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Detection methods for halite-embedded haloarchaea with potential relevance for extraterrestrial samples

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Abstract

Halophilic archaebacteria (haloarchaea) are found in environments with salt concentrations approaching saturation, such as natural brines, marine solar salterns and alkaline salt lakes; they have also been isolated from ancient subsurface salt sediments of great geological age (195–280 million years). Some of the strains from geologically old sediments were formally classified and described as novel species [1]. The characterization of subsurface microbial life is of astrobiological interest since extraterrestrial halite has been detected in meteorites and also on Mars. It is also conceivable that, due to the harsh surface conditions on Mars, microbial life, if existent, may have retreated into the subsurface. The renewed ExoMars plans of ESA and NASA call for an orbiter and two rovers, one of which will carry analytical instruments dedicated to exobiology and geochemistry research, including a subsurface drill [5]. Methods for the detection of microorganisms from subsurface locations are being developed; we are focussing on the detection of haloarchaea within natural or artificial halite samples. Three types of procedures were investigated here: Raman spectroscopy, staining of cells with fluorescent dyes, which provide also information on viability, and microarrays with a mixture of 300 antibodies against microbial cell components [4].
Raman spectroscopy is considered a very sensitive detection method for future astrobiological investigations on site. Its use for the detection of nine different extremely halophilic archaeal strains which had been embedded in laboratory-made halite crystals was explored [2]. The cells accumulated preferentially in tiny fluid inclusions of halite, as was demonstrated by pre-staining with the fluorescent green dye SYTO 9 (see Fig. 1). FT-Raman spectroscopy with laser excitation at 1064 nm yielded spectra with prominent peaks at 1507, 1152 and 1002 cm^{-1}, which were attributed to haloarchaeal C_{50} carotenoid compounds (mainly bacterioruberins) and whose intensity varied from strain to strain.
Analysis of cells following embedding in halite was based on the labeling with fluorescent dyes which are contained in the LIVE/DEAD *Bac*Light bacterial viability kit (Molecular Probes). The kit consists of two nucleic acid stains: SYTO 9, which penetrates most membranes freely, and propidium iodide, which is highly charged and normally cell-impermeant; it will, however, penetrate damaged membranes. Green fluorescence thus indicates viable cells with an intact membrane, whereas dead cells, due to a compromised membrane, show red fluorescence. A related method was staining of cells with the membrane potential kit, also from Molecular Probes, which provides information on the presence or absence of a membrane potential.
Antibody microarrays are immunosensors capable of detecting many different compounds from microbial species in complex mixtures such as environmental samples [4]. Positive signals were obtained with antibody mixtures and samples from Mediterranean sea salt or from the Dead Sea, which contained halophilic archaea and bacteria.

1. Introduction

The newly released plans for the ExoMars mission of ESA and NASA call for an orbiter, to be launched in 2016, dedicated to the characterisation of atmospheric trace gases of possible biological importance, and for a two-rover mission to be launched in 2018, one rover provided by NASA and the other by ESA. ESA's ExoMars rover will carry analytical instruments dedicated to exobiology and geochemistry research; it will travel several kilometres, collect and analyse samples from outcrops and from the subsurface with a drill down to a depth of 2 m [4].

The goals are the search for evidence of extinct or extant life on Mars. Methods for the detection of potential life forms are of interest for this endeavour and also for future extraterrestrial return samples.

2. Material and Methods

The following haloarchaeal strains were used (^{T} denotes type strain): *Halococcus dombrowskii* DSM 14522^T, *Hcc. morrhuae* DSM1307^T, *Halobacterium salinarum* NRC–1, *Hbt. salinarum* DSM 670, *Hbt. salinarum* DSM 3754^T, *Hbt. noricense* DSM 15987^T, *Haloarcula japonica* DSM 6131^T, *Halorubrum saccharovorum* DSM 1137, *Hrr. chaoviator* strain Naxos II. Embedding in halite, Raman spectroscopy and staining procedures were done as described previously [2, 3]. Microarray procedures were performed in collaboration with Dr. Parro and coworkers [4].

3. Figures

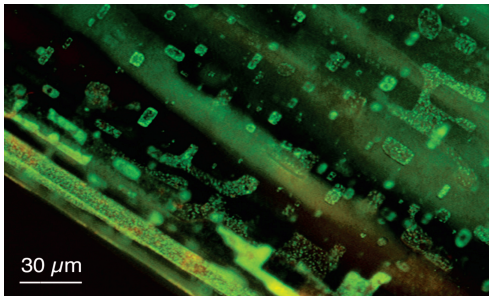

Figure 1. Localisation of pre-stained haloarchaea in fluid inclusions. Cells of *Halobacterium salinarum* NRC-1 were stained with the LIVE/DEAD BacLight kit, containing the green fluorescent dye SYTO 9, prior to embedding in artificial halite.

4. Conclusions

The metabolism of haloarchaeal strains from subsurface sediments is probably too slow to be seriously considered for the development of *in situ* activity tests for microorganisms on Mars or in return samples. Embedding in halite was found to render most cells inactive, possibly because they entered a dormant state. However, staining with fluorescent dyes, which give intense signals, could be envisaged for *in situ* detection procedures, especially when combined with specific antibodies. Raman spectroscopy is another promising tool for the detection of traces of organic molecules, e. g. characteristic pigments of extremophilic micro-organisms.

Acknowledgements

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References

[1] Fendrihan, S., Legat, A., Pfaffenhuemer, M., Gruber, C., Weidler, G.W., Gerbl, F.W. and Stan-Lotter, H.: Extremely halophilic archaea and the issue of long-term microbial survival, Reviews in Environmental Science and Bio/technology Vol. 5, pp. 1569–1605, 2006.

[2] Fendrihan, S., Musso, M. and Stan-Lotter, H.: Raman spectroscopy of extremely halophilic Archaea embedded in halite as a potential method for their detection in terrestrial and possibly extraterrestrial samples, J. Raman Spectroscopy Vol. 40, pp. 1996-2003, 2009a.

[3] Fendrihan, S., Berces, A., Lammer, H., Musso, M., Ronto, G., et al.: Investigating the effects of simulated Martian UV radiation on *Halococcus dombrowskii* and other extremely halophilic archaebacteria. Astrobiology Vol. 9, pp. 104-112, 2009b.

[4] Parro, V., Rodriguez-Manfredi, J. A., Briones, C., Compostizo, C., Herrero, P. L. Vez, E. et al.: Instrument development to search for biomarkers on mars. Terrestrial acidophile, iron-powered chemo-lithoautotrophic communities as model system. Planet. Space Sci., Vol. 53, pp. 729–737, 2005.

[5] Vago, J. L. The ExoMars rover mission to search for signs of life. Abstr. 5318, Astrobiology Science Conference, April 26-29, 2010, League City, TX, USA, 2010.