Survival of halobacteria in fluid inclusions as a model of possible biotic survival in Martian halite

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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 rovers; together with the previous discovery and isolation of several viable haloarchaea from Permo-Triassic rock salt (1-3), 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. The localization of halobacterial cells in the salt was almost exclusively in fluid inclusions, as could be shown by staining cells with fluorescent dyes (LIVE/DEAD *Bac*Light kit) prior to embedding. *Halobacterium 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. Storage of cells in crystals at minus 70°C for one month caused some reduction of CFU; however, epifluorescence microscopy showed still numerous viable cells following exposure to low temperatures. 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, and that potential microbial life in evaporites may occur as dormant forms, possibly with spherical morphology, and may be located in fluid inclusions.

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