Great Salt Lake Halophilic Archaea: A model for mineral-entrapment of life

WESTMINSTER COLLEGE
Great Salt Lake Institute

Adrik Z. Da Silva, Jessie Kochaver and Bonnie K. Baxter, Ph.D.

Abstract

NASA's Mars Exploration Rover, Opportunity, found veins of gypsum and layer of salt deposited by water in 2011(Figure 7). Gypsum has been detected on Mars as early as 2005 by the ESA's Mars Express Orbiter, which later found evidence of chloride salts in 2008. On Earth, gypsum is formed in hypersaline environments, in minerals left behind when water evaporates. During evaporation, gypsum deposits and salt crystals can trap microorganisms in fluid inclusions. Gypsum obtained from Great Salt Lake, along with salt crystals collected from the salt glands of pelicans, were used to develop a method to extract halophilic archaea and culture it in the lab. Our studies show that gypsum was difficult to dissolve in aqueous microbiological media. Various methods of dissolution involving mechanical crushing and different solvents including microbiological media were tested. We also employed a variety of cultivation methods. We will present data on best practices for obtaining halophilic microorganisms from gypsum and salt crystal samples. The method obtained could be used to isolate potential microorganisms present in these minerals from Mars.

Background

Nasa's Rover Opportunity discovered mineral deposits in 2011 at Meridiani Planum on Mars. The mineral deposits are suggestive of an ancient lake that is now evaporated. The area is covered with concentrated minerals and a thick salt crust, which indicate that Mars had salt lakes.[1,2] Halite and gypsum are known to create fluid inclusions during their formation which can trap and store biological materials, providing shelter from physical and chemical damage. [3, 4, 5]

Great Salt Lake (GSL) may serve as an analog to the evaporated salt lake regions of Mars. GSL has no outlets and contains large salt flats which are halite deposits formed by the evaporation of the ancient lake Bonneville.[6] Several studies indicate that halophiles can survive over geologic time when preserved in halite crystals.[7] This has been shown in the transport of halophilic archaea to geographical locations around the world in the salt glands and on the feathers of American White Pelicans.[8] Therefore, not only is the study of GSL mineral evaporites important to understanding the evaporites on Mars, but the study of the entrapped halophiles 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.

Materials

- Media: 23% Minimal Growth Media (MGM).[8]
- DNA extraction was done using the FastDNA Spin Kit for Soil.[9]
- Taq PCR kit.[10]
- Primers 1HK (5' ATTCCGGTTGATCCTGCCGG 3') and H589R (5' AGCTACGGTTTAGGC 3').[11, 12, 13, 14]

Methods

Various cultivation methods were performed for gypsum crystals comparing the effectiveness of halophilic archaea growth for non surface-sterilized gypsum and surface sterilized crystals with 100% ethanol. The methods tested included placing crystals directly into media; crushing the crystals with a mortar and pestle then placing in broth; rubbing gypsum crystals directly onto agar plates; sprinkling crushed crystals directly onto plates; and finally dissolving crushed gypsum in water based on its solubility value, then combining with broth in dilutions of 1:4, 1:2, and 1:1.

Pelican salt gland samples were collected by swabbing the inside of the birds nostril, and then incubating the swab in media for two weeks. Incubation temperature was 37°C for both pelican salt gland and gypsum samples.

The DNA from colonies was isolated. PCR amplification was done for the archaeal 16S rRNA gene using protocol.[15, 16] The PCR product was amplified and observed 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.[17]

Results

Figure 1. (Left) Gypsum crystal collected from Great Salt Lake North Arm near the artwork, Spiral Jetty (Robert Smithson, 1970). (Right) Infant pelican on the shores of the Great Salt Lake after being tagged. (Jaimi Butler, 2018).

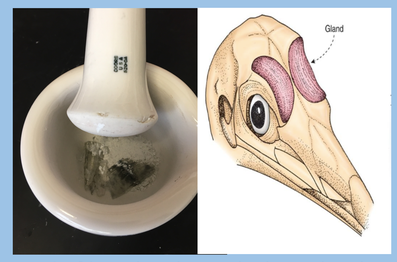

Figure 2: (Left) Neither the crushed samples placed in broth or sprinkled on plates showed any halophilic growth. The crushed samples dissolved in water and placed in broth did not show any growth either. (Right) The salt gland in the back of the pelican's throat was swabbed.

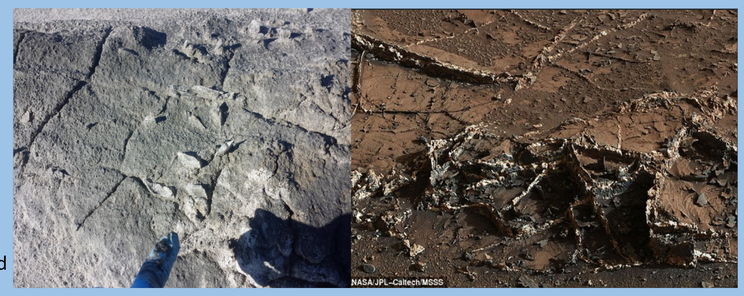

Figure 7: Comparison of gypsum deposits on Great Salt Lake (left, Photo Credit: Scott Perl) and Mars gypsum deposits imaged by NASA's Curiosity Rover (right).

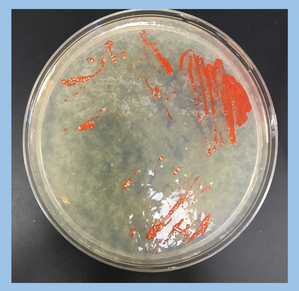

Figure 4: Plating the turbid samples of gypsum crystals and pelican swabs from the aqueous MGM resulted in growth of bright red colonies after incubation for about 3 weeks.

Figure 3: Gypsum crystal samples and pelican salt gland swabs placed directly into broth became turbid and light pink after about 2 weeks in the shaker. This was the only cultivation method that resulted in halophilic growth.

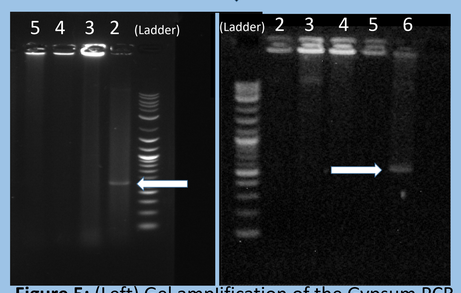

Figure 5: (Left) Gel amplification of the Gypsum PCR product indicated that the sample in lane 2 contained a roughly 550 bp product. Lane 1 contains a 100 bp Ladder (NEB). (Right) Lane 6 indicates a similar ~550 bp product from pelican samples determined by the 100 bp ladder (NEB). Both samples were sent in for sequencing.

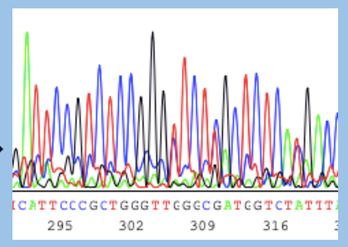

Figure 6: The above isolated pelican salt gland sample's sequence was entered into the Basic Alignment Search Tool of the National Library of Medicine (BLAST) to determine the identity of the microorganism.

Conclusions

Our studies indicate that gypsum is difficult to dissolve in aqueous microbiological media. Similarly, a consistent archaea cultivation method has not been identified due to variability in results. The most successful method noted was placement of a surface sterilized gypsum crystal into broth. This method resulted in the growth of microorganisms that could not be identified using the halophilic archaea primers (perhaps these are bacterial isolates). Further experiments will be done to improve halophilic archaea cultivation from gypsum, which will include methods such as isolation and culture from fluid inclusions in gypsum crystals. A *Halorubrum* species was isolated from salt crystals collected from the salt gland of a pelican. Collecting the crystals on a swab and placing them into media was successful in culture growth and isolation. This further proves that birds can serve to transport halophiles from one hypersaline habitat to another by carrying salt crystals containing microorganism filled fluid inclusions.[17] The successful method of halophilic archaea isolation can be used for analysis of gypsum and salt crystal samples returned from Mars, following the Mars 2020 Mission, to potentially cultivate any microorganisms present.

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