HU Bin and KONG Fanjing. 2014. Characterization and Recovery of the Halophiles in the Fluid Inclusions in Halite. *Acta Geologica Sinica* (English Edition), 88(supp. 1): 71-72.

Characterization and Recovery of the Halophiles in the Fluid Inclusions in Halite

HU Bin[1,2] and KONG Fanjing[1,2]

1 Institute of Mineral Resources, Chinese Academy of Geological Sciences, Beijing, 100037, China;
2 Key Laboratory of Saline Lake Resources and Environments, Ministry of Land and Resources, Beijing, 100037, China

1 Introduction

It is the focus of geology and biology that the creature preserved in the geological history and the organic evolution. The creature preserved in geological history by these things: sedimentary, frozen earth, chrysophoron and evaporation salt. Evaporation salt can preserve the microbe and biomarker compounds. As the recovery of the evaporation and the trace of water on Mars, the creature in the fluid inclusions in evaporation salt has become an important evidence of the signs of life on Mars. This study introduced a method to extraction the DNA of halophiles entrapped in the fluid inclusions in halite.

2 The halophiles in the fluid inclusions

We put the halophile of DL-S-2 into the solution of fluid inclusions in halite evaporated in the lab. DL-S-2 belongs to Halobacillussp, it is Cocci, and its diameter is 0.5μm (Fig 1).

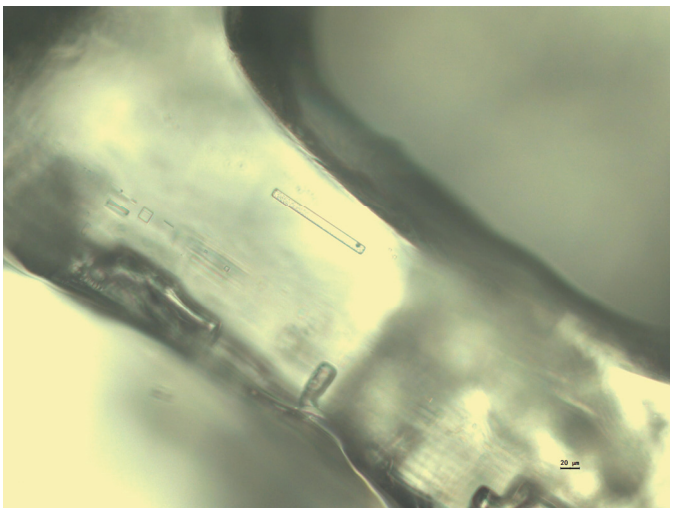

Fig. 1. Entrapped strain DL-S-2 in fluid inclusions (arrow heading to DL-S-2)

* Corresponding author. E-mail: hb.angel_1228@163.com

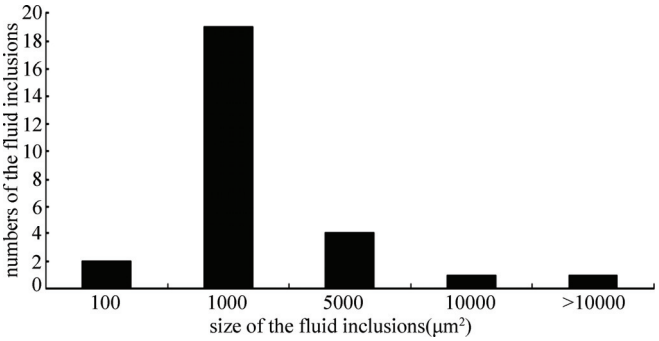

Fig. 2. Histograms of size of fluid inclusions which entrapped DL-S-2

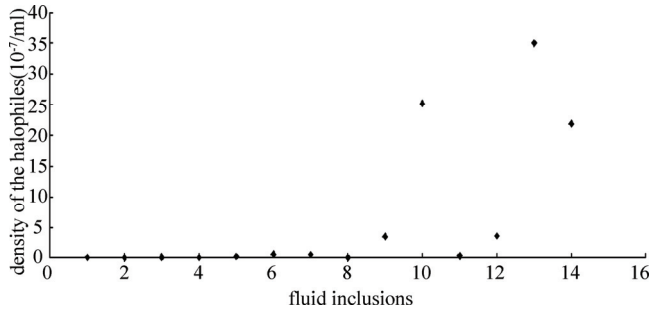

Fig. 3. Scatter diagram of density of fluid inclusions entrapped with DL-S-2

We analyses the sizes of the fluid inclusions under microscope. Among the 14 fluid inclusions within DL-S-2, there are 3 small fluid inclusions (less than 100μm2） and 8 middle fluid inclusions (100-1000μm2） and 4 large fluid inclusions (more than 1000μm2）(Fig2).

We also calculated the density of halophiles entrapped in the fluid inclusions. The lowest density is 0.03*107ind./ml and the highest one is 25.23*107 ind./ml. The average is 6.51*107 ind./ml (Fig 3).

3 Methods

First we do the surface sterilization for the sample to ensure that there no contamination DNA of the surface (Sankaranarayanan et al., 2011). Than we dissolve the

sample into the nano pure water. We use Amicon Ultra-0.5 Centrehall Filter Devices (500μl) (Carrigtwohill CO.) to collect the cells. And we use DNeasy Blood &Tissue (Qiagen CO.) to extract DNA. At last, we use PCR technique to amplify the DNA.

Tab 1 The testing result of DNA density by UV Spectrophotometer

Name of the samples	object	concentration(ng/ul)
0.5g sample（surface sterilization）	DNA	6.74
1g sample（surface sterilization）	DNA	8.79
0.5g sample（no surface sterilization）	DNA	12.85
1g sample（no surface sterilization）	DNA	12.18

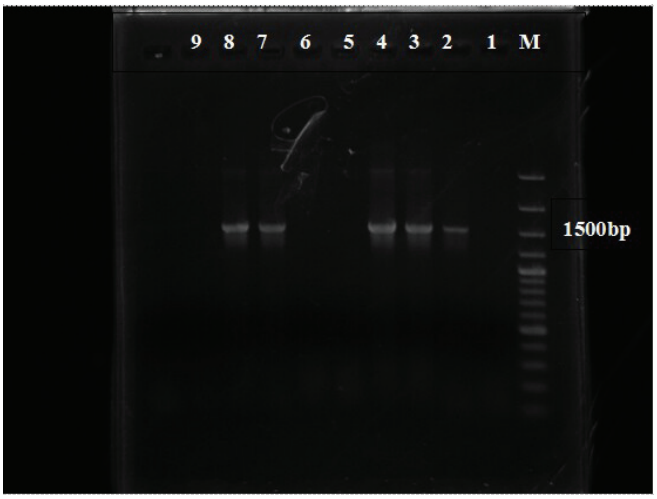

Fig. 4. Electrophoresis analysis of PCR
M: 100bp DNA ladder marker; 1: 2013-12-9 surface sterilization 0.5g sample; 2: 2013-12-9 surface sterilization 1g sample; 3: 2013-12-9 unsterilized 0.5g sample; 4: 2013-12-9 unsterilized 1g sample; 5: 2013-12-5 surface sterilization 0.5g sample; 6: 2013-12-5 surface sterilization 1g sample; 7: 2013-12-5 unsterilized 0.5g sample; 8: 2013-12-5 unsterilized 1g sample; 9: blank

4 Results

(1) We test the concentration of DNA by UV Spectrophotometer and the result is in Tab 1.

（2）We use the electrophoresis analysis to test the PCR result (Fig 4).

The BLAST results showed that the sequences of 16srRNA gene from Fluid inclusion in salt crystal has 99% homology with that of Dl-S-2 strain which was the original strain before the fluid inclusion formed. It demonstrated that this method was efficient to extract DNA from fluid inclusion of salt crystals.

5 Discussion

Using this method, we can successfully extract the DNA of DL-S-2 entrapped in the fluid inclusions. But we can't identify if there is any contaminate DNA. To ensure that, we may put an unrelated DNA on the surface of the sample. And detect this DNA after surface sterilization.

Key words: Fluid inclusions in halite; halophiles; DNA extraction

Acknowledgements

This study was supported by the China Geological Survey project (12120113019100), the project the Public Welfare Major Program from MLR(201011001-4). Thanks Pro. MP Zheng, Pro. KQ Cai, and Pro. J Lv for giving suggestions for modification.

References

Hebsgaard MB, Phillips M, Willerslev E. 2005. Geologically ancient DNA: fact or artefact? TRENDS Microbiol 13: 212–220.

Heide. 2002. Bacterial life in ancient salt[J]. 16(2):110-113.

James C. Adamski, Jennifer A. Roberts, and Robert H. Goldstein. 2006. Entrapment of Bacteria in Fluid Inclusions in laboratory-grown halite. Astrobiology 6(4): 552-562.

Johnson SS, Hebsgaard MB, Christensen TR, Mastepanov M, Nielsen R, et al. 2007. Ancient bacteria show evidence of DNA repair. Proc Natl Acad Sci U S A 104: 14401–14405.

Norton C F, Grant W D. 1988. Survival of Halobacterial within Fluid Inclusion in Salt Crystal[J]. Journal of General Microbiology, 134:1365-1373.

Sankaranarayanan K, Timofeeff MN, Spathis R, Lowenstein TK, Lum JK. 2011. Ancient microbes from halite fluid inclusion: optimized surface sterilization and DNA extraction. PLOS ONE, 6(6):1-8.

Vreeland R H, Rosenzweig W D, Powers D W. 2000. Isolation of a 250 million-year- halotolerant bacterium from a primary salt crystal[J]. Nature, 407:897-900.