Exploration of Life in Halite
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Abstract

Biological material surviving in modern halite may point to potential life in salt deposits on Mars. This project attempts to find an efficient method extracting cells and DNA from modern halite crystals to gain more insight into efficient methods of extracting DNA from ancient salt, considering potential Mars Rover techniques. Microorganisms were cultivated from Great Salt Lake halite and identified by 16S rRNA gene comparisons. We present data on the diversity of archaea isolated by this method. We made attempts at isolating DNA with various combinations of centrifugation columns and filters to extract and clean any DNA that may be trapped inside the halite fluid inclusions, using gel electrophoresis to analyze the purity and concentration of DNA. We noted inefficient yields in recovery of DNA, which must be improved upon before we apply the methods to ancient halite.

Introduction

Cell constituents and DNA can be preserved in fluid inclusions of halite crystals. Here we present methods for studying biological preservation in salt. Analyzing ancient DNA would reveal much about the biological history of Earth and provide methods for the search for life outside of Earth. Ancient DNA, however, is extremely sensitive to degradation, therefore, we sought to develop methods of DNA extraction from modern halite. Once our methods show the level of sensitivity required for ancient materials, we will apply them to halite collected from Permian deposits.

Methods

1. DNA extraction from halite

Sample of dissolved salt added

Wash with water

Remove fluid above the membrane

Place filter upside down

Centrifuge

Purified DNA

Run gel electrophoresis

Bind, wash, elute DNA

Centrifuge

2. Cultivation from halite

Cultures allowed to grow on petri dishes then isolated

Salt crystals were grown in growth media

Isolated colony was placed in growth media for one week

Figure 1. Electron microscopy of ancient DNA fragments from liquid inclusions of salt crystals (Baxter and Griffith, unpublished).

Figure 2. Amicon centrifugal filter and Qiagen spin column. Images from Amicon Ultra 0.5UL filter and Qiagen spin column manual.

Figure 3. Process of halite cultivation from isolation to sequencing. Images from R&D Systems.

Results & Conclusions

1. DNA Extraction by Amicon filter method shows about 50% recovery yield of starting material.
2. More than 1g of salt is required to detect DNA using this method.
3. This method can be used to detect whether a reasonable amount of DNA is present in ancient halite before proceeding onto stringent surface sterilization techniques, EM, and preservation methods to keep DNA from degrading.
4. Future research: at what level of DNA in environmental samples can we detect DNA using this method? How can we increase the recovery yield?

Acknowledgments

This project was funded by a NASA Space Grant research infrastructure competitive award. Also, we would like to thank the Biology 204 (Genetics) course students for their work in genetically identifying the isolates from our halite experiments.

Figure 4. Gel electrophoresis of DNA extraction from halite "UT untreated"

Figure 5. Densitometry of gel electrophoresis

Figure 6. Genetic identification of isolates from halite. Numbers represent a similarity score (closest match) in percent when the 16S rRNA gene sequence of our isolates was compared to 16S rRNA sequences in the GenBank database.

References

