

RESEARCH ARTICLE

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Key Points:

- The microbial diversity of brines from Salar de Atacama differs according to the concentration of lithium
- The brines with low lithium concentrations were dominated by Archaea, and Bacteria dominated lithium-concentrated brines
- Our research revealed the presence of microbial life in one of the most saline environments that has been described on Earth

Supporting Information:

- Supporting Information S1

Correspondence to:

C. Dorador,
cristina.dorador@uantof.cl

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Microbial Communities From the World's Largest Lithium Reserve, Salar de Atacama, Chile: Life at High LiCl Concentrations



Carolina F. Cubillos^{1,2,3} , Pablo Aguilar⁴ , Mario Grágeda² , and Cristina Dorador^{1,3,5}

¹Laboratorio de Complejidad Microbiana y Ecología Funcional, Instituto Antofagasta, Universidad de Antofagasta, Antofagasta, Chile, ²Department of Chemical Engineering and Mineral Process and Center for Advanced Study of Lithium and Industrial Minerals (CELiMIN), Universidad de Antofagasta, Antofagasta, Chile, ³Centre for Biotechnology and Bioengineering, Universidad de Chile, Santiago, Chile, ⁴Lake and Glacier Ecology Research Group, Department of Ecology, University of Innsbruck, Innsbruck, Austria, ⁵Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile

Abstract Salar de Atacama is one of the largest global reservoirs of natural lithium brines (mean lithium concentration = 1,500 ppm), enabling Chile to be a leading producer of lithium products. This large salar (3,000 km²), located in the Atacama Desert at 2,300 m above sea level, is dominated by microorganisms; however, little is known about the microbes present in the brines associated with this economically important mining process. Here we study lithium as a modulator of microbial richness and diversity in brines representing natural conditions (34.7% salinity) and conditions prior to lithium production with a concentrated brine (55.6% salinity). Brines only supported a single archaeal family (Halobacteriaceae): natural brines included the archaeal genera *Halovenus*, *Natronomonas*, *Haloarcula*, and *Halobacterium*. Concentrated brines included the archaeal genera *Halovenus*, *Halobacterium*, and *Halococcus*. The most abundant bacterial families in natural brine were Rhodothermaceae and Staphylococcaceae; Xanthomonadaceae dominated the bacterial community in the concentrated brine. A comparison of entire microbial community (Archaea and Bacteria) revealed that only seven operational taxonomic units were shared between samples, all of which were Archaea. Further, our results showed that Bacteria were phylogenetically more diverse and rich in the concentrated brine, while archaeal diversity was maximized in the natural brine. The concentrated lithium brines of the Salar de Atacama represent one of the most saline environments described to date (dominated by LiCl). We suggest that elevated concentrations of lithium could greatly modulate microbial diversity and give insights into the adaptive biology of microorganisms required to cope with extremely high concentrations of salts that extend beyond that of NaCl, a far more commonly studied salt.

Plain Language Summary Lithium is a main component of many of the batteries that we rely on for our daily use. In the last years, nearly 40% of all lithium obtained globally was from a single fragile salt-lake ecosystem: Salar de Atacama in the Atacama Desert of northern Chile. This salar has extremely saline waters called brines (dominated by NaCl, aka table salt), which are naturally highly concentrated in lithium and concentrated in evaporation ponds. In total, concentrated brines has a salinity of 55.6% (ocean salinity = 0.3%), representing one of the most saline environments described on Earth to date (dominated by lithium chloride). Our research has shown that concentrated brines support life and are dominated by hundreds of species of microorganisms. Due to saline stress these “extremophiles” have developed very special (and previously undescribed) strategies to survive in this lithium soup. These results have implications beyond Earth: they have marked implications for our understanding for the potential for life on Mars, where liquid water is known to occur as brine. Although lithium production has clear economic importance, our results show that we should consider how we will preserve these unusual ecosystems that act as reservoirs of unique microbial life.

1. Introduction

Located in the central Andes of northern Chile, the Salar de Atacama has a surface area of 3,000 km² (Lowenstein & Risacher, 2009). It was formed in the early Late Cretaceous as a consequence of the inversion of the Mesozoic backarc and is located at an elevation of 2,300 m above sea level (Mpodozis et al., 2005).

Aquatic habitats in the salar are highly saline, with brines being dominated by NaCl (Lowenstein & Risacher, 2009). Briefly, brines are defined as concentrated salt solutions that naturally occur in the form of natural coastal lagoons, salt and soda lakes, or as artificial salterns or saltworks (Trüper & Galinski, 1986) and are typically associated with NaCl. Brines from Salar de Atacama are formed under particular conditions that determine not only their physical and chemical characteristics but also the composition of its microbial communities. These saline systems include a number of different constituents: these include a liquid fraction, largely made up of chlorinated brines; a detrital fraction, consisting of sands, salts, and intertwined clays in the saline body; and a salt fraction, produced by the deposition of different salts resulting in areas dominated by carbonate, sulfate, and chlorides (Vila, 2010). Of these salts, lithium is important in the Salar de Atacama, which exhibits some of the highest reported concentrations of lithium in the world (Munk et al., 2016), with a mean concentration of 1,500 ppm (1.5 g/L) of Li (Habashi, 1997). The salar has been estimated to include total reserves of lithium in excess of 1,200,000 t (Vine, 1976).

Lithium is an alkali metallic element with a low melting point (Habashi, 1997) and is the 27th most abundant element in nature (Aral & Vecchio-Sadus, 2008; Habashi, 1997). The bulk of lithium is found in natural brines, and half of all world reserves are found in three countries in South America (Argentina, Bolivia, and Chile; Del Barco & Foladori, 2014). In recent years, extraction of lithium from natural brines has markedly increased: Chile dominates world production with the Salar de Atacama being the principal source. Here lithium is extracted by pumping brines into shallow evaporation ponds, followed by evaporation under controlled conditions that largely eliminates other components/ions (Gruber et al., 2011). In 2004, Garret showed that the chemical composition of natural brines from Salar de Atacama is dominated by sodium (91 ppm), potassium (23,600 ppm), lithium (1,570 ppm), and magnesium (9,650 ppm). The mining industry in the region takes advantage of high evaporation rates (3,700 mm/year) and solar radiation (6.3×10^6 cal·m⁻²·day), combined with low precipitation rates (10–25 mm/year), to allow the lithium extraction (Garcés, 2000; Garrett, 2004). During the initial phase of the extraction process (Figure S1), lithium is concentrated from 0.12% to 6% in solar evaporation ponds (Garcés, 2000; Garrett, 2004). This concentrated brine is subsequently transported to a chemical process plant, where lithium carbonate is obtained as a final product. Although the chemical composition of salty brines from Salar de Atacama has been extensively studied (e.g., Garcés, 2000; Garrett, 2004; Vila, 2010), little attention has been paid to microbial life associated with these brines. Particularly, studies examining the effects of high concentrations of lithium in microorganisms, as well the changes in microbial community structure in these brines during this economically important mining process of Chile are lacking.

Hypersaline environments have been widely studied, often with a focus on their saline compositions, high salinity and in some case, to understand microbial community composition. Some sites have been particularly well studied, including the Great Salt Lake (dominated by NaCl; Brock, 1975), Dead Sea (MgCl₂; Oren, 1992), Salar de Uyuni (NaCl/LiCl; Haferburg et al., 2017), and Abu Gabara Lake (Na₂CO₃; Imhoff et al., 1978). Halophile, or salt-loving organisms are classified as slight, moderate or extreme, depending in the concentrations of salts required for growth (DasSarma & DasSarma, 2012). Generally, the main bacterial groups detected in saline environments belong to Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, and Bacteroidetes (Oren et al., 2014; Ventosa et al., 2015), and Euryarchaeota and Nanoarchaeota in the Archaea (Mora-Ruiz et al., 2018; Ochsenreiter et al., 2002). In the case of lithium brines, only Halobacteriaceae, a family within Euryarchaeota (Haferburg et al., 2017), have been reported.

The biological role of lithium remains unclear, and it has been described as an element that does not appear to be essential for life (Wackett et al., 2004). It has a remarkably high capacity to replace other cations (Na⁺, K⁺, Mg⁺², and Ca⁺²) in living organisms, due to its reduced ionic radius and high polarizing strength (Sapse & Schleyer, 1995; Shahzad et al., 2017). In the case of prokaryotes, it has been suggested that some fungal groups have resistance or tolerance mechanisms against lithium (de Assunção, 2012). Further, other effects of lithium on microbial cells have been demonstrated including the stimulation of autolysis (Sugahara et al., 1983) and sporulation (Warburg et al., 1985), as well as growth inhibition (Kurita & Funabashi, 1984; Takenishi & Takada, 1984). Conversely, lithium has been used as a growth medium for the isolation of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bifidobacterium* sp. (Cox et al., 1990; Lapierre et al., 1992; Minor & Marth, 1976), and evidence exists of its accumulation in bacteria, actinomycetes, fungi, and yeasts (Perkins & Gadd, 1993; Tsuruta, 2005). In addition, it has been shown that *Micrococcus varians* ssp.

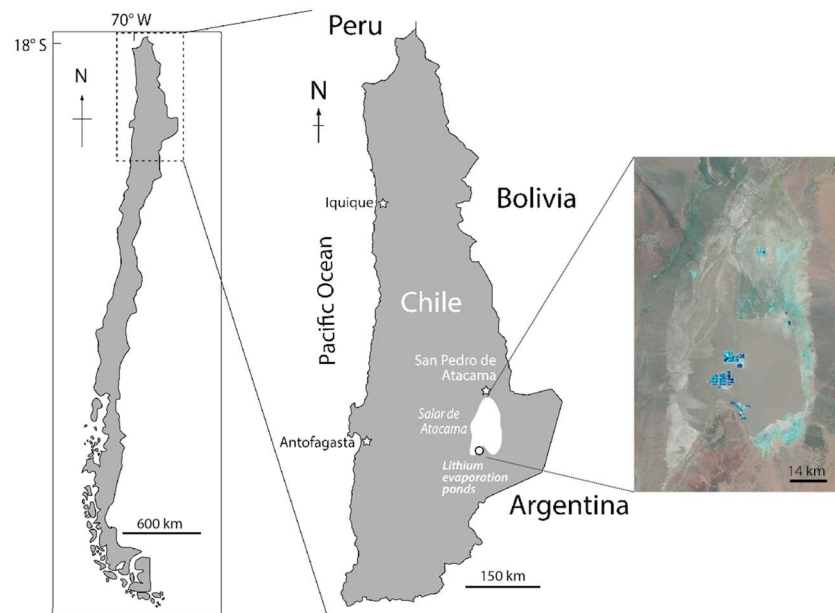


Figure 1. Map of Salar de Atacama and satellite image of evaporation ponds of lithium brines concentration process (blue squares; source EarthExplorer, U.S. Geological Survey, <https://earthexplorer.usgs.gov/>).

halophilus, a moderate halophilic bacterium, can grow in concentrations of up to 1.5 M of lithium chloride (Kamekura & Onishi, 1982), one of the highest values reported.

Despite the growing interest in the extraction and use of lithium to produce electrical appliances, there is little knowledge regarding the composition of microbial communities present in lithium brines, which can be considered as an underexplored microbial habitat. The aims of this study are to determine and to describe the diversity and composition of Archaea and Bacteria present in natural and concentrated, highly lithium-enriched brines from Salar de Atacama. We also aimed to clarify the role of lithium as a modulator of changes in microbial diversity associated with the concentration process of brines in the Salar de Atacama. Thus, our results described the microbial community composition from one environment with high concentrations of non-NaCl salts, which may be key for approaches in the search for extraterrestrial life, for example, on other planets or celestial bodies.

2. Methods

2.1. Study Area and Sampling

The industrial process of lithium extraction from Salar de Atacama brines begins with the extraction of natural brine from a depth of 50 m (>31.5 L/s) from the saline crust of the Salar (Figure 1). These brines are subsequently deposited in storage pools, where local environmental conditions such as the high evaporation rate (3,200 mm/year), minimal average rainfall (10 mm/year) and elevated concentrations of solar radiation (6.3×10^6 cal·m⁻²·day) allows the evaporation of water and crystallization/precipitation of salts over a sequential process of 12–14 months (Garcés, 2000). Here two samples of different types of brines were analyzed: a natural brine with 0.16% of lithium and a concentrated brine from the industrial process with 8.12% of lithium. Both brines are liquid (Figure S2); however, the concentrated brine was highly viscous with a yellow hue. Five liters of each brine were collected directly from the evaporation pools (initial and final stage respectively) in August 2014. Previous studies have shown that the different evaporation pools exhibit a growing salt concentration leading by lithium (Garrett, 2004). Due to the restricted access (only two main companies produce lithium in Chile) and sampling difficulties in the Salar de Atacama, it was unfortunately not possible to sample the entire process. However, this is one of the first descriptions of microbial communities associated with lithium evaporation ponds.

2.2. Characterization of Brines

Both brines were stored at 4 °C in the dark until processing. Lithium, magnesium, sodium, potassium, and calcium were measured by direct aspiration flame-air atomic absorption spectrophotometry (Model220FS-Varian, Inc.). Chloride measurements were made through the argentometric method, and finally, sulfate by sulfate determination through drying residue. Furthermore, we measured acidity (acid-base volumetric analysis), conductivity (Metrohm conductivity-five rings), density (Anton Paar, model DSA 5000 M), pH (781 pH/Ion Meter-Metrohm), salinity (absolute salinity calculated from chemical analysis), surface tension (Lauda TD-3), total dissolved salts (dried at 180 °C-SM 2540 C), total organic carbon (SM 5310 C), turbidity (Turbidimeter Zhifong, model WGZ-B), viscosity (viscometer AVS 310 Schott Gerate), and water activity (Novasina aw center, model AWC503-C; American Public Health Association, 1915). In parallel, we measured the presence of organic solvents, hydrocarbons, and oils in both brines (SM 5220 B).

2.3. DNA Extraction and 16S rRNA Gene Massive Sequencing

Two treatments were used to obtain genomic DNA from brine samples. The first consisted of filtering 50 mL of both brines through 0.22- μ m filters (Supor 200 Membrane Disc Filters, Pall Corporation). The second used 1 mL of brines, which was transferred directly to a 2-ml PowerBead tube (MoBio Lab, Inc). Samples were then homogenized using a bead beater with 5 repeated, 1-min cycles. Genomic DNA was subsequently extracted from each treatment for both brines using the PowerBiofilm DNA Isolation kit (MoBio Lab, Inc), following the manufacturer's protocol. The concentration and quality of DNA were measured with a Nanodrop spectrophotometer (Nanodrop 8000, Thermo Scientific). DNA concentration was higher in the filtered treatment for natural brines (8.8 ng/ μ l), and in the direct extraction for concentrated brines (5.6 ng/ μ l); therefore, these treatments were chosen for further analysis. DNA samples were sent to the Research and Testing Laboratory (Lubbock, Texas, USA) for Illumina sequencing. Samples were amplified for sequencing in a two-step process. The forward primer was constructed with (5'-3') the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAG ATGTGTATAAGAGACAG) and the primer 515F (GTGCCAGCMGCCGCGGTAA; Caporaso et al., 2011). The reverse primer was constructed with (5'-3') the Illumina i7 sequencing primer (GTCTCGTGGGCTCGGAGAT GTGTATAAGAGACAG) and the 806R primer (GGACTACHVGGGTWTCTAAT; Caporaso et al., 2011). Amplifications were performed in 25- μ l reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 μ l of each 5- μ M primer, and 1 μ l of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, California) under the following thermal profile: 95 °C for 5 min, then 35 cycles of 94 °C for 30 s, 54 °C for 40 s, 72 °C for 1 min, followed by one cycle of 72 °C for 10 min, and 4 °C hold. Products from the first stage amplification were added to a second PCR based on qualitatively determine concentrations. Primers for the second PCR were designed based on the Illumina Nextera PCR primers as follows: Forward: AATGATACGGCGACCACCGAGATCTACAC [i5index] TCGTCGGCAGCGTC; Reverse: CAAGCAGAAGACGGCATAACGAGAT [i7index]GTCTCGTGGGCTCGG. The second stage amplification was run the same as the first stage except for 10 cycles. Amplification products were visualized with eGels (Life Technologies, Grand Island, New York). Products were then pooled equimolar and each pool was size selected in two rounds using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana) in a 0.75 ratio for both rounds. Size selected pools were then quantified using the Quibit 2.0 fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2 \times 300 flow cell at 10 pM. Raw amplicons reads have been deposited in the Sequence Read Archive of NCBI under accession number SRP091434.

2.4. Data Analyses

Illumina reads were processed using Mothur version 1.27.0 (Schloss et al., 2009), following the standard operational procedure (www.mothur.org/wiki/Miseq_SOP) (Schloss et al., 2011). The screen.seqs command was used to cut off sequences and remove any sequence that was not within the range 100–400 pb. The sequences were aligned with the database SILVA compatible with Mothur. Chimeras were eliminated using UCHIME implemented in Mothur. The commands classify.seqs and remove.lineage were used to identify and remove pollutants classified as mitochondrial, chloroplast, Eukarya, and unknown. In total 47,865 reads from both brines studied were grouped into operational taxonomic units (OTUs), at the 3% level of divergence using the cluster.classic command.

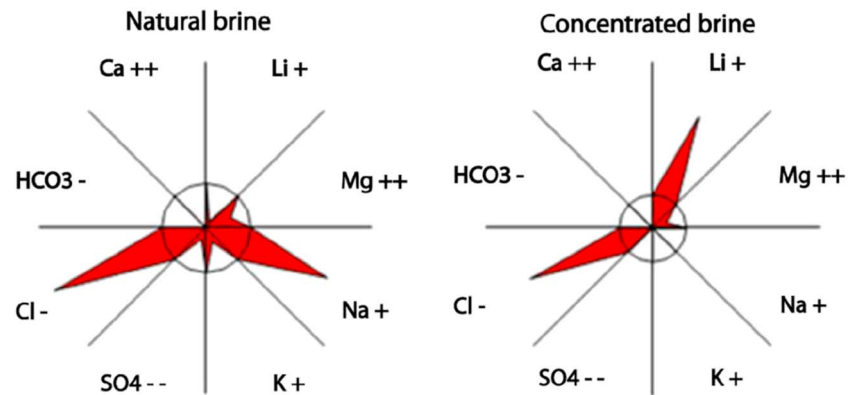


Figure 2. Maucha diagrams characterizing the anionic and cationic content of natural brine, with NaCl concentrations of 255 g/L; and concentrated brine, with LiCl concentrations of 495 g/L.

The exponential of Shannon entropy of the samples was used to calculate the alpha diversity of the samples, according to sample completeness instead of equal sample size (Chao & Jost, 2012) using the iNEXT package in R, to compute and plot seamless rarefaction and extrapolation sampling curves for this diversity index (Hsieh et al., 2016). To test for differences in microbial composition and relative abundances, samples were randomly subsampled to the same size according to the sample with the smallest number of reads. A relaxed neighbor joining phylogenetic tree was constructed using CLEARCUT (Evans et al., 2006) implemented in Mothur and visualized through iTOL (<http://itol.embl.de/>) (Letunic & Bork, 2011).

The Mothur shared and taxonomy files were converted to a Cytoscape network file using a custom R script (Neave et al., 2014). The data set was used without singletons. The network containing OTUs and samples as nodes, and edges were drawn between OTUs and brines in which they were detected. The weight of the edge was proportional to the abundance of the OTU. The networks were visualized using Cytoscape v3.5.0 (Shannon et al., 2003).

3. Results

3.1. Physicochemical Characteristics

The main ions detected in natural brine were sodium and chloride, while lithium and chloride dominated concentrated brines (Figure 2 and Table S1 in the supporting information) and ion concentrations were markedly different in the two brines. Both brines were circumneutral (natural pH = 6.9; concentrated = 7.3). The brines were both hypersaline (natural salinity = 347; concentrated = 556 g/L; Table 1), total dissolved solids (natural TDS = 348; concentrated = 566 g/L), and total organic carbon (natural TOC = 6.4; concentrated = 8.0 mg/L). Conductivity was 4 times higher in the natural than in the concentrated brines, reflecting the process of solar evaporation, which enriches the brines in solutes (salts) and decreases the amount of water present. Surface tension values were similar in both brines (natural = 80; concentrated = 86 mN/m). The turbidity of the natural brine (2 NTU) was lower than that recorded from concentrated brines (82 NTU), while concentrated brines (26 mPa-s) were more viscous than the natural one (2 mPa-s). The differences in composition and concentrations of ions highlight the effects of the industrial process involved in

Table 1
Physicochemical Parameters of Recorded From Concentrated and Natural Brines From the Salar de Atacama, Chile

Samples	Acidity (g/L)	Conductivity (mS/cm)	Density (g/ml)	Dinamic viscosity (mPa-s)	pH	Salinity (g/L)	Surface tension (mN/m)	Turbidity (NTU)	Total dissolved solids (g/L)	Total organic carbon (mg/L)	Water activity (a_w)
Concentrated brine	2 9.7	59	1.32	26.28	7.03	556.1	86.4	82	566.6	8.0	ND
Natural brine	ND	231	1.22	2.23	6.92	347.3	80.2	< 2	348.8	6.4	0.74

Note. Measuring temperature 22–25 °C. ND not detected.

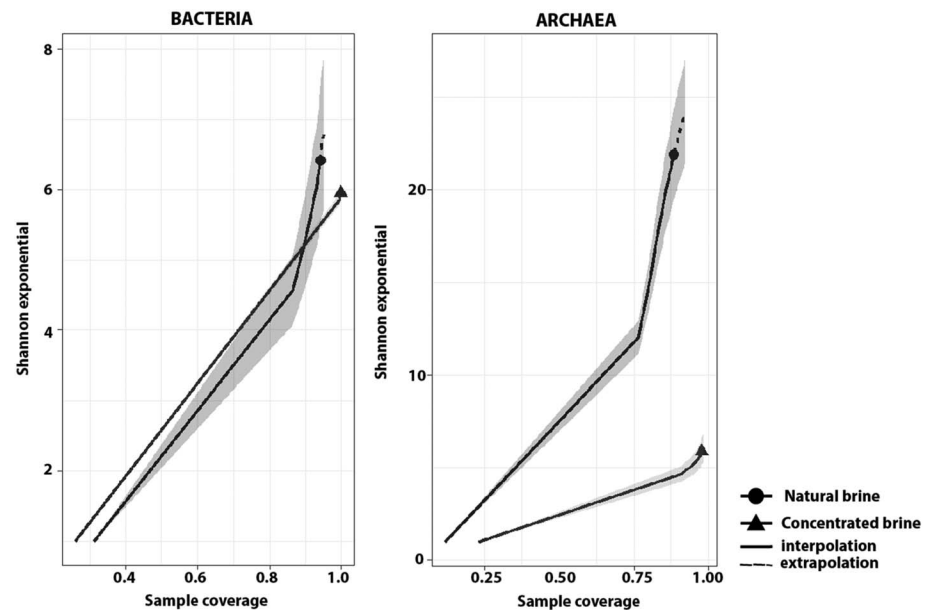


Figure 3. The Shannon exponential index between samples. The gray shadow shows estimated 95% confidence intervals.

concentrating the brines (Figure S1). There were no detectable concentrations of organic solvents, hydrocarbons, or oils in both brines.

3.2. Differences in Microbial Community Structure Between Brines

After postsequencing cleaning, 591 reads (grouped in 50 OTUs) were classified as Bacteria in the natural brine. A greater number of reads (45,731 grouped in 158 OTUs) was obtained from the concentrated brine. Conversely, in Archaea 1,194 reads (grouped in 193 OTUs) were obtained in the natural brine and 349 reads (grouped in 16 OTUs) from the concentrated brine. Bacterial richness was 3 times higher in the concentrated brine compared with the natural one, but bacterial diversity (Shannon exponential index) was similar in both brines (Figure 3). Archaeal diversity was lower in the concentrated brine (Shannon = 6) compared to natural brine (Shannon = 22). This change in brine microbial community composition was corroborated by quantification of 16S rRNA gene of Archaea through Q-PCR analysis (data not shown). The patterns of microbial richness shown in the samples was reflected by the results of phylogenetic comparisons: bacteria were more phylogenetically diverse in the concentrated brine (Figure 4a) and archaea was more phylogenetically diverse in natural brine (Figure 4b). These results show that natural brines are dominated by Archaea, while Bacteria dominate concentrated brines.

3.3. Microbial Community Composition

Natural and concentrated brines only supported a single halophilic archaeal family: the Halobacteriaceae, which differed in relative abundance at the genus level between the two brines (Figure S3a). This likely reflected differences in the concentration of salts of sodium chloride and lithium chloride. The most abundant archaeal genera (relative abundance) found in natural brine were *Halovenus* (26.8%), *Natronomonas* (20.1%), *Haloarcula* (14%), *Halobacterium* (13%), *Halorientalis* (4.6%), *Halomicroarcula* (1.7%), and *Halorubrum* (1.6%). In addition, the natural brine included a high relative abundance (17%) of unclassified reads in the family Halobacteriaceae. Other genera including *Candidatus Halobonum*, *Halobellus*, and *Halococcus* were present at low relative abundances (<1%).

In the concentrated brine, *Halovenus* was the most abundant archaeal genus (41%), followed by *Haloarcula*, *Halomicroarcula* and *Natronomonas*, which showed lower relative abundance relative to the natural brine. Further, unclassified reads within Halobacteriaceae family reached 2.3% of relative abundance in the concentrated brine. *Halobacterium* and *Halococcus* were the only genera displaying higher abundance in concentrated brine relative to natural brine. This was particularly marked in *Halococcus*, which represented 15% of reads in concentrated brines and only 0.5% in natural brines.

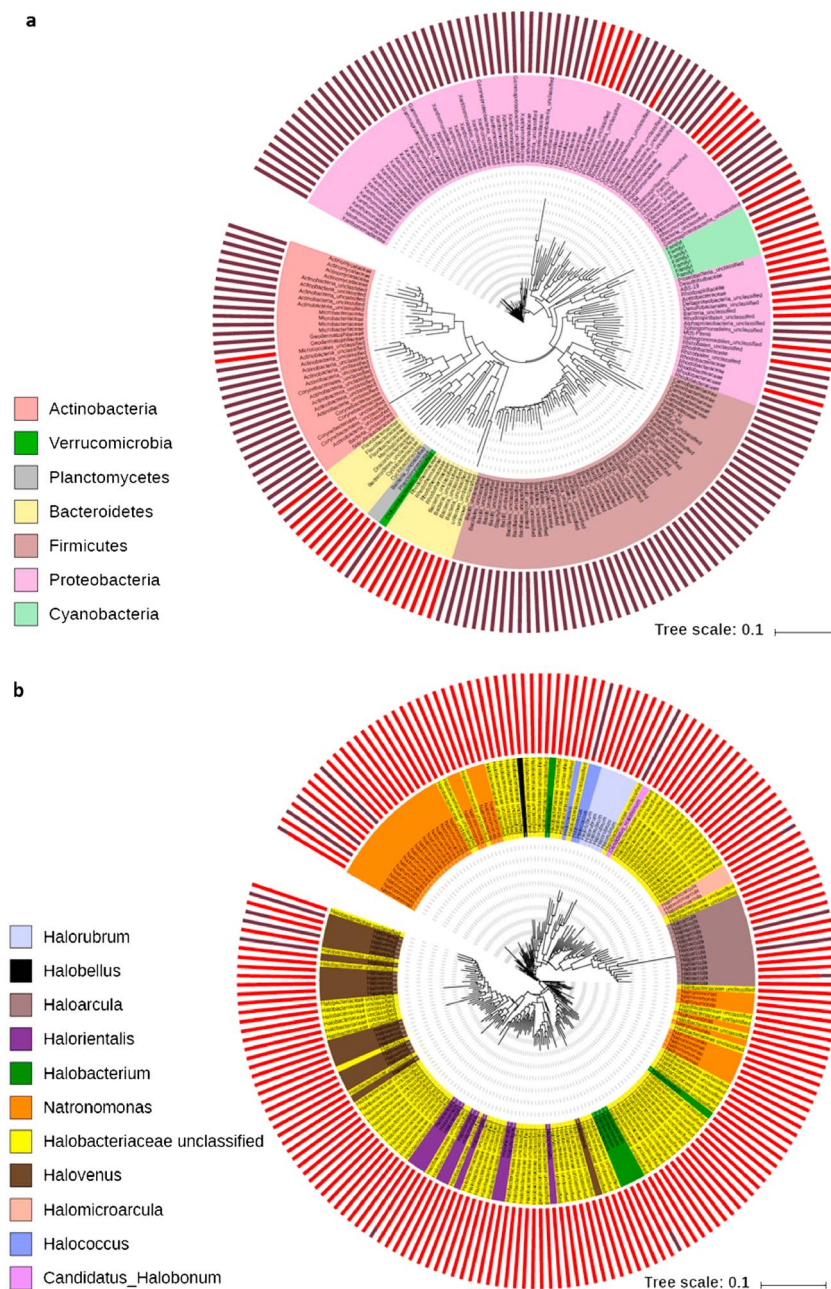


Figure 4. Phylogenetic relationship of taxa found in the samples. Barcharts in the outer ring show the abundance of OTUs in natural brine (red) and concentrated brine (brown). (a) Bacteria domain; (b) Archaea domain. Note that some OTUs occur in two samples. OTU = operational taxonomic unit.

We examined bacterial community composition at family level (Figure S3b), due to the large number of bacterial genera detected. Only 34 families were identified in total and 19 bacterial groups were considered as unclassified at order level. Together, both the Rhodothermaceae (Bacteroidetes) and 2B4 (Gammaproteobacteria) families contributed 65.1% of total bacterial composition in the natural brine. The family Rhodothermaceae (56% relative abundance) was solely represented by the genus *Salinibacter*: the family 2B4 (9.1% relative abundance) was represented by unclassified genera. However, other families also made considerable contributions including an undescribed family, represented by the genus *Thiohalorhabdus* made a contribution of 15.3% in the natural brine. A total of 8.9% was represented by unclassified reads associated with the order Oceanospirillales order, and 2.7% was represented by unclassified bacteria. Other families were detected but at a low relative abundance (<1%), including:

Rhodospirillaceae (0.8%), Family I (Cyanobacteria; 0.7%), Desulfobulbaceae (0.7%), Xanthomonadaceae (0.5%), Chromatiaceae (0.5%), Cyclobacteriaceae (0.3%), Draconibacteriaceae (0.3%), Rhodobacteraceae (0.3%), Flavobacteriaceae (0.3%), Alteromonadaceae (0.2%), Carnobacteriaceae (0.2%), Marinilabiaceae (0.2%), Oceanospirillaceae (0.2%), and Planctomycetaceae (0.2%).

A markedly different bacterial community composition was observed in concentrated brine relative to natural brine, for example, the Rhodothermaceae family was not detected, and the families Xanthomonadaceae (relative abundance = 39.8%) and Staphylococcaceae (26.9%) dominated the bacterial community. Within the Xanthomonadaceae, 99.9% of reads were unclassified; 99.9% of reads associated with the Staphylococcaceae were related with the genus *Staphylococcus*. In addition, unclassified Actinobacteria was detected with 17% of relative abundance. Other families including the Actinomycetaceae, Carnobacteriaceae, Family_XI (Cyanobacteria), and Rhodobacteraceae were detected at low relative abundances (between 1.8% and 2.4%). Fourteen other families were detected from concentrated brine with very low relative abundance (<1%). They were classified as Acetobacteraceae, Bacillaceae, Comamonadaceae, Corynebacteriaceae, Family_XIII (Cyanobacteria), Geodermatophilaceae, Litoricolaceae, Microbacteriaceae, Moraxellaceae, Paenibacillaceae, Pseudomonadaceae, Rhodocyclaceae, Solirubrobacteraceae, and Veillonellaceae. Of the reads classified at a family level, 61% were unclassified at genus level.

A comparison of the microbial communities (Archaea and Bacteria) retrieved from the two types of brine showed that only seven OTUs were found in common (Figure S4): all were Archaea (*Halobacterium*, *Natronomonas*, *Halomicroarcula*, *Halorientalis*, *Halococcus*, *Haloarcula*, and *Halovenus*).

4. Discussion

We examined two lithium brines (natural and concentrated brines): they showed markedly different compositions in terms of both their physiochemistry and their microbial communities. The main physicochemical differences between the two brines were viscosity and turbidity, where they were higher in the concentrated brine, giving it a yellow hue and an oily character. Elevated values in these parameters have been associated with high concentrations of salts (Jones & Talley, 1933; Onsager & Samaras, 1934). The Na⁺ and Li⁺ (principal ions of brines) are considered to be salting-out agents, as they decrease the solubility of nonpolar solutes in water (Breslow & Guo, 1990; Kunz et al., 2004) and induce the order in the water structure by increasing the number of hydrogen bonds, which increases the viscosity of the solution (Zangi, 2009). This last effect could be observed in the measurements of viscosity in brines (Table 1). Further, surface tension in both brines (80–86 mN/m) was high compared to values recorded from seawater (72.93 mN/m; Matubayasi et al., 2001).

The reduction in water availability in hypersaline environments represents a fundamental stress for cellular systems (Hallsworth et al., 2003). The lowest a_w value for growth recorded to date is 0.4 (Steinle et al., 2018). In this study, we observed that natural brine displayed values of a_w of 0.74. However, it was not possible to estimate this parameter from concentrated brine, due to the high concentration of salts (>500 g/L), a value 14 times higher than the mean salt concentration in contemporary oceans (35 g/L; Gargaud, 2011). In any given environment, salinity depends on several factors including the rate of evaporation (more evaporation resulting in an increase in salt concentration) and precipitation (more precipitation resulting in a decrease in salt concentration; Mahajan & Tuteja, 2005). The salinity values obtained in this study was compared with the most hypersaline lakes in the world (Table S2; Arabbeigi et al., 2014; Berube et al., 2007; Bratina et al., 1998; Brock, 1979; Craig, 1969; Dickson et al., 2013; Eder et al., 2001; Haynes & Hammer, 1978; Herbst, 1998; Jiang et al., 2006; Larsen, 1980; Quiroz et al., 2015; Rawson & Moore, 1944; Risacher & Fritz, 1991; Stivaletta et al., 2011). This revealed that natural brine from Salar de Atacama can be considered one of the world's most hypersaline natural environments (dominated by NaCl), in addition to Wadi Natrun Lakes (Zugm, Rizunia; Imhoff et al., 1979) and Lake Assal (Brisou et al., 1974). The concentrated lithium brines of the Salar de Atacama represent one of the most saline environments described to date (dominated by LiCl), followed by the Discovery brine, dominated by MgCl₂ (Yakimov et al., 2015). Furthermore, it has been reported that the lithium chloride shows a very high solubility (820 g/L at 20 °C) (Thies et al., 2002), explaining the extremely high salinities attained in these brines.

An extensive literature exists describing the presence of various taxa living in extremely saline conditions (e.g., Brisou et al., 1974; Imhoff et al., 1979; Pedrós-Alió, 2004; Trüper & Galinski, 1986; Ventosa et al., 1998); however, salinity in these studies typically reflects sodium salts. NaCl-dominated salterns have been reported to be dominated by Euryarchaeota (Kambourova et al., 2016; Maturrano et al., 2006) and

Gammaproteobacteria (Maturrano et al., 2006; Yeon et al., 2005), and to show high archaeal diversity (Andrei et al., 2012; Kambourova et al., 2016), similar to our results, where Euryarchaeota dominate the natural brine. Nevertheless, this does not occur in brines with other chemical compositions. For brines rich in CaCO_3 , microbial diversity is typically dominated by Deltaproteobacteria and Alphaproteobacteria (Foti et al., 2008). In the case of a deep-sea brine lake in the Mediterranean Sea rich in MgCl_2 , the environment was dominated by Bacteria from the Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria over Archaea (van der Wielen et al., 2005). In addition, a recent research has detected the presence of the first haloalkaliphilic members of the Candidate Phyla Radiation in soda lake sediments (Na_2CO_3), as well as the dominance of Firmicutes, Bacteroidetes, and Gammaproteobacteria over Archaea (Vavourakis et al., 2018).

It is likely that the relative composition of salts is not the only selective influence on microbial diversity in hypersaline environments but that physical state and water availability (e.g., brine or rock) also play important roles. In the case of the halite-rocks found in the Salar de Atacama, their saline composition (almost entirely NaCl) is similar to the natural brine studied here. However, their microbial composition is characteristically salt adapted, for example, *Cyanobacteria* sp., *Salinibacter* sp., *Halococcus* sp., and *Halorhabdus* sp. (Robinson et al., 2015) and is notably different from the high archaeal diversity seen in the natural brine.

Before this study, little was known regarding the microbiota associated with lithium evaporation ponds in Salar de Atacama. One report suggested that extremely saline (40% and 70% salinity) brines from the Salar de Atacama were devoid of life (Pedrós-Alió, 2004), and another report showed the presence of Archaea but not bacteria in this type of ponds (Demergasso et al., 2004). Recently, Haferburg et al. (2017) examined the microbial diversity in naturally lithium-rich brines from Salar de Uyuni, Bolivia (salinity ≤ 356 g/L), only reporting the presence of Archaea in its brines. These microbial groups were also found in our study, but we report the presence of both domains Bacteria and Archaea (Figures 4 and S3), in hypersaline lithium brines of Salar de Atacama (salinity = 556 g/L). The different results regarding the presence of bacteria likely reflect methodological differences, given that the results of high-throughput sequencing depend strongly on primer choice (Klinworth et al., 2013).

The high relative abundance of *Salinibacter* sp. (56%) found in the natural brine (dominated by NaCl) have been widely described, since it is a typical bacterium reported in hypersaline environments (Antón et al., 2000; del Mora-Ruiz et al., 2017; Maturrano et al., 2006). The principal families detected in the concentrated brines were Staphylococcaceae (99.9%) and Xanthomonadaceae-unclassified (99.9%). Some Staphylococcaceae have been reported as displaying halotolerance (Daoud et al., 2013). Furthermore, *Staphylococcus* spp. have been isolated from a hypersaline lake and solar salterns that have NaCl as a principal salt (Brisou et al., 1974; Ghozlan et al., 2006). However, to our understanding, the presence of *Staphylococcus* sp. in lithium hypersaline environments, as well as its halophilic capacity, has not been reported so far. Another striking result was the elevated number of unclassified reads from the family Xanthomonadaceae, as these groups are not typical groups in environments with an extremely high salt concentration, conversely the Xanthomonas strains has been isolated of contaminated soil and waste water (Chang & Zylstra, 2010).

The process of brine concentration via evaporation and subsequent harvesting of salts resulted in a change in the saline composition of the natural brine (high sodium/low lithium concentration) to a brine concentrated in lithium (low sodium/high lithium concentration). In addition, this change resulted in a marked shift in microbial diversity in initial/final brines of the process, with Archaea as the most phylogenetically diverse in natural brine, and Bacteria as the most phylogenetically diverse in concentrated lithium brine (Figures 4a and 4b). However, Archaea was the only group shared in both (Figure S4). We postulate that the factor driving tolerance to changes in salt composition and concentration in these microorganisms is their external structure. Halophilic Archaea such as *Halobacterium salinarum* have a surface layer (S layer), composed of single protein or glycoprotein species (Albers & Meyer, 2011). These cellular structures include large amounts of negatively charged amino acids including aspartate and glutamate (Bullock, 2000). When halophiles are present in saline environments such as brines, sodium ions neutralize the negative charges of the carboxyl groups, avoiding cell lysis (Trachtenberg et al., 2000). This could explain how natural brine has a higher richness in Archaea; however, this can be affected when the sodium concentrations decrease in the evaporation ponds, resulting in a decrease of Archaea and a subsequent proportional increase in Bacteria.

In evaporation pond brines, lithium can be considered as a stressing agent acting on microbial diversity selecting against lithium-intolerant microorganisms. This could explain the absence of extremely halophilic

bacteria such as *Salinibacter sp.* and *Halomonas sp.* (Haferburg et al., 2017) from concentrated brine. *Staphylococcus sp.*, *Xanthomonas sp.*, and Archaea present in the concentrated brine would likely developed new strategies to survive at high lithium concentrations. In the case of Bacteria, a lithium tolerant isolate classified as *Rhodococcus sp.* (Urbano et al., 2013) showed the overexpression of enzymes, such as degrader-Protease of misfolded or damaged proteins and proteins related a transcription and translation process, as response of osmotic stress (Belfiore et al., 2017). In parallel, intracellular accumulation of compatible solutes could be a key to survival, reflecting osmotic stress of sodium salt not only in halophiles (Oren, 2010) but also in the case of lithium. Halotolerant and halophilic aerobic bacteria utilize an osmoadaptation “salt-out” strategy, or the accumulation of osmotic solutes to avoid salt stress (Oren et al., 2002). Nevertheless, it has been reported that *Salinibacter ruber* display a “salt-in” osmoadaptation strategy (similar to the order Halobacteriales; Archaea). In growth conditions, when the concentration of NaCl is greater than 200 g/L, these bacterial cells accumulate high K^+ and Cl^- intracellular concentrations (Oren et al., 2002). This could explain the high abundance of *Salinibacter* in natural brines (where the concentration and ratio of Na^+/Cl^- are high and similar, respectively) and the absence of this genus in concentrated brines, where sodium concentrations decrease and chloride concentrations drastically increase. Furthermore, the salt-in strategy can be considered as an inflexible and very specific methods of osmoadaptation (Welsh, 2000), as all intracellular process must be functional at high NaCl concentrations; therefore, the change in chemical composition of brines (through evaporation process) could also act as a selective force on the microbial community. The presence of proteins spots for glutamine synthetase, glycerol-3-phosphate dehydrogenase (GDPH), and glycine dehydrogenase in *Rhodococcus sp.*, grown in lithium, could reveal information about lithium-resistance strategies, through of accumulation and synthesis of glutamine, glycerol and glycine, as compatible solute and chemical-chaperones for the correct folding of proteins (Belfiore et al., 2017; Diamant et al., 2001). Another possible strategy to survive in high lithium concentrations could be extracellular accumulation of lithium. This is supported by a previous study, which showed that Gram-positive bacteria are able to accumulate the highest concentrations of lithium, unlike gram-negative bacteria, fungi or yeasts, due to the presence of teichoic acids in their cell walls (Tsuruta, 2005). This accumulation of lithium by bacteria could be considered as a mechanism of lithium tolerance, as previously described for other metals (Lemire et al., 2013). However, to date, it has not reported a possible survival strategy to lithium in *Archaea*.

Microbial life found in contemporary extreme ecosystems can provide information about how early life evolved due to similarities with past environmental conditions (Gutiérrez-Preciado et al., 2018). For example, microbial mats located in the Atacama Desert have been considered as an analogue of major Precambrian environments (Gutiérrez-Preciado et al., 2018). Further, considering the dominant ions in this study (Cl^- , Li^+ , and Na^+) and the level of deliquescence of salts and water activity present in the saline systems (Davila et al., 2010), we suggest that each extreme saline environments represent a specific pattern of microbial life that can provide evidence regarding the ecology and geology of ancient ecosystems. Although extremely complicated, attempts to obtain ancient microbial life will likely provide important additional information about survival and adaptative mechanisms, for example, related to ionic composition of natural environments as Antarctic saline lakes (Matsubaya et al., 1979).

The salares (closed saline basins) of northern Chile are under continued threat, largely associated with water extraction to support mining, or the direct exploitation of their salts (Li and B). Salt extraction is in the early stages of development and will increase greatly in the near future to support the demands of commercial and domestic industrial development, especially those related with lithium-ion batteries for electric cars.

It is important to highlight that microbial life—representing the main organisms present in these extreme environments—requires protection within a framework of conservation. Our results clearly demonstrate the uniqueness of Salar de Atacama as an unusual and distinctive habitat for microorganisms. The unique features of these environments are important regarding not only the understanding of the microorganisms inhabiting high LiCl concentrations but also extend to other areas, including astrobiology. For instance, the presence of hydrated (hygroscopic) salts such as magnesium sulfate (Crisler et al., 2012), perchlorates of sodium, and calcium in Martian soils (Ojha et al., 2015) might be key to the development of life.

In recent decades, the Atacama Desert has been subject to an increased focus for astrobiological research, largely due to the presence of “Mars-like” soils (Bull et al., 2016; Navarro-González et al., 2003). These soils harbor active halophilic microbial communities (Ojha et al., 2015; Oren, 2013), specifically in areas with high

concentrations of NaCl, such as halite rocks (Robinson et al., 2015). The presence of abundant deposits of magnesium and sodium salts (major cations), as well as of sulfates and chlorides (major anions) on Mars (Clark & van Hart, 1981; Forte et al., 2016); and the abundant presence of lithium in meteorites (Shima & Honda, 1963) and stars including the Sun (Grevesse, 1968), potentially present important chemical similarities with the brines studied in this research.

Our results showing notably higher bacterial diversity in concentrated brines relative to natural brines, combined with the deliquesce effects of chloride in dry environments (like the Atacama Desert) in supporting an active microbial community in Halite (where at a specific relative humidity the crust is transformed to brines; Davila et al., 2010), have implications for potential life on Mars. It is not too difficult to believe that in deep brines (Orosei et al., 2018) and the chloride-rich deposits of Terra Sirenum (Glotch et al., 2010) of Mars could represent suitable habitat for extant or extinct Bacteria (similar than the concentrated lithium brines). If present, Martian-microbial life probably display a salt-out osmoadaptation strategy, allowing flexibility for variation in ionic composition and water availability.

Consequently, we propose that the brines of Salar de Atacama could represent a useful future model for studies examining biological resistance mechanisms under life-limiting conditions, as well as possible biological indicators for the search and probable development of extraterrestrial life.

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