WESTMINSTER COLLEGE

Great Salt Lake Institute

Identification of haloarchaea in gypsum from Great Salt Lake

Zhantao Du, Adrik D. Sliva & Dr. Bonnie K. Baxter, PhD*

*Bonnie K. Baxter, Ph.D., Director, Great Salt Lake Institute, Professor and Chair of Biology, Westminster College, Salt Lake City, Utah

Introduction

Gypsum ($CaSO_4 \cdot 2H_2O$) is a common precipitate at Great Salt Lake (GSL). On Earth, gypsum is formed in hypersaline environments, in minerals left behind when water evaporates. In the Autumn, as the water cools, mineral precipitation is favored since solubility is lowered. In this process, newly formed gypsum will trap microorganisms in fluid and clay inclusions. This study is to cultivate haloarchaea preserved in gypsum, and identify the species using PCR technique.

Background

Globally across Mars, there have been orbital and in-situ detections of evaporites, showing a wide diversity of minerals precipitated primarily from water or secondary modifications by water. The widespread evaporite mineralogy and closed basin nature of many of the regions on Mars have been observed by digital elevation models from the High Resolution Imaging Science Experiment (HiRISE) and the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM), which is onboard the Mars Reconnaissance Orbiter (MRO). These geochemical models and joint CRISM-HiRISE digital elevation models show how closed basin hydrogeological settings store these hydrated minerals. This suggests the existence of potentially hundreds of ancient salt lake sites on the Martian surface.

Studies have shown that the smectite clay minerals and hydrated sulfate minerals have similar minerology to the north shore of GSL. A spectacular example of this is the landing site of the Mars 2020 rover mission, Jezero Crater (Fig.1). Therefore, the study of the entrapped halophiles in GSL mineral evaporites will provide a model for the preservation of extant microorganisms trapped in Mars mineral evaporites that are resistant to desiccation, UV damage, and extreme osmotic conditions.

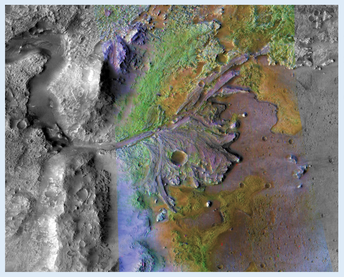

Figure 1. Jezero Crater, Mars, the chosen landing site for Mars 2020 rover mission and where the first samples are being collected for Mars Sample Return. HiRISE and CRISM map of a delta within Jezero Crater shows smectite clay minerals (green) and hydrated sulfate minerals in (warmer colors). image credit: NASA, JPL.

Materials and Methods

- Media: 23% Minimal Growth Media (MGM)
- DNA extraction was done using the QIAGEN® DNeasy® PowerSoil® Kit[1]
- PCR was done using the NEBNext® High-Fidelity 2X PCR Master Mix[2]
- Primers 1HK (5' ATTCCGGTTGATCCTGCCGG 3') and H589R (5' AGCTACGGTTTAGGC 3')[3,4,5,6]

Gypsum was surface sterilized using 100% ethanol and then crushed with a mortar and pestle to expose the halophiles inside the inclusion to salt liquid growth media. The culture was incubated at 37˚C for 3 months and streaking on solid growth media was done to isolate halophiles from different species. DNA from each individual colony was extracted and PCR amplification was done for the archaeal 16S rRNA gene using protocol[7,8]. The PCR product was observed and checked on a 1.2% agarose gel. The products were further cleaned with a QIAquick PCR Purification Kit and submitted to the Center for Integrated BioSystems at Utah State University for sequencing.

Result

Figure 2. Gypsum crystals collected from Great Salt Lake North Arm near the artwork, Spiral Jetty (Robert Smithson, 1970).

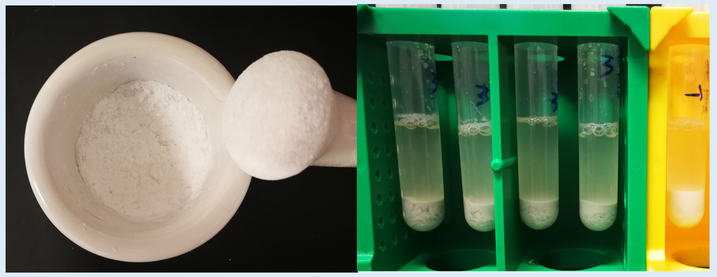

Figure 3. Gypsum was crushed into powder and placed in liquid growth media.

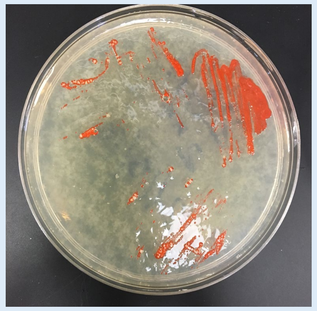

Figure 4. The growth of bright red colonies after incubation for about 3 weeks.

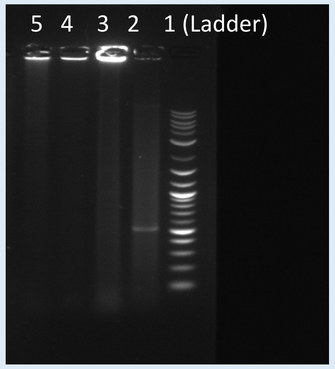

Figure 5. Gel amplification of the PCR product indicated that the sample in lane 2 contained a roughly 550 bp product. Lane 1 contains a 100 bp Ladder (NEB). The sample was sent in for sequencing. Results were input into NCBI BLAST which resulted in a 99% match with Halobacterium noricense strain DAB-31 isolated from Black Lake in China using 16s rRNA.

Discussion

This study indicates one effective way to cultivate halophiles from gypsum is to crush the gypsum into powder and place it into broth. Possible DNA extraction method and PCR assay are also tested. Further work will be done to preserve halophiles in liquid media for a longer period, which our current method can only keep them alive for less than 6 months. The successful method of halophilic archaea isolation and species identification can be used for analysis of gypsum or mirabilite returned from Mars, following the Mars 2020 Mission, to potentially cultivate any microorganisms present.

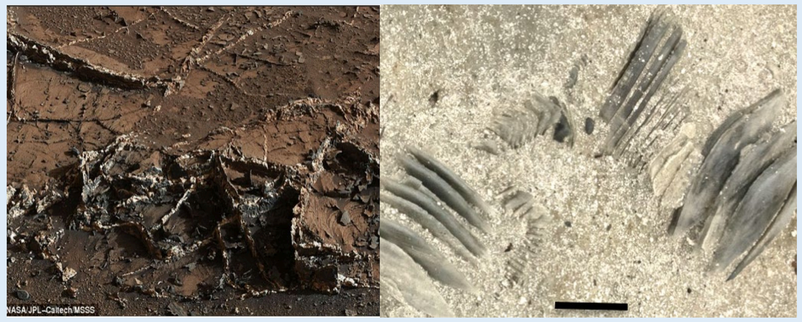

Figure 6. Comparison of gypsum deposits on Great Salt Lake (right) and Mars gypsum deposits imaged by Curiosity (left). Photo Credit: Scott Perl

Acknowledgement

Jaimi Butler and the GSLI for field trip support
Utah NASA Space Grant Consortium

References

1. QIAGEN, Hilden, Germany
2. New England BioLabs, Ipswich, MA
3. MP Biomedicals, Santa Ana, California 2019
4. New England Biolabs, Ipswis, Massachusetts.
5. Mankin, A. S., Kagramanova, V. K., Teterina, N. L., Rubtsov, P. M., Belova, E. N., Kopylov, A. M., ... & Bogdanov, A. A. (1985). The nucleotide sequence of the gene coding for the 16S rRNA from the archaebacterium Halobacterium halobium. Gene, 37(1-3), 181-189.
6. Martinez-Murcia, A. J., Acinas, S. G., & Rodriguez-Valera, F. (1995). Evaluation of prokaryotic diversity by restrictase digestion of 16S rDNA directly amplified from hypersaline environments. FEMS Microbiology Ecology, 17(4), 247-255.
7. Almeida-Dalmet, S., Sikaroodi, M., Gillevet, P., Litchfield, C., & Baxter, B. (2015). Temporal study of the microbial diversity of the North Arm of Great Salt Lake, Utah, US. Microorganisms, 3(3), 310-326.
8. Litchfield, C. D., Sikaroodi, M., & Gillevet, P. M. (2006). 21 Characterization of Natural Communities of Halophilic Microorganisms. In Methods in Microbiology (Vol. 35, pp. 513-533). Academic Press.