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Bacterial Diversity in Microbial Mats and Sediments from the Atacama Desert

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Abstract The Atacama Desert has extreme environmental conditions that allow the development of unique microbial communities. The present paper reports the bacterial diversity of microbial mats and sediments and its mineralogical components. Some physicochemical conditions of the water surrounding these ecosystems have also been studied trying to determine their influence on the diversity of these communities. In that way, mats and sediments distributed among different hypersaline lakes located in salt flats of the Atacama Desert were subjected to massive parallel sequencing of the V4 region of the 16S rRNA genes of *Bacteria*. A higher diversity in sediment than in mat samples have been found. Lakes that harbor microbial mats have higher salinity than lakes where mats are absent. *Proteobacteria* and/or *Bacteroidetes* are the major phyla represented in all samples. An interesting item is the finding of a low proportion or absence of *Cyanobacteria* sequences in the ecosystems studied, suggesting the possibility that other groups may be playing an essential role as primary producers in these extreme environments. Additionally, the large proportion of 16S rRNA gene sequences that could not be classified at the level of phylum indicates potential new phyla present in these ecosystems.

Keywords Microbial diversity · Atacama Desert · Mats · Sediments · Hypersaline lakes

Introduction

The Atacama Desert is the driest and oldest desert on Earth. Its surface conditions have remained relatively unchanged for millions of years [1, 2]. It has more than 100 basins with interior drainage, and most of them contain salt flats. These ecosystems are characterized by extreme physicochemical and climatological conditions like extreme aridity, strong winds, scarce but torrential rainfall, high rates of evaporation, high solar radiation, extreme daily temperature changes (e.g., −2 + 20 °C), negative water balance (e.g., precipitation rates of <10–300 mm year^{-1} versus evaporation rates 1000–1200 mm year^{-1}), combined with a wide range of salinity (from freshwater to saturated saltwater within the same basin) [1, 3–5]. The salt composition is typically dominated by sulfate, chloride, sodium, and divalent cations [3, 6–9]. In spite of these conditions, phototrophic and heterotrophic bacteria have been recently found inside halite and gypsum evaporites that form as bottom-growth crusts in the hyperarid core and microbial mats [9–14].

Microbial mats are sedimentary biofilms vertically laminated, and they are found in lagoons, intertidal and subtidal marine zones, hypersaline ponds, hot springs, and freshwater rivers and lakes [15]. They are considered to be the modern analogs of fossil stromatolites. The oldest stromatolites date back 3.5 billion years and therefore represent the oldest ecosystems known [16]. The various metabolic capacities of the microbial communities from modern stromatolites are

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responsible for the biogeochemical cycling of the elements. The classic view of a microbial mat is that each layer contains different microorganisms with distinct metabolic activities and they are often built by phototrophic organisms in the top layer, particularly *Cyanobacteria* [17].

Several studies on microbial diversity have been done in the evaporitic basins in northern Chile [7, 11, 12, 14, 18–25]. However, microbial mats have been investigated in these places only in a few cases [9, 10]. Furthermore, there is no study about diversity in sediment at these lakes. In addition, molecular diversity studies remain patchy, and efforts to couple local physicochemical data and the type of local microbial communities are still scarce.

The occurrence of microbial life associated with these particular environments opens up new perspectives regarding how communities are adapted and thrive in these hostile environments [14]. Our group has previously reported on the taxonomic diversity of microbial mats from Tebenquiche and Brava lakes at the Atacama Desert through sequencing of the V4 hypervariable region of 16S rRNA gene [9]. In this study, the knowledge about microbial mats and sediments of salt lakes from the Atacama Desert is extended, studying its bacterial diversity using independent culture tools as well as its physicochemical characteristics to discover if any physicochemical traits could be influencing its taxonomic composition.

Materials and Methods

Sample Sites and Sampling

Samples were taken from lagoons located in four salt flats in the Atacama Desert in January 2012: Salar de Atacama (Laguna Cejar), Salar de Llamara (Laguna Llamara), Salar de Coposa (Laguna Jachucoposa), and Salar de Pujsa (Laguna Pujsa) (Fig. 1). At 2300 m a.s.l., the Salar de Atacama is the largest Chilean salt flat, and it is characterized by the presence of shallow lakes with high salt concentrations, which are locally called *lagunas*. Laguna Cejar is a hypersaline lake, and it is located in the northern sector of the Salar de Atacama. Salar de Llamara is a salt flat basin in a region called Tarapacá at 850 m a.s.l.. Salar de Coposa is located in the First Region between the basin of Salar de Pintados in the Central Valley and the basins of the salt flats of Laguna Empexa in the Bolivian plateau, at 3730 m a.s.l.. A sample was obtained from Laguna Jachucoposa, which is the principal water body of this salt flat and is located at southwest within the Salar de Coposa. Finally, Salar de Pujsa is located to the southwest of Salar de Tara, at 83 km from San Pedro de Atacama and the northeast of Acamarachi volcano, at 4585 m a.s.l.. Laguna Pujsa is situated in the southwest

of this salt flat. All of these systems are springs and athalassohaline ponds in different salt flats of the Atacama Desert region.

Three mat and two sediment samples were collected. The mat samples were taken from Laguna Llamara (samples named LL1 and LL2) and Laguna Cejar (Cej), both have microbial mats forming at water's edge of these lakes. They were obtained at a depth of 10-cm water column. LL1 and LL2 were collected in different areas from Laguna Llamara, and they were chosen due to their visually distinct features. Sediments were taken from Laguna Jachucoposa and Laguna Pujsa where microbial mats are not present. A core of each sediment sample was collected at water's edge at a depth of 10-cm water column. Sample views are shown in Fig. 2. Their altitudes and locations are shown in Table 1. Mat and sediment cores have 10 cm^2 of surface and 3 cm of depth. For each section sample, three subsamples were taken and pooled. Triplicates of the water column over each mat and sediment were collected in 100-ml plastic bottles and stored at 4 °C.

Samples for scanning electron microscopy (SEM), lithogeochemistry, and water chemistry analyses were stored in the dark at 4 °C. Samples for DNA extraction were frozen in liquid nitrogen. All procedures were performed within a week. Temperature and pH measures of water samples were done by triplicate in situ. Dissolved oxygen, salinity, conductivity, total phosphorous, NO_3^-, NO_2^-, dissolved SiO_4, Ca^{+2}, Mg^{+2}, and major ions (K^+, SO_4^{2-}, and Na^+) were analyzed in the laboratory, according to the methodology described by the reference [26]. NH_4^+, orthophosphates, and total organic nitrogen (NOT) were analyzed using a Merck Nov. 60Spectrocuant instrument.

Scanning Electron Microscopy

For SEM, samples were first fixed overnight at 4 °C in Karnovsky's fixative, comprising formaldehyde (8 % *v/v*), glutaraldehyde (16 % *v/v*), and phosphate-buffered saline (PBS; 0.2 M, pH 7.4). The fixed samples were washed three times with phosphate buffer for 10 min. Later, they were fixed with 2 % *v/v* osmium tetroxide overnight. Finally, after washing twice with ethanol (30 % *v/v*) for 10 min, the samples were dried at critical point and sputtered with gold. Specimens were observed under vacuum using a Zeiss Supra 55VP (Carl ZeissNTS GmbH, Germany) scanning electron microscope.

Mineralogical Analysis by XRD

The mineral composition of the mats and sediments was obtained by X-ray diffraction (XRD) analyses following international conventional procedures [27, 28], which was carried out using finely ground sample material (<20 μm) and measured with a PANalyticalX'Pert PRO diffractometer, with Cu lamp (kα = 1.5403 Å) operated at 40 mÅ and 40 kV at the Centro de

Fig. 1 Location of studied area

Investigaciones Geológicas (La Plata, Argentina). The samples were measured from 2° to 40° 2θ, with a scan speed of 0.04°/s and a time per step of 0.50 s.

DNA Extraction

Total genomic DNA was isolated using the Power Biofilm DNA Isolation Kit (MO BIO Laboratories, inc.) according to the supplied protocol.

PCR and Pyrosequencing

The V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the Ribosomal Database Project (RDP)-suggested universal primers (http://pyro.cme.msu.edu/pyro/help.jsp) that contain the Roche 454 sequencing A and B adaptors and a 10 nucleotide "multiple identifier" (MID). Five independent PCRs were performed to reduce bias. The PCR mixture (final volume 25 μl) contained 2.5-μl FastStart High Fidelity 10X Reaction Buffer (Roche Applied Science, Mannheim, Germany), 20 ng of template DNA, 0.4 μM of each primer, 1.25 U FastStart High Fidelity Enzyme Blend (Roche Applied Science), and 0.2 mM dNTPs. The PCR conditions were 95 °C for 5 min for initial denaturation, followed by 95 °C for 45 s, 57 °C for 45 s, 72 °C for 60 s in 30 cycles, and a final elongation step at 72 °C for 4 min. Two negative control reactions containing all components except for the template were performed. The five reaction products were pooled and purified using AMPure beads XP. Quantification of the purified PCR product was performed

Fig. 2 Microbial mats and sediments from Lagunas (**a**) mat from Llamara (LL1) (**b**) mat from Llamara (LL2) (**c**) mat from Cejar (Cej) (**d**) sediment from Coposa (Cop) (**e**) sediment from Pujsa (Puj)
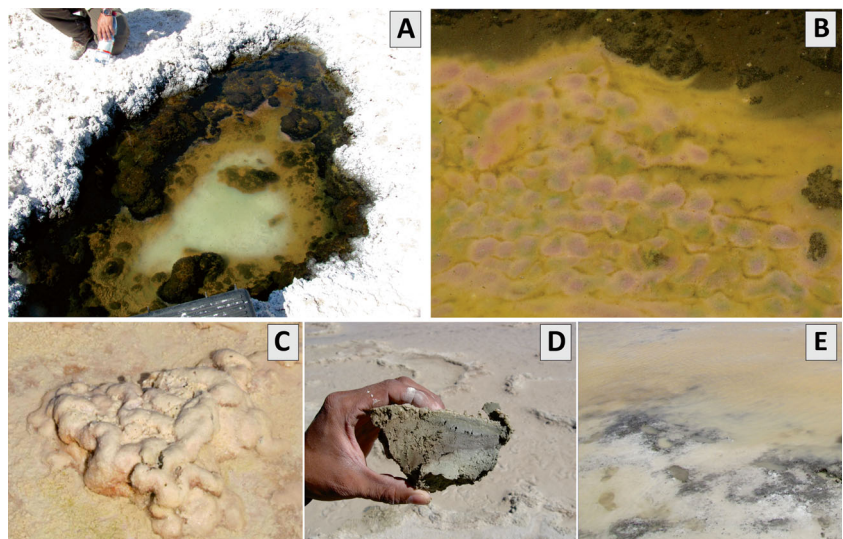
Table 1 Altitude and location of the systems

System	Altitude[a]	Location
Salar de Llamara	850	21° 23' S 69° 37' W
Salar de Atacama	2300	23° 12' S 67° 32' W
Salar de Coposa	3700	20° 38' S 68° 40' W
Salar de Pujsa	4500	23° 12' S 67° 30' W

[a] Meters above sea level

using the Quant-IT Pico Green dsDNA Kit (Invitrogen Molecular Probes Inc, Oregon, USA).

Purified PCR product was sequenced on a Genome Sequencer FLX (Roche Applied Science) using Titanium Chemistry according to the manufacturer's instructions. A total of 10,958 filtered sequences with an average length of 225 bp were obtained from five samples used in this study. Filter parameters were set to reject reads that had mean quality score <25, maximum homopolymer run >6, number of primer mismatches >0, and read length <200 or >1000 bp. Sequences were deposited as FASTAQ format in the NCBI Sequence Read Archive (SRA) under the following accession number SRP029444.

Taxonomy-Based on Alpha and Beta Diversity Analysis

Diversity of the microbial community was assessed by analyzing the sequences of the V4 hypervariable region of bacterial 16S rRNA using the QIIME software package v.1.7.0 [29]. Sequences were clustered into OTUs using UCLUST at the 97 % similarity level using the most abundant sequence as the representative sequence for each OTU. A table was compiled of the number of sequences per OTU. Each representative OTU sequence was characterized taxonomically using the Ribosomal Database Project (RDP) included in QIIME v1.7.0 software using a bootstrap confidence of 80.

Furthermore, from the ten most abundant OTUs in each sample, one representative sequence was selected. A maximum likelihood reference tree was constructed using RaxML, as implemented in ARB software package (Ludwig et al. 2004) using reference 16S rRNA gene sequences with near full length (>1300 nt) from cultured isolates. Later, partial 16S rRNA gene sequences from each sample and closely related environmental uncultured 16S rRNA gene sequences were inserted into reference tree without altering tree topology using maximum parsimony criterion and a 50 % base frequency filter. Bootstrap values greater than 50 % are indicated above nodes, and the scale bar represents 10 base substitutions per 100-nt positions.

In addition, OTU tables were subsampled using 10 replicates for each sampling effort at increasing intervals of 100 sequences, and so, alpha diversity indexes were calculated on each subsample of the rarefaction curve

and on the complete OTU table (including all sequences) using QIIME. Alpha diversity metrics calculated included Observed species (OTU number), CHAO1 (estimates the species richness), Shannon (the entropic information of the abundances of observed OTUs, accounting both richness and evenness), Equitability (Shannon index corrected for # species, pure evenness), Dominance (calculated as the sum of the squares of the frequencies of each OTU), and Simpson (1-Dominance) indices.

A beta diversity study was also made. It includes the phylum-level abundance based on 16S rRNA sequence classification using QIIME [29]. Samples from this study (LL1, LL2, Cej, Cop, and Puj samples) were compared to different mats and microbialites, including Tebenquiche mat (Teb), Brava mat (Bra) (SRA accession number SRP017289) [9], Socompa stromatolite (SRP007748) [30], Yellowstone stromatolites (http://inside.mines.edu/~jspear/resources.html), Highborne Cay thrombolites, SRX030166, and a Guerrero Negro microbial mat, (GenBank accession numbers DQ329539 to DQ331020 and DQ397339 to DQ397511). This comparison was analyzed using same procedures for comparative analysis.

Multivariate Analysis

A constrained ordination was carried out by a canonical correspondence analysis (CCA) to correlate environmental variables with prokaryotic phyla and samples. A Monte Carlo test with 499 permutations was made to ensure the significance of canonical axes. CANOCO 4.5 software package (Microcomputer Power, Ithaca, NY, USA) was used to perform the CCA and the tool CANODRAW for triplot visualization [31]

Results

General Water Physicochemical Parameters

Physicochemical parameters for water columns above samples are shown in Table 2. Mat samples (LL1, LL2, and Cej) show the lowest DO, organic matter (18 % of dry weight), and total phosphorous concentration. They also present the highest conductivity. The opposite is observed in sediment samples (Cop and Puj), being Puj the most oxic one. In addition, pH measurements show that sediment samples are strongly alkaline. All the samples have important concentrations of arsenic, with an extremely high amount in Puj. Concerning ion concentration, sodium and chloride were higher in Cej and Puj while in LL1 and LL2, sulfate and calcium were the highest. Also, magnesium, potassium, and lithium are higher in Cej and Puj.

Table 2 Physicochemical and biological characteristics of the water samples from the different places studied

Parameters		Units	LL1	LL2	Cej	Cop	Puj
Physicochemical	Temperature	°C	36.5	27.4	21.8	19.8	-
	pH	-	7.6	7.6	8.2	9.8	10.8
	Dissolved oxygen	mg/L	0.7	1.8	1.4	2.6	9.3
	Conductivity	mS/cm	152	143	161	6	70
Nutrients	Total phosphorous	mg/L	4.2	0.8	4.2	93	86
	Ortophosfate	mg/L	0.4	0.8	2.8	0	68.6
	Total organic nitrogen	mg/L	0.3	0.9	0.7	0.3	0.5
	Nitrate	mg/L	0.1	0.1	<0.04	0.2	0.1
	Nitrite	mg/L	<0.1	<0.1	<0.1	<0.1	<0.1
Majority ions	Dissolved silica	mg/L	98	94	99	68	138
	Calcium	mg/L	1088	1054	978	195	295
	Magnesium	mg/L	364	297	2559	127	1312
	Potassium	mg/L	668	552	1977	74	4404
	Sulfate	mg/L	13540	9780	18333	1177	14012
	Sodium	mg/L	20990	17330	49840	1683	38390
	Chloride	mg/L	31586	25467	73537	300	57807
	Lithium	mg/L	8	7	136	3	285
	Arsenic	mg/L	2	1.2	5.5	0.4	120
Other environmental parameters	Totala	mgCaCO3/L	159	191	7354	69	22663
	Hardness	mg/L	4213	3855	12977	1009	6138
	Organic matter	%	3.3	2.1	6.3	49.9	10.1
	Total sulfur	mg/L	<0.2	<0.2	<0.2	<0.2	<0.2
	Dissolved sulfur	mg/L	5119	4033	6681	482	4684

Scanning Electron Microscopy and Mineralogy

Electron microscopy revealed cyanobacterial filaments (Fig. 3 a–c), which in LL1 and LL2 appear empty and fossilized. Diatom frustules (Fig. 3c, d, e, f), microorganisms, and agglutinated mineral microcrystals are observed in all photos.

The XRD analysis (Supplementary Fig. S1) shows that halite and gypsum are the major minerals in the mat samples. The sediments are mainly composed of feldspar and halite, with carbonate minerals (calcite) found only in Cop.

Estimation of Richness and Diversity

Sequences were binned into operational taxonomic units (OTUs) based on a shared sequence threshold (97 % identity), and OTUs were used to calculate rarefaction and nonparametric estimators (Table 3). In Cop, the highest value of bacterial richness (the number of different OTUs in a sample) and diversity is obtained (Shannon's H index that considers the evenness of OTU distribution), in contrast to LL2 with the lowest value. Therefore based on the distribution of the sequences, Cop has a higher equitability and LL2 is more dominant. Besides, the largest diversity found in Cop is reflected

on the rarefaction curve with a raised slope while in the rest of the samples, the slopes are close to zero (Fig. 4). The low diversity in LL2 is confirmed with a gentle slope.

Taxonomy-Based on Alpha and Beta Diversity Analysis

The clusters formed by OTUs to determine the distribution of phylotypes in the samples were analyzed. Figure 5 displays the main phyla per sample, revealing large differences between them while Table 4 summarizes the most abundant taxonomic groups per sample classified at the highest possible taxonomic level. Among the classified OTUs, *Proteobacteria* and *Bacteroidetes* represent the most abundant phyla in all samples studied (20–40 and 2–30 % of 16S rRNA gene sequences, respectively). Many 16S rRNA gene sequences classified within *Bacteroidetes* show a high sequence identity percentage and are related to the family *Rodhothermaceae* in all samples except Puj, where they are associated to the genus *Balneola* (family *Chitinophagaceae*). Regarding proteobacterial sequences, the family *Desulfobacteraceae* or *Rhodospirillaceae* are the most representatives in all samples except Puj, where these taxa are absent, but there are sequences related to *Rhodobacteraceae* (*Alphaproteobacteria*)
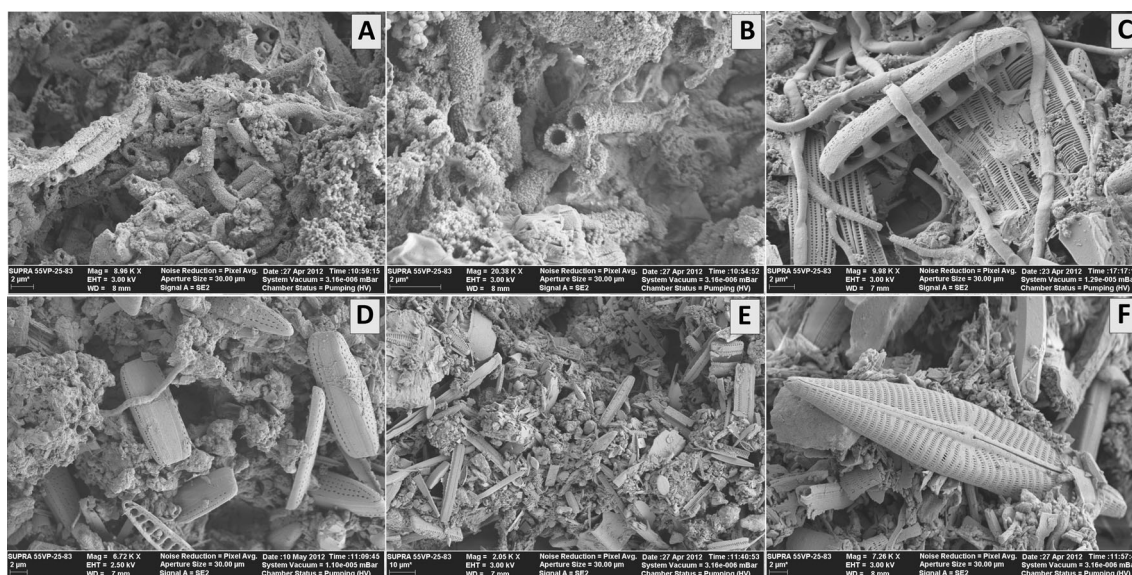
Fig. 3 SEM images. **a**, **b** Llamara 1 (mat LL1) (**c**), Llamara 2 (mat LL2) (**d**), Cejar (mat Cej) (**e**), Coposa (sediment Cop) (**f**), Pujsa (sediment Puj)

and *Epsilonproteobacteria*. 16S rRNA gene sequences assigned to the phylum *Spirochaetes* are also abundant in all samples, and they are mainly associated to the families *Spirochaetaceae* and *Leptospiraceae*. Lots of sequences related to the taxon *Caldithrix* are only detected in LL2, but they cannot be classified to a detailed taxonomic level. Sequences associated to *Tenericutes*, *Gemmatimonadetes*, and *Acidobacteria* are only observed in Cop, and related to *Actinobacteria* and *Gracilibacteria* are only found in Puj. Sequences related to *Deinococcus-Thermus* (*Deinococcaceae*) are observed in Cej, Cop, and Puj. Cyanobacterial sequences are present in a low proportion in LL2, Cej, and Puj, having only the last one an appreciable number of sequences. Sequences assigned to *Verrucomicrobia* are only present in a significant proportion in Puj. All samples have an important fraction of sequences that could not be classified at phylum level, and they are designated as "Other".

The beta diversity analysis comparing all samples with other microbial ecosystems clustered the samples in roughly three groups (Supplementary Fig. S2). The first one is composed by Cop, Cej, LL1, Socompa stromatolite, a mat sample from Brava and the Bahamas samples. But, the first five are more distantly related to the Bahamas samples.

The second group included on one side, mats from Gerrero Negro and Tebenquiche (Teb) together with LL2 and Puj. Finally, the Yellowstone thrombolite and stromatolite were included in the third group, placing completely separated from the rest of the samples.

Multivariate Analysis

CCA was carried out to analyze the potential relation between prokaryotic community samples and environmental parameters (Fig. 6). The CCA triplot revealed significant correlations to the ten most abundant phyla detected in each sample studied. Besides, the analysis of three evaporite samples was included, two from Laguna LLamara [8] and one from Laguna Tebenquiche [9], in order to observe differences among areas. CCA axes 1 and 2 explain 77 % of total variance of the data. The relation of a certain parameter with the community composition and the samples is provided by the length of a physicochemical parameter arrow in the ordination plot. The sediment sample Puj is strongly and positively correlated with physicochemical parameters as arsenic (As), orthophosphate (OP), and dissolved oxygen content (DO). This sample reveals the highest values for these physicochemical parameters causing

Table 3 Observed prokaryotic richness and diversity estimates based on 97 % OTU clusters

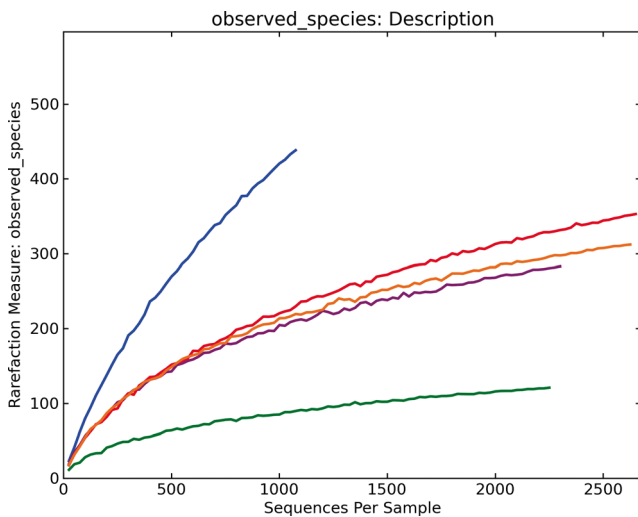| Sample	Number of reads	Seqs/Sample	Chao1	Dominance	Equitability	Observed species	Shannon	Simpson
LL1	2674	1000	333	0.047	0.80	210	6	0.95
LL2	2283	1000	130	0.138	0.62	86	4	0.86
Cej	2722	1000	393	0.032	0.80	220	6	0.97
Cop	1082	1000	863	0.007	0.92	419	8	0.99
Puj	2360	1000	297	0.024	0.84	200	6	0.98

Fig. 4 Rarefaction curves comparing bacterial diversity in all samples of this study. Cop (*line blue*), Cej (*line red*), LL1 (*line orange*), Puj (*line violet*) and LL2 (*line green*)

large differences among Puj and the remaining samples. Besides, the phyla *Actinobacteria* and *Cyanobacteria* are positively correlated with these parameters and with the sample Puj.

Discussion

This is the first report of bacterial diversity determined by pyrosequencing of the V4 region of the bacterial 16S rDNA

gene in microbial mats and sediments from these lakes. The high diversity found in Cop and Puj may be explained by the physicochemical conditions in the environment. They have a higher DO and proportion of organic matter and total phosphorous as well as a lower conductivity than mat samples. It is known that hypersaline environments have a low diversity, where halophilic microorganisms are able to survive to these extreme conditions because they have specific strategies to balance the osmotic pressure [32, 33]. Moreover, it has been seen that these systems usually have very low organic carbon and nitrogen content [8, 25, 30, 34]. The combination of these factors makes soils from these environments inhabitable only by a very low diversity of organisms, mostly bacteria and archaea, but also some fungi and protists [25, 34, 35]. An interesting point is that pH of sediments is very high. This, and the fact of sediment samples present poorly known taxa, would mean that new microorganisms have mechanisms to survive the alkalinity making these a possible source for biotechnological applications. Ion concentrations may explain the mineralogy of the samples, as they are consistent with the ionic composition of water at each site.

It should be noted that this study does not attempt to give absolute values for the presence of different phylogenetic groups due to potential PCR biases [36]. Regarding bacterial groups, *Proteobacteria* and *Bacteroidetes* phyla are quite high in almost all samples studied. Other phyla as *Spirochaetes*, *Chloroflexi*, or *Verrucomicrobia* are also found. These results agree with our previous studies where these bacterial phyla

Fig. 5 Diversity of the microbial community assessed by analyzing the sequences of the V4 hypervariable region of bacterial 16S rRNA using the QIIME software package v.1.7.0 [29]
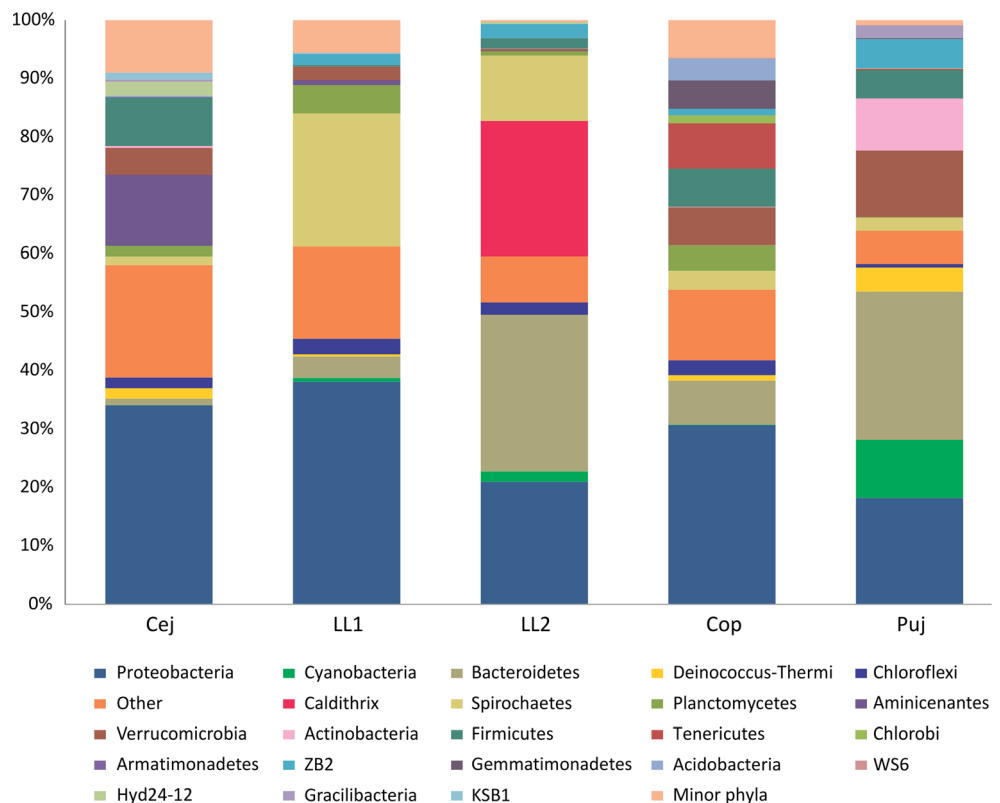
Table 4 Abundant bacterial groups per sample

LL1	LL2	Cej	Cop	Puj
23 % Desulfobacteraceae (Proteobacteria)	23 % Rhodothermaceae (Bacteroidetes)	26 % Unclassified Bacteria	21 % Acholeplasmataceae (Tenericutes)	18 % Balneola (Bacteroidetes)
17 % Spirochaetaceae (Spirochaetes)	17 % Caldithrix (no phylum rank)	23 % Rhodospirillaceae (Proteobacteria)	14 % Desulfobacteraceae (Proteobacteria)	10 % Stramenopiles (Cyanobacteria)
16 % Unclassified Bacteria	16 % Rhodospirillaceae (Proteobacteria)	11 % Desulfobacteraceae (Proteobacteria)	13 % Rhodothermaceae (Bacteroidetes)	9 % Euzebiaceae (Actinobacteria)
7 % Proteobacteria	7 % Spirochaetaceae (Spirochaetes)	11 % Uncultured clone HMMVPog-54 (Aminicenantes)	12 % Unclassified Bacteria	6 % Verrucomicrobiaceae (Verrucomicrobia)
5 % Rhodospirillaceae (Proteobacteria)	5 % Unclassified Bacteria	11 % Halanaerobiaceae (Firmicutes)	7 % Uncultured clone ZB2	5 % Unclassified Bacteria
4 % Leptospiraceae (Spirochaetes)	4 % Desulfohalobiaceae (Proteobacteria)	6 % Spartobacteriaceae (Verrucomicrobia)	3 % Gemmatimonadaceae (Gemmatimonadetes)	5 % Epsilonproteobacteria (Proteobacteria)
3 % Saprospiraceae (Bacteroidetes)	3 % Uncultured clone OPB11 (Chloroflexi)	2 % Ectothiorhodospiraceae (Proteobacteria)	2 % Desulfobulbaceae (Proteobacteria)	5 % Uncultured clone ZB2
3 % Phycisphaerae (Planctomycetes)	3 % Halanaerobiaceae (Firmicutes)	2 % Candidatedivision WS1	2 % Clostridiales (Firmicutes)	5 % Puniceicoccaceae (Verrucomicrobia)
2 % Uncultured clone OPB11 (Chloroflexi)	2 % Cyanobacteriaceae (Cyanobacteria)	2 % Uncultured clone Hyd24-12	2 % Pirellulaceae (Planctomycetes)	4 % Deinococcaceae (Thermi)
2 % Uncultured clone ZB2	2 % Desulfobacteraceae (Proteobacteria)	1 % Deinococcaceae (Thermi)	2 % Uncultured clone RB40 (Acidobacteria)	4 % Rhodobacteraceae (Proteobacteria)
2 % Uncultured clone IF025 (Spirochaetes)		Verrucomicrobia	2 % Verrucomicrobiaceae (Verrucomicrobia)	2 % Sphingobacteriales (Bacteroidetes)
1 % Hydrogenedetes		Spirochaetaceae (Spirochaetes)	1 % Uncultured clone A4b (Chloroflexi)	2 % Spirochaetaceae (Spirochaetes)
1 % Uncultured clone vadinHA49 (Planctomycetes)		Chromatiaceae (Proteobacteria)	1 % Uncultured clone TSBW08 (Hydrogenedentes)	2 % Gracilibacteria
1 % Uncultured clone TG3-1 (Chitinivibrionia)		1 % Uncultured clone GW-22 (Candidate division KSB1)	1 % Leptospiraceae (Spirochaetes)	2 % Flavobacteriaceae (Bacteroidetes)
			1 % Unculturedalphaproteobacteria clone BD7-3 (Proteobacteria)	1 % Clostridiales (Firmicutes)
				2 % Campylobacteraceae (Proteobacteria)
				1 % Uncultured clone ML615J-4 (Bacteroidetes)

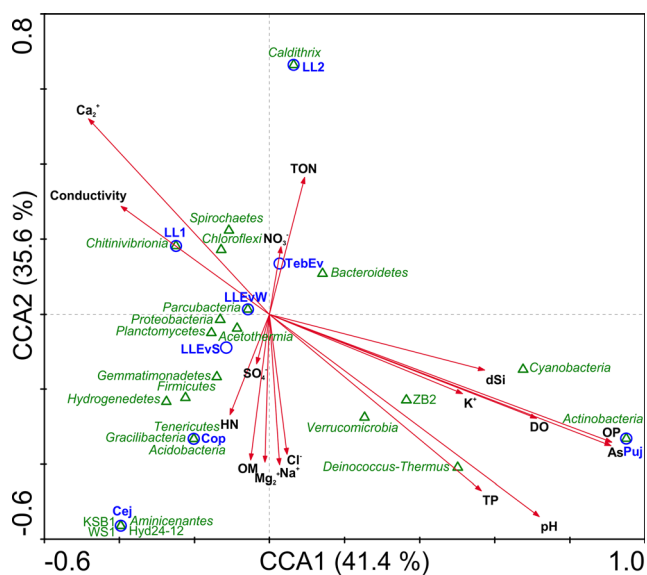OTUs are classified at the lowest possible taxonomic level

Fig. 6 Canonical correspondence analysis (CCA) of prokaryotic community, samples, and environmental parameters. *Arrows* indicate the direction and magnitude of environmental parameters associated with phyla and samples studied

appear to be dominant in these ecosystems [8, 9, 30] (Supplementary Fig. S2). All of these groups are well known to be abundant in marine ecosystems and also in extreme environments, such as microbial mats from hypersaline lakes [37–42].

Further, *Bacteroidetes* has been reported as a dominant phylum in hypersaline systems, including microbial mats [40, 43], hypersaline water and sediment samples [7, 19, 44, 45], and (endo)evaporitic samples [8, 9, 14, 46, 47]. LL2 and Puj display a very high amount of sequences related to this phylum, but in LL2, the family *Rhodothermaceae* dominates where the conductivity doubles that of Puj. This family contains the genus *Salinibacter* which is characterized for requiring high salt concentrations for its growth [48, 49]. Yet, in Puj, the most abundant sequences are associated to the genus *Balneola*, and although only two species of this have been reported, both grow at low salinity [50, 51]. However, the high conductivity samples LL1 and Cej exhibit lower number of sequences related to *Bacteroidetes*.

LL2 has a very abundant taxon, *Caldithrix* (23 % 16S rRNA gene sequences), which is absent in the rest of the samples, even in the other mat sample from Llamara, LL1. This group represents a lineage phylogenetically distinct within the *Bacteria* domain that is comparable to phylum status. This taxon contains only two species, *C. abyssi* and *C. palaeochoryensis*, isolated from a deep sea hydrothermal chimney on the Mid-Atlantic Ridge and a geothermally heated sediment of a marine hydrothermal system, respectively [52, 53]. In the last years, the number of 16S rRNA gene sequences deposited in public databases from environmental samples that are phylogenetically related to *C. abyssi* have increased. Some of them have been retrieved from hydrothermal waters

[54] or in sulfide and methane-rich cold seep sediments [55, 56]. However, the temperature measured in LL2 is lower than in LL1, and total phosphorous and organic matter are the only physicochemical parameters measured in the samples studied, with a lower concentration in LL2 than in the others. In addition, this phylum could not be associated to any specific taxonomic level, meaning that this/these member/s may belong to an unknown novel taxon.

It is considered that *Cyanobacteria* plays a fundamental role in microbial mats and sediments as primary producers [43, 57]. It was explained that the *Cyanobacteria* dominates microbial mats, working as the most important group in the organomineralization producing exopolisaccharide (EPS) into the mats which is a pliant matrix for structuring associations within microbial communities [58]. EPS can be produced by a wide array of microorganisms, photoautotrophic bacteria as well as heterotrophic bacteria, although *Cyanobacteria* are believed to generate the bulk of the quantity, and the content might vary with different stressors and/or environmental conditions [59–67]. However, we observed that the cyanobacterial OTUs found in our samples are very scarce or absent. This finding agrees with some previous studies of mats, microbialites, and evaporites in the Atacama Desert in which they also report a low abundance of *Cyanobacteria*, suggesting that it might be a common feature in them [8, 9, 30]. Additionally, when compared to mats and microbialite systems in other parts of the world, like Yellowstone and High borne Cay, Bahamas (Supplementary Fig. S2), the lower abundance of *Cyanobacteria* in all the studied Andean ecosystems is striking. McKay et al. (2003) suggested that *Cyanobacteria* might not be a dominant phylum in the Atacama Desert [4]. They measured the moisture under the stones and observed that it was not enough for the life of *Cyanobacteria*. Several reports indicated that primary production, usually performed by *Cyanobacteria*, could be partly substituted by other organisms such as diatoms or nonphototrophic carbon fixers, since dark carbon fixation by chemoautotrophic bacteria might be a large contributor to overall carbon fixation, especially in sediments with low organic matter content. But still, little is known about the importance of this process in lake systems, despite the assumption of a high chemoautotrophic potential of lake sediments [25, 34, 68–70].

Another interesting result is the considerable fraction of the 16S rRNA gene sequences (8–20 %) that could not be affiliated to any known bacterial phyla. This is evidence that they should belong to novel representatives of bacterial phyla not yet described. Some 16S rRNA gene sequences have been related to uncultured candidate divisions as ZB2 (LL1, Cop, and Puj), KSB1, WS1, and Hyd-24-12 (Cej) and to uncultured candidate phyla as *Aminicenantes* (Cej), *Chitinivibrionia* (LL1), *Gracilibacteria* (Cop), and *Hydrogenedetes* (LL1 and Cej). These results agree with a study of diversity in

stromatolites from Laguna Socompa (Salta-Argentina) [30]. Also, the beta diversity analysis unveils that Andean systems have a lot of new phyla compared with microbial ecosystems in other areas in the world (Supplementary Fig. S2). Other interesting finding is the elevated proportion of *Deinococcus-Thermus* in those lakes located at higher altitude (Puj, Cop, and Cej). It agrees with our previous papers [8, 9, 30], where a considerable proportion of sequences classified into this phylum was observed. Farias et al. (2013) argued that this phylum may be related to the protection role against UV radiation damage at high altitude [30].

The multivariate analysis indicates that some physicochemical parameters as arsenic, orthophosphate, and DO concentration contribute to explaining how the bacterial community from Puj differs more than the others among them. This sample has a high arsenic concentration and a great bacterial diversity. Arsenic is a very toxic compound with oncogenic effects, but some microorganisms are able to tolerate it in a variety of different ways: the responses include precipitation, chelation, compartmentalization, extrusion, or biochemical transformation [71–76]. Moreover, there are microorganisms that are not only able to resist but some of them can utilize, or even require arsenic for their ordinary physiology [74, 77]. All samples showed a significant arsenic concentration, but Puj had a very high amount of it. Microorganisms inhabiting this lake may be adapted to high amounts of arsenic, and it could be the case of some members of the phylum *Actinobacteria* due to actinobacterial OTUs are positively correlated with this sample and arsenic and orthophosphate concentration. More than that, at a high taxonomic level, the most abundant are represented by different groups than the rest of the samples, and very little known. This may mean that a selective population is harboring that sediment. Many isolates of the phylum *Actinobacteria* demonstrated to be resistant and/or tolerant to arsenic [78–80], and it is known that polyphosphates that are formed by tens or hundreds of orthophosphate molecules could have an important function in detoxification of metalloids like arsenic [81]. DO is widely accepted as a critical parameter on activity and microbial structure of communities from different habitats [82–85]. Puj is the sample with the highest DO concentration, and this is clearly favored by the large presence of *Cyanobacteria* in this sample, as a result of the product generated by oxygenic photosynthesis.

In conclusion, microbial mat formation appears to be related to hypersaline lakes, as other authors previously suggested. Also, the mineralogy of these mats seems to depend on the physicochemical conditions of surrounding water. The sediment samples (Cop and Puj) have a higher diversity, abundance, and equitability of OTUs than mat samples, and they contain phyla not observed in mat samples. In all the samples analyzed in this study, the most dominant phyla are *Proteobacteria* and *Bacteroidetes*. Also, these samples appear to have low proportion or absence of *Cyanobacteria*,

indicating that they might not be playing an essential role related to carbon fixation and as producers of EPS of microbial mats in these extreme environments. Finally, it was shown that a considerable fraction of the 16S rRNA gene sequences that could not be affiliated to any known bacterial phyla suggesting that in these ecosystems, there are potential novel representatives of bacterial phyla not yet described.

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