appl Chem* 73:761–770
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acids techniques in bacterial systematics*. Wiley, Chichester, pp 115–147
- Makhdoumi-Kakhki A, Amoozgar MA, Kazemi B, Pašić L, Ventosa A (2012) Prokaryotic diversity in Aran-Bidgol salt lake, the largest hypersaline playa in Iran. *Microbes Environ* 27:87–93. doi:10.1264/jsme2.ME11267
- Maturrano L, Santos F, Rosselló-Mora R, Antón J (2006) Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. *Appl Environ Microbiol* 72:3887–3895. doi:10.1128/AEM.02214-05
- Mezghani M, Alazard D, Karray F, Cayol JL, Joseph M, Postec A, Fardeau ML, Tholozan JL, Sayadi S (2012) *Halanaerobacter jeredensis* sp. nov., isolated from a hypersaline lake. *Int J Syst Evol Microbiol* 62:1970–1973. doi:10.1099/ijs.0.036301-0
- Mouné S, Caumette P, Matheron R, Willison JC (2003) Molecular sequence analysis of prokaryotic diversity in the anoxic sediments underlying cyanobacterial mats of two hypersaline ponds in Mediterranean salterns. *FEMS Microbiol Ecol* 44:117–130. doi:10.1016/S0168-6496(03)00017-5
- Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700
- Nadkarni MA, Elizabeth Martin F, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiol* 148:257–266. doi:10.1099/00221287-148-1-257
- Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology and applications. *J Ind Microbiol Biotechnol* 28:56–63. doi:10.1038/sj/jim/7000176
- Oren A (2010) Industrial and environmental applications of halophilic microorganisms. *Environ Technol* 31:825–834. doi:10.1080/09593330903370026

- Ori GG, Pascucci V, Gasmi N, Barbieri R (2009) Tunisian desert: a perfect place to simulate the landing on mars. In: 27th IAS meeting of sedimentology- field trips guide book, SASSARI, EDES, pp 315–342
- Ruff-Roberts AL, Kuenen JG, Ward DM (1994) Distribution of cultivated and uncultivated cyanobacteria and chloroflexus-like bacteria in hot spring microbial mats. *Appl Environ Microbiol* 60:697–704
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. doi:[10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197)
- Tsai CR, Garcia JL, Patel BKC, Cayol JL, Baresi L, Mah RA (1995) *Haloanaerobium alcaliphilum* sp. nov., an anaerobic moderate halophile from the sediments of Great Salt Lake, Utah. *Int J Syst Evol Microbiol* 45:301–307. doi:[10.1099/00207713-45-2-301](https://doi.org/10.1099/00207713-45-2-301)
- Ventosa A, Mellado E, Sanchez-Porro C, Marquez MC (2008) Halophilic and halotolerant micro-organisms from soils. In: Dion P, Nautiyal CS (eds) *Microbiology of extreme soils*. Springer, Berlin, pp 87–115. doi: [10.1007/978-3-540-74231-9_5](https://doi.org/10.1007/978-3-540-74231-9_5)
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703
- Wright ES, Safak Yilmaz L, Noguera DR (2012) DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. *Appl Environ Microbiol* 78:717–725. doi:[10.1128/AEM.06516-11](https://doi.org/10.1128/AEM.06516-11)