Endolithic cyanobacteria in soil gypsum: Occurrences in Atacama (Chile), Mojave (United States), and Al-Jafr Basin (Jordan) Deserts

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[1] Soil sulfates are present in arid and hyperarid environments on Earth and have been found to be abundant in soils on Mars. Examination of soil gypsum from the Atacama Desert, Chile, the Mojave Desert, United States, and Al-Jafr Basin, Jordan, revealed endolithic cyanobacteria communities just below the surface of soil gypsum samples. Optical and scanning electron microscope observations of the colonized layers indicated that the unicellular Chroococcidiopsis is the dominant cyanobacterium in all studied communities. 16S rRNA gene analysis revealed that in addition to Chroococcidiopsis, a few other cyanobacteria are present. Heterotrophic bacteria are also abundant in the colonized zones of the fine-grained gypsum from the Atacama and Mojave Desert, but insignificant in the fibrous gypsum from the Jordan Desert. Endolithic life forms similar to these described here may exist or have existed on Mars and should be targeted by the Mars Science Laboratory and future in situ missions.


1. Introduction

[2] Endolithic cyanobacteria have been reported to be present in a diverse range of environments on Earth, ranging from the frigid Dry Valleys of Antarctica to the hyperarid Atacama Desert of Chile [Friedmann, 1980; Friedmann et al., 1967; Wierczhos et al., 2006]. The types of rocks suitable for colonization by endolithic microorganisms are also diverse and include halite, sandstone, quartz, limestone, granite, and dolomite [Friedmann et al., 1967; Warren-Rhodes et al., 2006; Wierczhos et al., 2006]. Because these organisms typically inhabit cold and hot desert environments, some researchers have speculated that these or similar life forms may have existed on Mars (assuming life existed on the planet) [Friedmann and Ocampo-Friedmann, 1994; McKay, 1997; Warren-Rhodes et al., 2006; Wierczhos et al., 2006], as the planet changed from a warm, water-abundant world to a cold desert. However, some of these most preferred habitats for endolithic cyanobacteria on Earth (such as quartz and sandstone) are not likely widespread on Mars [Christensen et al., 2000], thus this Earth-based endolithic ecosystem model does not necessarily imply presence of similar life forms on Mars. There is a need to know whether the rock types known to be present on Mars are suitable for colonization by endolithic organisms.

[3] Recent reports indicate that gypsum rocks may also support endolithic communities. Parnell et al. [2004] observed cyanobacteria in impact-generated hydrothermal gypsum deposits in Devon Island, Canada. Hughes and Lawley [2003] reported a microbial endolithic community within gypsum crusts on sandstone boulders from Alexander Island, Antarctic Peninsula. Boison et al. [2004] studied N₂ fixation activities of cyanobacteria living in gypsum rock shards in the Harz Mountains in Germany. Gypsum and potentially other sulfates can provide a microenvironment that protects these organisms from exposure to extreme temperature, UV flux, and desiccation, yet they are sufficiently translucent to allow photosynthesis and N₂ fixation to occur in the rock [Boison et al., 2004; Rothschild et al., 1994]. Despite the fact that sulfate deposits are widespread on Mars [Clark et al., 1982; Clark and Van Hart, 1981; Cooper and Mustard, 2002; Klingelhofer et al., 2004; Gendrin et al., 2005; Langevin et al., 2005; McNamara et al., 2005; Squyres et al., 2004] and may be potentially important habitats for Martian life, endolithic life in soil gypsum has not been systematically studied, especially in dry desert environments.

[4] Sulfate minerals in the form of gyspic crusts are common within soils located in semiard to hyperarid deserts on Earth [Buck and Van Hoens, 2002; Buck et al., 2006a, 2006b; Herrero and Porta, 2000; Nettleton, 1991]. These crusts form as a result of either upward capillary migration and evaporation (per ascendum) and/or per descendum processes of downward leaching of soil waters. In both cases, sulfates accumulate in the soil because of a lack of sufficient water to remove the soluble salts. Sources of sulfate ions include in situ weathering of parent material or sulfide minerals [Carter and Insekip,
1988; Mermut and Arshad, 1987; Mrozek et al., 2006], fluvial or eolian input [Buck and Van Hoesen, 2002; Taimeh, 1992], and an atmospheric source from seawater [Podwojewski and Arnold, 1994; Rech et al., 2003; Toulkeridis et al., 1998].

In this study, we report endolithic cyanobacteria in soil gypsum crusts from several major deserts on Earth to identify if these surfaces may be a potential area to look for the presence of past life on Mars. Soil gypsum samples were collected from the Atacama, Jordan, and Mojave Deserts. Thin green layers (colonized zones) a few millimeters below the exposed surface were observed in all three samples. The microbial community within these colonized zones was studied by cultivation and molecular analysis. Our results indicate that photosynthetic cyanobacteria are either a predominant or a significant component in the total community, depending on the texture of gypsum. Heterotrophic bacteria are also present as important members in the community.

2. Methods

2.1. Samples and Study Areas

[6] Soil gypsum was examined for evidence of endolithic cyanobacteria during field work in the Atacama Desert, Chile, al-Jafar Basin, Jordan, and the Mojave Desert United States. At all locations, samples were collected after thin green layers (colonized zones) were observed within 1 cm from the top of the gypsum surface crust. Sample AT326b from the Atacama was collected in 2000 and samples JB1 (Jordan) and DG (Mojave) were collected in 2005. All samples were stored in sterile plastic bags until analysis in 2005. It is difficult to assess how much the microbial community in the Atacama sample changed during storage time, but we speculate any change should be minimal based on several reasons: (1) the sample had been stored dry at room temperature in a sealed zip-lock bag; (2) external layers were removed before any molecular analysis. Internal portions of the sample should have been isolated from any contact with air or moisture; (3) published literature documents that microbial community in low-permeability solid samples does not change in any significant way over an extended time period [Haldeman, 1997].

[7] The Atacama Desert is located in northern Chile between the Andes and the Pacific Ocean and is one of the driest locations on Earth. The core of the desert receives <10 mm/yr precipitation and lacks vascular plants [Houston and Hartley, 2003; Navarro-Gonzalez et al., 2003]. All soils in that region are composed of thick accumulations of soluble salts including sulfates, chlorides, and nitrates [Eriksen, 1981; Ewing et al., 2006; Buck et al., 2006a, 2006b]. Sulfates (gypsum and anhydrite) are most abundant at the top of soils. The Atacama sample was collected from a deflated surface crust of soil gypsum located on a mature alluvial fan in Llano de la Paciencia (22°49.045’S, 68°20.496’W), just north of the Salar de Atacama at an altitude of 2850 m. Colonized green layers were observable just below the surface of the gypsum in a limited area where the soil gypsum was exposed at the surface, but not in adjacent areas where the soil gypsum was covered by ~25 cm of overlying material. Because of limited exposure of soil gypsum, it was not possible to survey what percent of exposed soil gypsum was colonized. Precipitation at this locality is estimated to be ~10 mm/yr and average temperature to be ~13°C based on climate data from the Dirección General de Aguas, Chile, for stations at similar elevation along the Pacific slope of the Andes in this region of Chile. Vegetation cover is <2% and composed of Atriplex imbricata (alive) and Acantholippia desertica (dead).

[8] Al-Jafar Basin is a large internally-drained basin located in southeast Jordan. The landscape is largely devoid of vegetation and blanketed by a desert pavement of chert clasts with desert varnish. Sample JB-1 was collected from a secondary vein of fibrous gypsum within the Cretaceous Muwaqqar Chalk/Marl Formation that has been exposed by erosion on a pediment surface in the northern Al-Jafar Basin (30°30.479’N, 36°38.376’E) at an altitude of 940 m. Veins of fibrous gypsum and the green layers just below the surface were only found at this one location. Many fibrous gypsum rocks at this location are colonized, but in general, veins of fibrous gypsum at the surface are not widespread. Thus, it is difficult to assess heterogeneity of colonization patterns in the gypsum. Precipitation is estimated to be ~20 mm/yr and average temperature to be ~19°C, ranging from ~2°C to 35°C, based on limited climate data from the Jordan Meteorological Department for the nearby city of Al-Jafir. There is no vegetation in this area.

[9] The Mojave Desert sample DG was collected from the soil surface at the DryGyp site (36°23’10.60”N, 114°25’43.50”W) at an altitude of 474 m, located about 75 km northeast of Las Vegas, Nevada, United States. Colonized gypsum is commonly present in this region. Gypsum increases dramatically with depth, ranging from 14% in the surface to 90% within <20 cm, forming shallow petrogypsic horizons. Visual inspection reveals that the pattern of colonized gypsum is similar to one another, thus only one representative sample was analyzed. Soils in this area contain nearly pure gypsum that is cemented at depth to form petrogypsic horizons. Precipitation is ~150 mm/yr and the area has ~30% vegetation cover (Psorothamnus fremontii, Petalonyx parryi, Acacia greggii, and Eriogonum inflatum). Average temperature is ~20°C and ranges from ~3 to 48°C.

2.2. X-Ray Diffraction

[10] XRD analyses of powdered samples were performed by step scanning using a PANalytic X’Pert PRO diffraction system. CuKα radiation (K-alpha1 = 1.54060 A, K-alpha2 = 1.54443 A) was used at 40 kV, 40 mA with a 0.017° 2θ step interval and a step-counting time of 0.25 s. Diffraction patterns generated from a 6°–75° 2θ range were imported into X’Pert High Score software where sample mineralogy was determined by matching diffraction profile peaks via auto-identify and user defined database queries. Additional full mineralogical analyses using standard Soil Survey Methods (Soil Survey Staff, 2004) were performed on the Mojave Desert DryGyp sample at the National Soil Survey Center Soil Survey Laboratory in Lincoln, Nebraska.

2.3. Scanning Electron Microscopy (SEM)

[11] Freshly cleaved gypsum specimens were moistened in phosphate buffer saline (PBS) solution for 20 min, fixed in 0.5% formaldehyde (in PBS) and then in 1% glutaraldehyde (in PBS) each for 15 min, and dehydrated in ethanol series 15%, 30%, 50%, 75%, 95%, 100%, each for 15 min. After two additional changes in anhydrous ethanol, the
specimens underwent two soaks in hexamethyldisilazane (HMDS), each for 30 min. HMDS was decanted, and the specimens were air-dried. After gold-coating (75 s), the specimens were viewed under a JEOL scanning electron microscope JSM-5610 equipped with an Oxford ISIS energy dispersive spectrometer (EDS) at the University of Nevada Las Vegas Electron Microanalysis and Imaging Laboratory. SEM/EDS analyses were used to identify the relationship between gypsum crystals (with Ca and S peaks) and organic tissues (only C peaks).

2.4. Cultivation and Light Microscopy

[12] Experiments were set up to enrich cyanobacteria from the colonized layer of the Atacama and the Jordan sample using a general medium for cyanobacteria (i.e., BG11) according to a published procedure [Rippka et al., 1979]. Enrichment cultures were examined under light microscopy. DNA extraction was also performed on the enriched Jordan sample to confirm the identity of the enriched cyanobacteria.

2.5. Molecular Microbiology - Clone Library Construction

[13] Genomic DNA was extracted from the colonized zones of each sample and from an enrichment culture from the colonized layer of the Atacama sample. Extraction was performed with an Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA). The uncolonized zone of the Atacama sample was also analyzed as a background control. The samples were designated as follows: Atacama-col and Atacama-cont stand for colonized (green layer) and control (nongreen layer) Atacama gypsum, respectively, Jordan for the Jordan sample, and Mojave for the Mojave sample. Purified DNA was PCR-amplified according to the procedure of Failsafe Kit (Epicentre Biotechnologies, Madison, WI). Primer sequences for bacteria were Bac27F and Univ1492 [Jiang et al., 2006]. PCR conditions were the same as previously described [Jiang et al., 2006].

[14] The PCR product was ligated into pGEM-T vector (Promega Inc., Madison, WI) and transformed into E. Coli DH5α competent cells. Gene clone libraries of 16S rRNA were constructed, and a sufficient number of randomly chosen colonies per sample were analyzed for insert 16S rRNA gene sequences until rarefaction curves were fully saturated. However, only twelve clones were sequenced for the enrichment culture from the Jordan sample and rarefaction curve was not saturated. Plasmid DNA containing inserts of 16S rRNA gene was prepared using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA). Sequencing reactions were carried out with primer Bac27F for bacteria with a BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, CA, United States). The 16S rRNA gene sequence was determined with an ABI 3730 automated sequencer. Sequences were typically ~700–800 bp long. The sequences were aligned with ClustalW. Phylogenetic analyses of partial 16S rRNA gene sequences were conducted using molecular evolutionary genetics analysis (MEGA) version 2.1. Neighbor-joining phylogenies were constructed from dissimilatory distances and pair-wise comparisons with the Jukes-Cantor distance model. The sequences determined in this study have been deposited in the GenBank database under accession numbers EF071489–EF071546.

3. Results

3.1. Texture and Mineralogy of the Gypsum Samples

[15] The Atacama and Mojave samples are composed of massive, micritic to spar-sized gypsum. The colonized zone is located at 1–3 mm below the surface (Figures 1a and 1b). The Jordan sample is made of vertical bundles of clear, fibrous gypsum (selenite) (Figure 1c). In this sample the colonized zone is located deeper into gypsum, ~4–10 mm below the surface. It has a width of 3–14 mm and displays a more transitional (less contrast) appearance from the uncolonized to the colonized zone. The colonized zone also displays a blackish (dark-green) coloration (Figure 1c). In the Atacama and Mojave samples the colonized zones are concentrated around microtopographic lows or depressions in the soil gypsum crust.

[16] X-ray diffraction patterns revealed that the Jordan sample is pure gypsum with no detrital soil materials. The Atacama and Mojave samples are mostly gypsum (>95%), but also contain trace amounts of insoluble dust materials (quartz, feldspar, kaolinite, montmorillonite, and mica).

3.2. Light Microscopy and Scanning Electron Microscopy

[17] Light microscope observations revealed that cyanobacterium Chroococcidiopsis is a dominant organism in the enrichment culture from the colonized zone of the Atacama sample (Figure 1d). Similar observations were made for the Jordan enrichment culture. This identification was based on the characteristic morphology and texture of this organism.

[18] SEM observations of the colonized layers of all three gypsum samples revealed that all studied gypsum specimens contain abundant unicellular cyanobacteria, which range in diameter from 2.5 to 4.5 μm (Figures 2a–2e). The Mojave specimen contains, in addition to the unicellular forms, a small number of filamentous cyanobacteria (Figure 2d). In the Mojave specimen both the unicellular and filamentous cyanobacteria are covered by exopolymer polysaccharide (EPS), which obscure their morphology. In contrast, organisms in the other specimens appear to have no or little EPS and have a smooth appearance (Figures 2a, 2c, and 2e). In these specimens, evidence of binary cell divisions was observed. Small cocci and rod-shaped bacteria are present in all the communities, but are especially abundant in the Jordan specimen (Figures 2a and 2b). In all specimens, the organisms appear to occupy preexisting crevices and pore spaces, and exhibit no signs of boring. However, dissolution of gypsum crystals, possibly by organisms, was occasionally observed (Figure 2e).

3.3. Bacterial Diversity

3.3.1. Autotrophic Cyanobacteria

[19] Cyanobacteria are the most abundant organisms in the Jordan enrichment culture (3 out of 12 sequences) (Figure 3). In the three gypsum samples, cyanobacterial sequences constitute the most abundant group in the colonized zones of the Atacama and the Jordan sample (25 and 18 out of total 73 and 23 clone sequences, respectively) (Figures 3 and 4), but are a small component (9 out of 51) in
the colonized zone of the Jordan sample. The majority of clone sequences in this group (13, 13, and 9 out of those 25, 18 and 9 cyanobacterial sequences) are closely (96–99% similarity) related to hypolithic cyanobacterial clone sequences (AY644697) recovered from translucent quartz stones in the arid and hyperarid regions of the Atacama desert [Warren-Rhodes et al., 2006]. The second most abundant group of sequences forms a cluster with Chroococcidiopsis sp. BB96.1 (AJ344555) (Figure 3), a photo- biont of the lichen Peltula euploea in South Africa [Fever et al., 2002]. Seven sequences from the colonized zone of the Atacama sample form a cluster with uncultured Antarctic cyanobacteria (AY151733, DQ181678) [Taton et al., 2006, 2003].

3.3.2. Heterotrophic Bacteria

[20] Each group of the heterotrophic community is described below.

[21] Alphaproteobacteria: Members of the Alphaproteobacteria group are present in all samples, except in the Jordan sample (Figures 3 and 4). However, four sequences in the enrichment culture from the Jordan sample belong to this group. Most clone sequences from the colonized zone of the Atacama sample (Atacama-col) are related to the Rhizobiales group (such as Mycoplana dimorpha or Rhizobium tae- nense), bacterium of the rhizosphere (AJ252588), and Sphin- gomonas sp. from Lake Vostok accretion ice [Chrismer et al., 2001] (Figure 3). All sequences from the uncolonized regions of the Atacama sample (nongreen layer) are related to an uncultured alpha proteobacterium (X97079) from peat in Germany [Rheims et al., 1996]. The majority of clone sequences from the Mojave sample (along with 3 sequences from the Jordan enrichment culture) are nearly identical to Caulobacter sp. (AJ227770), a common bacterium in aquatic environments [Abraham et al., 1999]. Some sequences from the Mojave sample are related to Methylobacteri- um fujisawaense (AB175634) or iron-oxidizing acidophile Y005 (AY140237) (Figure 3). Members of the genus Methylobacterium are ubiquitous in nature [Green, 2001], including soil, dust, freshwater, and lake sediments. Strain Y005 was isolated from sites in Yellowstone National Park [Johnson et al., 2003].

[22] Gammaproteobacteria: The Gammaproteobacteria group is most abundant in the uncolonized region of the Atacama sample, a significant group in the Jordan sample, a minor group in the Mojave sample, and absent in the colonized layers of the Atacama sample (green layers) (Figures 3 and 4). The most dominant group of clone sequences (8 from the Atacama-cont and 1 from the Mojave sample) are >99% similar to gamma proteobacterium HTB082 isolated from deep-sea mud samples (2759 m) [Takami et al., 1999]. Another group of 3 sequences from the Atacama-cont sample are nearly identical to Stenotro- phonomas maltophilia. A group of 4 sequences from the Jordan sample are 95% similar to Pseudomonas plecoglos- sicida isolated from a rice field (GenBank description).

[23] Betaproteobacteria: One sequence from the Jordan enrichment culture is nearly identical to Ralstonia metallidurans.

[24] Deltaproteobacteria: Two sequences from the uncolonized zone of the Atacama sample are closely (>98% similarity) related to an uncultured hydrocarbon seep bacterium GCA017 (AF154102) (GenBank description).

[25] Verrucomicrobia: Three sequences from the colo- nized zone of the Atacama sample are related to (95% similarity) an uncultured Xiphinemato bacteriaceae bacterium from a pasture soil (GenBank description).

Figure 1. Samples of soil gypsum with cyanobacteria: (a) Soil gypsic crust sample AT326b from the Atacama Desert; (b) soil gypsic crust sample DG from the Mojave Desert; (c) fibrous gypsum sample JB1 from a secondary vein exposed by erosion at the surface from Al-Jafr Basin, Jordan; (d) light micrograph of cyanobacterium Chroococcidiopsis in an enrichment culture from sample AT326b from the Atacama Desert.
Firmicutes: This group is insignificant in the uncolonized region of the Atacama sample and in the Mojave sample, a minor component in the colonized zone of the Atacama sample, and absent in the Jordan sample (Figure 4). Three sequences from the Atacama-cont sample are related to unidentified Hailaer soda lake bacterium F27. The other three, along with two from the Mojave sample, are grouped with Bacillus sp. Z-0521 (DQ675454), an anaerobic alkaliphilic bacterium isolated from a soda lake (GenBank description) (Figure 3). Other Mojave sequences are related to either Desulfovosphorosinus sp. A10 (AJ582756) or Alkaliphilus transvaalensis (AB037677). A common nature of the Firmicutes sequences is that they are related to alkaliphilic bacteria (with the exception of Desulfovosphorosinus sp.) isolated from aquatic environments.

Figure 2. SEM micrographs of fractured soil gypsum specimens from (a) Jordan, revealing unicellular cyanobacteria and coccoid bacteria; (b) Jordan, showing rod-shaped bacteria; (c) Atacama site (AT326b), unicellular cyanobacteria and cell divisions (arrows); (d) Mojave, unicellular and filamentous cyanobacteria covered by exopolymer polysaccharide (arrows); (e) Jordan, unicellular cyanobacteria with crosscutting relationship between the cyanobacteria displaying cell division (arrow, lower right) and a cluster of tabular pseudohexagonal gypsum crystals indicating dissolution of gypsum crystals during the growth of the cyanobacteria cells. Scale bar is 5 μm.
Actinobacteria: The Actinobacteria group is minor or absent in all samples (Figures 3 and 4). The sequences in this group are related to uncultured bacteria from various soil environments [Lueders et al., 2006, 2004; Nemergut et al., 2004].

Gemmamonadetes: Four sequences from the Atacama-col sample are remotely related to (~90% similarity) uncultured Gemmimonadetes bacteria from soils [Mumey and Stahl, 2003].

Planctomycetes: Two sequences from the Atacama-col sample are remotely related to (85% similarity) uncultured Planctomycetes in river biofilms [Brummer et al., 2004].

Bacteroidetes: Three sequences from the Atacama-col sample are related to (97% similarity) uncultured Bacteroidetes bacterium in soils of Marble Point and Wright Valley, Victoria Land, Antarctica (GenBank description).

Unclassified bacteria: A small number of sequences in each clone library could not be classified. They were related to uncultured bacteria in the air of Texas (DQ129347) (GenBank description) and an agricultural soil (AY037635) [Furlong et al., 2002; Lu et al., 2006].

4. Discussion

4.1. Presence of Cyanobacteria in Gypsum Rocks

Several studies have documented endolithic cyanobacteria in gypsum from wet environments. Parnell et al. [2004] presented photographic evidence for the presence of cyanobacterial layers (possibly Gloeocapsa and Nostoc) in impact-generated hydrothermal gypsum deposits in Devon Island, Canada. Hughes and Lowley [2003] reported a microbial endolithic community within gypsum crusts found on the surface of sandstone boulders at Two Step Cliffs, Alexander Island, Antarctic Peninsula. The endolithic community was localized and only a few isolates were studied. Occasional snowmelt was the major source of water for these organisms. Boison et al. [2004] studied N₂ fixation activities of Chroococcidiopsis-dominated cyanobacteria in gypsum rock shards in the southern part of the Harz Mountains in Germany, but they did not characterize the

Figure 3. Neighbor-joining tree (partial sequences, ~700 bp) showing the phylogenetic relationships of bacterial 16S rRNA gene sequences cloned from three desert samples and one enrichment culture (Jordan) to closely related sequences from GenBank. One representative clone type within each phylotype is shown and the number of clones within each phylotype is shown at the end (after the GenBank accession number). If there is only one clone with a given phylotype, the number “1” is omitted. Clone sequences from this study are coded as follows, with Atacama-colB3 as an example: Atacama-col: colonized gypsum (green bands) from Atacama; B, bacteria; 3, clone number. Thus, it reads bacterial clone 3 from the colonized gypsum (green bands) of the Atacama sample. Scale bars indicate Jukes-Cantor distances. Bootstrap values of >50% (for 500 iterations) are shown. Aquifex pyrophilus is used as an outer group, and a single tree showing all bacterial sequences is created.

Figure 4. Pie charts showing frequencies of all phylotypes affiliated with the major phylogenetic groups in the bacterial clone libraries for all the samples.
cyanobacterial community and habitat. Warren-Rhodes et al. [2006] noted microbial colonization in soil gypsum in the Atacama Desert, but they did not analyze the community. Cyanobacterial colonization is commonly observed in gypsum crusts associated with evaporite deposits [Douglas and Yang, 2002; Douglas, 2004; Sanz-Montero et al., 2006] and salt evaporation ponds [Oren et al., 1995; Kedar et al., 2002; Ionescu et al., 2007], but environmental conditions at these locations are different from dry desert soils.

[33] In this study, we have demonstrated that soil gypsum crusts are an important substrate for cyanobacterial colonization in the dry deserts of Mojave, Jordan and Atacama. At the Atacama sampling site, occasional summer precipitation (~10 mm/yr) is an important source of moisture. Coastal fog does not penetrate into this region of the Atacama. To sustain a microbial community, gypsum crusts would have to retain sufficient moisture for a long period of time after a rainfall [Buck and Van Hoesen, 2002]. High humidity levels occur only during occasional precipitation events.

[34] Endolithic cyanobacteria typically grow a few millimeters below the exposed rock surface [Warren-Rhodes et al., 2006; Wieraczos et al., 2006]. The depth at which endolithic cyanobacteria colonize rocks appears to be determined by a physiological balance of several important factors. Whereas greater depth helps retain moisture and increases the level of protection from intolerable irradiation, high temperature, and arid surface condition, at the same time these microorganisms have to acquire a minimum amount of light and air (CO₂ and N₂) required for photosynthesis and N₂ fixation [Boisson et al., 2004; Rothschild et al., 1994]. Cockell et al. [2002] specifically investigated the shielding effect of rocks. A 5-min exposure of Chroococcidiopsis sp., a desiccation-tolerant, endolithic cyanobacterium, to martian-surface UV and visible light flux led to a 99% loss of cell viability. Under 1 mm of sandstone, however, the same organism could survive (and potentially grow) if water and nutrient requirements for growth were met.

[35] This physiological balance is apparently seen in our gypsum samples. Cyanobacteria colonize deeper into the clear form of gypsum (selenite) from the Jordan Desert than the massive microcrystalline form of gypsum from the Atacama and Mojave Deserts. The clear selenite form of gypsum is believed to facilitate the penetration of photosynthetically active radiation and UV radiation to the cyanobacterial colonies [Parnell et al., 2004]. Thus, in order to find their optimal niche (reduced light intensity and O₂ tension in addition to water), the organisms would have to reside deeper into the crystal. In order to cope with increased level of UV penetration into the clear selenite crystal, a black coloration (Figure 1c) may be developed by the synthesis of ultraviolet screening compounds [Cockell et al., 2002; Parnell et al., 2004]. In contrast, the massive and micritic form of gypsum from the Atacama and Mojave Desert do not exhibit any black coloration in the colonized zones and the colonization depth is shallow, only within a few mm from the surface, probably because of the poor light penetration through the massive gypsum. Although the observed colonization pattern (depth and coloration) appears to be correlated with the gypsum texture, it is cautioned that this observation is based on a limited number of samples. A better understanding of colonization and rock texture requires a systematic study with a larger sampling size.

4.2. Bacterial Diversity in Soil Gypsum Crusts from Desert Environments

[36] The clone library approach provides information on bacterial diversity, but the estimate of the relative proportion of each group (Figure 4) is qualitative. We note some degree of inconsistency in biomass estimation of cyanobacteria between visual observations (both optical and electron microscope observations) and molecular identification. Under optical and SEM, more than 95% of biomass appeared to be cyanobacteria, but 16S rRNA gene analysis revealed that cyanobacteria constitute a much lower percentage in the overall population. There are several possibilities that may be responsible for this inconsistency. Firstly, some amount of contamination by heterotrophic community from the uncolonized regions of gypsum (uncolonized by cyanobacteria) may be possible, thus lowering the percentage of cyanobacterial biomass in the colonized zone. Second, it may be difficult to release DNA from certain cyanobacterial cells, thus resulting in an underestimation of the cyanobacterial population by the molecular method. Thirdly, PCR may introduce some bias.

[37] Although the clone-library approach is qualitative in estimating the relative proportion of different microbial groups, it should be accurate to reveal diversity, especially when rarefaction curves are saturated. It is striking to note the difference in the microbial community structure among the studied gypsum samples (Figure 4). The community structure in the Atacama and the Mojave sample consists of 8 and 6 phyllogenetic groups, respectively. However, only 3 groups are present in the Jordan sample. One primary difference between the Jordan sample and the other two is the gypsum texture. The amount of precipitation between the Jordan and the Atacama sampling sites is similar, at 20 and 10 mm/yr, respectively, both of which are significantly lower than the Mojave Desert at 150 mm/yr. In both the Atacama and Mojave sample, gypsum occurs as fine-grained, massive assemblages, but in the Jordan sample, it occurs as clear crystal (selenite). It is possible that the clear form of gypsum allows more sunlight to penetrate so that photosynthetic cyanobacteria may be more competitive in this environment. Again, it is cautioned that this observed relationship between the microbial diversity and the gypsum texture is based on a limited number of samples.

[38] A secondary factor in controlling cyanobacterial abundance may be the amount of precipitation. Cyanobacteria are a significant group in the colonized zones of all three samples, but in different abundance (Figure 4). Qualitatively, the cyanobacteria group is more abundant in drier deserts (Atacama and Jordan), suggesting that a dry desert environment may favor cyanobacteria. In wetter climates where organic matter may be readily available, heterotrophic bacteria may be more dominant. In addition to the effect on the cyanobacterial abundance, the amount of precipitation apparently exerts an effect on the amount of EPS production. Our data indicate that in the samples from the drier sites (Atacama and Jordan), the amount of EPS is less than that in the wetter site (Mojave). EPS production may be related to the metabolic state of cells. When bacterial cells are near the limit of survival, they simply have no energy reserves to produce EPS. Stress level in the dry sites (especially at Atacama) may be near the limit because (1) cyanobacteria are dominated by Chroococci-
diopsis and there are no filamentous forms; (2) growth is binary instead of simultaneous divisions. Under such a stress condition, EPS production may be minimal. Under relatively wet conditions, such as the Mojave site of this study and Antarctic [de los Rios et al., 2004, 2007; Omelon et al., 2006], EPS are typically abundant in biofilms of endolithic microbial communities.

[39] The composition of the heterotrophic bacteria in the three desert environments is generally consistent with previously identified groups. Except in the Jordan sample, the Alphaproteobacteria group constitutes an important component in the overall community (Figure 4). Warren-Rhodes et al. [2006] identified clone sequences recovered from quartz stones in the Atacama Desert that belong to Alphaproteobacteria. The Alphaproteobacteria group occurs between Copiapo and Altamira with a precipitation of 21 and 4.7 mm/yr, respectively. Our gypsum sample was collected in an area that receives ~10 mm/yr precipitation and thus it is not surprising to observe this group in our gypsum sample. Abundant Alphaproteobacteria have also been detected in Beacon sandstone samples from the McMurdo Dry Valleys of Antarctic [de la Torre et al., 2003]. The authors report that some members of this group may potentially be capable of aerobic anoxygenic photosynthesis. However, caution must be exercised to infer any physiological functions based on relatedness of clone sequences to known cultures. Future work is needed to determine the functions of this important group in desert environments. Clone sequences that belong to Gammaproteobacteria have also been recovered from quartz stones in the core arid region of the Atacama Desert [Warren-Rhodes et al., 2006]. Our data show that this group may be significant in the normal Atacama soil gypsum (without cyanobacterial colonization).

[40] The other important groups, i.e., Gram positive Actinobacteria and Firmicutes, Gemmatimonadetes and Planctomycetes, have been detected in the Atacama Desert [Drees et al., 2006; Navarro-Gonzalez et al., 2003]. Navarro-Gonzalez et al. [2003] found that surface soil samples from the Atacama Desert, except for the driest Yungay region, contain 6 to 26 distinct taxa, most of which belong to the Gram positive Actinobacteria and Firmicutes. These two groups are detected in our normal gypsum sample without cyanobacterial colonization. Drees et al. [2006] detected Gemmatimonadetes and Planctomycetes phyla in the Atacama soil at a depth of 25–35 cm.

4.3. Endolithic Microbial Communities: The Last Stage of Life on Mars

[41] Life on Mars, assuming it ever arose there, probably originated about 3.5–4.0 Ga years ago, when the planet was warm and temperate [Carr, 1996; McKay, 1997; McKay and Davis, 1991]. As it dried and cooled, however, life would withdraw into protected refuges [Friedmann and Koriem, 1989; McKay et al., 1992]. The earliest such refuges were likely in ice-covered lakes, similar to those found in Antarctic Dry Valleys [Friedmann et al., 1987; McKay, 1997; Priscu and Christner, 2004]. The next stage of existence, after liquid water disappeared from the planet surface, would probably be endolithic microbial communities [McKay et al., 1992].

[42] The concept of endolithic life on Mars was initially based on the discovery of the cryptoendolithic communities in the Antarctic desert (high altitude regions of the Dry Valleys) [Friedmann et al., 1987]. These communities live inside sandstone, the most common endolithic substrate available in the Dry Valleys. The protective role of the substrate is well established. During the austral summer, the rocks are warmed by solar insolation and can melt snow. At the same time, sufficient sunlight penetrates the sandstone to support photosynthesis. It was suggested, therefore, that similar communities might have existed on Mars.

[43] In the original concept, the endolithic model was a general ecological model of survival on Mars [Friedmann et al., 1987; Friedmann and Ocampo, 1976]. However, it has been recently pointed out that quartz and sandstone are unlikely to occur on Mars [Christensen et al., 2000]. Quartz is a late stage mineral that requires a long geological period of plate tectonic activity, which is lacking on Mars [Head and Solomon, 1981; Solomon, 1978]. In light of these recent findings, the endolithic model is implied not to be directly applicable to study the biological history of Mars.

[44] Sulfate deposits have been detected as the predominant salts on Martian surface [Cooper and Mustard, 2002; Gendrin et al., 2005; Langevin et al., 2005; Squyres et al., 2004] and the presence of endolithic life in gypsum crystals thus revives the endolithic model. Understanding where life can and can not occur on Earth helps narrow the search of life on Mars. Sulfate soil crusts in the hyperarid Atacama Desert may serve as a potential Mars analog [Navarro-Gonzalez et al., 2003]. In addition to sulfate deposits and aridity, the hyperarid core region of the Atacama Desert has three other “Mars-like” characteristics, i.e., low levels of refractory organic material, low number of detectable soil bacteria, and the presence of one or more chemical oxidants [Navarro-Gonzalez et al., 2003; Wierzchos et al., 2006].

[45] Cockell and Raven (2004) used a radiative transfer model to study four micro-habitats in which damaging UV radiation could be reduced to levels tolerable to life, but where light in the photosynthetically active region would be above the minimum required for photosynthesis. These habitats include sediments containing ferric iron impurities, halite crystals, snow-ice covers, and impact-shocked crystaline rocks. Interestingly, massive halite was identified as one favorable habitat. Massive halite (sub-millimeter crystals) acts as an effective screen for UV radiation because halite crystals scatter radiation, not because NaCl is an effective UV absorber. Gypsum is not an effective UV absorber either, and the authors speculate that life protection under gypsum crusts would depend on its scattering property and/or presence of UV absorbing contaminants in gypsum such as iron. Despite the fact that gypsum as a potential habitat has not been demonstrated with such a model prediction, its role in life protection certainly can not be ruled out. Additionally, chemical data indicate that sulfate salts are more common than halite on Mars, so gypsum may be more likely to provide suitable widespread habitats on Mars.

[46] In addition to serving as a Mars analog, existence of life in salt deposit offers additional significance for exobiology research. On Earth, ancient microbial life, including some phototrophic organisms, can be preserved in sedimentary salt deposits [Bell, 1989; Coolen and Overmann, 1998; Kunte et al., 2002]. If inside fluid inclusions of salt crystals,
such microbial life may survive in salt for millions of years [Vreeland et al., 2000]. Thus, it may be possible to not only search for modern life on the planet, but also to study extinct Martian life and its evolution. Such investigations likely require a combination of multiple approaches, such as direct detection of microbial life, as well as biomolecules, geochemical, and mineralogical biosignatures left behind from past biological activity. Ancient soil sulfate crusts preserved in Miocene paleosols in the hyperarid Atacama Desert may provide valuable materials for studying ancient life on Mars.

Regardless of modern or ancient microbial life, microbial colonization under translucent rocks, such as gypsum and halite, offers advantage for detection by remote instrumentation. Depending on gypsum texture (massive vs. selenite), cyanobacterial community may occur only a few mm below the surface. If so, this community may be detectable by remote, nondestructive spectroscopy methods. Edwards et al. (2005) demonstrated that important biomolecular signatures from a cyanobacterial community a few millimeters below the surface can be detected by Raman spectroscopy. Before any Mars samples can be successfully returned to Earth, such remote-sensing based techniques continue to be an important source of information.

5. Conclusion

In this study, we have presented evidence that endolithic cyanobacteria colonize soil sulfate crusts in several major deserts around the world. Although only a limited number of samples were studied, our results show that the colonization pattern depends on the texture of the gypsum. The endolithic cyanobacteria preferentially colonize below microtopographic depressions where water can pond and infiltrate into the gypsum. The texture of the gypsum also appears to have a strong influence on the optical properties of the gypsum and the preferred habitat for endolithic cyanobacteria. In the micritic and massive gypsum from the Atacama and Mojave Desert, colonization occurs at a depth of only a few mm below the surface. In the fibrous gypsum from the Jordan Desert, the colonization depth is greater, up to 14 mm. The optimal colonization depth appears to be achieved by balancing acquisition of adequate light, O₂, and N₂ for photosynthesis with water retention and protection from adverse environmental conditions. In addition to cyanobacteria, heterotrophic bacteria are also an important component in the community. Both precipitation and gypsum texture are important in affecting bacterial diversity and the community structure. These results are important in defining future targets to look for modern and ancient life on Mars.

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