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Comparative analysis of the microbial communities inhabiting halite evaporites of the Atacama Desert

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Summary. Molecular biology and microscopy techniques were used to characterize the microbial communities inside halite evaporites from different parts of the Atacama Desert. Denaturing gradient gel electrophoresis (DGGE) analysis revealed that the evaporite rocks harbor communities predominantly made up of cyanobacteria, along with heterotrophic bacteria and archaea. Different DGGE profiles were obtained for the different sites, with the exception of the cyanobacterial profile, in which only one phylotype was detected across the three sites examined. *Chroococcidiopsis*-like cells were the only cyanobacterial components of the rock samples, although the phylogenetic study revealed their closer genetic affinity to *Halothece* genera. Gene sequences of the heterotrophic bacteria and archaea indicated their proximity to microorganisms found in other hypersaline environments. Microorganisms colonizing these halites formed microbial aggregates in the pore spaces between halite crystals, where microbial interactions occur. In this exceptional, salty, porous halite rock habitat, microbial consortia with a community structure probably conditioned by the environmental conditions occupy special microhabitats with physical and chemical properties that promote their survival. [**Int Microbiol** 2010; 13(2):79-89]

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Introduction

The Atacama Desert of Chile is the driest desert on Earth, with a surface that has been minimally disturbed by natural erosion for millions of years [25,33]. The most arid parts of the desert span towards northern Chile, between the rain shadows of the Andes Mountains and the Coastal Cordillera [34]. The present-day location of Atacama, within the dry subtropical climate belt, is the principal cause of its aridity. These conditions have been maintained for some 150 million years, making it also the world's oldest continuously arid desert [25]. Regions within the hyperarid core, or Central Depression, of the Atacama Desert were, until recently, considered the dry limit of photosynthetic activity and primary production [48], with extremely low levels of culturable organisms and oxidized organic species detected in the soils [12,36]. According to Connon et al. [12], most of the microorganisms found in the soils could have been preserved from a time when the climate was wetter and/or were recently transported from other areas by wind. The virtual absence of

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photosynthetic organisms in the soil supports this idea. However, phototrophic and heterotrophic bacteria have been recently found inside halite evaporites (95–99% by weight NaCl) that form as bottom-growth crusts in the hyperarid core [49]. These crusts are the surface expression of thick (tens of meters) evaporitic deposits that were formed during the upper Miocene-Pliocene, approximately 3–5 Ma ago [42], and which are not linked to any surface or subsurface water supply. Instead, the communities inside the salt crusts take advantage of the hygroscopic properties of halite, which enhance the moisture conditions and allow for primary productivity and thus the survival of complex community activity [13].

The major constraint for microorganisms living in deserts is the availability of liquid water [5]. Water maintains the structure of intracellular macromolecules and membranes whereas its removal from the cells of desiccation-sensitive organisms irreversibly aggregates essential macromolecules and disintegrates cell components. These organisms are able to tolerate drought by maintaining a constant imbalance between the internal water content and external water availability [1]. As an environmental factor, desiccation has been mostly addressed at the cellular and molecular levels [22,41], but has received little attention at the community level [43]. Desiccation resistance is not a simple process. Indeed, within a given aggregate, cells occur in different physiological states, which may influence their desiccation tolerance [7,17]. Regardless of the mechanisms causing the death of only a few cells within a dry aggregate, it may be assumed that they nonetheless contribute to the survival of the others by providing physical protection and/or nutrients upon rewetting [6]. All desert microorganisms undergo extended periods in a desiccated, metabolically inactive state, in which individual cells are subjected to a variety of chemical and physical stresses, resulting in damage that may not be repaired until metabolism restarts [37].

In addition to desiccation, microbial communities inside halite crusts have to deal with conditions of extreme salinity. The habitats examined in this study are clearly at the top of the salinity scale, and salinity controls the diversity and functions of the resident microbial communities. Studies conducted along salinity gradients have revealed a decreasing trend in the diversity of bacterial, archaeal, and eukaryotic communities as salinity rises [4,9,43].

Given the special features of this habitat and the lack of previous extensive reports on the microbiota of this very peculiar hypersaline environment, this study was designed to examine the microbial communities colonizing halites in an environmental gradient through light microscopy (LM), transmission electron microscopy (TEM), and low-temperature scanning electron microscopy (LTSEM). A second aim was to conduct a broad phylogenetic analysis of the communities present in the halite. This allowed us to identify the major components of the heterotrophic community, which are not easily discerned by microscopy only, and to compare its phylogenetic position with those of other microbial ecosystems. Denaturing gradient gel electrophoresis (DGGE) analysis of amplified products of the 16S rRNA gene of eubacteria, cyanobacteria, and archaea was employed to assess community structure along an environmental gradient.

Materials and methods

Samples. Microbial aggregates were studied in halite samples from three large *salares* (salt flats) within the hyperarid zone: Yungay (24°05′53″ S, 69°55′59″ W), Salar Grande (20°55′44″ S, 70°00´48″ W), and Salar Llamara (21º 16.191′ S, 69º 36.975′ W). Yungay is the southernmost site and is the same area where soil microbiology studies have reported very low numbers of microorganisms and a complete absence of phototrophs [12,29,36]. Salar Llamara lies in the central zone of the Central Depression and Salar Grande is close to the western coastal range. To compare cyanobacterial composition, halite crusts from a site close to the Pacific coast and Iquique (Cerro de los Ríos, 20°20′35″ S, 70°01′11″ W) were also analyzed. At each sampling site, air temperature and relative humidity data were collected over 12 months, starting from May 2008, using temperature (T) and relative humidity (RH) probes with data loggers (Onset, HOBO Pro v2). RH/T probes were placed on the soil adjacent to the crusts in the rain shadow. Halite samples were collected from the sites in February 2007 and dry-stored in the dark at room temperature until their preparation (within a week). The day before processing, the samples were left in a chamber under conditions of 100% relative humidity and constant light to allow for revitalization. *Chroococcidiopsis* cultures were obtained from the Culture Collection of Autotrophic Organisms (CCALA), Institute of Botany, Academy of Sciences of the Czech Republic.

Light microscopy. Halite samples from 3–5 mm below the crust surface containing microbial consortia were scraped and dissolved in an increasing series of NaCl from 0 (water) to 5 M. After brief (5 min) precipitation of the scarce mineral particles, the supernatant was centrifuged at 12,000× *g* for 10 min. The pellet of microorganisms was resuspensed in 20 µl of 5 M NaCl and cells were observed in bright field and by digital image correlation (DIC) using an AxioImager D1 Zeiss light microscope equipped with a CCD color camera (AxioCam MRc Zeiss).

TEM. Specimens were prepared by dissolving 0.295 g of representative colonized halite in distilled water. Following brief (5 min) precipitation of the scarce mineral particles, the supernatant was centrifuged at 12,000× *g* for 10 min. The microbial cells precipitated were fixed following the protocol described by de los Ríos & Ascaso [14], with modifications. In brief, precipitates containing microbial cells were fixed in 3% glutaraldehyde in 5 M NaCl at room temperature for 3 h and then in osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr's resin. Poststained ultrathin sections were observed in a Zeiss EM910 transmission electron microscope equipped with a Gatan CCD camera (1024 × 1024 p).

LTSEM. Small fragments of colonized halites were mechanically fixed onto the specimen holder of a cryotransfer system (Oxford CT1500), plunged into subcooled liquid nitrogen, transferred to the microscope's preparation unit via

an air-lock transfer device, and then cryofractured following the protocol described in de los Ríos et al. [15]. After ice sublimation, the etched surfaces were sputter-coated with gold in the preparation unit and the specimens then placed on the cold stage of the SEM chamber. Fractured surfaces were observed under a DSM960 Zeiss SEM microscope at –135°C.

DNA extraction, PCR amplification of cyanobacterial 16S rRNA genes, sequencing, and phylogenetic analysis. Total genomic DNA was extracted from the halite samples obtained from the different sites using the UltraClean Microbial DNA isolation kit (Mobio) for subsequent PCR amplification of 16S rRNA genes. The cyanobacterial 16S rRNA gene was amplified using the cyanobacterial-specific primer pair CYA781r and CYA106f [38]. Each 25-µl volume of PCR mix [75 mM Tris pH 9.0/50 mM KCl/20 mM $(NH_4)_2SO_4$] contained 1 unit of Taq polymerase, 0.2 mM of each of the four dNTPs, 0.4 µM of each primer, 100 µg of bovine serum albumin, 1.5 mM of $MgCl_2$, 5 µl of 5×Taq Master PCR enhancer (Prime), and ca. 10–50 ng genomic DNA. The annealing temperature was 60°C. Products were cleaned on a QIAGEN quick spin column (Qiagen). Both complementary DNA strands were sequenced separately at the SECUGEN sequencing company (S. L. Madrid, Spain).

Sequences were aligned using Clustal X [47] and G-blocks [10]. DNA sequence alignments were phylogenetically analyzed using MrBayes version 3 software [http://morphbank.ebc.uu.se/mrbayes]. For the Bayesian analysis, the general time reversible model was used, including site-specific rate heterogeneity and a fraction of invariant characters (GTR+I+G). This model was suggested both by hierarchical likelihood rate tests and the Akaike information criterion, as implemented in Modeltest [40]. Eight million generations were run, every 100th tree was sampled, and the first 10,000 generations were discarded as burn-in. A consensus phylogram showing mean branch lengths was calculated using the sumt command in MrBayes. *Chlorofllexus aggregans, Deinococcus radiodurans* and *Thermus* sp. were used as outgroups for the phylogenetic tree.

DGGE profiles. A fingerprinting technique (PCR-DGGE) and sequence analysis of the resulting 16S rRNA gene bands were used to survey the genetic diversity of the area's microbiota. Bacterial and archaeal fragments of 16S rRNA genes suitable for DGGE analysis were amplified from total genomic DNA using the following primer pair: CYA359fGC and CY781r for cyanobacteria [38], 341fGC and 907r for eubacteria [35], and 344f-GC and 915R for archaeal amplifications [9]. The PCR procedure for cyanobacterial 16S rRNA gene amplification was the same as described above. The eubacterial and archaeal 16S rRNA genes were PCR-amplified using a touch-down protocol. Acrylamide gels (6%) with a 30–60% urea-formamide denaturing gradient were prepared following the manufacturer's instructions. Lanes were loaded with 22 µl of PCR product, run at a constant 200 V for 7 h at 60°C, and stained with ethidium bromide to visualize and photograph the resultant bands. The main DGGE bands (in terms of intensity and frequency of appearance) were excised, reamplified (using the primers devoid of the GC clamp), and sequenced, although sequences of satisfactory quality were not always obtained.

Results

Environmental conditions. All sampling sites fell within the hyperarid core of the Atacama Desert, where mean annual precipitation is <1 mm [33]. Mean, maximum, and minimum temperatures were very similar at all sites, except at Salar Grande, where minimum temperatures never fell below freezing. There were larger differences between the sites with respect to relative humidity (Table 1). The Yungay site was the driest, with 15–20% less precipitation than at the other sites.

DGGE profiles. The specific eubacterial primers revealed the presence of different bacteria in the samples (Fig. 1A). Distinct DGGE profiles were obtained for each site: 3–4 DGGE bands per lane for Yungay and Salar Llamara and 4–6 bands for Salar Grande. Bacterial profiles varied among localities, although two bands were common to all the communities (Bact-B and Bact-D). One band appeared only in the samples from Salar Llamara (Bact-E) and two were exclusive to the Yungay area (Bact-A and Bact-G), although they were not present in all samples. Another band was common to Salar Grande and Salar Llamara but was lacking in samples from Yungay (Bact-F).

DGGE analysis using the primers specific for cyanobacteria yielded the same profiles for the three localities, Yungay, Salar Grande, and Salar Llamara (Fig. 1B), indicating the presence of only one cyanobacteria phylotype. The archaea-specific primers rendered different DGGE profiles for the three sites (Fig. 1C). The number of bands ranged from 7 to 8, but only some were dominant. No major band was common to all three localities. One band was common to Yungay and Salar Grande (Arch-A) and a further band was shared by samples from Salar Llamara and Salar Grande (Arch-B).

Table 1. Air temperature and relative humidity data recorded at the sampling sites from May 2008 to May 2009. Mean, maximum and minimun values for this period at the three localities

	Temperature (°C)			Relative humidity (%)		
Site	Max.	Min.	Mean	Max.	Min.	Mean
Yungay	46.51	–8.00	17.83	91.90	1.40	36.93
Salar Grande	44.57	4.97	20.24	93.01	2.57	53.97
Salar Llamara	43.19	–6.40	17.59	89.10	1.90	50.79

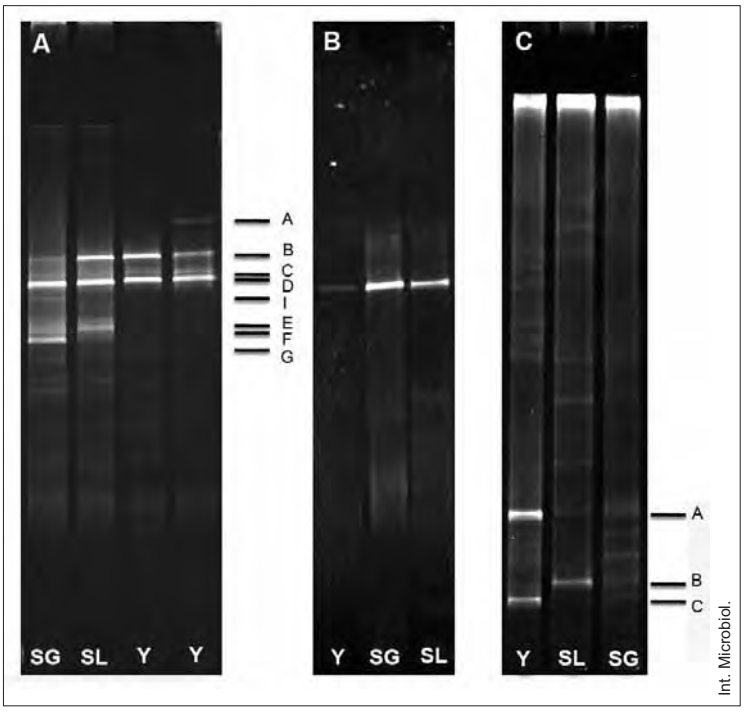

Fig. 1. Denaturing gradient gel electrophoretic profile of PCR-amplified 16S rRNA gene fragments obtained from halites sampled at different sites using: (**A**) the specific eubacterial primers 341fGC and 907r; (**B**) the specific cyanobacterial primers CYA359fGC and CYA781r; and (**C**) the specific archaeal primers 344fGC and 915r. Sampling sites: SG (Salar Grande), SL (Salar Llamara) and Y (Yungay).

Phylogenetic affiliations of the archaeal and bacterial community members. Five different sequences were obtained from the excised bacterial DGGE bands and three from the archaeal DGGE bands. Bacterial diversity showed a striking dominance of Bacteriodetes phyla from hypersaline environments (Table 2). One of the bands common to all three sites (Bact-D) corresponded to cyanobacteria. A band only occasionally observed in the Yungay samples (Bact-G) clustered with the actinobacteria sequences.

The only DGGE band obtained with cyanobacteria-specific primers in the three analyzed salterns corresponded to the Bact-D band in the eubacterial DGGE profile. BLAST analysis of archaeal sequences indicated that most band sequences corresponding to the three sites were related to those of uncultured unidentified archaea (Table 2). However, one band, Arch-C, which appeared only in samples from Yungay, showed 98% homology with *Halococcus salifodinae*.

Characterization of cyanobacteria according to the 16S rRNA gene sequences. Cyanobacterial DNA (coding for 16S rRNA) from genomic extracts of halite samples from each site was amplified to yield PCR products of approximately 592 bp. Through analysis of the resulting sequences, two distinct cyanobacterial sequences were detected. One of the sequences was amplified from DNA extracted from the Yungay, Salar Grande, and Salar Llamara samples and corresponded to sequences amplified from the DGGE cyanobacterial bands. The second sequence was obtained from specimens from Cerro de los Rios (locality close to the Pacific coast). The closest GenBank relatives were in both cases uncultured unidentified cyanobacterial clones and the identified microorganisms with highest 16S rRNA sequence similarity were *Halothece* sp. The position of the cyanobacteria found in the halite samples was resolved in a phylogenetic tree (Fig. 2). In the MrBayes SSU rRNA phylogenies, the sequences for samples from Yungay, Salar Grande, and Salar Llamara formed a separate, well-supported branch that also included an uncultured rock-inhabiting cyanobacterium and *Halothece*. The sequence for Cerro de los Ríos clustered together with that of a cyanobacterium isolated from lakes in western Australia and with the *Euhalothece* group sequences.

Light and electron transmission microscopy. In Yungay, Salar Grande, and Salar Llamara, only one morphotype of cyanobacterium was observed. Cells showing different developmental stages were observed after several hours of hydration (Fig. 3A). This cyanobacterium had features of *Chroococcidiopsis* cells (Fig. 3B). The cells divided by binary fission (Fig. 3C), which, as seen in three planes, produced more or less regular cubic-shaped cell aggregates individualized by a fibrous outer layer (Fig. 3A). Multiple fission occurred simultaneously in most of the aggregate's cells and was followed by the release of nonmotile baeocytes (Fig. 3D) similar in size to mature parental cells (diameter ~3 μm).

Table 2. Codes (DGGE bands), accession numbers, and closest relatives for the selected bacterial (Bact) and archaeal (Arch)

Band	Sampling site	Closest GenBank relative	Similarity
Bact-B [HM630144]	Yungay Salar Grande Salar Llamara	Uncultured Bacteroidetes bacterium clone from sediments of hypersaline lakes [DQ432319]	90%
Bact-D [HM630146]	Yungay Salar Grande Salar Llamara	Uncultured cyanobacterium from the Roman necropolis Carmona [FN298045]	95%
Bact-E [HM630143]	Salar Llamara	Uncultured bacterial clone SFA1G051 from a multipond solar saltern in Tunisia [CU467404] Uncultured *Salinibacter* [FN393499]	98% 98%
Bact-F [HM630142]	Salar Llamara Salar Grande	Uncultured bacterial clone from a hypersaline lake in the Sahara [EU869377] Uncultured *Salinibacter* [FN393499]	98% 98%
Bact-G [HM630145]	Yungay	Uncultured actinobacterial clone from saline brines in Cina [EU532513]	97%
Arch-C [HM630141]	Yungay	*Halococcus salifodinae* [AM159639] *Halococcus salifodinae* from Permo-Triassic rock salt [AJ13458]	98% 98%
Arch-A [HM630139]	Yungay Salar Grande	Uncultured archaeon from Permo-Triassic rock salt [AJ344320] *Natranobacterium* sp. [AJ878084]	98% 97%
Arch-B [HM630140]	Salar Llamara Salar Grande	Uncultured archaeon from the Dead Sea [GQ861364]	97%

Several cyanobacterial cells from Yungay had a denser outer fibrous layer (Fig. 3E) than that present in cyanobacteria from the other sites (Fig. 3F). This shell was observed in forms at different developmental stages. Cyanobacteria at different developmental stages were frequently accompanied by other microorganisms; perhaps the heterotrophic bacteria and archaea identified by the molecular techniques (arrows in Fig. 4A). These microorganisms were frequently associated with damaged cells (arrows in Fig. 4B) but also with living cyanobacterial cells (arrows in Fig. 4C). Heterotrophic microorganisms appeared closely associated with the outer cell wall layer (Fig. 4C) or the envelope of the multicellular aggregate (Fig. 3D). Algal and fungal cells were also occasionally observed in the Salar Grande samples but were not very common.

LTSEM revealed these microorganisms to be in close contact with halite crystals (Fig. 4D). The microorganisms formed aggregates in which extracellular polysaccharides were important structural components (arrow in Fig. 4E). These aggregates occupied the pore spaces, cracks, and narrow fissures among the NaCl crystals. In some places they appeared also in the proximity of areas harboring brine and empty pores that may have been occupied by air and water vapor (asterisks in Fig. 4F).

Discussion

Microbial communities inhabiting the hypersaline environment examined mainly comprise cyanobacteria along with heterotrophic bacteria and archaea. Ultrastructural features, observed by TEM, and DNA extraction from the halite samples confirmed the presence of viable microorganisms. Phylogenetic analysis of representative sequences of the domains *Bacteria* and *Archaea* indicated a close relationship between the microorganisms found in Atacama halite samples and those from other hypersaline environments. These results are consistent with the mixed microbial communities of cyanobacteria, heterotrophic bacteria, and archaea described in other rock substrates in deserts and saline environments [44,50].

Although all of the studied sites were within the hyperarid core of the Atacama Desert, their environmental conditions varied, mainly with respect to relative humidity.

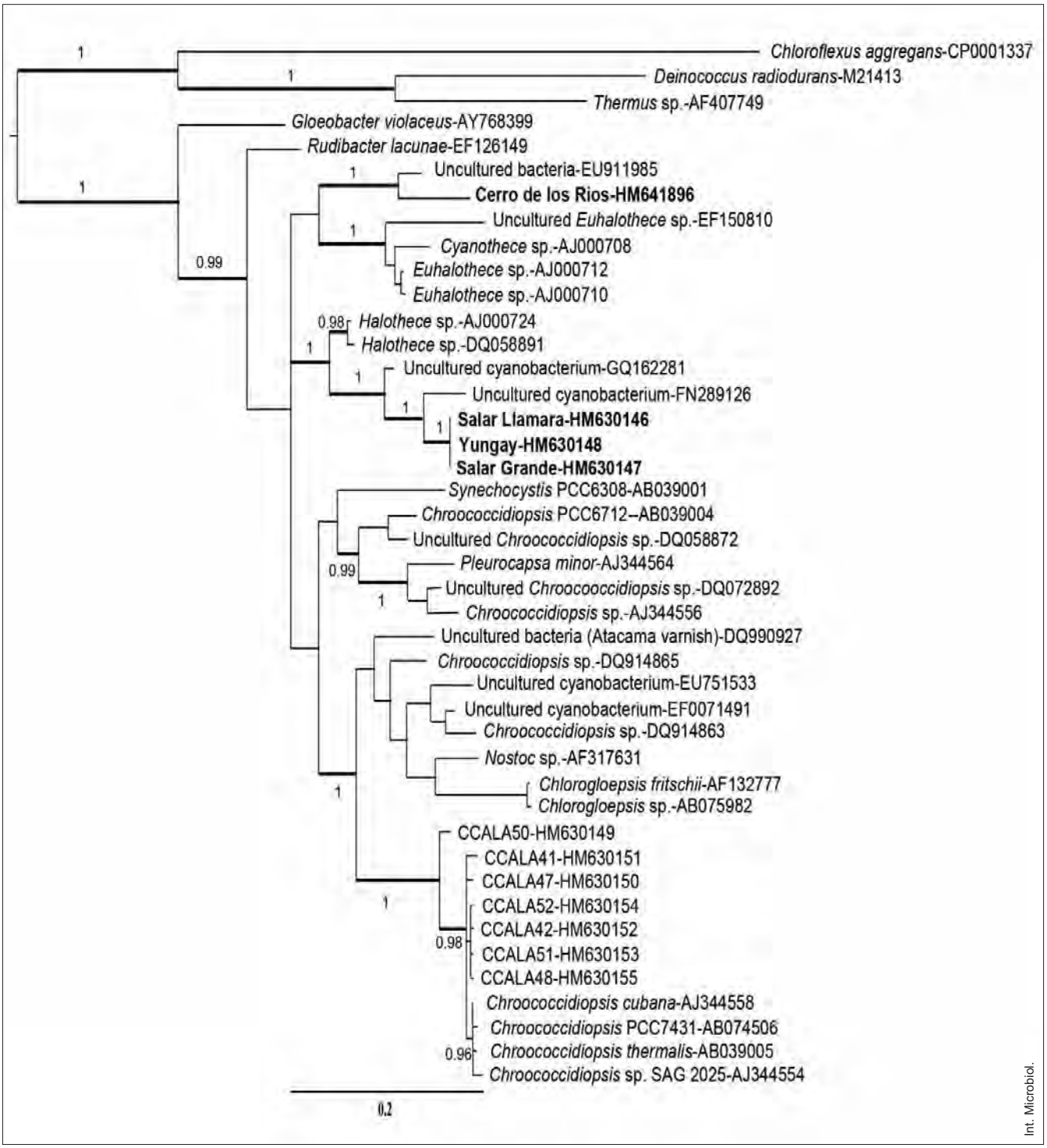

Fig 2. Consensus Bayesian phylogram based on the analysis of 592 nucleotides showing the relationships between the cyanobacterial 16S rRNA gene sequences of our Atacama halite isolates (bold), CCALA cultures, and those of 10 representantive *Chroococcidiopsis* and other known cyanobacteria retrieved from GenBank. Branches with strong support (probabilities >0.95) are in bold.

Lozupone et al. [30] argued that the main environmental determinant of microbial diversity is salinity. However, other authors [5,12] have claimed that water availability is the primary controlling factor for microbial activity and diversity along with community structure in desert soils. The halite crusts showed a low diversity that diminished across the sites

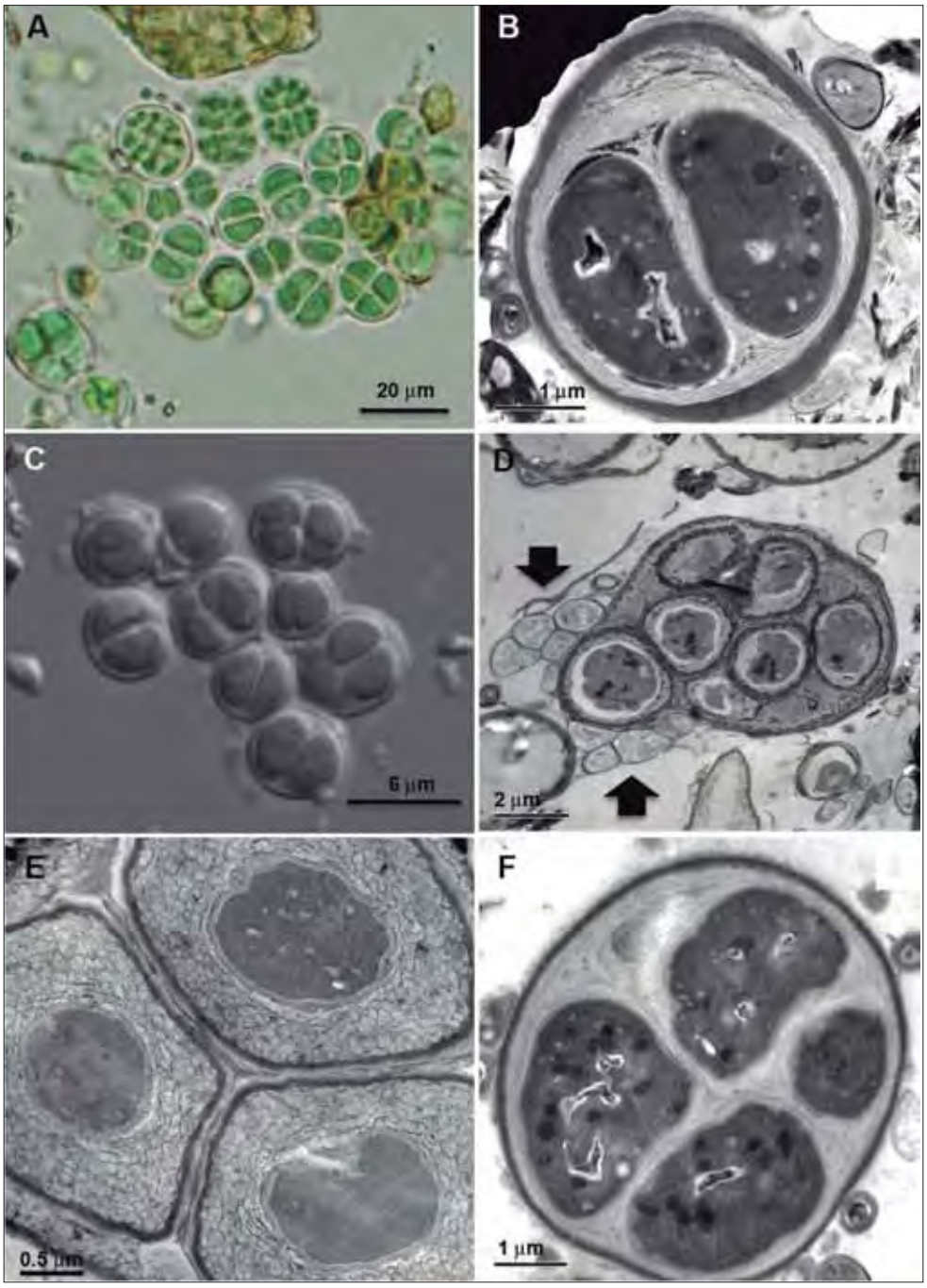

Fig. 3. Light (**A** and **C**) and transmission electron microscopy (**B, D, E,** and **F**) images of cyanobacteria colonizing Atacama halites. (**A**) Group of cells at different life cycle stages isolated from halite from Salar Grande. (**B**) Cells after binary fission in a sample from Salar Llamara. (**C**) DIC image of cyanobacteria in a sample from Salar Llamara showing binary fission in three planes. (**D**) Image showing the release of baeocytes from a multicellular aggregate with associated heterotrophic microorganisms (arrows). (**E**) Cell aggregate in a sample from Yungay with a dense outer fibrous layer. (**F**) Cell aggregate in a sample from Salar Llamara.

examined as aridity increased (measured by the mean annual RH), in agreement with the idea that, on the edge of life, diversity is reduced to a few adapted genotypes [27]. The fact that the composition of the halite crusts from all three sites was very similar suggests that differences in community structure are dictated, at least in part, by small differences in climate conditions, particularly moisture. However, differences between the microbial communities of saline environments can also be partially attributed to physical and chemical factors and the nutrient-enriched water source [3]. More exhaustive sampling is needed to establish which factors mainly affect microorganism distributions in different halite crusts.

Morphologically, cyanobacteria colonizing the halite crusts resembled *Chroococcidiopsis* cells. However, our phylogenetic data did not allow their inclusion in this genus since they had a closer relationship with the *Halothece* group (although, morphologically, these cyanobacteria were distant

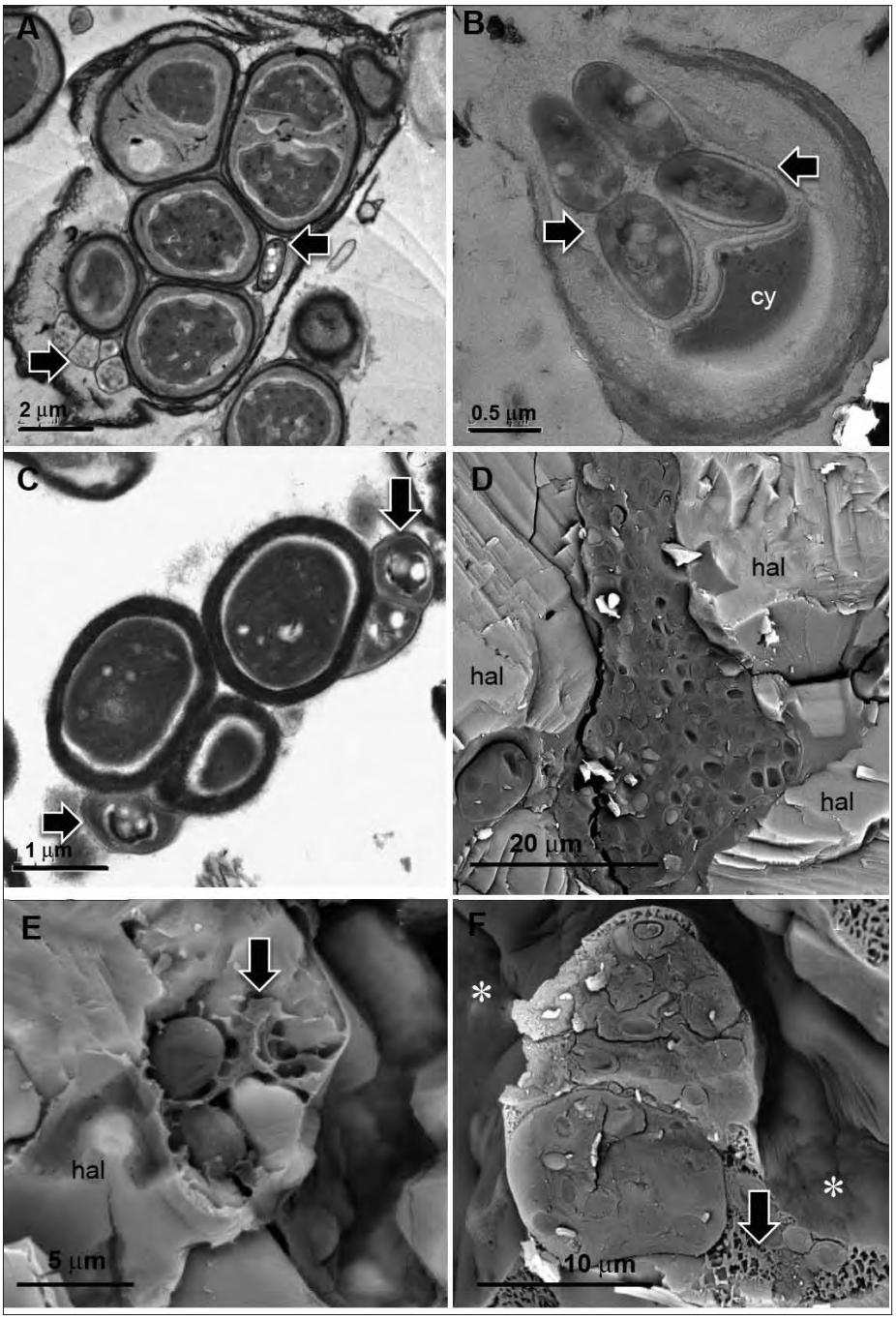

Fig. 4. TEM (**A, B**, and **C**) and LTSEM (**D, E**, and **F**) images of microbial aggregates colonizing halite from different sites. (**A**) Aggregate of cyanobacterial cells; note also the presence of different heterotrophic microorganisms (arrows). (**B**) Heterotrophic microorganisms (arrows) closely associated with a cyanobacterial (cy) damaged cell. (**C**) Heterotrophic microorganisms (arrows) closely associated with healthy cyanobacterial cells. (**D**) Cyanobacterial cells from Salar Llamara occupying a halite (hal) pore. (**E**) Cyanobacterial cells in a sample from Yungay showing the outer fibrous layer (arrow). (**F**) Microbial aggregate from Salar Grande, close to a high salt solution (arrow) and void (asterisks).

from the *Halothece* group). *Halothece* has been described as a monophyletic and monotypic taxon that thrives in hypersaline habitats [23,31]. These extremely halotolerant, unicellular cyanobacteria clearly differ from *Chroococcidiopsis* cells. *Halothece* are solitary or live in free groups of agglomerated cells but without a distinct enveloping slime sheath [31]. *Halothece*-like cells were detected in other halite samples from the Cerro de los Ríos (data not shown) whose 16S rRNA gene sequences clustered with *Euhalothece* group sequences (Fig. 2). The cyanobacteria in the halite crusts examined were not only genetically closely related but they also had ecological features, such as a preference for saline biotopes, in common with *Halothece* genera. Thus, genetic and ecological data need to be combined to resolve the taxonomy of the cyanobacterial strain colonizing these dry halite crusts. Ecological data cannot be ignored in assigning taxa to species, and at times they are critical even for assigning taxa to genera [26].

The presence of bacterial and archaeal phyla was detected by molecular analysis. Bacteriodetes closely related to *Salinibacter* was the dominant group of non-photosynthetic bacteria found in the crusts. In other saline environments, this group is among the dominant taxa and their relative abundance has been shown to increase with salinity [4,18,19,44]. Bacteriodetes was also previously found to be dominant in the hypersaline waters of the Salar Llamara [18].

While bacteria play an important role in some hypersaline environments [2], in others, although bacteria occur, archaea are dominant [3,8,32]. In salterns, an increase in archaea in parallel with salinity has been described. Most of the archaeal gene sequences obtained from the halite samples indicated a close relationship with an uncultured archaeon from hypersaline environments. The closest sequence was that of *Halococcus salifodinae*. Less similarity was found with sequences of the haloalkaliphilic *Natronobacterium*. Both sequences are related to those detected in Permo-Triassic salt rocks [20,24,46], which suggests an ancient origin of some of the archaea found in the Atacama deposits. However, our specimens also resembled archaea from another saline environment [39], such that an ancestral origin cannot yet be confirmed.

The bacterial and archaeal profiles recovered from the crusts appeared to be unique to that substrate. The microbial community found in these crusts most closely resembled that observed in the soil varnish of Yungay area. Clones belonging to eight major bacterial clades (including Cyanobacteria) were represented in the Yungay varnish [28], but *Eukarya* and *Archaea* were not detected. The dominant phylum in Yungay soils was Actinobacteria [12]; however, the actinobacteria found in the halite were more closely related to halotolerant actinobacteria than those described in the Atacama soils. Gypsum crusts are another colonized substrate in the arid core, and microbial communities containing cyanobacteria, accompanied by heterotrophic bacteria with a predominance of Proteobacteria, have been reported [21]. Remarkably, the cyanobacteria found in the gypsum crusts are not related to those in the halite crusts, although the two environments are separated by less than 100 km. Yet another example of a populated environment in the hyper arid core are small ponds in the Salar Llamara [18]. The bacterial profile of microbial mats from the ponds, both in terms of eubacteria and cyanobacteria, differs from that of the dry halite crusts of the same area, which show a lower diversity in terms of the number of gene sequence bands recovered and of the phylogenetic groups represented. Conversely, sequences closely related to *Salinibacter* that were extracted from the halite were not recovered in the microbial mats from nearby ponds.

Taken together, our results suggest that the communities found inside the halite are truly native to that substrate, and have adapted to the particular environmental conditions by colonizing this niche. Similar to the advantages provided by weathering-resistant varnish, which retains moisture and provides shelter from UV radiation [28], halite crusts may provide additional benefits that favor their colonization. Indeed, the hygroscopic properties of halite determine the presence of liquid water, the limiting factor for life in the Atacama Desert [13,15]. Simultaneously, halite crusts permit the passage of photosynthetically active radiation (PAR) and act as UV-light scatterers when precipitated as a mass of small crystals [11]. Thus, the interior of halite crusts seems to have unique microhabitats whose micro-environmental conditions cannot be found in soils or other lithic substrates of the area. Microbial activity in desert soils is highly dependent on environmental characteristics, such as temperature, moisture, and availability of organic carbon, but in lithic microhabitats these features usually differ from those of the external environment [16,45]. The particular characteristics of the halite microhabitat are determined essentially by its hygroscopic nature, although the special features of the intercrystalline spaces, occupied by air and/or high salt solutions, might also play an important role in conditioning the distribution and survival of microbial colonies.

The microorganisms colonizing the halites take the form of microbial aggregates associated with halite crystals. The close relationships observed among the components of these aggregates suggest interactions among them that could help in nutrient recycling and the functioning of this microbial consortium. The death of some members of the consortium may promote the life of another component [3,6]. Thus, organization as microbial consortia may be essential for the survival of the microorganisms analyzed.

In summary, our combined microscopy/DGGE analysis allowed characterization of the microbial consortia present in halites of the Atacama Desert and estimation of their diversity at different sites. Further studies based on cloning or massive sequencing techniques will be needed to determine the true biodiversity present in these Atacama halites and to complete this initial approach. The endoevaporite rock communities of the Atacama Desert grow in a salinity range that excludes a large number of physiological groups; yet this microhabitat emerges as a reservoir of microbial life adapted to a very hostile environment that may contribute to biogeochemical cycling in the system. Our results have important implications for the habitability and the potential for life in extremely dry environments on Earth and other planets. Since chloride salts have the same physical properties on Earth and on Mars, it is possible that the same deposits on Mars provided habit-

able conditions for microorganisms adapting to the increasingly arid conditions.

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