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ORIGINAL PAPER

# Microbiome analysis and bacterial isolation from Lejía Lake soil in Atacama Desert

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## Abstract

As a consequence of the severe climatic change affecting our entire world, many lakes in the Andes Cordillera are likely to disappear within a few decades. One of these lakes is Lejía Lake, located in the central Atacama Desert. The objectives of this study were: (1) to characterize the bacterial community from Lejía Lake shore soil (LLS) using 16S rRNA sequencing and (2) to test a culture-based approach using a soil extract medium (SEM) to recover soil bacteria. This extreme ecosystem was dominated by three phyla: *Bacteroidetes*, *Proteobacteria,* and *Firmicutes* with 29.2, 28.2 and 28.1% of the relative abundance, respectively. Using SEM, we recovered 7.4% of the operational taxonomic units from LLS, all of which belonged to the same three dominant phyla from LLS (6.9% of *Bacteroidetes*, 77.6% of *Proteobacteria,* and 15.3% of *Firmicutes*). In addition, we used SEM to recover isolates from LLS and supplemented the culture medium with increasing salt concentrations to isolate microbial representatives of salt tolerance (*Halomonas* spp.). The results of this study complement the list of microbial taxa diversity from the Atacama Desert and assess a pipeline to isolate selective bacteria that could represent useful elements for biotechnological approaches.

**Keywords** Atacama Desert · Microbiome · Soil extract medium (SEM) · Isolation and characterization

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## Introduction

The Atacama Desert is recognized as an extreme environment, being considered the most arid desert on Earth (Gómez-Silva et al. 2008). It displays intense UV radiation and severe temperature fluctuations during the day and night, environmental conditions that are exacerbated in the high-elevated saline lakes from the Altiplano region (Azua-Bustos et al. 2012; Cordero et al. 2014; Crits-Christoph et al. 2013; Davila et al. 2013; McKay et al. 2003). As a consequence of the severe climatic change affecting our entire world, many lakes in the Altiplano region are likely to disappear within a few decades (Cabrol et al. 2009; Parro et al. 2016), hence, this seems as an appropriate moment to document and study these ecosystems.

One of these saline lakes is Lejía Lake, located at 4314 m a.s.l., has 1.9 $km^2$, nested at the base of Lascar Volcano and comprises high contents of total dissolved solids (1.2–7.0 g/l) of cations Na–Mg and anions $SO_4$–Cl (Risacher et al. 1999). Despite its extreme conditions, a diverse group of bacteria have been found to reside in the water and sediment samples of Lejía Lake by bacterial 16S rRNA clone

libraries, detecting *Proteobacteria* and *Bacteroidetes* as the most dominant phyla (Demergasso et al. 2010). These microorganisms become interesting to investigate considering that they are adapted to live under adverse environmental shifts, and could serve to search and identify molecular components and regulatory mechanisms linked to the management of these extreme physicochemical variables. Demergasso and coworkers (Demergasso et al. 2010) explored the community composition of water and sediment samples obtained from Lejía Lake, observing the relative abundance of dominant phyla by 16S rRNA gene clone library sequencing methodology. Today, the current implementation of next-generation sequencing (NGS) permits better yields in depth and precision of the microbial representatives from an environmental sample, with reduced bias in revealing only the most abundant microorganisms present in a community.

The first aim of this study was to survey the bacterial 16S rRNA structure (microbiome) by NGS technology of the soil bacterial microbiota from Atacama Desert Altiplano Lejía Lake dry shore, a zone located under a salt crust at the interphase between water and un-vegetated arid soils, while the second aim was to test a culture-based approach using a soil extract medium (SEM) to recover salt-tolerant soil bacteria. The results of this study complement the list of microbial taxa diversity from the Atacama Desert and assess a pipeline to isolate selective bacteria that could represent useful elements for biotechnological approaches.

## Materials and methods

### Site description, sample collection, and processing

The research site is located on the shore of Lejía Lake (23°50′S and 67°69′W), a saline lake located close to Lascar Volcano at 4314 m a.s.l. at the Chilean Altiplano in the Atacama Desert. Soil samples were obtained from the southwestside of Lejía Lake shore under a salt crust during a sampling day of April 2014 (Fig. 1). Lejía Lake soil samples (LLS1, LLS2, and LLS3, ~ 200 g each) were obtained at 5–10 cm depth in the soil to avoid contamination from other surfaces. LLS1, LLS2, and LLS3 were used separately as biological replicates. Sub-samples from LLS samples were used for: (1) community DNA extraction, which were placed into sterile plastic bags and immediately stored in dry ice for transport to the laboratory where they were frozen at − 80 °C

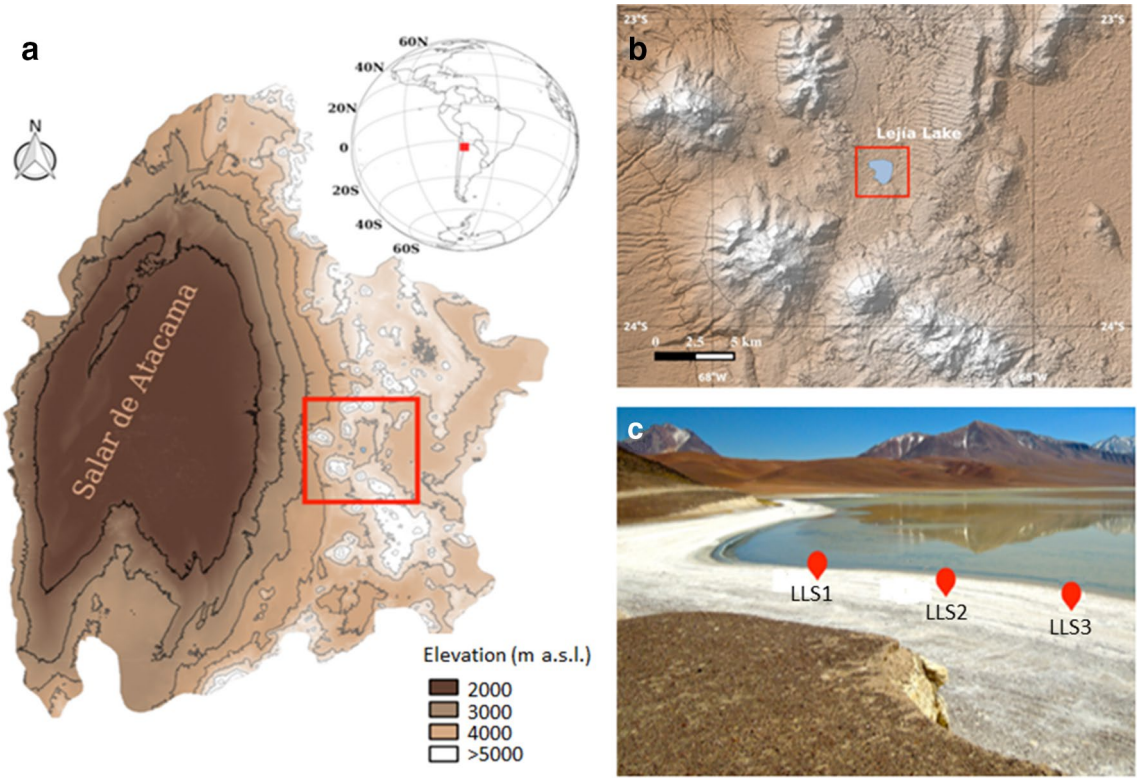


**Fig. 1** Study site. **a** Regional context of Northern Chile showing location of the Salar de Atacama and a digital elevation model indicating where Lejía Lake is located (4314 m a.s.l.). **b** Zoom of the location of Lejía Lake at 23°50′S and 67°69′W in the Chilean Altiplano. **c** Photograph showing the specific sampling sites. *LLS1* Lejía Lake shore soil sample 1, *LLS2* Lejía Lake shore soil sample 2, *LLS3* Lejía Lake shore soil sample 3

until DNA extraction; (2) soil extract medium (SEM) preparation, which were placed into sterile plastic bags and stored at 4 °C; (3) bacterial growth, which were placed into sterile plastic bags and immediately stored at 4 °C for transport to the laboratory where they were plated a few days after sampling; and (4) physicochemical analyses, which were stored at room temperature until use.

## Local environmental measurements and soil physicochemical analyses

Soil temperature was measured in the field with portable iButton DS1923 Hygrochron sensors located between 5 and 10 cm depth in the soil. Soil samples used for physicochemical analyses were sieved (2 mm). Soil pH was analyzed in deionized water at 1:1 w/w ratio (Thomas and Wimpenny 1996). The soil elemental composition was measured by total reflection X-Ray fluorescence (TXRF) in a Bruker S2 PICOFOX following a previously published protocol (De La Calle et al. 2013) and adding gallium as an internal standard element. Total organic carbon and nitrogen contents were determined with an isotope-ratio mass spectrometry (IRMS, Thermo Delta Advantage) coupled to an elemental analyzer Flash EA2000. All the measured carbon was considered organic, since no effervescence was observed when tested with 4 M HCl (Nelson and Sommers 1996).

## Soil DNA extraction

DNA was extracted using CTAB-based method described by (Zhou et al. 1996), and modified by (Prestel et al. 2008) with modifications using the DNeasy Blood & Tissue Kit (Qiagen). For soil DNA extractions, 5 g of soil was re-suspended in 5 mL extraction buffer [100 mM Tris–HCl; pH 8, 100 mM Na EDTA; pH 8, 100 mM $Na_2HPO_4$, 1.5 M NaCl, 1% (w/v) CTAB], and then, 10 mg/mL of lysozyme (final concentration) was added and mixed by vortexing, followed by incubation at 37 °C for 1 h with shaking. The mixture was subjected to centrifugation at 2000×*g* for 5 min at room temperature and the supernatant fluid was transferred to a new tube. Then, 3 µl of pronase (100 mg/mL) was added, followed by incubation at 37 °C for 1 h with gentle shaking. Afterwards, 1 mL of 20% (w/v) SDS was added and incubation continued at 65 °C for another 1 h with gentle shaking. The mixture was then subjected to centrifugation at 6000×*g* for 10 min at room temperature and the supernatant fluid was transferred to a new tube and mixed by vortexing with 1 mL of AL Binding Buffer from the kit. The mixture was incubated at 65 °C for 10 min with gentle shaking. Then, 1 mL ethanol 100% was added and the mixture was transferred into DNeasy mini-spin column, where the protocol continued according to the manufacturer's indications. Extracted DNA was visualized in Tape Station 2200 (Agilent Technologies) using Genomic DNA Screen Tape, Agilent Kit plus Genomic DNA Reagents according to the manufacturer's indications.

## Soil DNA sequencing

For 16S rRNA gene amplification and sequencing, microbial DNA extracted from each soil sample was amplified using a bacteria-specific primer set 28F (5′-GA GTT TGA TCM TGG CTC AG-3′) and 519R (5′-GWA TTA CCG CGG CKG CTG-3′), flanking variable regions V1–V3 of the 16S rRNA gene (Turner et al. 1999) with barcode on the forward primer. Amplification was performed using the Qiagen Kit HotStarTaq Plus Master Mix under the following conditions: initial denaturation at 94 °C for 3 min followed by 28 cycles, each set at 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, with a final elongation step at 72 °C for 5 min. After amplification, PCR products were checked in a 2% agarose gel to determine the success of the amplification and the relative intensity of the bands. At this point, the three barcoded samples for microbial analyses were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Agencourt AMPure XP beads. These processed PCR products were used to prepare DNA libraries following Illumina TruSeq DNA library preparation protocol. Sequencings were performed at the molecular research DNA laboratory (Shallowater, TX, USA) on an Illumina MiSeq platform in an overlapping 2 × 300 bp configuration with a minimum throughput of 20,000 reads per sample.

16S rRNA raw amplicon sequences were processed and analyzed following the previously described protocols (Dowd et al. 2008; Handl et al. 2011). Briefly, sequences were joined (overlapping pairs) and grouped by samples following the barcodes and then the barcodes were removed. Then, sequences < 150 bp or with ambiguous base calls were removed. Sequences were filtered using the USEARCH clustering algorithm at 4% sequence divergence to remove chimeras and clusters consisting of only one sequence (i.e. singletons) (Edgar 2010; Edgar et al. 2011). Finally, sequences were quality filtered with Mothur v.1.22.2 (Schloss et al. 2009) with the minimal quality average set to 30. Sequences were analyzed with the software quantitative insights into microbial ecology [QIIME v1.8.0, (Caporaso et al. 2010)]. Briefly, we used QIIME script 'pick_closed_reference_otus.py' to extract all 16S rRNA reads from the amplicon data that matched GreenGenes r16S database release gg_otus_13_08 (McDonald et al. 2012) at 97% of similarity or 3% divergence, with the taxonomy of the resulting operational taxonomic units (OTUs) assigned directly from the closest reference sequence match. The OTU picking process was done with USEARCH v6.1.544 (Edgar 2010; Edgar et al. 2011) using QIIME default parameter values (-s

0.97 –z True –max_accepts 1 –max_rejects 8 –word_length 8 –minlen 64 –usearch61_sort_method abundance). OTUs unassigned or assigned to mitochondria and chloroplast were removed. Singlet OTUs were also removed.

To characterize microbial diversity patterns, we calculated alpha OTU diversity by randomly sub-sampling (without replacement) each soil sample using the alpha_rarefaction.py script in QIIME. The Shannon and Faith's phylogenetic diversity (PD) indices, along with the observed number of OTUs ('richness'), were calculated. Rarefaction curves for each of these metrics were obtained by serial subsampling in increments of 1299 sequences and 10 iterations per increment, to standardized 13,000 sequences per sample (Supplementary Fig. 1). This number represented the lowest number of curated sequences obtained across our samples.

## Soil extract medium and cultivable-community growth conditions

Soil extract medium (SEM) was prepared by mixing agar with the water soluble portion of soil obtained from Lejía Lake shore soil according to SEM from Leibniz-Institut DSMZ Media Catalogue. SEM pH was not adjusted, since it had the same pH as the one described for Lejía Lake shore soil (pH=8.5). Fungizone (2.5 µg/mL) was added to SEM to recover only bacteria from the cultures and avoid the growth of fungi on the plates.

To obtain the cultivable-community, 1.5 g of soil was re-suspended in 2 mL of sterile PBS for 2 h, tubes were centrifuged for 5 min at 500 rpm, and 100 µl of supernatant was plated in triplicates in SEM and incubated at 25 °C for 7 days. At day 7, all grown bacteria were manually scraped for DNA extraction. It is important to mention that at day 4, isolated colonies were observed in the plates, while at day 7, a heterogeneous bacterial lawn was formed. In addition, for growth controls, supernatant from soil samples re-suspended with PBS was sterilized, plated onto culture media and maintained in the same growth culture conditions, showing no microbial growth.

## Cultivable-community DNA extraction and sequencing

DNA extraction of the cultivable-community was based on Rettedal and coworkers (Rettedal et al. 2014) with some modifications. Briefly, each plate was manually scraped off the media surface following a rinse with 1.5 mL of sterile PBS, homogenization, and collection into sterile tubes. The collection tubes were centrifuged at 5000*g* for 30 min and remnants of solid media were carefully removed. The pelleted samples from the cultivable community were used for DNA extraction using the same protocol for soil DNA extraction and visualization.

Cultivable-community DNA sequencing was performed same as the soil 16S rRNA gene amplification and sequencing methodology. In addition, the microbial diversity patterns were also calculated as mentioned before for soil samples.

## Bacterial isolation, molecular identification, and tolerance tests

To obtain bacterial isolates from Lejía Lake, 1.5 g of soil was solubilized in 2 mL of sterile PBS for 2 h. Tubes were centrifuged for 5 min at 500 rpm; and 100 µl of supernatant diluted 1/100 was plated in triplicates in SEM and incubated at 25 °C for 4 days. At day 4, all morphologically different colonies observed in SEM were isolated ($n = 30$). Bacterial DNA from these isolates was purified from 1 mL of exponential phase growth cultures (OD600 ~ 0.5) using the DNeasy Blood & Tissue Kit for DNA (Qiagen). Bacterial culture samples were centrifuged for 10 min at 8000 rpm, supernatant was discarded, and the pellet obtained was lysed according to the manufacturer's instructions. For 16S rRNA PCR amplifications, primers 27F (5′-AGAGTTTGATCA TGGCTCAG-3′) and 1492R (5′-TACGGTTACCTTGTT ACGACTT-3′) were used (Jiang et al. 2006), giving PCR products of around 1400 bp. 16S rRNA PCR amplifications were carried out in 25 µl volumes containing 200 ng (~4 µl) of bacterial DNA, 12.5 µl of GoTaq mix (Promega), 5.5 µl of nuclease-free water, and 1 µl of each primer (10 mM). The PCR amplification was performed in MJ research, Inc. Thermal cycling controller with the following protocol: 10 min at 95 °C, 30 cycles of 95 °C for 60 s, 58 °C for 30 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were kept at 4 °C until use. PCR products were visualized in 2% (w/v) agarose gel electrophoresis in Tris-acetate-EDTA (TAE) buffer (1X) and stained with ethidium bromide. PCR products from 16S rRNA amplifications of the strains used in this study (of ~ 1400 bp) were sequenced in Macrogen USA, while the identification of the genus was based on the best sequence match obtained using EzBioCloud database (Yoon et al. 2017). Only the isolates with different 16S rRNA sequences ($n = 9$) were used in this study and submitted to NCBI.

Each strain was screened for salinity (NaCl 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20%) and pH tolerance (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14) three times by salt supplementation or pH adjustment (Mandakovic et al. 2015) in SEM plates after 7 days of culture.

To obtain bacterial isolates highly tolerant to salt concentrations from Lejía Lake, 1.5 g of soil was solubilized in 2 mL of sterile PBS for 2 h. Tubes were centrifuged for 5 min at 500 rpm, and 100 µl of supernatant was plated in triplicates in SEM with different concentrations of salt supplementation (5, 10, 15, and 20% NaCl) to promote the

isolation of the most salt-tolerant members and incubated at 25 °C for 7 days. The two colonies obtained at 15% NaCl supplementation were isolated. Bacterial DNA from these isolates was purified, amplified, and sequenced as mentioned previously for the other isolates. Again, the identification of the genus was based on the best sequence match obtained using EzBioCloud database (Yoon et al. 2017).

The datasets supporting the microbiome results of this article are available in the National Centre for Biotechnology Information sequence read archive under the study accession number SRP070518 and BioProject ID PRJNA312391. Isolates 16S rRNA sequences obtained in this study are available in NCBI database under GenBank codes: *Carnobacterium* sp. ALS4 (KU714718.1), *Paenibacillus* sp. ALS5 (KU714719.1), *Bacillus* sp. ALS1 (KU714720.1), *Planococcus* sp. ALS7 (KU714721.1), *Planococcus* sp. ALS8 (KU714722.1), *Streptomyces* sp. ALS2 (KU714723.1), *Streptomyces* sp. ALS3 (KU714725.1), *Streptomyces* sp. ALS6 (KU714724.1), *Microbacterium* sp. CGR2 (KU714726.1), and *Halomonas* sp. ALS9 (KU714727.1).

## Results and discussion

### Lejía Lake soil physicochemical features and bacterial community composition

Lejía Lake soil replicates (LLS1, LLS2 and LLS3) were obtained under a salt crust from the dried shore of the southwest side of the lake in April 2014 (autumn, Southern Hemisphere) (Fig. 1). The pH of the soil was alkaline, with an average value of 8.5 ± 0.1, and the soil temperature during the sampling period fluctuated between −0.5 and 18.7 °C (averaged daily temperature of 8.5 ± 6.4 °C). When analyzing LLS elemental composition (Supplementary Table 1), we could observe that the most concentrated element measured was Na (4380 ± 147 mg/kg), which was an expected outcome considering that this lake is highly saline (Cabrol et al. 2009; Demergasso et al. 2010). In contrast, micronutrient's contents at our research site were under the detection limit (Cu, Zn, and Mn < 0.001 mg/kg) or lower in two orders of magnitude (Fe 681 ± 102 mg/kg) (Supplementary Table 1) than the values reported for soils close to Lejía Lake (Neilson et al. 2012). Hence, LLS samples were taken from alkaline, saline and nutritionally poor soils that display a wide temperature range during the day, which are extreme features comparable to other zones of Lejía Lake and to other Altiplano saline lakes (Cabrol et al. 2009; Demergasso et al. 2010; Escudero et al. 2007).

To determine the bacterial community members present in LLS, we applied an NGS approach to survey the bacterial 16S rRNA gene. From this analysis, 35,313 ± 915 good-quality (clean) reads were obtained in average from LLS1, LLS2, and LLS3 microbiomes, while 14,504 ± 1449 were mapped to 16S rRNA sequences (Supplementary Table 2). In addition, the microbial diversity estimates (Richness, Shannon and PD indices) present in LLS were calculated, showing values of 674 ± 5.8 for Richness, 6.5 ± 0.1 for Shannon, and 40.8 ± 0.3 for PD. In the case of the number of OTUs, 945 were detected in LLS microbiomes (Supplementary Table 2), from which the most abundant belonged to *Proteobacteria*, *Bacteroidetes,* and *Firmicutes* (Supplementary Table 3; Fig. 2a), phyla that accounted for more than the 80% of the bacterial relative abundance present in LLS. At family level, the three most abundant from each dominant phyla were: *Desulfobulbaceae* (21.7 ± 1.6%), *Rhodobacteraceae* (13.8 ± 2.3%) and *Desulfuromonadaceae* (10.7 ± 1.0%) for *Proteobacteria*; *Balneolaceae* (53.3 ± 0.8%), *Flavobacteriaceae* (18.6 ± 1.0%) and SB-1 (7.0 ± 0.6%) for *Bacteroidetes*; and *Halanaerobiaceae* (89.1 ± 2.5%), *Aerococcaceae* (2.84 ± 0.8%) and *Acidaminobacteraceae* (2.4 ± 0.4%) for *Firmicutes*. Consistently, these same phyla were found in high abundance in the water and sediments of Lejía Lake when analyzed by bacterial 16S rRNA clone libraries (Demergasso et al. 2010). Remarkably, by NGS, we were able to distinguish all five bacterial phyla described by Demergasso and coworkers (Demergasso et al. 2010), together with 18 additional phyla, mostly of low-relative abundance, that have not been previously described for this environment. These results show that the relative abundance of the total identified OTUs from LLS displays the typical pattern found in most soil microbial communities, which is a power-law distribution (Bailey et al. 2013) composed by a few number of highly abundant OTUs and a large number of low-abundant OTUs (Supplementary Fig. 2).

Furthermore, we observed in LLS microbiomes, the presence of eight phyla (GN02, NKB19, OP3, OP8, OP9, TM6, TM7, and WS3) that form a part of a group of microorganisms that have never been cultivated (Supplementary Table 3), termed as "microbial dark matter" (Gasc et al. 2015; Nobu et al. 2015). Thus, our genomic analysis allows complementing the list of rare microbial taxa inhabiting extreme environments. As has been suggested, the ecological roles of these rare bacteria remain to be determined, but as reservoirs of genetic and functional diversity in extreme environments, such as the Altiplano Atacama Desert, they are of potential biotechnological importance (Bull et al. 2016).

### Culture-based strategies to recover bacteria from Lejía Lake soil

Since interventional studies on natural harsh habitats are technically difficult, especially over 4000 m a.s.l., the use of in vitro studies are required to study members of microbial communities that could give insights into molecular

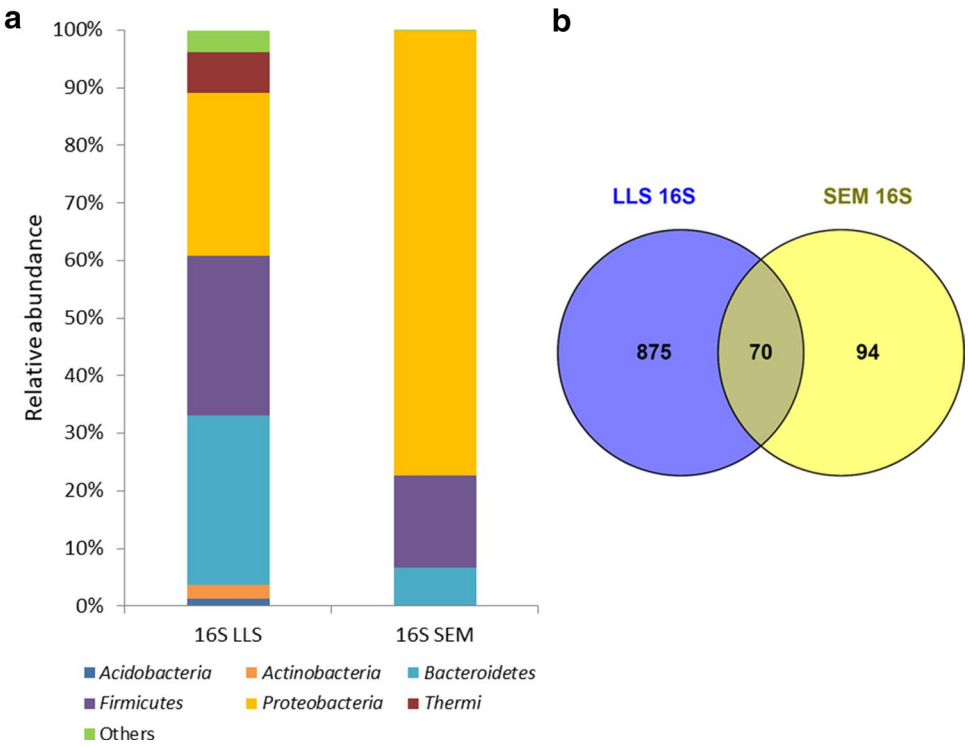


**Fig. 2** Phyla relative abundances and OTUs distribution LLS microbiome (16S LLS), and 16S rRNA amplicon sequencing of SEM (16S SEM). **a** Bar chart of phyla relative abundances. "Others" include phyla with relative abundances < 1% in all samples and correspond to *Armatimonadetes*, *Chlorobi*, *Chloroflexi*, *Cyanobacteria*, *Fusobacteria*, *Gemmatimonadetes*, GNO2, H-178, NKB19, *Nitrospirae*, OD1, OP11, OP3, OP8, OP9, PAUC34f, *Planctomycetes*, SBR1093, SR1, *Spirochaetes*, *Synergistetes*, TM6, TM7, *Tenericutes*, *Verrucomicrobia*, WS3, and WWE1. **b** Venn diagram showing OTUs exclusive and shared between 16S LLS and 16S SEM

components and regulatory mechanisms linked to the management of extreme environmental variables. For this reason, we applied culture-based approaches that simulated the chemical composition of LLS to improve the recovery of its bacterial community in the laboratory (Bakken 1985; Davis et al. 2005; Ellis 2004; James 1958; Liebeke et al. 2009). As a first step, we generated a SEM that consisted of a mixture of agar and the water soluble fractions from LLS samples (for details, see Materials and methods). After that, we sequenced the 16S rRNA amplicon of LLS bacterial cultivable-community growing during 7 days in this culture medium.

When comparing the microbial diversity estimates among LLS and SEM microbiomes, we could observe that they differed quite markedly (Supplementary Table 2). All parameters measured were higher in LLS than in SEM, showing that a higher richness and diversity was displayed in the OTUs obtained from the soil sample than from the cultivable-community.

In addition, we analyzed the OTUs present in LLS and cultivable-community microbiomes, and detected 875 unique OTUs in LLS replicates, whereas 94 OTUs were exclusively recovered from SEM (Fig. 2b, Supplementary Table 4). This result suggests an enrichment of some bacteria in the culture media that exhibited a very low abundance in the original soil samples and, therefore, were not detected by the sequencing strategy directly from the soil. This may be due to the differential supply of nutritional requirements in the culture media than in the soil, the removal of growth inhibitory components and/or the reduction of competitive interactions with members of the community, such as competitive bacteria–bacteria interactions (Auld et al. 2013). The exclusive LLS OTUs belonged to 22 different phyla, while exclusive SEM OTUs contained only 5 phyla. Most of the exclusive OTUs that belonged to LLS were *Proteobacteria* (47.9%; 419 out of 875), mainly Gamma- and Alpha- *Proteobacteria* classes, and in second place to *Bacteroidetes* (13.8%; 121 out of 875) (Supplementary Table 4). Interestingly, though it was the third most abundant phylum in LLS samples, *Firmicutes* (77 out of 875) contained less exclusive LLS OTUs than *Actinobacteria* (101 out of 875), result that was not observed in the shared or exclusive SEM OTUs (Supplementary Tables 3, 4), indicating a significant variety of uncultured *Actinobacteria* members present in the soil.

On the other hand, a comparison of the composition of the microbiomes showed that most of the 16S rRNA sequences recovered using SEM belonged to the same three most dominant phyla found in LLS (*Proteobacteria*, *Bacteroidetes* and *Firmicutes*) (Fig. 2a; Supplementary Table 3). Nevertheless, at family level, the abundances were very dissimilar between LLS and SEM. The three most abundant families from each dominant phyla from SEM microbiome were: *Comamonadaceae* (97.0 ± 1.3%), *Erythrobacteraceae* (0.9 ± 0.6%) and *Rhizobiaceae* (0.6 ± 0.0%) for *Proteobacteria*; *Cyclobacteriaceae* (99.7 ± 0.0%), *Flavobacteriaceae* (0.1 ± 0.1%) and *Marinilabiaceae* (0.1 ± 0.1%) for *Bacteroidetes*; and *Paenibacillaceae* (96.6 ± 2.0%), *Planococcaceae* (3.3 ± 2.0%) and *Bacillaceae* (0.1 ± 0.1%) for *Firmicutes*.

Thus, only the family *Flavobacteriaceae* from *Bacteroidetes* was dominant in SEM and LLS microbiomes. Despite this, the number of OTUs shared between 16S LLS and SEM was 70 (Supplementary Table 4), indicating a 7.4% (70 out of 945 OTUs from 16S LLS) of recovery rate from the soil sample in the culture medium. This corresponds to a satisfactory recovery accomplishment considering that most culture strategies allow the cultivation of less than 1% of the members of the bacterial community (Giovannoni 2000; Grimes et al. 2000; Liesack et al. 1997). Thus, this result gives insight into the importance of physicochemical and biotic factors present in the environment that are relevant at the moment of culturing, which many times are unknown or difficult to supplement or include into the culture media. The utilization of an appropriate culture medium combined with the use of NGS to identify the total cultivable-community becomes a promising approach to be implemented in studies of exceptionally diverse microbial communities such as soil environmental samples.

In SEM, a lawn of bacteria was obtained; therefore, we decided to make dilutions (1/100) to recover isolates from LLS to investigate whether their physiological characteristics of salinity and pH tolerance features are involved in the organism ability to tolerate/adapt to the extreme conditions present at Lejía Lake shore soil. The salinity and pH tolerance ranges were evaluated, because these variables are described as the principal modulators of soil bacterial communities (Fierer and Jackson 2006; Lauber et al. 2009; Rousk et al. 2010).

From a group of around 30 isolates, nine of them that had different 16S rRNA sequences were selected for further analyses. Five corresponded to the phylum *Firmicutes* (*Carnobacterium* sp. ALS4, *Paenibacillus* sp. ALS5, *Bacillus* sp. ALS1, *Planococcus* sp. ALS7 and *Planococcus* sp. ALS8) and four strains to the phylum *Actinobacteria* (*Streptomyces* sp. ALS2, *Streptomyces* sp. ALS3, *Streptomyces* sp. ALS6 and *Microbacterium* sp. CGR2). Although some of these strains belonged to *Firmicutes*, a greatly represented phylum in the SEM microbiome, we also isolated low-abundant bacteria from *Actinobacteria*. The representation of only two phyla in the isolates from the nine phyla obtained in SEM (Supplementary Tables 3, 4) indicated that the isolation approach is disfavored when compared to the study of the cultivable microbiome, suggesting a high impact produced by the community–ecological interactions (Mandakovic et al. 2018).

The adaptive capacity of the isolates to salinity ranges showed high variability, ranging between 3 and 10% of salt supplementation (slight to moderate halophiles) (Fig. 3). Two of them tolerated up to 10% of NaCl supplementation, both belonging to *Planococcus* genus, while seven isolates tolerated less salt concentrations. The pH tolerance ranges among isolates were between pH 6 and 12 (Fig. 3), with a

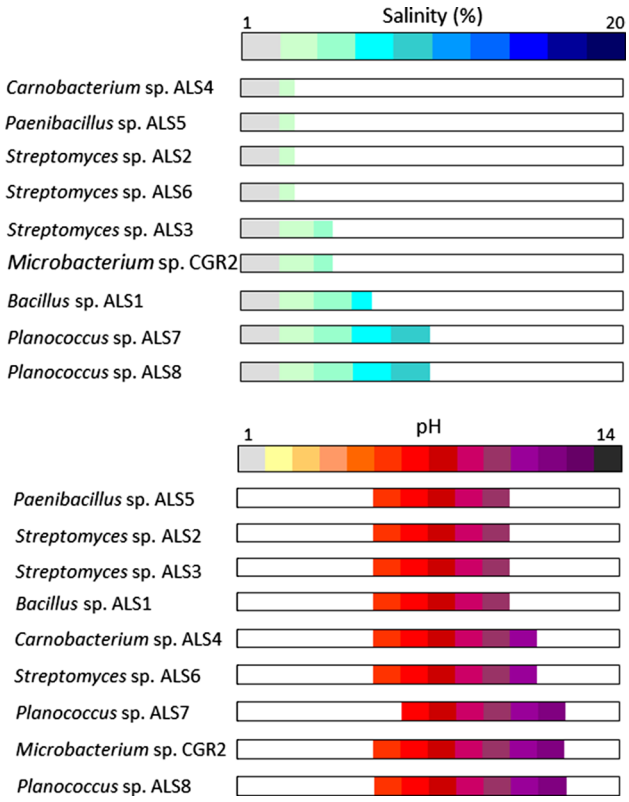


**Fig. 3** Salinity and pH tolerance tests. Nine isolates obtained from LLS were tested for salinity (1–20% NaCl) and pH (1–14) tolerances

tendency to alkalinity, concordant to the pH level displayed in LLS. The higher pH levels were reached by three bacteria (*Microbacterium* sp. CGR2, *Planococcus* sp. ALS7 and *Planococcus* sp. ALS8), confirming the high adaptive capacity of *Planococcus* genus to salt and pH levels. These results indicate that SEM was useful for bacterial isolation and characterization, allowing challenging the isolates and determining their tolerance capacities in a matrix close to the original soil composition.

Another strategy that we used to recover isolates from LLS was to challenge the cultivable-community grown in SEM to different concentrations of salt supplementation (5, 10, 15 and 20% NaCl) in the medium preparation to promote the isolation of the most salt-tolerant members. We observed a decrease in the number of colony forming units (CFU) as the salt concentration augmented, growing $403 \pm 23$ and $62 \pm 7$ CFU at 5 and 10% NaCl, respectively. At 15% NaCl, only two CFU were cultured in the three replicates used, while at 20% NaCl, no bacteria were observed after 7 days of growth. Since the higher salt tolerance was observed at 15% NaCl, we selected both colonies cultivated at that supplemented salt concentration and identified them by sequencing their 16S rRNA gene. Both bacteria gave the same best match by BLAST alignment at NCBI database with *Halomonas alkaliantarctica*

strain CRSS (99% sequence identity), suggesting that these strains from the *Halomonas* genus are autonomous, and do not need the growth factors and/or stress-relieving effects provided by their community to grow under salt exposure.

In summary, we analyzed the complete bacterial community present in the extreme Lejía Lake shore soil by 16S rRNA gene amplification and NGS technology. In addition, we applied a culture-based approach that simulated the chemical composition of the original soil sample to examine the complete cultivable-community protocol that increased the recovery rate obtained by standard culturing procedures. Therefore, our results contribute to determine the magnitude of bacterial species inhabiting this extreme environment, and, along with other studies (Bull et al. 2016), support the notion that the Atacama Desert exhibits a broad system of ecological niches containing high microbial diversity. Moreover, we supplemented the culture medium with increasing salt concentrations to isolate microbial representatives of salt tolerance from LLS, strategy that could be useful to isolate other microorganisms tolerant to other environmental variables.

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