Published in final edited form as:

Antonie Van Leeuwenhoek. 2019 July ; 112(7): 1105–1119. doi:10.1007/s10482-019-01244-0.

Cultivation and Characterization of the Bacterial Assemblage of Epsomic Basque Lake, BC

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Abstract

Athalassohaline waters that are rich in divalent ions are good analogues for the chemical environments of Mars and the ocean worlds. Sulfate salts, along with chlorides, are important in Mars regolith with Ca, Fe, Mg, and Na counterions. Certain lakes in the Pacific Northwest are saturated with MgSO₄ as epsomite. Here we report on the microbial community of Basque Lake, BC, a group of playas that is saturated with MgSO₄. More than 60 bacterial isolates were obtained from Basque Lake soils by enrichment culture and repetitive streak-plating using media containing 10% (~1.7 M) NaCl or 50% (~2 M) MgSO₄. Most of the isolates (~75%) were Gram-positive, motile, and produced endospores. Isolates related to *Marinococcus halophilus* and *Virgibacillus marismortui* dominated the collection. *Halomonas* and *Salinivibrio* were Gram-negative genera found at Basque Lake. Nearly all of the Basque Lake isolates grew at 50% MgSO₄, with 65% growing at 60% MgSO₄. Several isolates could grow in saturated (67%) MgSO₄ ($a_w = 0.90$). All of the isolates grew at 10% NaCl with 70% growing at 20% salinity (~3.5 M NaCl; $a_w = 0.82$). Basque Lake isolates grew better at basic pH than acidic pH, with 80% growing at pH 9 and 30% growing at pH 10. Only 20% of the isolates grew at pH 5. Numerical taxonomy dendrograms based on 44 phenetic characteristics showed a strong correspondence to phylogenetic trees constructed from 16S rRNA gene sequences. Pyrosequencing of 16S rRNA gene sequences from direct DNA extracts of Basque Lake soils recovered predominantly *Proteobacteria* (60%), Firmicutes (11%), and unclassified bacteria (27%). Microbes capable of growth under the extreme chemical conditions of Mars are a particular concern for forward planetary protection should they contaminate a spacecraft.

Keywords

astrobiology; epsomite; extremophiles; halotolerance; Mars; salinotolerance

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Authors' Contributions

JDC, FC, BCC & MAS conceived and designed the study. JDC, FC & MAS performed research and analyzed data. JDC and MAS wrote the paper.

Compliance with Ethical Standards

Human and animal rights statement Neither animal nor human subjects were part of this work.

Introduction

Athalassohaline lakes of the Canadian Plains are rich in sulfate salts including Glauber's salt and epsomite (Hammer, 1978, 1986; Hammer and Haynes, 1978; Last and Slezak 1988; Nesbitt 2004; Last and Ginn 2005). The vast majority of the lakes in this region (53 of 60) are dominated by sulfate anions, with most of these exceeding 80 eq. %. Epsomic lakes are relatively rare natural environments that are hypersaline (high in salts), but not hyperhaline (high in NaCl). Microbiologists have mainly worked in environments rich in NaCl and few studies have investigated epsomic environments. Early observational work at Hot Lake, WA, an area permanently saturated with MgSO_4 , reported green sulfidic mats, consistent with *Chlorobium*, *Oscillatoria*, and *Plectonema* (Handy 1916; Anderson 1958; Hammer 1978, 1986). Microbes able to grow at high concentrations of MgSO_4 , those that are epsotolerant, have been reported from soils and constructed environments (Markovitz 1961; Markovitz and Sylvan 1962; Laiz et al. 2000; Mandrioli and Saiz-Jimenez 2002).

Our community analysis at Hot Lake (Kilmer et al. 2014) was preceded by a study of the epsotolerance of a diverse collection of halotolerant bacteria from the Great Salt Plains (GSP) of Oklahoma, a hypersaline NaCl environment (Crisler et al. 2012). While selected for their halotolerance, the GSP isolates showed remarkable epsotolerance. Strong growth was observed in the presence of 50% MgSO_4 (~2 M as epsomite) by the vast majority of GSP bacterial isolates, including *Bacillus* and *Halomonas*. The isolate collection from Hot Lake soils was rich in Gram-negative bacteria, including *Halomonas*, *Idiomarina*, and *Marinobacter* (Kilmer et al. 2014). *Bacillus*, *Marinococcus*, *Planococcus*, and *Virgibacillus* were observed among the Gram-positive bacteria, as were the Actinomycetes *Nesterenkonia* and *Nocardiopsis*. No archaea were isolated, which may not be surprising given that halophilic archaea require higher NaCl concentrations than present in Hot Lake (Schneegurt 2012). Hot Lake bacteria were tolerant to 50% MgSO_4 and many grew at higher concentrations, including saturated MgSO_4 (Kilmer et al. 2014; Wilks et al. 2019). None of the isolates were epsophilic, requiring high MgSO_4 concentrations for growth. Random 16S rRNA gene clone libraries constructed from direct DNA extracts of Hot Lake soils recovered Gram-positive bacteria (e.g. *Bacillus*, *Clostridia*) and many clones related to uncultured Actinomycetes. The Gram-negative clones included *Acidovorax*, *Coxiella*, *Legionella*, and *Deltaproteobacteria*. Nearly one-third of the clones from Hot Lake were from uncultured candidate divisions. Deep sequencing of Hot Lake mat communities showed a predominance of cyanobacteria (*Tychonema*), Chlorobia, Chromatia (*Halochromatium* and *Thiohalocapsa*), Halomonads, and Verrucomicrobia (Lindemann et al. 2013).

Basque Lake is similar to Hot Lake in several respects, particularly in being an environment saturated with MgSO_4 . It is a series of epsomic playas, with small pools interspersed by muddy soils. Due to the similarity of its composition to potential brines on Mars, it was the subject of a study using IR spectroscopy to detect the signatures of life (Hyde et al. 2007; Foster et al. 2010). While enrichment cultures for halotolerant microbes were positive, no isolates or microbiological analyses were reported. A study on the survival of microbes in evaporating brines of relevance to Mars, included inocula from Basque Lake (Fox-Powell et al. 2016). The initial community in sulfate-rich medium had moderate diversity, with *Arthrobacter*, *Bacillus*, and *Virgibacillus*. No microbes from Basque Lake formed colonies

after the evaporation of sulfate-rich brines. A recent study used molecular methods to describe the microbial community of Spotted Lake, an epsomic lake in the same region as Basque Lake (Pontefract et al. 2017).

The microbial populations of Basque Lake were characterized in the current report using cultivation and molecular techniques. Sixty-two salinotolerant bacterial isolates were purified and then identified through phylogenetic analysis of 16S rRNA gene sequences. These isolates were further characterized by measuring their epsotolerance, halotolerance, and metabolic capabilities. Deep sequencing of rRNA genes from soil extracts revealed additional microbial diversity. The microbial community at Basque Lake is compared to those found at epsomic Hot Lake and the hyperhaline GSP. Our findings are placed in the astrobiology context of planetary protection and life detection.

Materials and Methods

Site Description and Sample Collection

Water and lake margin soils (~100 g from top 4 cm) were collected during summer using sterile tools and containers from Basque Lake, BC at three locations: S1 (50° 36' 02.5" N 121° 21' 32.2" W), S2 (50° 35' 59.8" N 121° 21' 27.6" W) and S3 (50° 35' 58.9" N 121° 21' 27.2" W) (Fig. 1). Composite samples were collected from five spots within a 1-m² areas of the raised mud that separates the epsomic playa pools and not from sediments or algal mats. Samples were mixed and divided into an aliquot for molecular work that was frozen in the field and kept frozen during transport on dry ice and an aliquot for live culture work that remained fresh at ambient temperature. The soils were predominantly a grey and black fine clay and silt, often with a strong sulfidic odor, and saturated with salts. The geochemistry and hydrology of Basque Lake has been reported previously (Hammer, 1978, 1986; Hammer and Haynes, 1978; Last and Slezak 1988; Nesbitt 2004; Last and Ginn 2005).

Microbial Enrichment and Isolation

Direct plating, liquid enrichment, and dilution plating were used to isolate halotolerant and epsotolerant microbes from Hot Lake waters and soils. The media used were based on SP medium (Caton et al. 2004), a nutrient-rich and moderately saline (10%, ~1.7 M) medium containing per liter: NaCl, 98 g; KCl, 2.0 g; MgSO₄·7H₂O, 1.0 g; CaCl₂·2H₂O, 0.36 g; NaHCO₃, 0.06 g; NaBr, 0.23 g; FeCl₃·6H₂O, 1.0 mg; trace minerals, 0.5 ml; tryptone, 5.0 g; yeast extract, 10.0 g; glucose, 1.0 g; final pH 7.0. This was prepared with either 10% NaCl, 10% (~0.4 M) MgSO₄, or 50% MgSO₄. While these media were useful for liquid enrichments, agar plates with 50% MgSO₄ would not gel sufficiently, so isolates from liquid enrichments in this medium were transferred to plates with 10% NaCl medium. Enrichment cultures (100 ml) were maintained as shake-flasks on a rotary shaking platform (2.5-cm stroke dia) at 150 rpm and incubated at 7, 25, or 37 °C, before aliquots (100 µl) were plated at 24 or 48 h after inoculation. For dilution plating, samples (10 g) were diluted 10-fold with appropriate media and serially diluted prior to plating. In some cases, soil aliquots (approximately 1 g) were spread directly onto the surface of plates. Culture plates were maintained in moist boxes and colonies were collected after several days or more.

Colonies arising on the plates were selected for isolation based on gross colony morphological and physiological features, including pigmentation, size, margin, or rate of growth. Colonies were transferred to fresh SP agar plates with 10% NaCl or 10% MgSO₄ and isolated using the streak plate method. Each isolate was subjected to at least five successive streak platings to ensure clonal purity. The isolates were archived as 50% glycerol stocks at -80 °C and as agar slants.

Characterization of Isolates

SP medium composition was modified with different concentrations of NaCl or MgSO₄ to measure salinity tolerances in liquid shake-tubes. Similarly, the pH of SP medium was modified to determine growth tolerances. Results are reported as the maximum and minimum conditions for growth. A threshold of 0.05 OD units was used for positive growth and inconclusive tests were repeated. Pearson correlation coefficients were calculated using 2-tailed tests within SPSS.

Characterization of the bacterial isolates followed protocols outlined previously (Caton et al. 2004; Litzner et al. 2006). All isolates were Gram-stained using the Protocol Gram-staining kit (Fisher Diagnostics) following the manufacturer's instructions. Acid-fast staining used carbolfuchsin and endospores were stained with malachite green. Motility was assessed by examining wet mounts of 24-h cultures at 1000X and by stab inoculation of Sulfur-Indole-Motility medium (SIM; BBL). The addition of 3% hydrogen peroxide solution to confluent plates or smears of confluent culture on slides was used to detect catalase. Oxidase testing was performed using the BBL DrySlide system according to the manufacturer's instructions. Amylase was determined after a 5-d incubation on Starch agar (Difco), followed by the flooding of plates with 25% stabilized Gram's iodine solution. Hydrogen sulfide production was assayed using SIM medium; Kovac's reagent was added after incubation to test for the production of indole. Urease activity was determined at 5 d using urea broth with phenol red indicator. Production of acid and gas from carbohydrates was tested using 0.5% (w/v) glucose, lactose, or sucrose in culture tubes containing inverted Durham tubes, using a 10% NaCl solution supplemented with tryptone (10 g l⁻¹) and phenol red (0.018 g l⁻¹; pH 7.3).

Phenetic trees of relatedness were generated using the numerical taxonomy package NTSYSpc 2.1 (Applied Biostatistics, Inc., NY), as previously described (Litzner et al. 2006). Each characteristic (44 total) was introduced as binary data to determine Jaccard similarity coefficients. The similarity matrix was converted into phenetic dendrograms using a UPGMA method (SAHN).

PCR, DNA Sequencing, and Phylogenetic Analyses

Crude DNA extracts from each isolate were prepared using a freeze-thaw technique as described in Caton et al. (2004). Genomic DNA in the supernatant was the target for PCR amplification of nearly complete 16S rRNA gene fragments using primers specific for the domain *Bacteria* (EUBPA: 5'-AGAGTTTGATCCTGGCTCAG-3' and EUBPH: 5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards et al. 1989). PCR was performed in a thermal cycler (Eppendorf Mastercycler) as 25-μL reactions containing 0.2 μM of each primer, 1 U of ExTaq DNA polymerase and associated master mix (Takara), and 5 μL of cell

extract. DNA was denatured at 95 °C for 2 min, followed by 40 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final 5-min extension at 72 °C. PCR amplicons were single-pass sequenced by a commercial vendor (Eurofins Genomics, Louisville, KY) using the EUBPA primer. Raw sequences were trimmed to remove remaining vector regions. All sequences appear in GenBank with accession numbers MG461380 to MG461441.

Sequences were aligned using SILVA and maximum likelihood analysis was used to build trees in MEGA v7.0 (Kumar et al. 2016). Known contextual 16S rRNA gene sequences from type organisms were identified in GenBank using BLAST. The trees were rooted using a functional outgroup.

Pyrosequencing was performed using 454 Life Sciences technology by a commercial vendor. Direct DNA extracts were obtained from composite soil samples using a bead-beating and freeze-thaw method as previously described and amplified with 28F (5'-GAGTTTGATCNTGGCTCAG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') bacterial primers. Over 19,000 reads were obtained and analyzed. Sequences were processed using MOTHUR v.1.39.3 according to the 454 SOP (https://www.mothur.org/wiki/454_SOP [accessed 5/9/2018]) with the following modifications (Schloss et al. 2009; Schloss and Westcott 2011). Denoised sequences were screened to permit a maximum homopolymer of 9 and a minimum length of 250 nts. Sequences were aligned to the MOTHUR-formatted version of the SILVA seed alignment, v. 132 (Quast et al. 2013). Aligned sequences were screened for a start position of 1046 within the alignment and optimized in total length to retain 80% of the sequences. Chimera checking was performed using the VSEARCH algorithm implemented in MOTHUR. Sequences were classified using the MOTHUR-formatted Ribosomal Database Project training set (version 16). Sequences classified within domain *Eukarya*, as mitochondrial sequences, or with unknown classification at the domain level, were removed from further processing. Ecological α -diversity metrics were calculated using the nseqs, coverage, sobs, invsimpson, simpson even, Chao, and Shannon calculators as implemented in MOTHUR.

Results

Collection and Characterization of Bacterial Isolates

All three of the composite soil samples from Basque Lake produced bacterial isolates through enrichment cultures. Sampling site and enrichment conditions are indicated within the phylogenetic trees (Figs. S1 and S2). Nearly all of the isolates were obtained from enrichments at 25 °C and 10% NaCl. Seven isolates were obtained at 37 °C and one at 30 °C. Eight isolates were collected from enrichments at 50% MgSO₄ (~2 M). While *Marinococcus* isolates produced characteristically orange colonies, none of the colonies arising from even eutrophic high-salt media enrichments at 37 °C were red, a trait often associated with halophiles, particularly haloarchaea that contain the photopigment bacteriorhodopsin.

Biochemical and physiological characteristics of the Basque Lake isolates are shown on Figs. 2–3 and Tables S1–S6. The vast majority of Basque Lake isolates (77%) stained Gram-positive and no isolates assigned to Gram-negative phyla by 16S rRNA sequencing stained

Gram-positive (Fig. 2a, Table S1). All of the *Virgibacillus* isolates, and no others, produced endospores. Nearly all of the isolates were motile, with only two Gram-positive isolates (*Marinococcus* sp. str. BL17 and *Staphylococcus* sp. str. BL61) being nonmotile. While all but one (*Staphylococcus* sp. str. BL61) of the Gram-positive isolates were oxidase-positive, only 29% of the Gram-negative isolates were oxidase-positive. All isolates were catalase-positive. No isolates produced indole from tryptophan and only three expressed amylase (*Halomonas* sp. str. BL1, *Salinivibrio* sp. str. BL46, and *Staphylococcus* sp. str. BL61). The *Marinococcus* and *Staphylococcus* isolates expressed urease. Sulfide production was nearly absent for Gram-positive isolates (except *Virgibacillus* sp. str. BL14, *Salinivibrio* sp. str. BL46, and *Staphylococcus* sp. str. BL61), but was present for ~80% of Gram-negative isolates. Fermentation was widespread among the Basque Lake isolate collection, with the vast majority of isolates fermenting glucose, lactose, and sucrose (Fig. 2b, Table S2). *Marinococcus* isolates showed no fermentation, five *Halomonas* strains (BL 30, 48, 50, 58, and 59) only fermented glucose, and *Salinivibrio* sp. str. BL46 did not ferment sucrose. Only two isolates produced gas during fermentation, *Salinivibrio* sp. str. BL46 with glucose and *Staphylococcus* sp. str. BL 61 with lactose.

Basque Lake bacterial isolates generally grew better at basic pH than at acidic pH (Fig. 3a, Table S3). All of the isolates grew at pH 8. Nearly 80% of the isolates grew at pH 9 and about one-third (31%) grew at pH 10. Only three isolates, *Halomonas* sp. str. BL 48, 58, and 59, grew at pH 11, the highest pH tested. All three of these, along with BL31, were *Halomonas* that did not grow below pH 7. Twelve isolates (19%) grew at pH 5 and none of the isolates grew at pH 4. Cold tolerance has particular relevance to astrobiology, given the physical conditions of Mars and the icy worlds. All isolates, except *Staphylococcus* sp. str. BL61, grew at 10 °C (Table S6). Of the 62 Basque Lake isolates, 55 (89%) grew at 7 °C and 45 (73%) grew at 4 °C.

Epsotolerance and Halotolerance in Bacterial Isolates

The Basque Lake isolate collection was screened for growth tolerance to high NaCl and MgSO₄ concentrations. Salinity tolerance ranges of bacterial isolates from Basque Lake are shown in Figs. 3b–3c and Tables S4–S5. Nearly all (85%) of the bacterial isolates grew at 50% MgSO₄ and about two-thirds (65%) grew at 60% MgSO₄ (Fig. 3b). Five isolates (*Marinococcus* sp. str. BL 10, 37, 38, and 57 and *Virgibacillus* sp. str. BL13) grew at the saturation point (67%) for MgSO₄ at room temperature. One *Virgibacillus* isolate (BL42) could not grow above 30% MgSO₄. Two isolates (*Marinococcus* sp. str. BL 12 and 43) either did not grow or grew particularly poorly at 1% MgSO₄.

The Basque Lake isolates showed remarkable growth tolerance to high NaCl concentrations, although this saline environment is not dominated by NaCl or other monovalent cations. All isolates grew at 10% NaCl and 71% of the isolates grew at 20% NaCl (~3.5 M NaCl) (Fig. 3c). Note that the water activity of 20% NaCl (0.82) is substantially lower than that of saturated MgSO₄ (0.90). This is due in part to limited dissociation of MgSO₄ in solution. Some of the isolates (19%) showed growth at 30% NaCl, near saturation. Two isolates (*Marinococcus* sp. str. BL 12 and 43) either did not grow or grew poorly at 1% NaCl.

Halotolerance and epsotolerance were positively correlated ($r = 0.609$, $p = 0.010$) for the 62 Basque isolates. However, there was not always a direct correspondence for individual isolates. For instance, *Virgibacillus* sp. str. BL6 grows at 60% MgSO_4 , but only at 10% NaCl (degree of saturation of 90 and 31%, respectively). The correlations between tolerances to these two salts were greater than those seen previously for bacterial growth tolerances to high salts and sugars (Fredsgaard et al. 2017a). The conclusion there was that specific solute effects, and not simply water activity, were working to set degrees of tolerance.

Numerical Taxonomy of Isolates

Characterization of the Basque Lake isolate collection included 44 phenotypic parameters that were used to construct dendrograms (Fig. 4). Six phenoms can be distinguished at a coefficient of 0.40. With few exceptions bacterial isolates clustered on the numerical taxonomy dendrograms within the phylogenetic groups determined by 16S rRNA gene sequencing. The lone Gram-negative *Salinivibrio* isolate (BL46) was a singleton on the numerical taxonomy dendrogram and clustered within the Gram-positive clade. Two *Marinococcus* isolates (BL 12 and 43) formed a cluster distinct from the other *Marinococcus* and *Virgibacillus* isolates. These two isolates grew poorly (or not at all) at 1% salt (Fig. 3c).

Phylogenetic Groups Recovered

Representatives of the most common colony types from each Basque Lake sample were collected, although this increased the degree of duplication in the overall collection. Phylogenetic analysis was performed on 16S rRNA gene sequences to identify the taxonomic placement of each isolate (Figs. 5 and S1–S2). Gram-positive bacteria dominate the Basque Lake culture collection, however, representatives of only three clades were recovered (Figs. 5a and S1). Isolates related to *Marinococcus halophilus* and *Virgibacillus marismortui* accounted for all but one of the Gram-positive isolates. The *Marinococcus* isolates produced BLAST correspondences to bacteria found in hypersaline environments (Tang et al. 2011; Kilmer et al. 2014; Li and Yu 2015) and to our previous isolates from epsomic Hot Lake (e.g., HL 6–9, 29, 60, 72, and 88). The *Virgibacillus* isolates had BLAST similarities to isolates obtained from hypersaline environments and to our previous isolates from the Great Salt Plains (e.g., GSP17) (Caton et al. 2004; Caton and Schneegurt 2012; Kim et al., 2012; Li and Yu 2015). It is interesting to note that many of the bacterial isolates had sequences nearly identical to those obtained from Death Valley soils or from Yuncheng Lake in China, which is rich in SO_4 and Na, with elevated Mg and reduced Cl.

The Gram-negative isolates from Basque Lake (Figs. 5b and S2) include a *Salinivibrio* and species of *Halomonas*, which are widely found in hypersaline environments (Caton et al. 2004; Tang et al. 2011; Kilmer et al. 2014; Li and Yu 2015). The *Halomonas* group that includes BL 48, 58, and 59 showed BLAST correspondence to isolates from haloalkaline environments, some rich in sulfates (Montoya et al. 2013; Kilmer et al. 2014). The isolates in this clade did not grow below pH 7 and were the only isolates to grow at pH 11 (Fig. 3a).

Conflicts of Interest The authors have no conflicts of interest.

The closest BLAST matches for *Salinivibrio* sp. str. BL46 are again from Yuncheng Lake, which is rich in Na₂SO₄ (Li and Yu 2015).

Culture-Independent Community Analysis

Pyrosequencing of PCR-amplified 16S rRNA genes in direct DNA extracts was used to examine the bacterial community in Basque Lake soils. This represents a snapshot of the bacterial community at a particular location and time. There is likely considerable variability among different areas of Basque Lake and across temporal and seasonal scales. Samples were taken from muddy soils rising between epsomic playa pools. The distribution of amplicons into phyla indicated that *Proteobacteria* were the predominant bacterial group, encompassing 60% of the sequences recovered (Figs. 6 and S7). The abundance of *Betaproteobacteria* (27%) was greater than the abundance of *Alphaproteobacteria* (19%) and more than twice that of the *Gammaproteobacteria* (12%). *Betaproteobacteria* were mainly within Burkholderiales. Rhizobiales, Rhodobacterales and Sphingomonadales were the main contributors to the *Alphaproteobacteria*. *Gammaproteobacteria* were predominantly Chromatiales and Oceanospirillales. Other Gram-negative bacterial groups were minor components of the community. Sulfate-reducing *Deltaproteobacteria* were observed. Gram-positive bacteria represented 11% of the library, with 95% clustering within Firmicutes, mainly Bacillales and Clostridiales. Bacteroidetes were minor populations (1.3%) within this community. Unclassified bacterial sequences represented 27% of the library.

At the 97% identity level for 16S rRNA gene sequences, 955 species were observed. Chao estimators predict that the bacterial community contains 4354 species (95% CI of 3573 – 5369) and Good's coverage was 0.79. Bacterial diversity appears high for the extreme environment of Basque Lake based on a Shannon index of 5.07 (95% CI of 5.00 – 5.14) and an inverse Simpson index of 32.37 (95% CI 29.62 – 35.68). Simpson's E value was low (0.034) indicating that species are not evenly distributed within the community.

Discussion

Environments rich in NaCl are abundant on Earth, from the oceans to solar salterns to hyperhaline lakes and salt flats. The communities and growth tolerances of microbes from hyperhaline environments have seen extensive study for over a century (Grant 2004; Schneegurt 2012). Natural environments dominated by salts besides NaCl are relatively rare and consequently have been investigated far less frequently (Handy 1916; Anderson 1958; Markovitz 1961; Markovitz and Sylvan 1962; Boring et al. 1963; Hammer 1978, 1986; Laiz et al. 2000; Mandrioli and Saiz-Jimenez 2002; Nesbitt 2004; Hyde et al. 2007; Foster et al. 2010; Crisler et al. 2012; Lindemann et al. 2013; Kilmer et al. 2014; Pontefract et al. 2017). Epsomic lakes and mud flats rich in MgSO₄ present a different challenge to microbes, since the ions in these environments are predominantly divalent. It is not clear what impact divalent ions have on microbes, how microbes respond and adapt to high concentrations of divalent salts, and how microbial responses compare to those observed with high NaCl.

A fundamental difference between solutions rich in NaCl and those rich in MgSO₄ is the result of differing levels of dissociation among these salts. Water activity is much more greatly reduced by NaCl than MgSO₄ at the same concentration (or degree of saturation),

since MgSO_4 does not fully dissociate in water (Crisler et al. 2012; Kilmer et al. 2014). Previous work has demonstrated that water activity is not the sole physical parameter limiting microbial growth in media with very high solute concentrations, whether these are salts or sugars (Fredsgaard et al. 2017a). Our studies of bacteria isolated from epsomic Hot Lake have revealed broad tolerances to a wide range of MgSO_4 concentrations, with strong growth for most isolates to at least 2 M salt (Kilmer et al. 2014). Basque Lake bacteria have exhibited broad tolerances to MgSO_4 exposure in the same way. Broad tolerances may indicate that the cells are exposed to variable salt concentrations in their environment, such as might occur with snowmelt that dilutes briny playa pools.

Basque Lake may have the highest concentrations and greatest dominance of divalent ions of any surface waters on Earth (Hammer 1978, 1986; Haynes and Hammer 1978; Last and Slezak 1988; Last and Ginn 2005). Both Basque Lake and Hot Lake present environments that are saturated with respect to epsomite. Our earlier work demonstrated growth of GSP and Hot Lake isolates in 60% MgSO_4 (Crisler et al. 2012; Kilmer et al. 2014). Here we have extended this range to demonstrate microbial growth in media essentially saturated with MgSO_4 (~67%). MgSO_4 is the only salt besides NaCl where microbial growth has been observed in a saturated salt solution and this is the highest concentration of Mg (~2.7 M) for which growth has been demonstrated (Schneegurt 2012). The astrobiology implications of this observation for deliquescent salts on Mars are discussed below. While Basque Lake is an environment rich in divalent ions, the isolates from Basque Lake grew well in high concentrations of NaCl. Certain isolates were able to grow in NaCl near saturation, as well as saturated MgSO_4 (~67%). Overall, there was a positive statistical correlation between halotolerance and epsitolerance for the Basque Lake isolates. However, this was not the case for every individual isolate.

The isolate collection from Basque Lake was not as diverse as a similar collection from Hot Lake (Kilmer et al. 2014). This could reflect true differences in these communities or biases in the enrichment and selection of colonies for purification. Gram-negative *Proteobacteria* seem to be particularly undersampled. However, the predominant genera captured at Basque Lake also were abundant members of the Hot Lake community, as well as found in the GSP community (Caton et al. 2004; Kilmer et al. 2014). *Halomonas*, *Marinococcus*, and *Virgibacillus* (and other Bacilli) dominate the culturable community of these hypersaline sites. Basque Lake and GSP did not yield the Actinomycetes recovered from Hot Lake. Initial deep sequencing of Basque Lake amplicons yielded 16S rRNA sequences from cyanobacteria, Clostridia, and sulfate-reducing bacteria that were not targeted in the current enrichment scheme. Similar analysis of epsomic Spotted Lake found a predominance of Proteobacteria, Firmicutes, and Bacteroidetes, with similarities to the Basque community (Pontefract et al. 2017). Community analysis performed through cultivation and culture-independent analyses do not match in the current study given their very different criteria. The cultivated isolates have corresponding representatives in the deep sequence libraries, but many of the taxa observed were not captured in the cultivated collection. Identification of isolates by phenetic characteristics was not pursued the necessary characteristics for taxonomic assignment of microbial isolates were not all assayed.

While archaea, as haloarchaea, are often in high abundance in hyperhaline environments, this does not seem to be the case for all epsomic environments. No archaea were recovered from either Hot Lake or Basque Lake, despite using enrichment conditions known to select for archaea (increased Mg, high amino acid content, 37 °C; Schneegurt 2012). Archaea are not entirely absent from these locations since archaeal sequences can be PCR-amplified using primers specific for the domain *Archaea*. Initial studies indicated that these were nearly all within the methanogens, and not the haloarchaea (Vaishampayan et al. 2013; Chen et al. 2016). Archaea were reported from epsomic Spotted Lake (Pontefract et al. 2017). Haloarchaea typically require at least 1.5 M NaCl for growth and the NaCl cannot be replaced by another salt (Mohr and Larsen 1963; Mullakhanbhai and Larsen 1975; Onishi et al. 1980; Vreeland and Martin 1980). Hot Lake and Basque Lake do not contain this level of NaCl.

Salts in the regolith of Mars appear to be dominated by divalent ions, with sulfates being more abundant than chlorides, and generally the sulfate has Ca, Fe, and Mg counterions (Clark and van Hart 1981; Clark 1993; Wänke et al. 2001; Vaniman et al. 2004; Clark et al. 2005; Gendrin et al. 2005; Altheide et al. 2009). Liquid water is a key requirement for life and given the cold environment of Mars, ephemeral liquid water is expected to be hypersaline, whether generated from melting permafrost or by deliquescing salts (Cull et al. 2010; Lanza et al. 2010; McEwen et al. 2011; Möhlmann and Thomsen 2011; McKay et al. 2013). The subsurface oceans of Ceres, Enceladus, Europa, and the other icy worlds also appear to be salty and perhaps rich in MgSO₄ (Kargel et al. 2000; Marion et al. 2003; Hussmann et al. 2006; Mottl et al. 2007; Postberg et al. 2011; Nimmo and Pappalardo 2016).

Heavy brines on Mars formed through deliquescence may exist as eutectic solutions with very low freezing points (Chevrier and Altheide 2008; Möhlmann and Thomsen 2011; Clark and Kounaves 2016; Rivera-Valentin et al. 2018). The eutectic conditions for epsomite are 43% (w/w) at -4 °C. Initial studies with psychrotolerant isolates obtained from Basque Lake by enrichment cultures at 4 °C and 50% MgSO₄, showed slow growth under eutectic conditions (Wilks et al. 2018). The Curiosity rover has found that atmospheric humidity is high enough for (per)chlorate salts to deliquesce and these may be the most likely sources of liquid water on Mars (Chevrier et al. 2009; Martín-Torres et al. 2015; Ojha et al. 2015). The eutectic points for these salts are ~40% and -40 °C (or lower), conditions unlikely to support microbial growth. However, initial studies have demonstrated bacterial growth at the potassium chlorate eutectic (3% at -4 °C) and room temperature growth was observed at >25% (per)chlorates (Al Soudi et al. 2017; Wilks et al. 2018). Our findings will inform planetary protection efforts that seek to reduce contamination on robotic spacecraft, especially for life detection missions (Kargel et al. 2000; Marion et al. 2003; Hussmann et al. 2006; Mottl et al. 2007; Tosca et al. 2008; Fox-Powell et al. 2016). On this note, it also appears that epsotolerant microbes are found in common soils and spacecraft assembly facilities (Fredsgaard et al. 2017b; Eberl and Schneegurt, unpublished observations).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors are thankful for the preliminary and supportive work done by Amer Al Soudi, Bishal Bista, John Dille, Timothy Eberl, Casper Fredsgaard, Brian Kilmer, Tony Mai, Donald Moore, Trista Newville, Kyle Rowe, Namrata Shrestha, and Karen Woltersdorf. We are grateful to Bruce Madu and Jim Britton (British Columbia Ministry of Energy and Mines; BCMEM) for collecting and documenting Basque Lake samples. We thank Fadi Aramouni (Kansas State University) for performing water activity measurements and Stephen Lindemann (Purdue University) for deep sequencing analyses. Preliminary accounts of this work have been presented previously (Crisler et al. 2010a, b, 2018; Kilmer et al. 2012). This work was supported by awards from National Aeronautics and Space Administration (NASA), Research Opportunities in Space and Earth Science (ROSES), Planetary Protection Research (09-PPR09-0004 and 14-PPR14-2-0002). Additional student support was from Kansas Institutional Development Award (IDeA) Networks of Biomedical Research Excellence (KINBRE), National Institute of General Medical Sciences (NIGMS), National Institutes of Health (NIH) (P20 GM103418). The content is solely the responsibility of the authors and does not necessarily represent the official views of BCMEM, KINBRE, NASA, NIGMS or NIH.

References

- Al Soudi AF, Farhat O, Chen F, Clark BC, Schneegurt MA (2017) Bacterial growth tolerance to concentrations of chlorate and perchlorate salts relevant to Mars. *Int J Astrobiol* 16:229–235
- Altheide T, Chevrier V, Nicholson C, Denson J (2009) Experimental investigation of the stability and evaporation of sulfate and chloride brines on Mars. *Earth Planet Sci Lett* 282:69–78
- Anderson GC (1958) Some limnological features of a shallow saline meromictic lake. *Limnol Oceanogr* 3:259–270
- Boring J, Kushner DJ, Gibbons NE (1963) Specificity of the salt requirement of *Halobacterium cutirubrum*. *Can J Microbiol* 2:143–154
- Caton TM, Witte LR, Ngyuen HD, Buchheim JA, Buchheim MA, Schneegurt MA (2004) Halotolerant aerobic heterotrophic bacteria from the Great Salt Plains of Oklahoma. *Microb Ecol* 48:449–462 [PubMed: 15696379]
- Caton IR, Schneegurt MA (2012) Culture-independent analysis of the soil bacterial assemblage at the Great Salt Plains of Oklahoma. *J Basic Microbiol* 52:16–26 [PubMed: 21953014]
- Chen F, Vaishampayan P, La Duc M, Probst A, Schneegurt MA, Clark B (2016) Comparative phylogenetic analysis of the microbial communities in a spacecraft assembly facility and in epsomite lakes. Abstracts and program, 116th Annual Meeting of the American Society for Microbiology, Boston, June 2016
- Chevrier VF, Altheide TS (2008) Low temperature aqueous ferric sulfate solutions on the surface of Mars. *Geophys Res Lett* 35:L22101
- Chevrier VF, Hanley J, Altheide TS (2009) Stability of perchlorate hydrates and their liquid solutions at the Phoenix landing site, Mars. *Geophys Res Lett* 36:L10202
- Clark BC (1993) Geochemical components in Martian soil. *Geochim Cosmochim Acta* 57:4575–4581
- Clark BC, Kounaves SP (2016) Evidence for the distribution of perchlorates on Mars. *Int J Astrobiol* 15:311–318
- Clark BC, van Hart D (1981) The salts of Mars. *Icarus* 45:370–378
- Clark BC, Morris RV, McLennan SM, Gellert R, Jolliff B, Knoll AH, Squyres SW, Lowenstein TK, Ming DW, Tosca NJ, Yen A, Christensen PR, Gorevan S, Brückner J, Calvin W, Dreibus G, Farrand W, Klingelhoefer G, Waenke H, Zipfel J, Bell JF III, Grotzinger J, McSween HY, Rieder R (2005) Chemistry and mineralogy of outcrops at Meridiani Planum. *Earth Planet Sci Lett* 240:73–94
- Crisler JD, Kilmer BR, Cunderla B, Madu BE, Schneegurt MA (2010a) Isolation and characterization of microbes from Basque Lake, BC, and Hot Lake, WA, environments with high magnesium sulfate concentrations. Abstracts and program, 110th General Meeting of the American Society for Microbiology, San Diego, May 2010
- Crisler JD, Kilmer BR, Rowe K, Cunderla B, Madu BE, Schneegurt MA (2010b) Isolation and characterization of microbes from Basque Lake, BC, and Hot Lake, WA, environments with high magnesium sulfate concentrations. 142nd Annual Meeting of the Kansas Academy of Science, Hays, April 2010. *Trans KS Acad Sci* 113:122

- Crisler JD, Newville TM, Chen F, Clark BC, Schneegurt MA (2012) Bacterial growth at the high concentrations of magnesium sulfate found in Martian soils. *Astrobiology* 12:98–106 [PubMed: 22248384]
- Crisler JD, Chen F, Clark BC, Schneegurt MA (2018) Numerical taxonomy and phylogenetic analyses of the microbial community of epsomic Basque Lake, BC. Abstracts and program, 118th General Meeting of the American Society for Microbiology, Atlanta, June 2018
- Cull SC, Arvidson RE, Catalano JG, Ming DW, Morris RV, Mellon MT, Lemmon M (2010) Concentrated perchlorate at the Mars Phoenix landing site: Evidence for thin film liquid water on Mars. *Geophys Res Lett* 37:L22203
- Edwards U, Rogall H, Blöcker H, Emde M, Böttger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucl Acids Res* 17:7843–7853 [PubMed: 2798131]
- Foster IS, King PL, Hyde BC, Southam G (2010) Characterization of halophiles in natural MgSO₄ salts and laboratory enrichment samples: Astrobiological implications for Mars. *Planet Space Sci* 58:599–615
- Fox-Powell MG, Hallsworth JE, Cousins CR, Cockell CS (2016) Ionic strength is a barrier to habitability of Mars. *Astrobiology* 16:427–442 [PubMed: 27213516]
- Fredsgaard C, Moore DB, Al Soudi AF, Crisler JD, Chen F, Clark BC, Schneegurt MA (2017a) Relationships between sucrotolerance and salinotolerance in bacteria from hypersaline environments and their implications for the exploration of Mars and the icy worlds. *Int J Astrobiol* 16:156–162
- Fredsgaard C, Moore DB, Chen F, Clark BC, Schneegurt MA (2017b) Prevalence of sucrotolerant bacteria in common soils and their isolation and characterization. *Ant Leeuwen* 110:995–1005
- Gendrin A, Mangold N, Bibring JP, Langevin Y (2005) Sulfates in Martian layered terrains: the OMEGA/Mars express view. *Science* 307:1587–1591 [PubMed: 15718429]
- Grant WD (2004) Life at low water activity. *Phil Trans R Soc Lond B* 359:1249–1267 [PubMed: 15306380]
- Hammer UT (1978) The saline lakes of Saskatchewan. III. Chemical characterization. *Int Rev Hydrobiol* 63:311–335
- Hammer UT (1986) Saline Lake Ecosystems of the World Junk, Dordrecht
- Hammer UT, Haynes RC (1978) The saline lakes of Saskatchewan. 2. Locale, hydrology and other physical aspects. *Int Rev Ges Hydrobiol Hydrog* 63:179–203
- Handy FM (1916) An investigation of the mineral deposits of northern Okanogan County. Department of Geology Bulletin 100, State College of Washington, Pullman, WA, 27 pp
- Haynes RC, Hammer UT (1978) The saline lakes of Saskatchewan. IV. Primary production by phytoplankton in selected saline ecosystems. *Int Rev Hydrobiol* 63:337–351
- Husmann H, Sohl F, Spohn T (2006) Subsurface oceans and deep interiors of medium-sized outer planet satellites and large trans-neptunian objects. *Icarus* 185:258–273
- Hyde BC, Foster IS, King PL, Southam G, Nushaj D (2007) Limits of detection for life on Mars: An example using IR spectroscopy of sulfate salts and halophiles from lakes in British Columbia, Canada. *Lunar Planet Sci Conf* 38:2278
- Kargel JS, Kaye JZ, Head JW III, Marion GM, Sassen R, Crowley JK, Ballesteros OP, Grant SA, Hogenbloom DL (2000) Europa's crust and ocean: Origin, composition, and the prospects for life. *Icarus* 148:226–265
- Kilmer BR, Eberl TC, Crisler JD, Cunderla B, Madu BE, Schneegurt MA (2012) Cultivation and molecular analysis of the microbial community in epsomite lakes of the Pacific Northwest. Abstracts and program, 112th General Meeting of the American Society for Microbiology, San Francisco, June 2012
- Kilmer BR, Eberl TC, Cunderla B, Chen F, Clark BC, Schneegurt MA (2014) Molecular and phenetic characterization of the bacterial assemblage of Hot Lake, WA, an environment with high concentrations of magnesium sulphate, and its relevance to Mars. *Int J Astrobiol* 13:69–80 [PubMed: 24748851]

- Kim JS, Makama M, Petito J, Park NH, Cohan FM, Dugan RS (2012) Diversity of bacteria and archaea in hypersaline sediment from Death Valley National Park, California. *MicrobiologyOpen* 1:135–148 [PubMed: 22950020]
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molec Biol Evol* 33:1870–1874 [PubMed: 27004904]
- Laiz L, Recio D, Hermosin B, Saiz-Jimenez C (2000) Microbial communities in salt efflorescences. In: Ciferri O, Tiano P, Mastromei G (eds.), *Of Microbes and Art: The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage*, Kluwer, New York, pp 77–88
- Lanza NL, Meyer GA, Okubo CH, Newson HE, Wiens RC (2010) Evidence for debris flow gully formation initiated by shallow subsurface water on Mars. *Icarus* 205:103–112
- Last WM, Slezak LA (1988) The salt lakes of western Canada: A paleolimnological overview. *Hydrobiologia* 158:310–316
- Last WM, Ginn FM (2005) Saline systems of the Great Plains of western Canada: an overview of the limnogeology and paleolimnology. *Saline Syst* 1:10 [PubMed: 16297237]
- Li X, Yu YH (2015) Biodiversity and screening of halophilic bacteria with hydrolytic and antimicrobial activities from Yuncheng Salt Lake, China. *Biologia* 70:151–156
- Lindemann SR, Moran JJ, Stegen JC, Renslow RS, Hutchison JR, Cole JK, Dohnalkova AC, Tremblay J, Singh K, Malfatti SA, Chen F, Tringe SG, Beyenal H, Fredrickson JK (2013) The epsomitic phototrophic microbial mat of Hot Lake, Washington: community structural responses to seasonal cycling. *Front Microbiol* 4:323 [PubMed: 24312082]
- Litzner BR, Caton TM, Schneegurt MA (2006) Carbon substrate utilization, antibiotic sensitivity, and numerical taxonomy of bacterial isolates from the Great Salt Plains of Oklahoma. *Arch Microbiol* 185:286–296 [PubMed: 16518618]
- Mandrioli P, Saiz-Jimenez C (2002) Biodeterioration: Macromonitoring and microeffects on cultural heritage and the potential benefits of research to society. *EC Advanced Study Course Technical Notes, Sessions 7–8*, pp. 1–5
- Marion GM, Fritsen CH, Eicken H, Payne MC (2003) The search for life on Europa: limiting environmental factors, potential habitats, and Earth analogues. *Astrobiology* 3:785–811 [PubMed: 14987483]
- Markovitz A (1961) Method for the selection of bacteria that synthesize uronic acid-containing polysaccharides. *J Bacteriol* 82:436–441 [PubMed: 13766927]
- Markovitz A, Sylvan S (1962) Effect of sodium sulfate and magnesium sulfate on heteropolysaccharide synthesis in gram-negative soil bacteria. *J Bacteriol* 83:483–489 [PubMed: 14470034]
- Martín-Torres FJ, Zorzano M-P, Valentín-Serrano P, Harri A-M, Genzer M, Kempainen O, Rivera-Valentin EG, Jun I, Wray J, Madsen MB, Goetz W, McEwen AS, Hardgrove C, Renno N, Chevrier VF, Mischna M, Navarro-González R, Martínez-Frías J, Conrad P, McConnochie T, Cockell C, Berger G, Vasavada AR, Sumner D, Vaniman D (2015) Transient liquid water and water activity at Gale crater on Mars. *Nature Geosci* 8:357–361
- McEwen AS, Ojha L, Dundas CM, Mattson SS, Byrne S, Wray JJ, Cull SC, Murchie SL, Thomas N, Gulick VC (2011) Seasonal flows on warm Martian slopes. *Science* 333:740–743 [PubMed: 21817049]
- McKay CP, Stoker CR, Glass BJ, Davé AI, Davila AF, Heldmann JL, Marinova MM, Fairen AG, Quinn RC, Zacny KA, Paulsen G, Smith PH, Parro V, Andersen DT, Hecht MH, Lacelle D, Pollard WH (2013) The Icebreaker Life Mission to Mars: a search for biomolecular evidence of life. *Astrobiology* 13:334–353 [PubMed: 23560417]
- Möhlmann D, Thomsen K (2011) Properties of cryobrines on Mars. *Icarus* 212:123–130
- Mohr V, Larsen H (1963) On the structural transformations and lysis of *Halobacterium salinarum* in hypotonic and isotonic solutions. *J Gen Microbiol* 31:267–280
- Montoya L, Vizioli C, Rodríguez N, Rastoll MJ, Amils R, Marin I (2013) Microbial community composition of Tirez lagoon (Spain), a highly sulfated athalassohaline environment. *Aquat Biosyst* 9:19 [PubMed: 24083554]
- Mottl MJ, Glazer BT, Kaiser RI, Meech KJ (2007) Water and astrobiology. *Chem Erde* 67:253–282

- Mullakhanbhai MF, Larsen H (1975) *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. Arch Microbiol 104:207–214 [PubMed: 1190944]
- Nesbitt HW (2004) Groundwater evolution, authigenic carbonates and sulfates, of the Basque Lake No. 2 basin, Canada. In: Spencer RJ and Chou I-M (eds.), Fluid-Mineral Interactions: a tribute to H.P. Eugster, Special Publication, Vol 2, Geochemical Society, pp 355–371
- Nimmo F, Pappalardo RT (2016) Ocean worlds in the outer solar system. J Geophys Res Plan 121:1378–1399
- Ojha L, Wilhelm MB, Murchie SL, McEwen AS, Wray JJ, Hanley J, Massé M, Chojnacki M (2015) Spectral evidence for hydrated salts in recurring slope lineae on Mars. Nature Geosci 8:828–832
- Onishi H, Fuchi H, Konomi K, Hidaka O, Kamekura M (1980) Isolation and distribution of a variety of halophilic bacteria and their classification by salt-response. Agric Biol Chem 44:1253–1258
- Pontefract A, Zhu TF, Walker VK, Hepburn H, Lui C, Zuber MT, Ruvkun G, Carr CE (2017) Microbial diversity in a hypersaline sulfate lake: A terrestrial analog of ancient Mars. Front Microbiol 8:1819 [PubMed: 29018418]
- Postberg F, Schmidt J, Hillier J, Kempf S, Srama R (2011) A salt-water reservoir as the source of a compositionally stratified plume on Enceladus. Nature 474:620–622 [PubMed: 21697830]
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596 [PubMed: 23193283]
- Rivera-Valentin EG, Gough RV, Chevrier VF, Primm KM, Martinez GM, Tolbert M (2018) Constraining the potential liquid water environment at Gale Crater, Mars throughout MSL's traverse. Lunar Planet Sci Conf 2018:2752
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. (2009) Introducing MOTHUR: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541 [PubMed: 19801464]
- Schloss PD, Westcott SL (2011) Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. Appl Environ Microbiol 77:3219–3226 [PubMed: 21421784]
- Schneegurt MA (2012) Media and conditions for the growth of halophilic and halotolerant bacteria and archaea. In: Vreeland RH (ed.), Advances in Understanding the Biology of Halophilic Microorganisms, Springer, Dordrecht, pp 35–58
- Tang J, Zheng A, Bromfield ESP, Zhu J, Li S, Wang S, Deng Q, Li P (2011) 16S rRNA gene sequence analysis of halophilic and halotolerant bacteria isolated from a hypersaline pond in Sichuan, China. Ann Microbiol 61:375–381
- Tosca NJ, Knoll AH, McLennan SM (2008) Water activity and the challenge for life on early Mars. Science 320:1204–1207 [PubMed: 18511686]
- Vaishampayan P, Schneegurt M, Clark B, Chen F (2013) Comparative phylogenetic analysis of the microbial community in spacecraft assembly facility and in epsomite lakes. Abstracts and program, 113th General Meeting of the American Society for Microbiology, Denver, May 2013
- Vaniman DT, Bish DL, Chipera SJ, Fialips CI, Carey JW, Feldman WC (2004) Magnesium sulphate salts and the history of water on Mars. Nature 431:663–665 [PubMed: 15470421]
- Vreeland RH, Martin EL (1980) Growth characteristics, effects of temperature, and ion specificity of the halotolerant bacterium *Halomonas elongata*. Can J Microbiol 26:746–752
- Wänke H, Brückner G, Dreibus G, Rieder R, Ryabchikov I (2001) Chemical composition of rocks and soils at the Pathfinder site. Space Sci Rev 96:317–330
- Wilks J, Chen F, Clark BC, Schneegurt MA (2018) Bacterial growth in saturated and eutectic solutions of magnesium sulfate and potassium chlorate with relevance to Mars and the ocean worlds. Int J Astrobiol 1–8. 10.1017/S14735504180000502

Figure 1.
Contextual map of Basque Lake showing sampling sites.

Figure 2.

Biochemical and physiological assays of Basque Lake bacterial isolates. The value within each segment of the column is the percentage of positive organisms within the Gram-positive (solid) and Gram-negative (open) isolates. The value above each column is the percentage of positive organisms among all of the isolates. a: assays of amylase, catalase, endospore formation, Gram reaction, motility, oxidase, sulfide production, and urease. b: assays of fermentation of glucose, lactose, and sucrose.

Figure 3.

Tolerances of Basque Lake isolates to environmental conditions as determined by growth in liquid culture. BL46 is a *Salinivibrio* and BL61 is a *Staphylococcus*. The threshold for positive growth was 0.05 OD. a: pH tolerance; none of the isolates grew at pH 4. b: epsotolerance. c: halotolerance.

Figure 4. Dendrograms showing the relatedness of Basque Lake bacterial isolates based on 44 phenetic characteristics. Taxonomic assignments were determined by phylogenetic analysis of 16S rRNA gene sequences.

Figure 5.

Phylogenetic trees of bacteria isolated from Basque Lake based on most likelihood analysis of aligned 16S rRNA gene sequences. Expanded trees with collateral data and GenBank accession numbers are given in Figs. S1 and S2. a: Gram-positive bacterial isolates. b: Gram-negative bacterial isolates.

Figure 6.

Relative abundance of major bacterial phyla recovered through pyrosequencing of 16S rRNA gene sequences in direct DNA extracts from a composite sample of Basque Lake soil. Numerical data is presented in Table S7.