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Bacterial Diversity in Çamaltı Saltern, Turkey

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Abstract

A combination of culture-dependent and culture-independent approaches was employed to identify the bacterial diversity of Çamaltı solar saltern in Turkey. The bacterial communities of Çamaltı Saltern were analyzed by molecular techniques that included denaturing gradient gel electrophoresis of 16S rRNA gene fragments PCR amplified from DNA extracted from the water samples of the saltern and 16S rRNA gene library analysis. A total of 42 isolates were identified at the genus/species level and 17 of them were found to belong to the *Bacteria* domain. All bacterial isolates were phylogenetically related to *Halobacillus, Virgibacillus* and *Halomonas* genus. A total of 50 clones from 16S rRNA gene library were analyzed by ARDRA. 16S rRNA sequence analysisof these clones revealed that most (85%) of the bacterial clones were related to *Salinibacter* genus members of the *Bacteroidetes*. The sequences of DGGE bands were related to the uncultured *Salinibacter*, uncultured halophilic bacterium and *Halomonas* sp. This work highlights the halophilic bacterial diversity of Çamaltı marine solar saltern.

Key words: bacterial diversity, halophilic bacteria, solar salterns

Introduction

Salts are necessary for all organisms but halophiles require high salt concentrations for growth and thus thrive in saline environments (DasSarma and DasSarma, 2012). Microorganisms adapted to life at high salt concentrations are widespread, both within the bacterial and the archaeal domain. As a result, highly diverse prokaryote communities can be found at all salt concentrations, from seawater up to about 340–350 g/liter (brines saturated with NaCl), in both thalassohaline and athalassohaline environments (Oren, 2006). The crystallizer ponds of solar salterns are reflecting characteristics of thalassic environment. Despite the prevailing extreme environment, a great diversity of microbial life has been observed in hypersaline areas of greater than 3.5 mol/l NaCl, a point at which only a few extreme halophiles can grow (DasSarma and DasSarma, 2012). These extreme halophiles grow best at the highest salinities (3.4–5 mol/L NaCl), forming dense blooms, and resulting in the red colour of many salterns (Guixa-Boixereu *et al*., 1996). There are three major groups of organisms in brines containing more than 12% salt. These include the halophilic (salt loving) members of the domain Bacteria which generally have a broad salt tolerance and the halophilic Archaea, the salt-loving prokaryotes, with a requirement for highly elevated concentrations of salt, often up to 10 times the salinity of seawater (Litchfield *et al*., 2001).

Çamaltı Saltern is the biggest artificial marine solar saltern in Turkey. It is a multipond system consisting of 182 ponds covering 58 km^2 and located about 38°35′N and 26°57′E on the east cost of the Aegean sea (Fig. 1). Sea salt extraction has been carried out in the area since 1863. It is divided into several evaporation ponds connected by pipes and channels along a 18 km seacoast. Çamaltı Saltern have been functioning with the system of successive evaporation basins (Tıraş, 2007). In this saltern, solar irradiance and wind are main factors contributing to water evaporation and salt crystallization. The brines originate by evaporation of seawater (so-called thalassohaline brines) and reflect the ionic composition of the sea which Na^+ is the predominant cation, Cl^- the main anion, followed by SO_4^{2-} (Oren, 2006).

Microbial diversity of the different salterns around the world have been examined both by culture-independent and culture-dependent techniques. These techniques have been used to analyse the microbial diversity of Salterns in Santa Pola, Spain (Anton *et al*., 1999; Anton *et al*., 2000), and coastal Australia (Burns *et al*., 2004a), Peru (Maturrano *et al*., 2006), Turkey (Mutlu *et al*., 2008), Croatia (Pašić *et al*., 2007), Korea (Park *et al*., 2006) and Tunisia (Hedi *et al*., 2009). Litchfield *et al*. (2001) examined and compared whole metabolic diversity of two different solar salterns. Polar lipids and pigments were also used as biomarkers to study microbial communities of solar salterns (Litchfield and Oren, 2001).The Çamaltı Saltern, the largest saltern in

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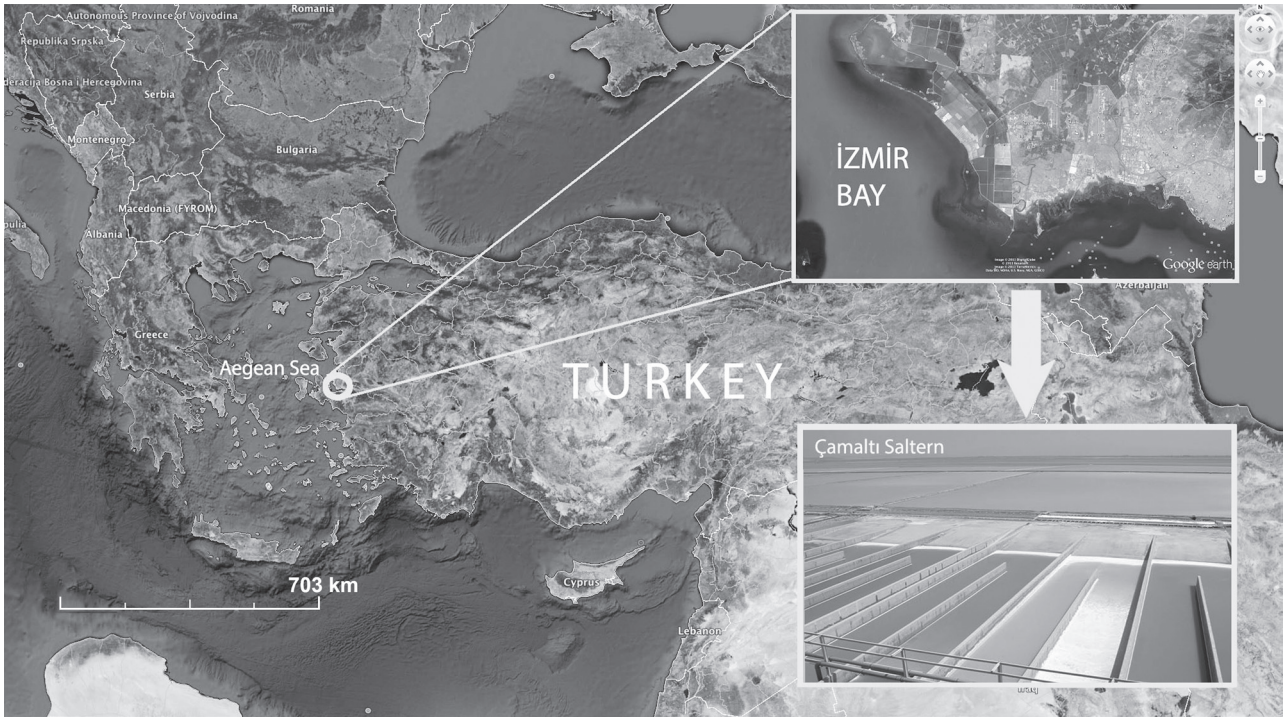

Fig. 1. Detailed map showing Çamaltı Saltern, in Turkey. Sampling points were shown with circle on the map. GPS coordinates of the sampling points were 38°28′47N–26°56′11E; 38°29'57N–26°53'37E. (Satellite imagery: Google/Google Earth).

Turkey, is an important source of salt for food. Two culture independent techniques namely, real time PCR and fluorescence *in-situ* hybridization (FISH) were used in a preliminary study which gave us some perspective to reveal the prokaryotic diversity of this hypersaline area (Mutlu and Guven, 2011). Given it's economic value for the region as a salt source, we have proposed to conduct a survey to gain better knowledge of the prokaryotic diversity thriving in this extreme ecosystem not only at a domain level, but also at genus, as well as species level. In a previous study, we determined PAH degrading archaeal isolates from the Çamaltı saltern (Erdogmuş *et al*., 2013) and *Haloferax* sp., *Halorubrum* sp., *Halobacterium* sp., and *Haloarcula* sp. were seen as dominant genera. Therefore, we only present the bacterial populations of largest Turkish saltern using both culture-dependent and culture-independent approach that includes denaturing gradient gel electrophoresis of PCR-amplified fragments of 16S rRNA gene from DNA extracted from the saltern in this study.

Experimental

Materials and Methods

Sample collection. Brine samples were taken from 10 different locations (38°28′47N–2656′11E; 38°29'57N–26°53'37E) of the Çamaltı Saltern in July 2007. A circle containing the sampling area is indicated on the map in Fig. 1. The total salt concentration of each sample was determined *in situ* with a hand refractometer (Eclipse) and the pH was measured with TOA WQC water analyser at the sampling point. The total salt concentration of these samples were measured between 6% and 32% and the pH values were between 6.5 and 7.5.

Isolation and selection of microorganisms by ARDRA (Amplified Ribosomal DNA Restriction Analysis). The following medium was used for isolation: to a liter of a solution of salts, named as 25% Sea Water (SW) and containing ($g\,l^{-1}$): NaBr 0.65, $NaHCO_3$ 0.17, KCl 5, $CaCl_2$ 0.72, $MgSO_4$_$7H_2O$ 49.5, $MgCl_2$_$6H_2O$ 34.6, NaCl 195, 1 g yeast extract, and 20 g of agar were added. Several dilutions (from 10^{-1} to 10^{-5}) of the original water sample were used to inoculate the plates by a plate spread technique. Two hundred µl of water samples were plated in duplicate onto 25% Sea Water Medium. Samples were incubated at 37°C for 3–4 weeks. Selected colonies were analyzed by 16S rRNA gene PCR amplification. Isolates were screened for redundancies by ARDRA (Amplified Ribosomal DNA Restriction Analysis) with the enzymes *Hinf*I and *Mbo*I (Vaneechoutte *et al*., 1992). Enzymatic digestions were performed by incubating 10 µl of the PCR product with 5U of enzyme and the corresponding enzyme buffer. The digestion products were analyzed in 2% agarose gels in 0.5X Tris-boric acid-EDTA (TBE) buffer. The gels were stained using ethidium bromide (0.2 µg/ml) and visualized and photographed under a UV transilluminator.

Identification bacterial isolates by 16S rRNA gene sequencing. Pure cultures were lysed in 100 µl MQ water and boiled for 10 min. Cell debris were pelleted by centrifugation at 13 000 × g for 10 min. One µl cell lysate was used in a PCR reaction containing (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 0.2 mM dNTPs, 3 mM $MgCl_2$, 20 pmol forward primer, 20 pmol reverse primer, 2.5 U Taq polymerase and MQ water to a final volume of 50 µl. To amplify the 16S rRNA genes, bacteria domain specific primer sets were used. The sequence of the forward primer was 27f (5'-AGAGTTTGATCATGGCTCAG-3'). The reverse primer was 1492r 5'-GTTACCTTGTTACGACTT-3' (Lane *et al*., 1985). The following conditions were used for amplification: a cycle of 94°C for 3 min, 30 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 2 min; plus an extension step of 7 min at 72°C. Negative controls were included with no addition of template DNA. Five µl of PCR product was loaded onto 1% agarose gel in 1X Tris Acetic acid-EDTA (TAE) buffer. The gel was stained using ethidium bromide (0.2 µg/ml), visualized and photographed under a UV transilluminator. PCR products were purified using the Wizard PCR and Gel Purification Kit (Promega) and stored at –20°C until required. DNA sequencing was performed by Beckman CEQ 8000 genetic analyser. The DNA sequences were analyzed using the BLASTN homology search program, which is available at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov) to identify close matches (Altschul *et al*., 1990). Multiple gene alignments were performed using MUSCLE 3.7 and Gblocks 0.91 b modules of "Phylogeny.fr". Phylogenetic analysis was performed using PhyML 3.0 a LRT and cladograms were generated using TreeDyn 198.3 module of Phylogeny.fr. All the above software and modules are freely available at http://www.phylogeny.fr/ (Dereeper *et al*., 2008).

Nucleic acid extraction. Microorganisms were collected by filtration of 50 ml of a brine sample on a 0.22-µm pore size GV filter (Durapore, Millipore). The filter was cut into small pieces with sterile scissors, and placed in RNAse- and DNAse-free 2-mL cryotubes containing 600 µl of extraction buffer (100 mM Tris-HCl, 100 mM EDTA pH 8.0). Six µl of lysozyme (3 mg ml^{-1}) was added and incubated at 37°C for 15 min. Then, 9 µl of proteinase K (150 mg ml^{-1}) and 60 µL of 10% sodium dodecyl sulfate (SDS) were added to the tubes and incubated at 37°C for 30 more minutes. After the addition of 120 µl 5M NaCl and 90 µl CTAB solution (10% CTAB, 0.7 M NaCl), the tubes were incubated at 65°C for 10 min., immersed into liquid nitrogen for 2 min., and incubated again for 2 min. at 65°C. The freeze-and-thaw steps were repeated three times. Nine hundred µl of phenol:chloroform:isoamylalcohol (25:24:1) (PCI) was added, mixed, and centrifuged at 16 000 g for 5 min. at 4°C. The aqueous phase was transferredto a new tube and one volume of PCI was added, vortexed, and centrifuged again (two to three times) until a clear interphase between the aqueous and the organic phases was observed. Finally, nucleic acids were precipitated with ethanol and resuspended in 50 µl of sterile deionized water. To check the quality of nucleic acids, they were run in 1% agarose (LE, FMC Products, Rockland, ME) gel and visualized under UV light after ethidium bromide staining. Extracts were stored at –85°C until used.

Cloning of PCR products. Ligation of the PCR products with the pCRII-TOPO vector, transformation of *Escherichia coli* TOP10, and selection of the transformants were carried out using the TOPO TA cloning kit (Invitrogen) according to the manufacturer's protocol. 16S rRNA gene library was generated with the pooled products of at least three independent PCR reactions. Clones were screened for redundancies by amplified rDNA restriction analysis (ARDRA) (Vaneechoutte *et al*., 1992) with the enzymes *Hinf*I and *Mbo*I (New England Biolabs) as described before. Clones representing the different restriction patterns were selected for sequencing.

DGGE analysis. 16S rRNA gene fragments were PCR amplified from pooled samples for DGGE analysis with the following primer sets: 341F-GC (5'-GCclamp-CCTACGGGAGGCAGCAG-3') and 907R (5'-CCGTCAATTCCTTTRAGTTT-3') (Muyzer *et al*., 1993). The forward primer was supplied with a GC-clamp (CGCCCGCCGCGCGCG-GCGGGCGGGGCGGGGGCACGG GGGG) at the 5' end. The PCR program was: 94°C for 5 min., 65°C 1 min., 72°C 3 min., and nine touchdown cycles of: 94°C for 1 min., 65°C (with the decreasing 1°C each cycle) 1 min., 72°C 3 min., followed by 20 cycles of: 94°C for 1 min., 55°C 1 min., and 72°C 3 min. During the final cycle, the length of the extension step was increased to 10 min.

The PCR products were separated by DGGE on a Ingeny system. Two stock solutions were prepared, representing 0 and 100% denaturing agent, respectively. The 0% solution consisted of 10% (wt/vol) acrylamide-bisacrylamide (37.5:1) in 1X Tris-acetic acid-EDTA buffer (TAE), and the 100% solution consisted of 10% (wt/vol) acrylamide-bisacrylamide, 420 g of urea per liter, and 400 ml of formamide per liter in 1X TAE. The DGGE gels were cast by using mixtures of these stock solutions in linear denaturing gradients with 40% denaturing agent in the top and 70% in the bottom of the gels. The wells in each gel were loaded with 15 µl of PCR products, and the gels were run for 18 h at 70 V and 60°C. The gels were stained for 30 min. in ethidium bromide solution and evaluated on a transilluminator (Uvitec). Individual bands were excised and

Table I.
Bacterial isolates and their closest GenBank Matches.

ARDRA Pattern[a]	Selected Isolate	No. of Isolates	Closest GenBank Match		
			% Identity	Taxon	Accession no.
IB	C12	2	99%	*Halobacillus* sp.	AB189301
IIB	C13	1	99%	*Halomonas halophila*	042050
IIIB	C15	2	97%	*Virgibacillus marismortui*	GU213159
VIB	C17	2	94%	*Halobacillus* sp.	FM992846
VIIB	C18	2	97%	*Halomonas halophila*	FN257740
VIIIB	C20	3	98%	*Halomonas halophila*	FN257740
IVB	C22	2	99%	*Halobacillus* sp.	AB189301
VB	C25	3	99%	*Halobacillus* sp.	JX992844

[a] The ARDRA pattern is indicated by a roman numeral followed by B (Bacterial).

resuspend in 20 microliters Milli Q water and incubated at 4°C overnight. An aliquot of the supernatant was used for PCR reamplification with the original primer set, and 50 ng of reamplified PCR products were used for the sequencing reaction.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined in this study have been deposited in the GenBank database under accession numbers KF863788 to KF863800 and KF938670 to KF938672.

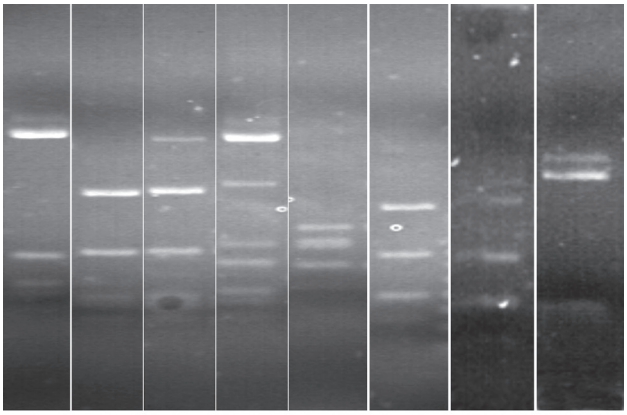

Fig. 2. ARDRA profiles of the Çamaltı Saltern isolates. *Hinf* I restriction products of *16S rRNA* gene PCR amplified DNA obtained from the isolates.

Results

Selection of bacterial isolates by ARDRA (Amplified Ribosomal DNA Restriction Analysis) and their identification. Fourty two colonies were selected according to their morphological characteristics such as size, shape and colour. Seventeen of them were found to be belonging to the *Bacteria* domain by PCR using *Eubacteria* specific primers. They were then characterized by 16S rRNA gene analysis. ARDRA profiles showed that these 17 Bacterial colonies yielded 8 different patterns (Table I). Fig. 2 shows *Hinf* I restriction products of 16S rRNA gene PCR amplified DNA obtained from the isolates. ARDRA profiles of isolates and their closest genbank matches were shown in Table I. High similarities to previously cultured halophilic bacteria, such as *Halobacillus* (99%), *Virgibacillus* (97%) and *Halomonas* (98%) were obtained in Gene-Bank. The sequences of isolates C12 and C25 were 99% identities with the genus *Halobacillus* sp. isolated from the Sahara Desert in Tunisia (Hua and Naganuma, 2007), and sea water in China (Acc. Number JX992844) respectively. The sequence of C17 was 94% identity with the genus *Halobacillus* sp. isolated from sediment in Greece (Gärtner *et al*., 2011). The sequence of the isolate C15 was related (97%) to *Virgibacillus* sp. isolated from Ebro Delta microbial mat in Spain (Villanueva *et al*., 2010). The sequences of the isolates C13, C18 and C20 were afiliated to the genus *Halomonas* with 99%, 97% and 98% similarity, respectively (Table I). Phylo-

Table II.
Clones and their closest GenBank Matches.

Selected Clone	No. of Clones	Closest GenBank Match		
		% Identity	Taxon	Accession no.
1A1	32	99%	*Salinibacter* sp. 5Sm6	AY987851.1
1A4	11	98%	*Salinibacter ruber* strain POLA 18	AF323502.1
1A5	7	92%	Uncultured bacterium clone 4–48B	EF459714.1

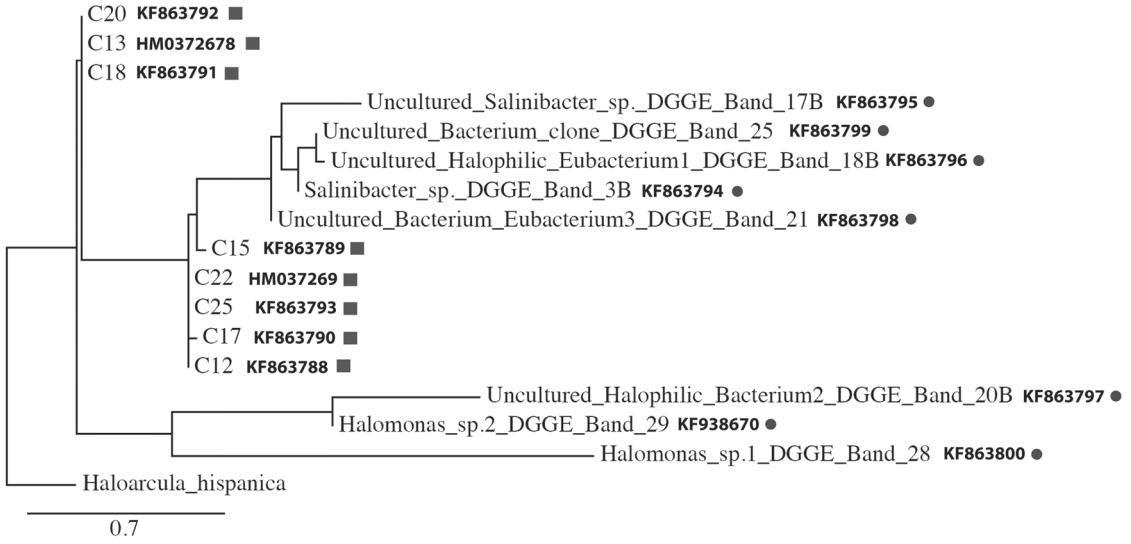

Fig. 3. Phylogenetic inferences based on *16S rRNA* gene sequences from isolates (indicated by green circle) and DGGE bands (indicated by blue circle) belonging to the halophilic bacteria.

genetic inferences based on 16S rRNA gene sequences from isolates (indicated by green circles) belonging to the halophilic bacteria were given in Fig. 3.

16S rRNA gene library construction. A total of 50 clones were analyzed by ARDRA, which yielded a total of three different patterns for Bacteria. At least one clone per restriction pattern was chosen for partial sequencing. The best match with the sequences in databases was obtained by BLAST analysis of the selected clones (Table II). Most (85%) of the bacterial clones were related to *Salinibacter* genus (*Bacteroidetes*).

DGGE fingerprint analyses. A total of 7 bands were identified using DGGE (Fig. 4). Most of the DGGE bands halted at between 50% and 60% denaturant concentrations. The sequences obtained from the bands yielded similarities to uncultured halophilic bacterium (bands 20B and 21B in Fig. 4) with high percentages of similarity (95% and 93% respectively) and three bands were closely related to the uncultured bacterium clone (bands 17B, 18B and 25B (72%, 98% and 97% respectively) (Fig. 4). Two bands yieldedsimilarity to *Halomonas* sp. and their sequences (28B and 29B) shared (83% and 99% respectively) sequence identity with the *Halomonas* sp. (Tsiamis *et al*., 2008) that was isolated from a Greek solar saltern. The sequencefrom Band 3B was affiliated to the genus *Salinibacter* and displayed relatedness (88%) to the sequence retrieved from the North Armof Great Salt Lake, USA (Acc. Number KF569484). The sequences from Band 17B and Band 18B were affiliated to the uncultured *Salinibacter* clone from Chula Vista Saltern California in USA (Zhaxybayeva *et al*., 2013) and uncultured bacterium clone from Guerrero Negro Solar Saltern in Mexico (Acc. Number KF741593) (82% and 98% similarity respectively). The sequence of the band 25B shared

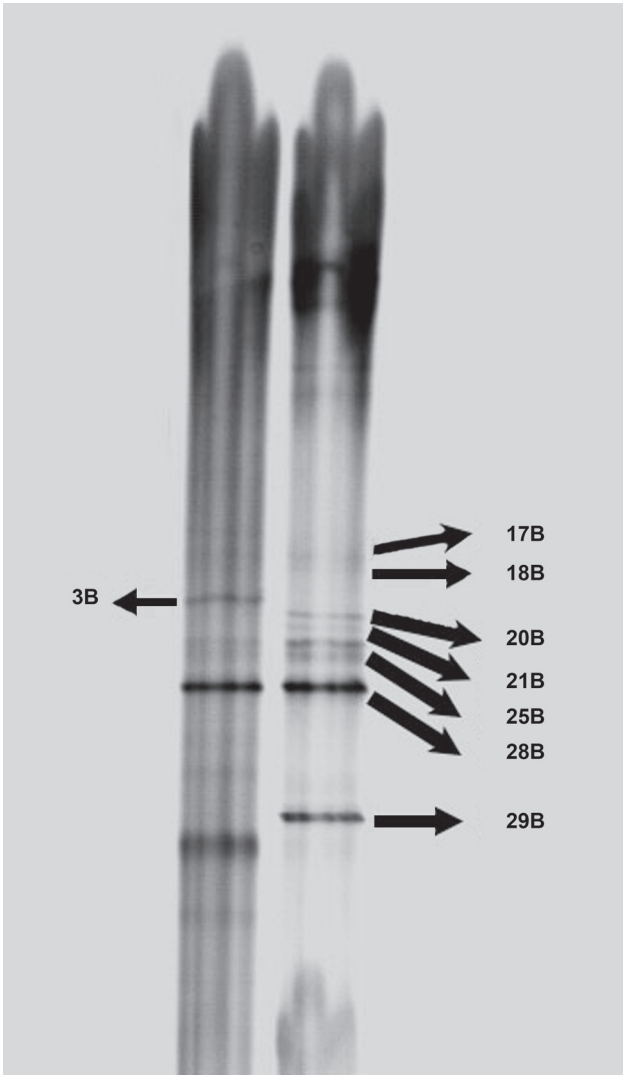

Fig. 4. DGGE analyses of partial *16S rRNA* gene fragments amplified with the general bacterial DGGE primer set, as described in the text. Sequenced bands are marked.

Table III
Obtained DGGE bands and their closest relatives in GenBank.

DGGE bands	% Similarity Closest relative in BLAST search of Gen Bank
Bacteria	
3B	88% KF569486 *Salinibacter* sp.
17B	82% JN839857 uncultured *Salinibacter* clone
18B	98% KF234381 uncultured bacterium clone
20B	95% FN994933 uncultured halophilic bacterium
21B	93% FN994932 uncultured halophilic bacterium
25B	92% JX881795 uncultured bacterium clone
28B	83% DQ873739 *Halomonas* sp.
29B	99% EU308363 *Halomonas* sp.

92% identity with the uncultured bacterium clone from Lake Tyrrel in Victoria Australia (Podell *et al.*, 2013). The sequences of Band 20B and Band 21B were 95% and 93 related respectively to the uncultured halophilic bacterium from Solar Saltern in Tunus (Baati *et al.*, 2008) (Table III). Phylogenetic inferences based on 16S rRNA gene sequences from DGGE bands (indicated by blue circles) belonging to the halophilic bacteria were given in Fig. 3.

Discussion

Multi-pond solar salterns represent ideal candidate model systems due to their managed nature, in which salt concentrations are kept relatively constant over time, in contrast to natural systems which are more susceptible to external variables such as climatic variation. Additionally, salterns exist around the world, albeit under somewhat different conditions. This provides a greater degree of international comparability than most natural systems (Burns *et al.*, 2007). This work contributes to our knowledge of prokaryotic communities of Çamaltı solar saltern located in the Aegean region of Turkey together with our previous studies (Mutlu and Güven 2011; Erdogmus *et al.*, 2013).

Several studies examined solar salterns by comparisons of polar lipid and pigment profiles (Litchfield *et al.*, 2001); comparisons of metabolic properties (Litchfield *et al.*, 2001); 16S rDNA sequencing from both denaturing gradient gel electrophoresis (DGGE) and clone libraries and FISH technique (Anton *et al.*, 1999; Anton *et al.*, 2000; Casamayor *et al.*, 2002; Burns *et al.*, 2004ab; Pašić *et al.*, 2007; Mutlu *et al.*, 2008; Hedi *et al.*, 2009). There have been many investigations of the archaeal, bacterial, and eukaryal inhabitants in these environments using both culture and culture-independent techniques (Javor *et al.*, 1982; Javor, 1984; Diez *et al.*, 2000; Benlloch *et al.*, 2002; Casamayor *et al.*, 2002; Litchfield and Gillevet, 2002; Ovreas *et al.*, 2003; Burns *et al.*, 2004ab; Maturrano *et al.*, 2006; Mutlu *et al.*, 2008; Rossello-Mora *et al.*, 2008).

The analysis of microbial diversity has shifted in the last two decades from cultivation-dependent approaches to 16S rDNA-based cultivation-independent approaches, which led to the discovery of many novel microbial taxa. Nevertheless, this approach also has important limitations and is often confined to naming 16S rDNA clones through sequence similarity and speculation on their ecophysiology on the grounds of this similarity. Therefore, cultivation is still the method of choice to understand fully the physiology and complex ecological interactions in which microorganisms engage (Gunde-Cimmerman *et al.*, 2005). Litchfield *et al.* (2009) reported that the microbial community in the waters of a solar saltern is variable and represantatives of the *Archaea* and *Bacteria* domains can be found throughout the saltworks.

The prokaryotic community of Çamaltı saltern was already investigated by two culture-independent methods, fluorescence*in-situ* hybridization (FISH) and Real Time PCR (Mutlu and Güven, 2011). DAPI counts of the samples fell in the range of $1.21–3.2 \times 10^7$ cells mL^{-1} indicating a variety of morphologies of cells (straight rods, curved rods, and cocci) and high salinity samples contained higher numbers of *Archaea*. FISH indicated that cells hybridized with the *Eubacteria* specific probe (EUB338) ranged from 48% to 67% of all DAPI-stained cells, and from 33% to 57% of all DAPI stained cells hybridized with the ARC915 probe (*Archaea* spesific probe) in the Çamaltı samples (Mutlu and Güven, 2011). However, limited archaeal diversity, *Haloferax* sp., *Halorubrum* sp., *Halobacterium* sp., and *Haloarcula* sp., were observed in culture-dependent assay (Erdogmus *et al.*, 2013).

The bacterial diversity described in Çamaltı saltern is similar to that described in other coastal solar salterns in the world. Since the saline water of solar salterns were found to be thalassohaline Lim *et al.* (2004), Tsiamis *et al.* (2008) and Hedi *et al.* (2009) detected *Halobacillus* sp. and *Halomonas* sp. in their samples from solar saltern in Korea, Greece and Tunisia respectively.

In this study, nine out of seventeen of the bacterial strains grown as pure colonies were related to *Halobacillus* sp., six out of seventeen were *Halomonas* sp. and only two strains were related to *Virgibacillus* sp. known as extreme halophiles growing in the presence of 10 to 30% total salts. The sequence of our isolate C17 had 94% identity with the genus *Halobacillus* sp. isolated from sediment in Greece (Gärtner *et al.*, 2011). If a threshold value of 16S rDNA similarity considered to be 90% for assignment to the genus *Halobacillus*, 16S rDNA sequence similarity (94%) of C17 indicate that this strain might be a novel species of the genus. There-

fore, further studies *e.g.* DNA:DNA homology should be carried out with different *Halobacillus* species.

Banding patterns identified in DGGE provide good insights in understanding the composition change of microbial communities. It is well known that most of the Bacteroidetes group is predominantly aerobic and is generally found in natural environments such as water and soil (Benlloch *et al*., 2002). In this study, most of the sequences obtained from DGGE for *Bacteria* and clone library yielded similarities to uncultured species of *Salinibacter* sp. which is a member of the Bacteroidetes group. However, we did not succeed incultivating any *Salinibacter*. *Salinibacter* representatives have been detected in saline environment using different techniques, with different levels of sensitivity (Anton *et al*., 2008). Contradictory results of same samples were obtained when analyzing the bacterial community inhabiting the hypersaline Tuz Lake in central Anatolia, Turkey. Although FISH counts gave very low numbers, sequences related to *Salinibacter* dominated bacterial 16S rRNA gene clone libraries and DGGE profiles (Mutlu *et al*., 2008).

It was reported that certain haloarchaea (*e.g. Halorubrum*) can inhibit *Salinibacter* growth (Anton *et al*., 2008). In a previous study (Erdogmuş *et al*., 2013), *Haloferax* sp., *Halorubrum* sp., *Halobacterium* sp. and *Haloarcula* sp. strains have been cultivated in Çamaltı Saltern and detected as dominant archaeal genera may have antogonistic effect on the cultivation of *Salinibacter* in this study.

More diversity among the bacterial isolates (*Halobacillus* sp., *Halomonas* sp., *Virgicacillus* sp.) than among the clones (*Salinibacter* sp.) was obtained in this study, supporting the observation that has been made previously for hypersaline environments by Benlloch *et al.* (2002) and Maturrano *et al.* (2006).

It is known that the microbiology of saltern systems in various parts of the world suggests a high degree of similarity (Oren, 1993) but some differences must occur as the result of changes in incident radiation, temperature, nutrient availability, residence time in the ponds (Litchfield *et al*., 2001). Tsiamis *et al.* (2008) observed microbial diversity by using a high-density oligonucleotide microarray (PhyloChip) as the part of their culture-independent studies of a Greek solar saltern located on the Agean coast just opposite of Çamaltı saltern at the same latitude. Similar to our results, most of the prokaryotic isolates recovered from hypersaline water (26% salinity) were extremely halophilic bacteria which were phylogenetically related to *Actinobacteria*, *Firmicutes* and δ-*Proteobacteria*in their study.

The genus *Halomonas* consists currently of 82 species (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2745) although Vreeland *et al.* (1980) originally described the genus *Halomonas* with only one species as *H. elongata*.

In this study, extremely low diversity in terms of genera was obtained by culture independent assay results, whereas a high number of different species within the single genus may occur as it was reported by Benlloch *et al.* (2001).

The recent development of sequencing technologies generating massive amount of bioinformatic data has enabled us to assess much deeper layers of microbial communities at lower costs (Kim *et al*., 2013). Whole-genome molecular techniques offer a more comprehensive view of genetic diversity compared to PCR-based molecular approaches that target only a single or few genes. These techniques attempt to analyze all the genetic information present in total DNA extracted from an environmental sample or pure culture (Rastogi and Sani, 2011). New approaches such as pyrosequencing which is considered as PCR and cloning bias-free method have contributed significantly to the development of microbial ecology. Ghai *et al.* (2011) recently described the microbiota of two hypersaline saltern ponds, one of intermediate salinity (19%) and a NaCl saturated crystallizer pond (37%) using pyrosequencing. The analyses of these metagenomes (nearly 784 Mb) reaffirmed the vast dominance of *Haloquadratum walsbyi* but also revealed novel, abundant and previously unsuspected microbial groups such as of low GC *Actinobacteria*. Metagenomic assembly revealed three new abundant microbes: a low-GC euryarchaeon with the lowest GC content described for any euryarchaeon, a high-GC euryarchaeon and a gammaproteobacterium related to *Alkalilimnicola* and *Nitrococcus*. These discoveries showed the combined power of an unbiased metagenomic and single cell genomic approach (Ghai *et al*., 2011). Therefore these new PCR and cloning bias-free technique could be used to reveal prokaryotic communities in Çamaltı Saltern in future studies.

This is the first study in which both culture-dependent and culture-independent techniques have been used simultaneously to target unique regions of the 16S rRNA gene in samples obtained from Çamaltı solar saltern and *Halobacillus* sp. *Halomonas* sp. and *Virgibacillus* sp. isolates.

Since, halophilic prokaryotes have great potential in industrial use such as production of compatible solutes, biopolymers, and bioremediation processes (Ventosa *et al*., 1998; Margesin and Schinner, 2001; Mellado nad Ventosa, 2003) and aromatic hydrocarbon degradation by halophilic archaea (Erdogmus *et al*., 2013) prompts us to screen our collection of halophilic bacteria isolated from Çamaltı saltern in future studies of biodegration and other biotechnological applications.

Acknowledgements

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Literature

Altschul S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol*. 215: 3389–3402.

Anton J., E. Llobet-Brossa, F. Rodríguez-Valera and R.I. Amann. 1999. Fluorescence *in situ* hybridization analysis of the prokaryotic community inhabiting crystallizer ponds. *Environ. Microbiol*. 1: 517–523.

Anton J., R. Rosselló-Mora, F. Rodríguez-Valera and R. Amann. 2000. Extremely halophilic Bacteria in crystallizer ponds from solar salterns. *Appl. Environ. Microbiol*. 66: 3052–3057.

Anton J., A. Pena, F. Santos, M. Martinez-Garcia, P. Schmitt-Kopplin and R. Rossello-Mora. 2008. Distribution, abundance and diversity of the extremely halophilic bacterium *Salinibacter ruber*. *Saline Systems*. 4:15.

Baati H., S. Guermazi, R. Amdouni, N. Gharsallah, A. Sghir and E. Ammar. 2008. Prokaryotic diversity of a Tunisian multipond solar saltern. *Extremophiles*. 12: 505–518.

Benlloch S., A. López-López, E.O. Casamayor, L. Ovreas, V. Goddard, F.L. Daae G. Smerdon, R. Massana, I. Joint, F. Thingstad and others. 2002. Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. *Environ. Microbiol*. 4: 349–360.

Burns D.G., H.M. Camakaris, P.H. Janssen and M.L. Dyall-Smith. 2004a. Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. *Appl. Environ. Microbiol*. 70: 5258–5265.

Burns D.G., H.M. Camakaris, P.H. Janssen and M.L. Dyall-Smith. 2004b. Cultivation of Walsby's square haloarchaeon. *FEMS Microbiol. Lett*. 238: 469–473.

Burns D.G., P.H. Janssen, T. Itoh, M. Kamekura, Z. Li, G. Jensen, F. Rodríguez-Valera, H. Bolhuis and M.L. Dyall-Smith. 2007. *Haloquadratum walsbyi* gen nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. *Int. J. Syst. Evol. Microbiol*. 57: 387–392.

Casamayor E.O., R. Massana, S. Benlloch, L. Ovreas, B. Diez, V.J. Goddard, J.M. Gasol, I. Joint, F. Rodriguez-Valera and C. Pedro's-Alio. 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.

DasSarma S. and P. DasSarma. 2012. Halophiles. http://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0000394.pub3, 2012.03.30.

Dereeper A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot and others. 2008. Phylogeny. fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res*. 36: W465–469.

Diez B., J. Anton, N. Guixa-Boixereu, C. Pedros-Alio and F. Rodriguez-Valera. 2000. Pulsed-field gel electrophoresis analysis of virus assemblages present in a hypersaline environment. *Int. Microbiol*. 3: 159–164.

Erdogmus S.F., M.B. Mutlu, S.E. Korcan, K. Guven and M. Konuk. 2013. Aromatic Hydrocarbon Degradation by Halophilic Archaea Isolated from Camalti Saltern, Turkey, pp. 224:1449. In: *Water Air and Soil Pollution*.

Gärtner A., M. Blümel, J. Wiese and J.F. Imhoff. 2011. Isolation and characterisation of bacteria from the Eastern Mediterranean deep sea. *Antonie Van Leeuwenhoek*. 100: 421–435.

Ghai R., L. Pašić, A.B. Fernández, A.B. Martin-Cuadrado, C.M. Mizuno, K.D. McMahon, R.T. Papke, R. Stepanauskas, B. Rodriguez-Brito, F. Rohwer and others. 2011. New abundant microbial groups in aquatic hypersaline environments. *Sci. Rep.* 1: 135.

Guixa-Boixereu N., J.I. Calderon-Paz, M. Heldal, G. Bratbak and C. Pedros-Alio. 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. *Aquat. Microb. Ecol*. 11: 215–227.

Gunde-Cimerman N., A. Oren and A. Plemenitas. 2005. Adaptation to life at high salt concentrations in *Archaea*, *Bacteria*, and *Eukarya* – Introduction. *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya* 9: 1–6.

Hua N.P., A. Kanekiyo, K. Fujikura, H. Yasuda and T.Naganuma. 2007 *Halobacillus profundi* sp. nov. and *Halobacillus kuroshimensis* sp.nov., moderately halophilic bacteria isolated from a deep-sea methane cold seep. *Int. J. Syst. Evol. Microbiol*. 57: 1243–1249

Hedi A., N. Sadfi, M.L. Fardeau, H. Rebib, J.L. Cayol, B. Ollivier and A. Boudabous. 2009. Studies on the Biodiversity of Halophilic Microorganisms Isolated from El-Djerid Salt Lake (Tunisia) under Aerobic Conditions. *Int. J. Microbiol*. 2009:731786.

Javor B.J., C. Requadt and W. Stoeckenius. 1982. Box-shaped halophilic bacteria. *J. Bacteriol*. 151:1532–1542.

Javor B.J. 1984. Growth potential of halophilic bacteria isolated from solar salt environments: carbon sources and salt requirements. *Appl. Environ. Microbiol*. 48:352–360.

Kim M., K.H. Lee, S.W. Yoon, B.S. Kim, J. Chun and H. Yi. 2013. Analytical tools and databases for metagenomics in the next-generation sequencing era. *Genomics Inform*. 11(3):102–113

Lane D.J., B. Pace, G.J. Olsen, D. Stahl, M. Sogin and N.R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. USA*. 82: 6955–6959.

Lim J.M., J.H. Yoon, J.C. Lee, C.O. Jeon, D.J. Park, C.K. Sung and C.J. Kim. 2004. *Halomonas koreensis* sp. nov., a moderately halophilic bacterium isolated from a solar saltern in Korea. *Int. J. Syst. Evol. Microbiol*. 54: 2037–2042.

Litchfield C.D. and A. Oren. 2001. Polar lipids and pigments as biomarkers for the study of the microbial community structure of solar salterns. *Hydrobiologia*. 466:81–89.

Litchfield C.D., A. Irby, T. Kis-Papo and A. Oren. 2001. Comparative metabolic diversity in two solar salterns. *Hydrobiologia* 466: 73–80.

Litchfield C. and P. Gillevet. 2002. Microbial diversity and complexity in hypersaline environments: a preliminary assessment. *J. Ind. Microbiol. Biotechnol*. 28: 48–55.

Litchfield C., A. Oren, A. Irby, M. Sikaroodi and P.M. Gillevet. 2009. Temporal and salinity impacts on the microbial diversity at the Eilat, Israel solar salt plant. *Global NEST Journal*. 11: 86–90.

Margesin R. and F. Schinner. 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles* 5: 73–83.

Maturrano L., F. Santos, R. Rosselló-Mora and J. Antón. 2006. Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. *Appl. Environ. Microbiol*. 72: 3887–3895.

Mellado E. and A. Ventosa. 2003. Biotechnological potential of moderately and extremely halophilic microorganisms, pp. 233–256. In: Barredo J.L. (ed) *Microorganisms for* Health Care, Food and Enzyme Production. Research Signpost, Kerala.

Mutlu M.B., M. Martínez-García, F. Santos, A. Peña, K. Guven and J. Antón. 2008. Prokaryotic diversity in Tuz Lake, a hypersaline environment in inland Turkey. *FEMS Microbiol. Ecol*. 65: 474–483.

Mutlu M.B. and K. Güven. 2011. Detection of prokaryotic microbial communities of Çamaltı Saltern-Turkey by Fluorescein *In Situ* Hybridization (FISH) and Real Time PCR *Turkish Journal of Biology*. 35:687–695.

Muyzer G., E.C. De Waal and A.G. Uitterrlinden. 1993. Profiling in complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reactionamplified genes coding for 16S rRNA. *Appl. Environ. Microbiol*. 59: 695–700.

Oren A. 1993. Ecology of extremely halophilic microorganisms, pp. 25–53 In: Vreeland R.H., L.I. Hochstein (eds.), TheBbiology of Halophilic Bacteria. CRC Press, Boca Raton.

Oren A. 2006. Life at high salt concentrations. *Prokaryotes* 2: 263–282.

Ovreas L., F.L. Daae, V. Torsvik and F. Rodriguez-Valera. 2003. Characterization of microbial diversity in hypersaline environments by melting profiles and reassociation kinetics in combination with terminal restriction fragment length polymorphism (T-RFLP). *Microb. Ecol*. 46: 291–301.

Pašić L., N.P. Ulrih, M. Črnigoj, M. Grabnar and B.H. Velikonja. 2007. Haloarchaeal communities in the crystallizers of two Adriatic solar salterns. *Can. J. Microbiol*. 53:8–18.

Park S.J., C.H. Kang and S.K. Rhee. 2006. Characterization of microbial diversity in a solar saltern of Korea based on 16S rRNA gene analysis. *J. Microbiol. Biotechnol*. 16:1640–1645.

Podell S., J.A. Ugalde, P. Narasingarao, J.F. Banfield, K.B. Heidelberg and E.E. Allen. 2013. Assembly-driven community genomics of a hypersaline microbial ecosystem. *PLoS One.* 8(4): e61692.

Rastogi G. and R.K. Sani. 2011. Molecular Techniques to Assess Microbial Community Structure, Function, and Dynamics in the Environment, pp. 29–57. In: Ahmad I., F. Ahmad, J. Pichtel (eds) Microbes and Microbial Technology: Agricultural and Environmental Applications. Springer, New York..

Rossello-Mora R., M. Lucio, A. Pena, J. Brito-Echeverria, A. Lopez-Lopez, M. Valens-Vadell, M. Frommberger, J. Anton and P. Schmitt-Kopplin. 2008. Metabolic evidence for biogeographic isolation of the extremophilic bacterium Salinibacter ruber. *ISME J*. 2: 242–253.

Tıraş M. 2007. Çamaltı Tuzlası. *Doğu Coğrafya Dergisi*. Cilt 12, 18:291–300 (In Turkish). (English Translation: Traş M. 2007. Çamaltı Saltern. *East Geography Journal*. Vol. 12, 18: 291–300)

Tsiamis G., K. Katsaveli, S. Ntougias, N. Kyrpides, G. Andersen, Y. Piceno and K. Bourtzis. 2008. Prokaryotic community profiles at different operational stages of a Greek solar saltern. *Res. Microbiol*. 159: 609–627.

Vaneechoutte M., R. Rossau, P. De Vos, M. Gillis, D. Janssens, N. Paepe, A. De Rouck, T. Fiers, G. Claeys and K. Kersters. 1992. Rapid identification of bacteria of the Comamonadaceae with amplified ribosomal DNA-restriction analysis (ARDRA). *FEMS Microbiology Letters* 93:227–234.

Ventosa A., J.J. Nieto and A. Oren. 1998. Biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev*. 62: 504–544.

Villanueva L., J. Del Campo and R. Guerrero. 2010. Diversity and physiology of polyhydroxyalkanoate-producing and -degrading strains in microbial mats. *FEMS Microbiol. Ecol*. 74: 42e54.

Vreeland R.H., C.D. Litchfield, E.L. Martin and E. Elliot. 1980. Halomonas elongata, a new genus and species of extremely salt-tolerant bacteria. *Int. J. Syst. Bacteriol*. 30485495.

http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2745. Accessed 10 December 2013.

Zhaxybayeva O., R. Stepanauskas, N.R. Mohan and R.T. Papke. 2013. Cell sorting analysis of geographically separated hypersaline environments. *Extremophiles* 17(2): 265–275.