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Survival of halobacteria (haloarchaea) in fluid inclusions as a model of biotic survival in subterranean and extraterrestrial halite

H. Stan-Lotter (1), S. Fendrihan (1), S. Leuko (2), A. Legat (1), C. Gruber (1), M. Pfaffenhuemer (1), G. Weidler (1), H.-J. Busse (3)

(1) Department of Microbiology, Hellbrunnerstr. 34, A-5020 Salzburg, Austria, (2) Cyanobacteria and Astrobiology Research Laboratory, Biological Science Building, NSW Sydney 2052, Australia, (3) Institute of Bacteriology, Mycology and Hygiene, Veterinärplatz 1, A-1210 Vienna, Austria (helga.stan-lotter@sbg.ac.at, FAX 00436628044144, Phone 004366280445756)

From alpine rock salt deposits of Permian and Triassic age viable, extremely halophilic archaea (haloarchaea) have been isolated and some of them were described as novel species (1-3). Amplification of 16S rRNA genes, using the polymerase chain reaction with bacterial and archaeal primers, indicated evidence for the presence of a large haloarchaeal community in the salt deposits (4). Halite was discovered in the SNC meteorites, which stem from Mars, and recently evidence for the former presence of salt water on the Martian surface was obtained by the US rovers. If Mars and Earth had a similar geological past, as has been suggested by several authors, then microbial life, or the remnants of it, could still be present on Mars. Together with the previously documented presence of viable haloarchaea in ancient rock salt, a reconsideration of survival conditions of microorganisms and the possibility of preservation for very long time periods appears warranted. Strains of *Halobacterium salinarum* and *Halococcus dombrowskii* were grown in complex media to cell densities of about 10^9 colony forming units (CFU) per ml. Cells were embedded in salt crystals under conditions, which simulated the natural formation of evaporites. Recovery of viable cells following dissolution of salt crystals was between 10^7 and 10^8 CFU/ml (0.4 to 16 %), depending on the strains. Staining of cells with fluorescent dyes (LIVE/DEAD *Bac*Light kit from Molecular Probes) prior to embedding allowed easy visualization and suggested a preferential localization of haloarchaea in fluid inclusions. *Halobac-*

terium salinarum NRC-1 and related rod-shaped strains were found to undergo morphological changes, mainly a transformation to spherical shapes, when embedded in salt crystals. Responses of cells towards simulated Martian conditions, using either a simulation chamber (at the Austrian Academy of Science, Graz) or deep freezers and vacuum chambers, were tested. Storage of cells in crystals at minus 70^{o}C for one month caused reduction of CFU; however, epifluorescence microscopy showed still numerous viable cells following exposure to low temperatures. Exposure to the Mars simulation chamber produced damaged DNA with increasing dryness of samples. The results suggested that fluorescent labeling in the presence of 4 M NaCl can be used for testing the response of haloarchaeal cells to simulated environmental extremes, which resemble the conditions on Mars. In addition, potential microbial life in evaporites may occur as dormant forms, possibly with spherical morphology, which are located mainly in fluid inclusions. Detection methods of dormant cellular forms in dry materials such as salt must be improved and optimized before attempts for identification in valuable extraterrestrial materials are made.

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