Extremophiles (2012) 16:523–538
DOI 10.1007/s00792-012-0452-1

ORIGINAL PAPER

Enrichment of arsenic transforming and resistant heterotrophic bacteria from sediments of two salt lakes in Northern Chile

José Lara · Lorena Escudero González · Marcela Ferrero · Guillermo Chong Díaz · Carlos Pedrós-Alió · Cecilia Demergasso

Received: 13 January 2012 / Accepted: 2 April 2012 / Published online: 4 May 2012

Abstract Microbial populations are involved in the arsenic biogeochemical cycle in catalyzing arsenic transformations and playing indirect roles. To investigate which ecotypes among the diverse microbial communities could have a role in cycling arsenic in salt lakes in Northern Chile and to obtain clues to facilitate their isolation in pure culture, sediment samples from Salar de Ascotán and Salar de Atacama were cultured in diluted LB medium amended with NaCl and arsenic, at different incubation conditions. The samples and the cultures were analyzed by nucleic acid extraction, fingerprinting analysis, and sequencing. Microbial reduction of As was evidenced in all the enrichments carried out in anaerobiosis. The results revealed that the incubation factors were more important for determining the microbial community structure than arsenic species and concentrations. The predominant microorganisms in enrichments from both sediments belonged to the Firmicutes and Proteobacteria phyla, but most of the bacterial ecotypes were confined to only one system. The occurrence of an active arsenic biogeochemical cycle was suggested in the system with the highest arsenic content that included populations compatible with microorganisms able to transform arsenic for energy conservation, accumulate arsenic, produce H_2, H_2S and acetic acid (potential sources of electrons for arsenic reduction) and tolerate high arsenic levels.

Keywords Arsenic microbial metabolisms · Arsenic-resistant microorganisms · Northern Chile

Communicated by A. Oren.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-012-0452-1) contains supplementary material, which is available to authorized users.

J. Lara · M. Ferrero
Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Tucumán, Argentina

L. Escudero González · C. Demergasso (✉)
Centro de Biotecnología "Profesor Alberto Ruiz", Universidad Católica del Norte, Avda. Angamos 0610, Antofagasta, Chile
e-mail: cdemerga@ucn.cl

L. Escudero González · C. Demergasso
Centro de Investigación Científica y Tecnológica para Minería (CICITEM), Antofagasta, Chile

G. Chong Díaz
Departamento de Ciencias Geológicas, Universidad Católica del Norte, Antofagasta, Chile

C. Pedrós-Alió
Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, ES-08003 Barcelona, Spain

Introduction

Arsenic is widely present in water, rocks, and soils in many different areas of the world, including the Antofagasta region, Northern Chile. It is generally assumed that arsenic has mainly a magmatic genesis trough volcanic activity and ore deposits with high arsenic. Endogenous and exogenous agents, such as meteorological effects on the soil, and the circulation of subterranean water and wind, distribute arsenic through the atmosphere, soil and water. Additionally, the Atacama region is one of the largest mining areas in the world and this type of activity also releases arsenic (Flynn et al. 2002; Romero et al. 2003). Such natural and industrial processes shape the global geochemical cycling of arsenic in the region.

Arsenic is a highly toxic element that supports a surprising range of biogeochemical transformations. The biochemical basis of these microbial interactions lies in energy yielding redox biotransformations that cycle between the As(V) and As(III) oxidation states. Those biotransformations have a subsequent impact on the chemistry and the microbiology of studied environments (Lloyd and Oremland 2006). Microbes primarily metabolize inorganic arsenic either for resistance or for energy generation. The transformations may involve oxidation, reduction, methylation or demethylation (Slyemi et al. 2011).

The microbial communities involved in arsenic metabolism have been extensively studied in saline alkaline (pH 8.5–9.8) environments in the US (Oremland et al. 2004; Kulp et al. 2008), but poorly investigated in saline environments at lower pH (7.8–8.6), like Salar de Ascotán, Chile (pH 7.8–8.6) (Chong et al. 2000; Demergasso et al. 2007). It is worth considering that no alkaline brines are known in Chilean salt flats because of the higher sulfate and the lower alkalinity of inflow waters, as a consequence of the suspected higher sulfur content in Chilean volcanic rocks (Risacher and Fritz 2009).

Local cycling of arsenic was described in saline soda lakes located in the Mojave Desert: Mono Lake and Searles Lake (Oremland et al. 2004, 2005). Those transformations included heterotrophic and lithotrophic arsenate respiring microorganisms, as well as arsenic oxidizing microorganisms. Recently, anoxygenic photosynthetic microorganisms fueled by As have been isolated (Kulp et al. 2008). Organic matter, sulfide, hydrogen, and As(III) (Hoeft et al. 2004) were postulated as the main electron donors in such environments, while As(V), Se (VI), sulfate, low O_2 and nitrate were the electron acceptors (Oremland et al. 2004, 2005). It was shown that borate also plays an important role in the arsenic cycle because of its apparent effect on sulfate reduction (Kulp et al. 2007). The formation of arsenic III/sulfide soluble species also drives the arsenic cycle in soda lakes (Oremland et al. 2004). Considering the impact of the pH on the borate and arsenic III/sulfide species solubility, a different structure of the arsenic cycle should be expected at lower pH.

In the brines of the Salar de Ascotán arsenic concentrations reach levels up to 2.5 mM. The occurrence of arsenic microbial reduction associated to the formation of arsenic sulfides (Demergasso et al. 2007), as well as the arsenite-oxidizing microbial activity (Blamey, J. Bioscience F., personal communication) have been already described in this environment.

The aim of this study was to investigate the key microorganisms associated with the arsenic cycle present in the sediments of the Salar de Ascotán and Salar de Atacama and the conditions to grow those populations. A cultivation-based approach using organic electron donors was used in that stage of the research. The composition of the arsenic resistant and metabolizing bacterial populations in aerobic and anaerobic enrichment cultures was analyzed by 16S rDNA PCR-DGGE.

Materials and methods

Site description, sampling and measurements

A wide range of hypersaline environments with different characteristics can be found in the Atacama Desert area, Northern Chile. From west to east, deposits from the Coastal Range, the Central Depression, the Pre-Andean Depression, and the Altiplano Salt Deposits are found in succession (Chong 1984). All these basins receive their main water input from the east, which has a high percentage of salts from the leaching of volcanic rocks. The combination of both arid climate and closed basins in these salt flats produces a high rate of evaporation and the consequent increase in salt content of the brines.

The evaporitic basins of the Salar de Ascotán (21°29′S/68°19′W, 3.720 masl) and the Salar de Atacama (23°23′S/68°21′W, 2.305 masl) have been described as Andean and Pre-Andean salt flats, respectively (Stoertz and Ericksen 1974; Chong 1984). Ascotán (234 km^2) is in the lowest part of a tectonic basin surrounded to the east and west by volcanic chains, including some active volcanoes over 5000 m high, with the highest peaks of about 6000 m. The geological setting is dominated by volcanic structures that include acidic (rhyolites) and intermediate (andesites) rocks of Tertiary and Quaternary age. It is characterized by its concentric mineralogy, with a very irregular distribution of shallow "evaporites" (Herrera et al. 1997). The salt flat exhibits both permanent and temporary shallow ponds, and salt crusts, with a wide range of salinities and chemical compositions.

The Salar de Atacama Depression is the oldest and the largest evaporitic basin in Chile. It is located in a tectonic intramontane basin filled with Tertiary/Quaternary clastic and evaporitic sediments of continental origin. Its geological setting is quite complex and receives both surface and underground water inputs, mainly from the east. The main input is related to the leaching and direct contribution of acidic to basic volcanic material, mainly Tertiary and Quaternary. However, there is also an important input from a geological setting of different lithology as well as varied age.

These salt flats were visited in June 2006 during an intensive sampling expedition. Water and sediment samples were collected to perform physicochemical analysis and culture enrichments of arsenic-resistant and arsenic-metabolizing bacteria. The four different sites sampled in

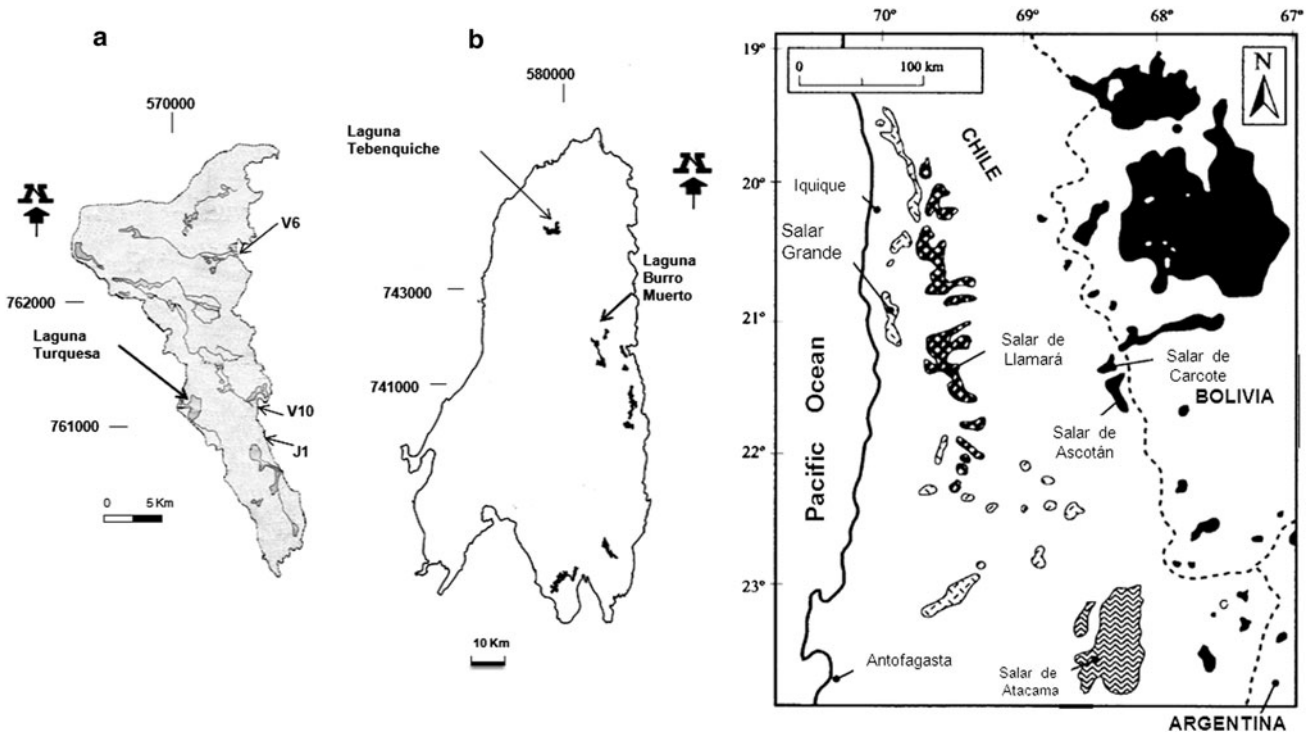

Fig. 1 Map of Salar de Ascotán (*left*), showing the location of Laguna Turquesa, J1, V6 and V10, and Salar de Atacama (*middle*) showing the location of Laguna Burro Muerto and Tebenquiche. *Inset* the salt flats sampled are shown in a map of Northern Chile (*right*)

Salar de Ascotán were two ponds (Laguna Turquesa and point J1) and two springs (V6 and V10) (Fig. 1). Meanwhile, two ponds were sampled in the Salar de Atacama: Laguna de Tebenquiche and Laguna Burro Muerto (Risacher and Alonso 1996) (Fig. 1). Two sites were selected for the enrichments, one from each system, considering the As content.

An Orion model 290 pH metre was used to measure temperature and pH in situ. Salinity, conductivity, and total dissolved solids (TDS) were determined using an Orion model 115 conductivity meter. Dissolved oxygen was measured with a Thermo Orion model 9708. Water samples and surface sediment (0–3 cm) were transferred to plastic bottles and kept in an icebox with ice until processing. Water samples for chemical analysis were filtered through 0.45 μm pore-size 25 mm diameter filter (Millipore, Billerica, MA, USA). Approximately 1 L of water for each data point was filtered through several nitrocellulose 0.22 μm pore size, 47 mm diameter filters for DNA and RNA extraction. Additional samples were fixed (3.7 % formaldehyde) and filtered through polycarbonate 0.22 μm pore size, 47 mm diameter filter (Millipore, Billerica, MA, USA) for bacterial total counts. Sediment samples for DNA and RNA extractions and for 4′, 6-diamidino-2-phenylindole (DAPI)-cell counting were treated as follows: sediment (10 g) was homogenized in a 150-mL Erlenmeyer flask with 50 mL of 0.9 % NaCl and 60 μL of 100 % Tween 20 for 30 min in a rotatory shaker (150 rpm) at room temperature. Subsequently, the suspension was transferred to a 50 mL-centrifuge tube and centrifuged at $5000\times g$ for 5 min. Supernatant was filtered through several nitrocellulose 0.22 μm pore size, 47 mm diameter filters (Millipore, Billerica, MA, USA). Filters for DNA extraction and for bacteria counting with DAPI-stain were kept at −80 °C. Total arsenic was determined by hydride generation atomic absorption spectroscopy (HGAAS), and the other cation concentrations using an atomic absorption spectrophotometer (AAnalyst 800; Perkin-Elmer, Norwalk, CT, USA). Sulfate and chloride contents were measured by gravimetric and Mohr method (APHA et al. 1998), respectively.

Counting of in situ bacteria and culturable arsenic-reducing bacteria

Bacteria were counted by epifluorescence using DNA-specific dye DAPI with a Leica DMSL microscope. Culturable arsenic-reducing bacteria (AsRB) were detected by most-probable-number (MPN) incubations using fresh minimal medium (Newman et al. 1997) modified by addition of 0.008 % yeast extract and amended after autoclaving with sterile 20 mM sodium lactate, 10 mM sodium sulfate (Na_2SO_4), 1 mM L-Cistein ($C_3H_7NO_2S$) and 1 mM dibasic sodium arsenate ($Na_2HAsO_4.7H2O$)

under N_2:CO_2:H_2 atmosphere (80:15:5, v/v) (Demergasso et al. 2007). To obtain a similar salinity to that of Laguna Turquesa, NaCl was added to obtain a value of TDS close to 30 g L^{-1}. The highest decimal dilution was 10^{-6} and five tubes were analyzed for each data point. The tubes were incubated in the dark at 28 °C. An abiotic control was carried out in sterile medium without inoculum. The presence of yellow precipitate was considered as a positive result. These cultures took between 3 and 4 weeks to precipitate arsenic sulfides as a result of the reduction of arsenate and sulfate.

Enrichment cultures

The sediment samples from Laguna Turquesa (Salar de Ascotán) were chosen for enrichments because they showed the highest concentration of total arsenic (28 mg L^{-1}). Burro Muerto sediment was used for the enrichments of Salar de Atacama samples. Sediment (4 g) was dispensed into 40-mL bottles filled to the top with 50 % diluted LB medium with appropriate concentration of NaCl (according to the salinity of the sampling point) for anaerobic enrichments. The average content of organic matter measured in Salar de Ascotán sediment samples in different sampling expeditions was 1.25 %, similar to the final concentration in the diluted LB medium used in the enrichments. For aerobic cultures, 125 mL bottles were used with 30 mL of culture medium. The bottles were then amended either with Na_2AsO_2 (0.5 or 10 mM) or $Na_2HAsO_4.7H_2O$ (13 or 100 mM). Henceforth, the terms As(III) and As(V) will be used in the text, the tables and the figures to refer to the two arsenic species, respectively. For anaerobic cultures, the bottles were capped and bubbled with N_2 gas across the butyl cap with a syringe for a few minutes to displace the oxygen and then they were incubated in the dark, without agitation. The incubation was done in a rotary shaker at 180 rpm for aerobic cultures. Both cultures, anaerobic and aerobic, were maintained at 4 or 28 °C. Samples (1 mL) were withdrawn after 10 days and after 30 days of incubation and they were transferred to microcentrifuge tubes for subsequent DNA extraction. The enrichment cultures in which sodium arsenate was present showed some yellow precipitate after 15 days of incubation, and precipitates became abundant after 30 days of incubation, showing that reduction of arsenate had taken place.

DNA extraction and PCR amplifications

High molecular mass DNA of high purity was extracted from sediment samples. Sediments were previously treated to separate cells from sediment particles. Sediment (10 g) was dispensed into 100 mL bottles with 50 mL of NaCl (0.9 %) and 60 μL of Tween 20 (100 %). The bottles were incubated at room temperature for 30 min at 180 rpm. Then, sediment was allowed to settle and the supernatant was filtered through a nitrocellulose 0.22 μm pore size, 47 mm diameter filter (Whatman®). DNA was extracted from filters using High Pure PCR Template Preparation Kit (Roche). DNA extraction from cultures was performed by the CTAB method (Ellis et al. 1999) using 2 mL of each enrichment culture. The quality and quantity of DNA from the several sources were checked in a 0.8 % agarose gel after staining with ethidium bromide. DGGE oligonucleotide primers 341F-GC (*Escherichia coli* 16S rDNA positions 341–357) and 518R (*E. coli* 16S rDNA positions 518–534) (Muyzer et al. 1993) were used to amplified the V3 region of eubacterial 16S rDNA from whole enrichment cultures and those from sediment samples. The PCR cycle followed previously described conditions (Becker et al. 2006) and the enzyme GoTaq™ DNA Polymerase (Promega Co., Madison, WI, USA) was used.

DGGE analysis

DGGE was performed using a D-Code system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) (Ferrero et al. 2010). About 20 μL (approximately 800 ng) of PCR-product was loaded for most of the samples and the gels were run at a constant voltage of 120 V at 60 °C for 4.5 h. The gel was stained with SYBR® Gold (Molecular Probes, Eugene, OR) and visualized with a Bio-Rad UV transilluminator. Digital images of the gels were captured with the Quantity One software (version 4.3.1; Bio-Rad Laboratories, Inc., Hercules, CA) for use in comparative image analysis. The gel image analyses were performed with Cross Checker (version 2.91; Wageningen UR Plant Breeding, The Netherlands) to analyze the bacterial community structure.

The Range-weighted richness (Rr) values were calculated based on the total number of bands (*N*) and the denaturing gradient between the first and the last band of each pattern (Dg) (Marzorati et al. 2008). The values obtained were compared pairwise between conditions and systems, and hypothesis tests were run to identify significant differences. The band pattern distributions of the samples were used to construct a binary matrix. Cluster analyses (WPGMA), based on percent similarity between the samples, were conducted using the SPSS.

The bands selected for identification were carefully excised from the gel with a razor blade under UV illumination. Then, they were placed in 30 μL TE buffer and they were incubated for 15 min at −70 °C or overnight at −20 °C to elute the DNA from the gel; 0.5–2.0 μL of eluate was used as a template for PCR amplification with the original primer set, 341F and 518R (without a GC clamp).

Some PCR products obtained from the excised bands were randomly re-run in DGGE gel to confirm their relative position to the bands from which they were excised.

Sequencing was performed directly on PCR products with the 341F primer in Macrogen (Macrogen Inc., Korea).

Phylogenetic analysis

The identity and similarity to the nearest neighbor of DGGE band sequences were obtained using the BLAST (Basic Local Alignment Search Tool) algorithm (Altschul et al. 1997) at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/BLAST/). Phylotypes were selected at four different levels of minimal sequence identity: 98, 95, 92 and 89 % using cd-hit (Li and Godzik 2006). The sequences were aligned using the alignment tool in Greengenes (http://www.greengenes.lbl.gov).

Partial sequences were inserted into the optimized and validated tree available in ARB (derived from complete sequence data) using the maximum-parsimony criterion and a special ARB parsimony tool that did not affect the initial tree topology. The respective ARB tools were used to perform maximum parsimony (MP), neighbor-joining (NJ) and maximum likelihood (ML) analyses for full sequences. The results from the three types of analyses were essentially identical and only the maximum likelihood trees are shown. Bootstrap resampling analysis for 500 replicates was carried out to estimate the confidence of tree topologies.

The relative abundance of the major groups (Gammaproteobacteria and Firmicutes) was calculated adding the percentage of intensity of the DGGE bands with sequences affiliated to those groups after the phylogenetic analysis. Pairwise comparisons between samples were conducted using paired *t* tests.

Sequence accession number

The nucleotide sequences identified in this study were deposited in the EMBL nucleotide sequence database (GenBank/EMBL/DDBJ) under the accession numbers from FR751005 to FR751037, from FR796494 to FR796542 and from FR839347 to FR839372 for DGGE bands.

Results

The geographical location and the results of the physicochemical and microbial analysis of the brines and the sediments from the studied salt lakes are summarized in Table 1. The differences in temperature between the pond and the spring samples from the Ascotán system were due to thermalism (Risacher et al. 1999). The pH of the

Table 1 Geographical location, physicochemical and biological parameters from Salar de Ascotán and Salar de Atacama

Sample	Coordinates	Date	Altitude	UV-B W/m^2 (280–320 nm)	O_2 (mg L^{-1})	pH	Temp. (°C)	Conductivity (mS cm^{-1})	TDS (mg L^{-1})	As (mg L^{-1})	SO_4^{2-} (mg L^{-1})	Cl^- (mg L^{-1})	MPN count (Cells g^{-1})	DAPI count (Cells mL^{-1} Cells g^{-1})
J-1 Pond	577921E/7609328N	22/06/2006	3744	1.3	8.9	8.3	0	3.90	1750	3.4	683	4561	790	3.93E+06 6.03E+07
Spring10	577782E/7610573N	22/06/2006	3740	1.6	8.8	8	18	2.85	1390	1.6	366	1849	230	1.50E+06 1.92E+08
Spring 6	577180E/7622870N	22/06/2006	3738	1	10.8	ND	16	1.044	493	0.9	181	1139	230	1.20E+06 5.47E+07
Laguna Turquesa	572171E/7610653N	22/06/2006	3741	1.2	7.5	8.3	3	18.5	10200	28	3650	3009	490	4.25E+07 5.12E+07
Laguna Burro Muerto	5044353E/7424617N	01/05/2006	2350	0.155	4	7.8	9.6	83.3	55800	9.62	16500	28000	ND	1.40E+06
Laguna Tebenquiche	577338E/7441804	01/05/2006	2350	0.103	2.96	7.08	24.2	177.1	113000	1.11	16100	42000	ND	6.40E+06

ND not determined

Ascotán brines associated with the sediments ranged between 8.0 and 8.6 while the pH of the Atacama brines was lower (≤7.8). The Ascotán brines have been reported to evolve following different evaporation pathways. Laguna Turquesa is located in the border between two zones described as following the sulfate neutral and the sulfate alkaline evolution pathways (Risacher et al. 1999). Meanwhile, the water evolution in Salar de Atacama was reported to follow that of neutral brines (pH lower than 7.8) (Risacher and Alonso 1996). The arsenic concentration in Laguna Turquesa was the highest detected in this study.

Arsenic reduction activity

Arsenic sulfide precipitation (yellow precipitate) was observed in all of the anaerobic enrichments inoculated with both Laguna Turquesa and Burro Muerto sediments while it was not observed in the uninoculated controls or in the aerobic enrichments. This demonstrates the occurrence of arsenic reduction microbial activity in the sediments analyzed (Kuai et al. 2001).

DGGE pattern analysis

The DGGE analysis of 16S rRNA gene fragments from the enrichments of sediment samples from the Ascotán (Fig. 2a) and Atacama (gel not shown) salt lakes showed the development of different bacterial communities from each environment at the different culture conditions. Between 3 and 25 bands per sample were found in each enrichment culture.

The cluster analysis (UPGMA) of DGGE bands showed that the enrichments carried out at the same temperature clustered together (for example, Fig. 2b shows the dendrogram analysis with the relatedness of DGGE bands obtained from the Ascotán and Atacama sediments in anaerobic enrichments). A second level of clustering was observed between the enrichments supplemented with the same arsenic species (Fig. 2b).

The ranged-weighted richness (Rr), useful to reflect the carrying capacity of the system (Marzorati et al. 2008), was calculated to compare DGGE analyses from different gels. The Rr observed in the original sample from the Ascotán system and from the enrichments ranged between 2 and 35 (Fig. 3), which corresponds to environments whose richness ranges from a particularly restricted to medium range-weighted richness environments (Marzorati et al. 2008). The Rr parameter range in Atacama (0.18–155.5), observed in the original sample and in the enrichments (Fig. 3), corresponded to environments in which richness ranged from particularly restricted to high range-weighted richness (Marzorati et al. 2008). Pairwise comparisons between samples using paired *t* tests showed significant higher (<0.05) Rr values in the Atacama than in the Ascotán samples.

In the anaerobic experiments inoculated with the Ascotán sediments and supplemented with As(V) at 28 °C, the Rr value was higher than 30 during the 30 days of experiment. This is the limit established between a medium range-weighted and a typical or very habitable richness environment (Fig. 3).

Phylogenetic diversity

The identities of members of the bacterial communities selected by the different enrichment conditions were obtained by excision and sequencing of both representative (e.g., most frequent) and rare bands from the DGGE gels. Approximately 70 % of DGGE band sequences recovered were similar to "uncultured bacteria" sequences. About 90 and 66 % of the OTUs found in each system (at 98 and 95 % sequence identity, respectively) were only detected in that system.

According to phylogenetic analysis, the bacterial community in the original sediment sample from Laguna Turquesa was composed of unidentified microorganisms, *Bacteroidetes*, Proteobacteria and Firmicutes. Meanwhile, the predominant microbial populations found in the sediments from Laguna Burro Muerto were related to Proteobacteria, unidentified microorganisms, High GC Gram positive and Firmicutes.

Firmicutes

The Firmicutes populations from Salar de Ascotán were significantly ($p < 0.001$) richer in anaerobiosis compared to aerobiosis, considering similar physicochemical conditions (Table 2). It was also richer at 4 °C than at 28 °C ($p < 0.05$) (Table 2). Firmicutes grew in all the conditions assayed (arsenic species and concentrations, temperature and incubation times) during anaerobic incubations. The Firmicutes population in the Salar de Atacama samples was also significantly ($p < 0.001$) enriched in anaerobiosis compared to aerobiosis (Table 2), considering similar physicochemical conditions. Firmicutes grew better in 28 °C enrichments in all the conditions assayed (arsenic species and concentrations, and incubation times) during anaerobic incubations compared to enrichments at 4 °C ($p < 0.05$) (Table 2). In addition, the Firmicutes populations in the cultures inoculated with the Salar de Atacama samples decreased significantly ($p < 0.05$) at higher arsenic concentration. This feature was not observed in the cultures inoculated with the Ascotán samples.

According to DGGE band sequencing, four bacterial sequences (102, 107, 108 and 86) recovered from enrichment cultures (Tables 2 and S1) were closely

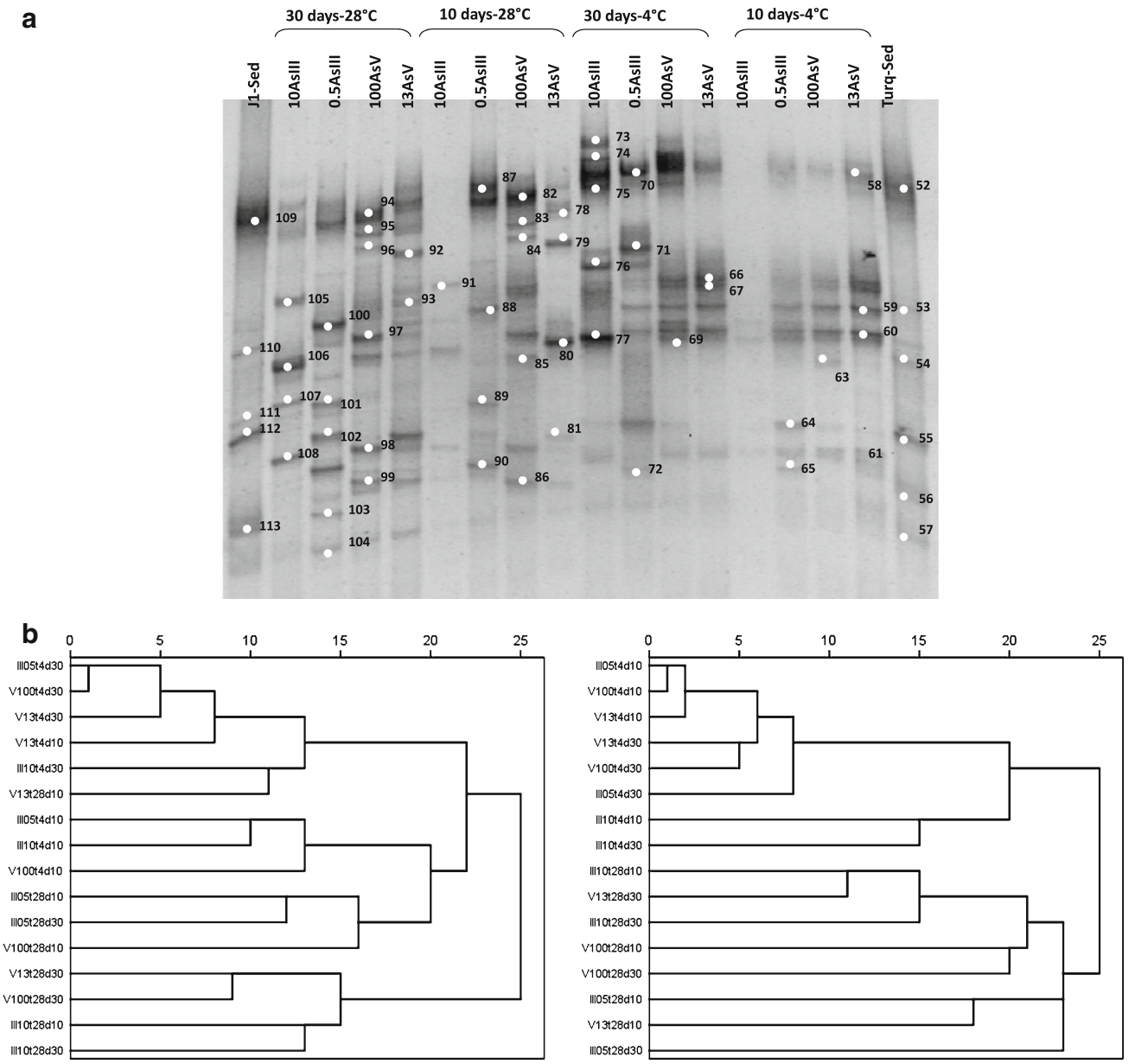

Fig. 2 **a** Negative image of a denaturing gradient gel electrophoresis (DGGE) of bacterial community in cultures supplemented with As(III) or As(V) inoculated with the sediment samples from Laguna Turquesa. Enrichment conditions are indicated at each lane. Bands that were cut off from the gel are labeled with the same number as in Table S1. When bands across several lanes could be identified as being the same, they all have the same number. Turq and J1-Sed correspond to original sediment samples from Laguna Turquesa and J1 from Salar de Ascotán, respectively. **b** Relationship between bacterial community structures represented by a dendrogram (UPGMA cluster analysis) obtained from anaerobic enrichments inoculated with the Ascotán (*left*) and the Atacama (*right*) sediments

related to cultured species from the *Alkaliphilus* genus (Fig. 4a), whose type species is *Alkaliphilus transvaalensis* (Takai et al. 2001). The *Alkaliphilus*-related sequences recovered from the DGGE gel showed between 96 and 97 % similarities to the *Alkaliphilus transvaalensis and Alkaliphilus metalliredigens* QYMF and they were relatives of *Alkaliphilus oremlandii* (*Clostridium* sp. OhILAs), a microorganism able to metabolize arsenic (Fisher et al. 2008).

The closest cultured relative of two other sequences (14 and 90) was *Sporacetigenium* sp (Tables 2 and S1, Fig. 4a). These sequences were also related to *Peptostreptococcus* sp. Ch5, isolated from the Andean salt lakes (Fig. 4a). The *Clostridiaceae* bacterium CAa338 clustered with six sequences (106, 80, 262, 224, 227 and 223) retrieved from the experiments (Tables 2 and S1). The strain CAa338 is an alkaliphilic bacterium isolated from tufa columns from Greenland (Schmidt et al. 2006). Four

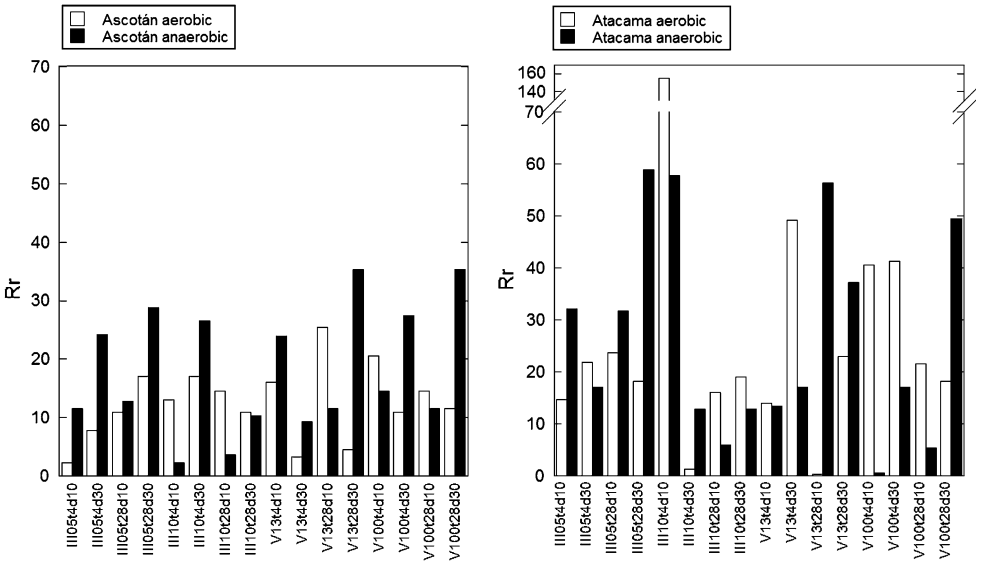

Fig. 3 Range-weighted richness (Rr) of the denaturing gradient gel electrophoresis (DGGE) profiles of enrichments inoculated with the Ascotán (*left*) and the Atacama (*right*) sediments and amended with organic matter and As in aerobic and anaerobic conditions

other sequences (64, 65 70 and 283) clustered together with a psychrophilic anaerobic bacterium isolated from rice paddy soil (*Clostridium* sp. C5S18). One of the closest cultured relatives of such clusters is a representative of *Clostridiaceae* family, *Caloramator* sp. (Fig. 4a). Three sequences (37, 39 and 61) formed a cluster (Bacillus 1) with *Bacillus agaradhaerens* (Table S1, Fig. 4a). *B. agaradhaerens* is a starch-hydrolyzing alkaliphilic bacterium isolated from soda lakes (Martins et al. 2001). This bacterium was described as an halotolerant, aerobic, heterotrophic and alkaliphilic dissimilatory Fe(III)-reducing bacterium, which was isolated from the Great Salt Plains of Oklahoma (Caton et al. 2004).

In the aerobic enrichments inoculated with the Ascotán samples, *B. agaradhaerens*-related sequences (37 and 39) were the most abundant from the Firmicutes phylum in almost all the conditions tested.

Another cluster (Bacillus 2) is formed by sequences (13, 17, 18 and 29) isolated from the enrichments (Table 2) and a microorganism that has been previously found in saline lakes (*Bacillus* sp. N29) (Ordonez et al. 2009). The closest cultured relatives were the strict aerobes *B. horikoshii* (Nielsen et al. 1995), *B. arsenicus* (Shivaji et al. 2005) and the facultative anaerobe *B. selenatarsenatis* (Fig. 4a). The abundance of these sequences was higher at 4 °C.

B. arseniciselenatis (Switzer Blum et al. 1998) isolated from Mono Lake formed a cluster (Fig. 4a) with five more sequences from the enrichments (69, 73, 77, 97 and 252). Most of these (69, 73 and 77) were retrieved only in the experiments at 4 °C. Only one sequence (band 74) related to *Paraliobacillus* (similarity of 96 %) was found in the enrichments. Another phylotype (277 and 163) that was related to *Halolactibacillus miurensis* (Table S1, Fig. 4a) was obtained from enrichments and from the original sediment. *Halolactibacillus* and *Paraliobacillus* genera are included in a phylogenetic group composed of halophilic/halotolerant/alkaliphilic and/or alkalitolerant species in Bacillus rRNA group 1. The genus *Paraliobacillus* was first proposed (Ishikawa et al. 2002) to accommodate a Gram positive, facultatively anaerobic, chemo-organotrophic, spore-forming and slightly halophilic bacterium (Chen et al. 2009).

Another sequence (271) was distantly related to microorganism from the arsenate-reducing sulfide-oxidizing population of Mono Lake (Hollibaugh et al. 2006). This sequence was not included in the phylogenetic tree in Fig. 4a for clarity.

Proteobacteria

Gammaproteobacteria-related sequences were predominant in most of the aerobic enrichments inoculated with the Ascotán sediments ($p < 0.01$) (Table 2). In the anaerobic cultures, the sequences closely related to Gammaproteobacteria were less abundant than the sequences related to other groups (mainly Firmicutes) (Table 2).

There were also significant differences between the Gammaproteobacteria populations in aerobic and anaerobic conditions in the experiments inoculated with the Atacama samples. Gammaproteobacteria populations from Salar de Atacama were significantly ($p < 0.05$) enriched in aerobic compared to anaerobic environments (Table 2).

The uncultured marine bacteria (clone A3 and A50 from GenBank) formed a cluster with sequences obtained from the enrichments with the Ascotán (40, 41, 42, 59, 60 and 205) and the Atacama sediments (Fig. 4b). Several Gammaproteobacteria sequences from the cultures (6, 7, 8, 34, 35, 21, 12, 171 and 182) had *Halomonas* as their closest relative (Table S1, Fig. 4b) and they also clustered with the

Table 2 Distribution of the retrieved sequences among the enrichment cultures inoculated with the Ascotán and the Atacama sediments

Enrichment →	Inoculated sediment →	Ascotán								Atacama							
	Oxygen availability →	Aerobic				Anaerobic				Aerobic				Anaerobic			
	Temperature of incubation(°C) →	4	28	4	28	4	28	4	28	4	28	4	28	4	28	4	28
Bands ↓	Relative ↓	AsIII	AsIII	As V	As V	AsIII	AsIII	As V	As V	AsIII	AsIII	As V	As V	AsIII	AsIII	As V	As V
	Firmicutes	+	+	+	+	+++	++	+++	++	+	+	+	+	++	+++	++	+++
102, 107, 108, 86	*Alkaliphilus*																
69, 73, 77, 97	*B.arseniciselenatis*																
106, 80, 262, 224, 227, 223	Clostridiaceae bacterium CAa338																
64, 65, 70	*Clostridium* sp. C5S18																
271	Arsenate-reducing, sulfide-oxidizing population																
14, 90	*Sporacetigenium*																
37, 39, 61	*Bacillus agaradhaerens*																
13, 17, 18, 29	*B. horikoshii*																
74	*Paraliobacillus*																
277, 163	*Halolactibacillus*																
	Gammaproteobacteria	+++	+++	+++	+++	++	++	++	++	+++	+++	+++	+++	++	+	++	+
40, 41, 42, 59, 60, 205	uncultured marine bacterium																
6, 7, 8, 34, 35, 21, 12, 171, 182	*Halomonas*																
127, 173,162,147,129,164,165, 135	*Marinobacter*																
91, 105, 274	*Pseudomonadales*																
276	*Xanthomonadales*																
	Other Phyla																
22	Alphaproteobacteria																
215, 217, 219, 294, 292, 297, 222	*Rhodobacteracea*																
24, 31	*Burkholderia* sp.																
100, 123,172, 263	Epsilonproteobacteria, clone RH.208-35-22																
195, 148,196, 170, 150	*Arcobacter*																
175	Uncultured bacterium clone RL311																
241	*Desulfovibrionaceae*																
244	*Dethiosulfovibrio acidaminovorans*																
103	*Spirochaetes*																

The number of + indicates significant differences in the relative abundances of Firmicutes and Gammaproteobacteria sequences

The dark cells mean the presence of those sequences in the culture condition

strain GFAJ-1 isolated from Mono Lake (Wolfe-Simon et al. 2010). One *Halomonas*-related sequence was also obtained from the original Ascotán sediment sample.

One more cluster was formed by eight sequences from Atacama enrichments (127, 173,162,147,129,164,165 and 135) plus cultured and uncultured representatives of *Marinobacter* genus (Table S1, Fig. 4b), some of them isolated from hydrothermal vents (Takai et al. 2005).

The closest relatives of three other sequences of the Gammaproteobacteria group retrieved from the cultures (91, 105 and 274) were the uncultured *Pseudomonas* sp. clone ESP450-K6IV-53, isolated from the deep oxycline (450 m depth) of the eastern tropical South Pacific (Stevens and Ulloa 2008).

A few *Vibrio*-related sequences (146 and 145), not included in the phylogenetic tree for clarity, were also isolated from the aerobic enrichments inoculated with the Atacama sediments (Table S1).

The sequence related to Alphaproteobacteria group (22) showed the highest phylogenetic similarity with a soil microorganism that is able to fix nitrogen (Saikia et al. 2007). The nearest neighbors of the predominant Alphaproteobacteria sequences (215, 217, 219, 294, 292, 297 and 222) were related to *Rhodobacteracea*.

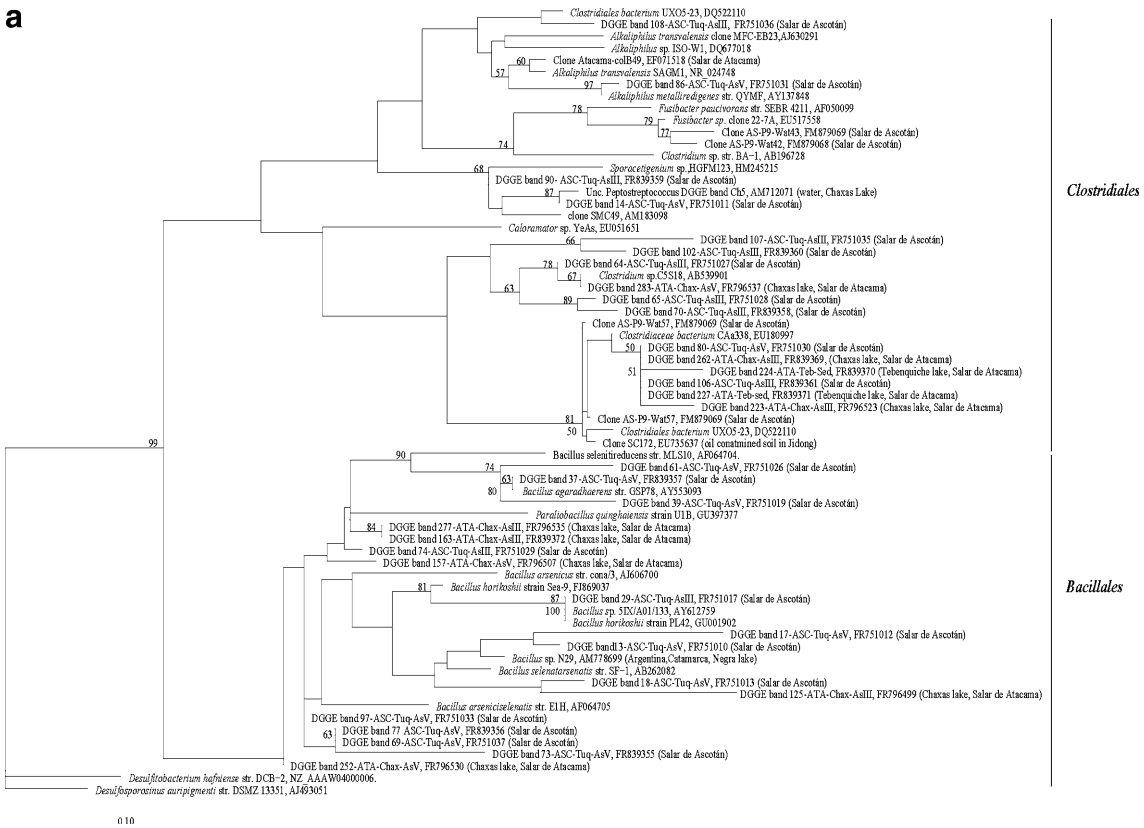

Fig. 4 Phylogenetic tree that includes partial sequences from DGGE bands and clones affiliated to the predominant bacterial phyla, Firmicutes (**a**) and Proteobacteria (**b**). *Bar* corresponds to 0.1 substitutions per nucleotide position

The closest relative of one sequence (100) (Table S1, Fig. 4b) was an uncultured microorganism (clone RH.208-35-22) isolated from hypersaline industrial water (GenBanK information). The most closely related (93–98 %) cultured microorganisms were *Arcobacter marinus* (Kim et al. 2010). The Epsilonproteobacteria-related sequences were more abundant in the aerobic enrichments inoculated with the Atacama samples. Three other sequences (123,172 and 263) clustered together with that group (Fig. 4b). Five sequences (195, 148,196, 170 and 150) formed another cluster without cultured relatives (Fig. 4b). The closest cultured organisms also belonged to the *Arcobacter* genus (Donachie et al. 2005; Kim et al. 2010). Another sequence from the Epsilonproteobacteria group (175, not included in the phylogenetic analysis) was retrieved and its closest relative (Table S1) was found in a metagenome of a ubiquitous and abundant but uncultivated oxygen minimum zone microbe (SUP05) (Walsh et al. 2009).

Two other sequences (24 and 41) belonged to the Betaproteobacteria group (Table S1, Fig. 4b) and the closest culture relative is *Burkholderia* sp., isolated from Vietnamese soils (Huong et al. 2007). One of these sequences was also retrieved from the original Ascotán sample and it represented 30 % of the bacterial community.

One *Desulfovibrionaceae* (Deltaproteobacteria) related sequence (241) was also found and the closest cultured microorganism was a sulfate reducing bacteria *Desulfovibrio* sp. AR1201/00 (Table S1, Fig. 4b), isolated from sediment samples (GenBank information).

Other groups

The Bacteroidetes (Table S1, DGGE bands 1, 47, 53, 55, 110, 112 and 113) group was present in point J1 and in Laguna Turquesa sediment samples (Ascotán). However, no sequences from this particular group were enriched in cultures supplemented with arsenic, neither aerobic nor anaerobic.

High GC-related sequences (218, 221, 115 and 118) were retrieved from Tebenquiche sediment samples but not from the Laguna Burro Muerto sediments. One sequence (244) related to *Synergistetes* Phylum (Vartoukian et al. 2007) was found in the cultures. The closest cultured (97 %) is *Dethiosulfovibrio acidaminovorans* strain sr15 (Table S1) isolated from sulfur mats in saline environments (Surkov et al. 2001). That strain was reported as strictly anaerobic, sulfur- and thiosulfate-reducing bacteria.

Another sequence (103) affiliated to *Spirochaetes* was retrieved. The closest relative of this sequence (Table S1)

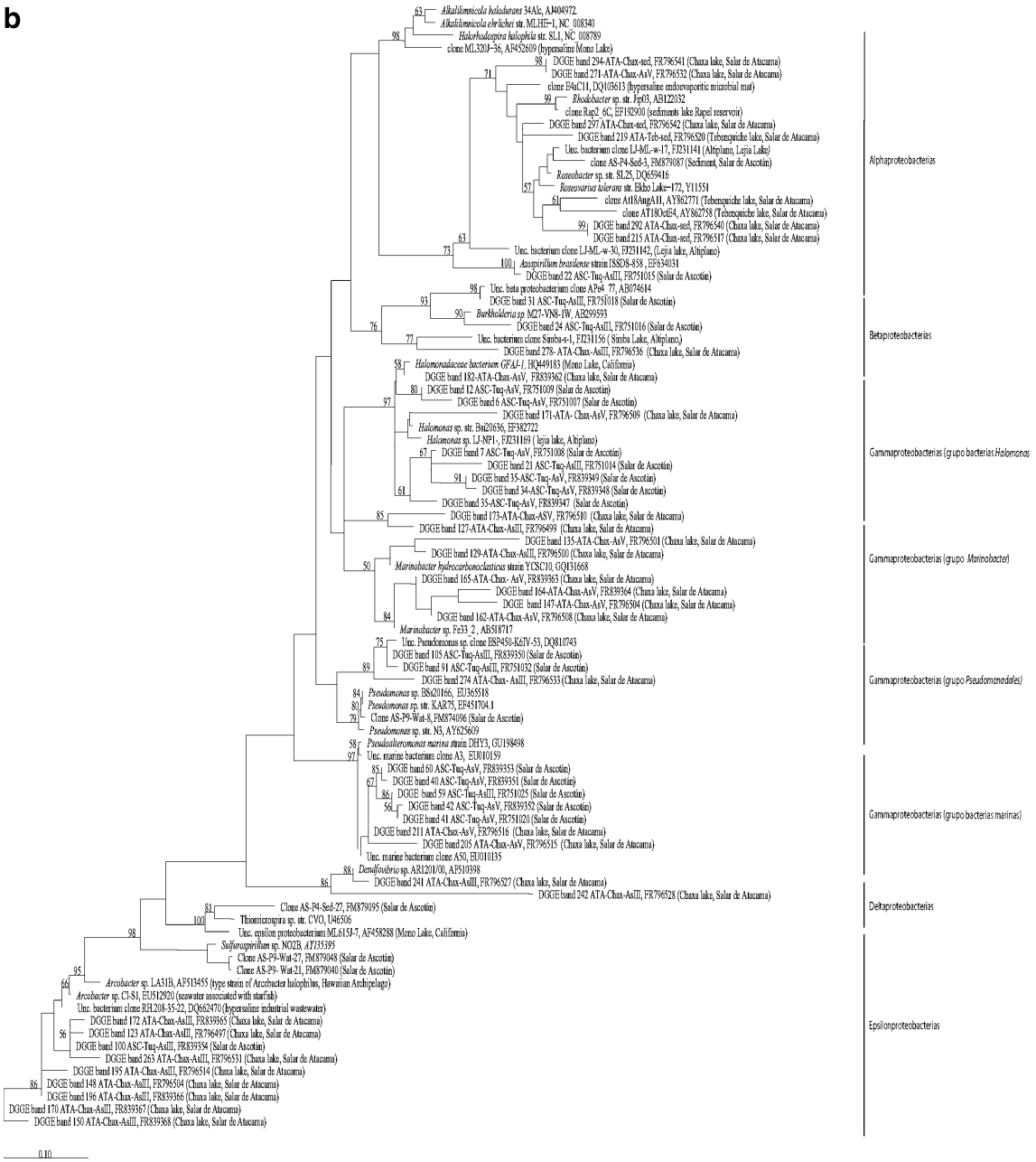

Fig. 4 continued

was isolated from deep sea sediments (Toffin et al. 2004) as a dominant community member together with Firmicutes and Proteobacteria sequences. The members of the *Spirochaetes* were reported to inhabit biotopes with high salinity, alkalinity, pressure, and temperature in methanogenic reactors and in sediments under sulfate-reducing conditions (Fernandez et al. 1999; Hoover et al. 2003; Toffin et al. 2004).

Sequences without relatives in the databases (2, 125, 185, 225, 226, 233, 242, 243, 276 and 278) were also detected (Table S1). The occurrence of these sequences in the culture experiments is summarized in Table 2.

Discussion

To understand the microbiome associated with the As cycle in saline circumneutral environments, we compared the behavior of the microbial communities from two similar systems amended with As and organic matter. The enrichment conditions had different effects on the Ascotán and the Atacama communities.

Molecular fingerprinting techniques—such as DGGE—are useful to study the structure and the composition of the microbial communities. The need to interpret and compare the information obtained from such type of techniques has

led to the development of a three stage tool set successfully used in several environments (Read et al. 2011). The tool was designed for the DGGE pattern analysis (Marzorati et al. 2008) and it allows analysis of microbial fingerprints obtained with different gel runs. The interpretation is based on three parameters: (1) the ranged-weighted richness (Rr), useful to reflect the carrying capacity of the system (Marzorati et al. 2008); (2) the dynamics (Dy) reflecting the specific rate of species coming to significance and (3) the functional organization (Fo), recently renamed as community organization (Co) and defined as the ability of the community to organize itself in an adequate distribution of dominant microorganisms and resilient ones, condition that should assure the potentiality of counteracting the effect of a sudden stress exposure (Marzorati et al. 2008). This provides an ecological interpretation of the raw DGGE data describing the structure of the community (Read et al. 2011). Thus, it was possible to derive conclusions about the relationships between the structure of the microbial community and its functionality.

In spite of the higher values of the Rr parameter in the Atacama microbial communities, the Rr parameter of specialized populations in the Ascotán samples increased under anaerobic conditions (Fig. 3) during the incubation period (from 10 to 30 days). These results indicate a stimulation of the native microbial community by organic matter and As(V) in anaerobiosis.

The salinity level of both enrichment media seems to be one of the causes of the differences between the cultures inoculated with the Ascotán and the Atacama sediments. Following Oren's theory (Oren 1999), anaerobic processes, such as sulfate reduction, yield insufficient energy to support the metabolism of these microorganisms under salt-saturated conditions. In similar analyses performed in sediments from two salt lakes in the US (Kulp et al. 2007), the authors concluded that when salt concentration approaches salt saturation levels, microbial metabolisms based on bioenergetically favorable electron acceptors (such as arsenate) may become more important (Kulp et al. 2007).

Firmicutes-related sequences from the Ascotán and the Atacama environments showed differential traits in anaerobic enrichments.

The increase of the Rr parameter between 10 and 30 days of incubation in anaerobic enrichments inoculated with the Ascotán sediments was mainly due to the appearance of new Firmicutes sequences. The changes in abundance of major Firmicutes sequences suggest that psychrophilic, and mesophilic or psychrotolerant organisms occur in the Ascotán and the Atacama samples, respectively (Table 2). On the other hand, the arsenic concentration introduces a most important shift in the abundance of the major sequences in the Atacama compared to the Ascotán anaerobic enrichments (data not shown). Those features could be due to the higher arsenic concentrations and the lower temperatures usually registered in Ascotán.

The Gammaproteobacteria-related sequences seemed to be responsible for most of the microbial growth with arsenic occurrence in aerobic enrichments inoculated with the Ascotán and the Atacama samples. The most abundant sequences from Gammaproteobacteria phylum were related to *Marinobacter* and *Halomonas* in the Atacama and the Ascotán enrichments, respectively (Table 2). *Marinobacter* is restricted to the enrichments from the Atacama sediments; meanwhile, Halomonas grew better in the cultures inoculated with the sediments from Ascotán (Table 2). *Halomonas* and *Marinobacter* have been reported to be cosmopolitan genera commonly found in the deep sea and in hydrothermal vent settings (Kaye et al. 2010). The most abundant Gammaproteobacteria sequences in Ascotán, the system with higher As levels in the aerobic and the anaerobic enrichments, belonged to *Halomonas*, *Pseudomonas* and an uncultured marine bacteria. It is worth mentioning that reports about arsenic resistance in *Halomonas* (Takeuchi et al. 2007), as well as in *Pseudomonas* (Cai et al. 1998, 2009), have suggested the presence of an unknown resistance mechanism. The accumulation of As has been reported in members of the *Halomonadaceae* family (Takeuchi et al. 2007). The *Halomonas*-related sequences disappeared at 100 mM As(V) in aerobic experiments with the Ascotán samples. Only the sequences related with marine bacteria (clone A3 and A50 from GenBank) remained in those enrichments. The analysis of the ecotype distribution of microorganisms from the *Marinobacter* and *Halomonas* lineages in hydrothermal settings suggested an antagonistic effect of higher concentrations of hydrogen sulfide on *Marinobacter* (Kaye et al. 2010).

The results suggest that *Halomonas*-related microorganisms were psychrophilic (significant—<0.01—higher abundances were observed at 4 °C) and that *Marinobacter* related microorganisms were psychrotolerant (Fig. 5). The range of growth temperature reported for several microorganisms from *Marinobacter* genus is between 0 and 45 °C (Montes et al. 2008; Zhang et al. 2008). On the other hand, *Halomonas*-related sequences increases their abundance preferentially in experiments supplemented with As III (data not shown). Meanwhile, *Marinobacter* strains grew in both As(III) and As(V) supplemented enrichments. In addition, Gammaproteobacteria closely related to *Halomonas* have been reported to be abundant in contaminated (de Souza et al. 2001; Dib et al. 2010) and cold environments (Reddy et al. 2003). Comparative studies suggest that most of the members of *Halomonas* are strictly aerobic organisms (Bouchotroch et al. 2001).

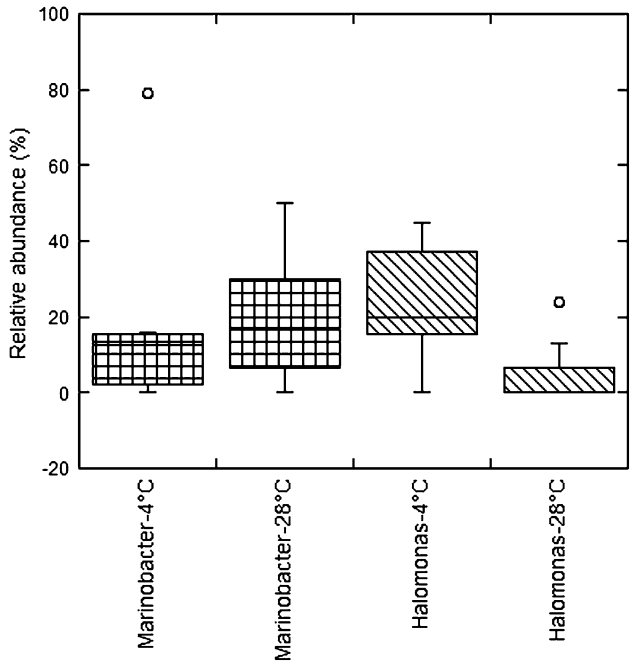

Fig. 5 *Marinobacter* and *Halomonas* relative abundance percentages in the enrichments inoculated with the Atacama and Ascotán sediments, respectively. Each box encloses 50 % of the data with the median value of the variable displayed as a line. The *top* and *bottom* of the box mark the limits of ±25 % of the variable population. The *lines* extending from the *top* and *bottom* of each box mark the minimum and maximum values within the data set that falls within an acceptable range. Any value outside of this range, called an outlier, is displayed as an individual point (KaleidaGraph tool)

Most of the sequences associated to known arsenic respiring microorganisms were obtained from the enrichments inoculated with the Ascotán sediments and belonged to the Firmicutes phylum (Table 2). The related and known arsenic respiring microorganisms from the Firmicutes phylum are *Alkaliphilus oremlandii*, *Caloramator sp.*, *Bacillus selenatarsenatis* and *Bacillus arseniciselenatis*. *Alkaliphilus oremlandii* (*Clostridium* sp. OhILAs) is able to use arsenate and thiosulfate as terminal electron acceptors, and it has the *arr*A gen, involved in anaerobic-arsenate-reducing metabolism (Fisher et al. 2008) (Fig. 4a; Table 2). *Caloramator* sp. metabolizes arsenic and produces beta-realgar (arsenic sulfide), a mineral that has been recently observed as a by-product of arsenic microbial metabolism (Ledbetter et al. 2007a; Ledbetter et al. 2007b). The characteristics of this mineral were similar to those of pararealgar, which was previously identified as a by-product of microbial precipitation of arsenic sulfides using bacterial cultures from Ascotán (Demergasso et al. 2007). Some microorganisms from the *Caloramator* genus have been also reported to be acetogenic (Drake et al. 2008). *B. selenatarsenatis* is a selenate and arsenate-reducing bacterium (Yamamura et al. 2007). *B.arseniciselenatis* grows by dissimilatory reduction of As(V) to As(III) with the concomitant oxidation of lactate to acetate plus CO_2.

Only two sequences compatible with arsenate-reducing microorganisms were isolated from the enrichments inoculated with the Atacama sediments (Table 2). In addition, the reports of an arsenate-respiring and arsenite-oxidizing marine species, *Marinobacter santoriniensis* sp. isolated from hydrothermal sediments (Handley et al. 2009), allow us to infer that Marinobacter-related sequences could be also associated to arsenic-metabolizing microorganisms.

Other sequences inside the Firmicutes phylum are related to cultured microorganisms with known metabolisms that could play indirect roles in the arsenic biogeochemical cycle like *Sporacetigenium* sp and *Halolactibacillus* (Table 2). The isolated strains of the *Sporacetigenium* genus produced H_2, acetic acid and ethanol from glucose fermentation (Chen et al. 2006). Acetogens had traditionally been considered to be strict anaerobes. Recently, however, these microorganisms have been also found in environments subject to fluctuations in O_2, such as aerated soils (Gossner et al. 2008). *Halolactibacillus miurensis* is a halophilic and alkaliphilic marine lactic acid bacterium (Ishikawa et al. 2005). Other fermentative microorganisms from the Proteobacteria phylum could be represented by the sequences clustered with *Arcobacter marinus* (Kim et al. 2010). *A. marinus* grows well under either aerobic or microaerobic conditions and forms a group with the fermentative and facultative anaerobic Epsilonproteobacterium, *Arcobacter halophilus* (Donachie et al. 2005).

Those fermentation products could be potential sources of electrons for arsenic reduction.

The Alphaproteobacteria-related sequences clustered with Rhodobacteraceae that are able to decompose organic compounds under visible light with simultaneous evolution of hydrogen and carbon dioxide (Seifert et al. 2010).

In addition, sulfate-reducing and sulfur- and thiosulfate-reducing bacteria like *Desulfovibrio* sp. (Deltaproteobacteria) and *Dethiosulfovibrio acidaminovorans* strain sr15 (Synergistetes) (Table 2) could also play indirect roles in the arsenic biogeochemical cycle.

The phylogenetic analysis also revealed the occurrence of sequences clustering with each other and without close cultured relatives, mainly from Firmicutes and Proteobacteria phyla in both the Ascotán and the Atacama enrichments (Fig. 4).

The phylogenetic analysis of the sequences retrieved from the Ascotán and the Atacama enrichment experiments showed that the populations involved belonged to the Phyla represented in the phylogenetic tree of the arsenotrophs (Oremland et al. 2009) (Table 2; Fig. 4). In addition, Spirochaetes and Synergistetes sequences were also retrieved and they could be new targets of isolation efforts together with *Halomonas* and *Marinobacter* in the Gammaproteobacteria

Phylum, which were found to be a predominant group in the Ascotán and the Atacama communities, respectively. Isolation of such microorganisms will allow the confirmation of their hypothetical participation in the arsenic biogeochemical cycle or its arsenic tolerance.

Conclusions

Fingerprint techniques were useful to preliminary assess the microbial community structure and function in the arsenic bearing salt lakes of circumneutral pH, and to design the research strategy to understand the biogeochemical cycle of that element.

Based on the stimulation of populations from the Ascotán microbial community in the enrichment conditions and on the phylogenetic relationship of some of those populations with reported sequences, we can conclude that a better established arsenic cycle should occur in Ascotán. Microbial populations compatible with microorganisms able to transform arsenic to conserve energy, accumulate arsenic, produce H_2, H_2S and acetic acid—potential sources of electrons for arsenic reduction—, and to tolerate high arsenic levels is evidence for a fully developed As cycle.

The predominant ecotypes that were specifically associated with transformation of organic carbon in those arsenic rich salt lakes in Northern Chile belonged to the Firmicutes and Proteobacteria phyla. Further culturing efforts should be directed toward the isolation of representatives of those predominant. In accordance with the results obtained, salinity, temperature, and oxygen availability are some of the selective conditions that should be used to isolate the target populations. Similar enrichment experiments are needed to investigate the autotrophic and phototrophic populations involved in the arsenic cycle in these systems.

Acknowledgments This work was supported by funds provided by the BBVA Foundation project BIOARSENICO and the Chilean Government through FONDECYT Project 1100795 from the Science and Technology Chilean Commission (CONICYT). We also would like to thank the A. v. Humboldt Stiftung for the donation of part of the equipment used in this project, as well as V. Iturriaga, M. J. Demergasso and M.V. Coalova for their collaboration.

References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, 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

APHA, AWWA, WEF (1998) Cl B Argentometric method. In: Clescerl LS, Greenberg AE, Eaton AD (eds) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, p Method 4500Cl B

Becker J, Parkin T, Nakatsu C, Wilbur J, Konopka A (2006) Bacterial activity, community structure, and centimeter-scale spatial heterogeneity in contaminated soil. Microb Ecol 51:220–231

Bouchotroch S, Quesada E, del Moral A, Llamas I, Bejar V (2001) *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. Int J Syst Evol Microbiol 51:1625–1632

Cai J, Salmon K, DuBow MS (1998) A chromosomal *ars* operon homologue of *Pseudomonas aeruginosa* confers increased resistance to arsenic and antimony in *Escherichia coli*. Microbiol-SGM 144:2705–2713

Cai L, Rensing C, Li XY, Wang GJ (2009) Novel gene clusters involved in arsenite oxidation and resistance in two arsenite oxidizers: *Achromobacter* sp SY8 and *Pseudomonas* sp TS44. Appl Microbiol Biotechnol 83:715–725

Caton TM, Witte LR, Ngyuen HD, Buchheim JA, Buchheim MA, Schneegurt MA (2004) Halotolerant aerobic heterotrophic bacteria from the Great Salt Plains of Oklahoma. Microb Ecol 48:449–462

Chen S, Song L, Dong X (2006) *Sporacetigenium mesophilum* gen. nov., sp. nov., isolated from an anaerobic digester treating municipal solid waste and sewage. Int J Syst Evol Microbiol 56:721–725

Chen YG, Cui XL, Zhang YQ, Li WJ, Wang YX, Xu LH, Wen ML, Peng Q, Jiang CL (2009) *Paraliobacillus quinghaiensis* sp nov., isolated from salt-lake sediment in China. Int J Syst Evol Microbiol 59:28–33

Chong G (1984) Die Salare in Nordchile—Geologie. Struktur und Geochimie. 67:1–146

Chong G, Pueyo JJ, Demergasso C (2000) The borate deposits in Chile. Rev Geol Chile 27:99–119

de Souza MP, Amini A, Dojka MA, Pickering IJ, Dawson SC, Pace NR, Terry N (2001) Identification and characterization of bacteria in a selenium-contaminated hypersaline evaporation pond. Appl Environ Microbiol 67:3785–3794

Demergasso C, Chong G, Escudero L, Pueyo J, Pedrós-Alió C (2007) Microbial precipitation of arsenic sulfides in Andean salt flats. Geomicrobiol J 24:111–123

Dib J, Motok J, Zenoff VF, Ordoñez O, Farías ME (2010) Occurrence of resistance to antibiotics, UV-B and arsenic in bacteria isolated from extreme environments in high altitude (above 4400 m) Andean wetlands. Curr Microbiol 56:510–517

Donachie SP, Bowman JP, On SL, Alam M (2005) *Arcobacter halophilus* sp. nov., the first obligate halophile in the genus Arcobacter. Int J Syst Evol Microbiol 55:1271–1277

Drake HL, Gossner AS, Daniel SL (2008) Old acetogens, new light. In: Wiegel J, Maier RJ, Adams MWW (eds) Incredible anaerobes: from physiology to genomics to fuels, pp 100–128

Ellis RJ, Thompson IP, Bailey MJ (1999) Temporal fluctuations in the pseudomonad population associated with sugar beet leaves FEMS. Microb Ecol 28:345–356

Fernandez A, Huang SY, Seston S, Xing J, Hickey R, Criddle C, Tiedje J (1999) How stable is stable? Function versus community composition. Appl Environ Microbiol 65:3697–3704

Ferrero M, Menoyo E, Lugo M, Negritto M, Antón A, Siñeriz F (2010) Molecular characterization and in situ detection of bacterial communities associated with rhizosphere soil of high altitude native *poaceae* from the Andean puna region. J Arid Environ 74:1177–1185

Fisher E, Dawson AM, Polshyna G, Lisak J, Crable B, Perera E, Ranganathan M, Thangavelu M, Basu P, Stolz JF (2008) Transformation of inorganic and organic arsenic by *Alkaliphilus*

oremlandii sp nov strain OhILAs. In: Incredible anaerobes: from physiology to genomics to fuels, pp 230–241

Flynn HC, McMahon V, Chong Diaz G, Demergasso CS, Corbisier P, Meharg AA, Paton GI (2002) Assessment of bioavailable arsenic and copper in soils and sediments from the Antofagasta region of Northern Chile. Sci Total Environ 286:51–59

Gossner AS, Picardal F, Tanner RS, Drake HL (2008) Carbon metabolism of the moderately acid-tolerant acetogen *Clostridium drakei* isolated from peat. FEMS Microbiol Lett 287: 236–242

Handley KM, Hery M, Lloyd JR (2009) *Marinobacter santoriniensis* sp nov., an arsenate-respiring and arsenite-oxidizing bacterium isolated from hydrothermal sediment. Int J Syst Evol Microbiol 59:886–892

Herrera C, Guerra N, Pereira M, Guerrero C (1997) Características geológicas y geoquímicas del Salar de Ascotán. In: Chong G (ed) VII Congreso Geológico Chileno. Universidad Católica del Norte, Antofagasta, pp 732–737

Hoeft SE, Kulp TR, Stolz JF, Hollibaugh JT, Oremland RS (2004) Dissimilatory arsenate reduction with sulfide as electron donor: experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. Appl Environ Microbiol 70:2741–2747

Hollibaugh JT, Budinoff C, Hollibaugh RA, Ransom B, Bano N (2006) Sulfide oxidation coupled to arsenate reduction by a diverse microbial community in a Soda Lake. Appl Environ Microbiol 72:2043–2049

Hoover RB, Pikuta EV, Bej AK, Marsic D, Whitman WB, Tang J, Krader P (2003) *Spirochaeta americana* sp nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California. Int J Syst Evol Microbiol 53:815–821

Huong NL, Itoh K, Suyama K (2007) Diversity of 2,4-dichlorophenoxyacetic ccid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)-degrading bacteria in vietnamese soils. Microbes Environ 22:243–256

Ishikawa M, Ishizaki S, Yamamoto Y, Yamasato K (2002) *Paraliobacillus ryukyuensis* gen. nov., sp nov., a new Gram-positive, slightly halophilic, extremely halotolerant, facultative anaerobe isolated from a decomposing marine alga. J Gen Appl Microbiol 48:269–279

Ishikawa M, Nakajima K, Itamiya Y, Furukawa S, Yamamoto Y, Yamasato K (2005) *Halolactibacillus halophilus* gen. nov., sp nov and *Halolactibacillus miurensis* sp nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in Bacillus rRNA group 1. Int J Syst Evol Microbiol 55:2427–2439

Kaye JZ, Sylvan JB, Edwards KJ, Baross JA (2010) *Halomonas* and *Marinobacter* ecotypes from hydrothermal vent, subseafloor and deep-sea environments. FEMS Microbiol Ecol 75:123–133

Kim HM, Hwang CY, Cho BC (2010) *Arcobacter marinus* sp. nov. Int J Syst Evol Microbiol 60:531–536

Kuai L, Nair AA, Polz MF (2001) Rapid and simple method for the most-probable-number estimation of arsenic-reducing bacteria 67:3168–3173

Kulp TR, Han S, Saltikov CW, Lanoil BD, Zargar K, Oremland RS (2007) Effects of imposed salinity gradients on dissimilatory arsenate reduction, sulfate reduction, and other microbial processes in sediments from two California soda lakes. Appl Environ Microbiol 73:5130–5137

Kulp TR, Hoeft SE, Asao M, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic (III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. Science 321:967–970

Ledbetter RN, Connon SA, Neal AL, Dohnalkova A, Magnuson TS (2007a) Biogenic mineral production by a novel arsenic-metabolizing thermophilic bacterium from the Alvord Basin, Oregon. Appl Environ Microbiol 73:5928–5936

Ledbetter RN, Deobald LA, Paszczynsk AJ, Magnuson TS (2007b) Directed proteomics applied to the detection and characterization of arsenic-transforming enzymes in complex communities from the Alvord Basin hydrothermal system. Geochim Cosmochim Acta 71:A552–A552

Li W, Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659

Lloyd JR, Oremland RS (2006) Microbial transformations of arsenic in the environment: from soda lakes to aquifers. Elements 2:85–90

Martins RF, Davids W, Abu Al-Soud W, Levander F, Radstrom P, Hatti-Kaul R (2001) Starch-hydrolyzing bacteria from Ethiopian soda lakes. Extremophiles 5:135–144

Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10:1571–1581

Montes MJ, Bozal N, Mercade E (2008) *Marinobacter guineae* sp. nov., a novel moderately halophilic bacterium from an Antarctic environment. Int J Syst Evol Microbiol 58:1346–1349

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

Newman DK, Kennedy EK, Coates JD, Ahmann D, Ellis DJ, Lovley DR, Morel FM (1997) Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. Arch Microbiol 168:380–388

Nielsen P, Fritze D, Priest FG (1995) Phenetic diversity of alkaliphilic bacillus strains—proposal for 9 new species. Microbiology 141:1745–1761

Ordonez OF, Flores MR, Dib JR, Paz A, Farias ME (2009) Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. Microb Ecol 58:461–473

Oremland RS, Stolz JF, Hollibaugh JT (2004) The microbial arsenic cycle in Mono Lake, California. FEMS Microbiol Ecol 48:15–27

Oremland RS, Kulp TR, Blum JS, Hoeft SE, Baesman S, Miller LG, Stolz JF (2005) A microbial arsenic cycle in a salt-saturated, extreme environment. Science 308:1305–1308

Oremland RS, Saltikov CW, Wolfe-Simon F, Stolz JF (2009) Arsenic in the evolution of earth and extraterrestrial ecosystems. Geomicrobiol J 26:522–536

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334–348

Read S, Marzorati M, Guimaraes BCM, Boon N (2011) Microbial resource management revisited: successful parameters and new concepts. Appl Microbiol Biotechnol 90:861–871

Reddy Raghavan, Sarita Prakash, Nagesh N, Delille D, Shivaji (2003) *Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica. Extremophiles 7:55–61

Risacher F, Alonso H (1996) Geochemistry of the Salar de Atacama. 2. Water evolution. Rev Geol Chile 23:123–134

Risacher F, Fritz B (2009) Origin of Salts and Brine Evolution of Bolivian and Chilean Salars. Aquat Geochem 15:123–157

Risacher F, Alonso H, Salazar C (1999) Geoquímica de aguas en cuencas cerradas: I, II y III Regiones—Chile. Technical Report S.I.T. No. 51. In: Convenio de Cooperación DGA, UCN, IRD. Santiago, Chile

Romero L, Alonso H, Campano P, Fanfani L, Cidu R, Dadea C, Keegan T, Thornton I, Farago M (2003) Arsenic enrichment in waters and sediments of the Rio Loa (Second Region, Chile). Appl Geochem 18:1399–1416

Saikia SP, Jain V, Khetarpal S, Aravind S (2007) Dinitrogen fixation activity of *Azospirillum brasilense* in maize (*Zea mays*). Curr Sci India 93:1296–1300

Schmidt M, Prieme A, Stougaard P (2006) Bacterial diversity in permanently cold and alkaline ikaite columns from Greenland. Extremophiles 10:551–562

Seifert K, Waligorska M, Laniecki M (2010) Brewery wastewaters in photobiological hydrogen generation in presence of *Rhodobacter sphaeroides* O.U. 001. Int J Hydrogen Energ 35:4085–4091

Shivaji S, Suresh K, Chaturvedi P, Dube S, Sengupta S (2005) *Bacillus arsenicus* sp. nov., an arsenic-resistant bacterium isolated from a siderite concretion in West Bengal, India. Int J Syst Evol Microbiol 55:1123–1127

Slyemi D, Moinier D, Brochier-Armanet C, Bonnefoy V, Johnson DB (2011) Characteristics of a phylogenetically ambiguous, arsenic-oxidizing Thiomonas sp., Thiomonas arsenitoxydans strain 3As(T) sp. nov. Arch Microbiol 193:439–449

Stevens H, Ulloa O (2008) Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol 10:1244–1259

Stoertz GE, Ericksen GE (1974) Geology of salars in Northern Chile. Geol Surv Prof Paper 811–876

Surkov A, Dubinina G, Lysenko A, Glockner F, Kuever J (2001) *Dethiosulfovibrio russensis* sp. nov., *Dethiosulfovibrio marinus* sp. nov. and *Dethiosulfovibrio acidaminovorans* sp. nov., novel anaerobic, thiosulfate- and sulfur-reducing bacteria isolated from 'Thiodendron' sulfur mats in different saline environments. Int J Syst Evol Microbiol 51:327–337

Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS (1998) *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30

Takai K, Moser DP, Onstott TC, Spoelstra N, Pfiffner SM, Dohnalkova A, Fredrickson JK (2001) *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. Int J Syst Evol Microbiol 51:1245–1256

Takai K, Moyer CL, Miyazaki M, Nogi Y, Hirayama H, Nealson KH, Horikoshi K (2005) *Marinobacter alkaliphilus* sp. nov., a novel alkaliphilic bacterium isolated from subseafloor alkaline serpentine mud from Ocean Drilling Program Site 1200 at South Chamorro Seamount, Mariana Forearc. Extremophiles 9:17–27

Takeuchi M, Kawahata H, Gupta LP, Kita N, Morishita Y, Ono Y, Komai T (2007) Arsenic resistance and removal by marine and non-marine bacteria. J. Biotechnol 127:434–442

Toffin L, Webster G, Weightman AJ, Fry JC, Prieur D (2004) Molecular monitoring of culturable bacteria from deep-sea sediment of the Nankai Trough, Leg 190 Ocean Drilling Program. FEMS Microbiol Ecol 48:357–367

Vartoukian SR, Palmer RM, Wade WG (2007) The division *Synergistes*. Anaerobe 13:99–106

Walsh DA, Zaikova E, Howes CG, Song YC, Wright JJ, Tringe SG, Tortell PD, Hallam SJ (2009) Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. Science 326:578–582

Wolfe-Simon F, Blum JS, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, Weber PK, Davies PC, Anbar AD, Oremland RS (2010) A bacterium that can grow by using arsenic instead of phosphorus. Science 332:1163–1166

Yamamura S, Yamashita M, Fujimoto N, Kuroda M, Kashiwa M, Sei K, Fujita M, Ike M (2007) *Bacillus selenatarsenatis* sp nov., a selenate- and arsenate-reducing bacterium isolated from the effluent drain of a glass-manufacturing plant. Int J Syst Evol Microbiol 57:1060–1064

Zhang DC, Li HR, Xin YH, Liu HC, Chen B, Ch ZM, Zhou PJ, Yu Y (2008) *Marinomonas arctica* sp nov., a psychrotolerant bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1715–1718