

Dissertation Anja Bauermeister im Jahr 2013 (mit Auszeichnung)

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Abstract

In the last decades, our neighboring planet Mars has received much attention as one of the most promising targets close to Earth on which life may have developed in the past or might even exist at present. Recent NASA and ESA missions have provided detailed information on the physical and geochemical environment of Mars that can help to identify potential habitats and organisms that could be able to grow with the energy sources present on the planet. Due to the abundance of iron and sulfur minerals on Mars, iron-sulfur transforming microorganisms are considered likely models for putative Martian life forms (e.g. Amils et al. 2007; Fernández-Remolar et al. 2005; Nixon et al. 2012). For this study, two species of acidophilic iron-sulfur bacteria were chosen: the chemolithoautotrophic *Acidithiobacillus ferrooxidans* and the spore-forming *Sulfobacillus thermosulfidooxidans*. The goal of this thesis was to study their metabolic capacities, especially regarding the ability to grow with *in situ* resources that could be expected on Mars, and their tolerance to environmental stress conditions. A subsurface environment was assumed as the most likely habitat in these experiments, because it has a higher probability for liquid water, can provide different sources of energy for lithotrophic metabolism, and afford protection from the harsh surface physical conditions.

It was shown that acidophilic iron-sulfur bacteria such as *A. ferrooxidans* may play an important role in iron redox cycling on Mars, as they can both oxidize ferrous iron (Fe^{2+}) using O_2 as an electron acceptor, and reduce ferric iron (Fe^{3+}) using H_2 , which could be generated by several processes in the subsurface of Mars. The threshold for aerobic respiration in the experiments with *A. ferrooxidans* and *S. thermosulfidooxidans* was 0.05% of dissolved O_2 . Oxygen partial pressure in the Martian atmosphere was too low to yield any measurable iron oxidation activity of *A. ferrooxidans* after 1 week of incubation in Mars gas at low pressure (15 hPa). However, abiotic processes in the subsurface involving the interactions of liquid water with iron-bearing minerals could generate O_2 in sufficient quantities. Furthermore, *A. ferrooxidans* was incubated on two synthetic mixtures of Mars regolith minerals under aerobic and anaerobic conditions. Growth was observed with either O_2 as an external electron acceptor or H_2 as an external electron donor, and minerals provided not only Fe^{2+} and Fe^{3+} for energy gain, but probably also other essential nutrients for lithoautotrophic growth. The bacteria also seemed contribute to the reductive dissolution of Fe^{3+} -containing minerals like goethite and hematite, which are characterized by a high thermodynamic stability.

Because the Martian environment is not a static one, but will exhibit fluctuations in physical conditions, potential life forms would have to cope with stress factors like desiccation, high salt concentrations, low temperature, and radiation. A set of viability indicators was used to compare the effect of the stress conditions on different aspects of cellular viability such as reproduction, metabolic activity, and integrity of cellular compounds (membrane, DNA, rRNA). Reproductive ability (culturability) of *A.*

ferrooxidans after exposure to different stressors was often more severely affected than other markers of cell viability such as iron oxidation activity, membrane integrity, and rRNA integrity, which might suggest that cells entered a viable-but-nonculturable state. At low temperatures (4°C), *A. ferrooxidans* did not grow, but remained metabolically active, pointing to a state of maintenance metabolism. Based on culturability, *A. ferrooxidans* and *S. thermosulfidooxidans* exhibited only moderate tolerance to most of these stress factors. However, desiccation tolerance was improved if the cells were kept under low oxygen tension, grown as a biofilm, or embedded within an external matrix of compatible solutes such as sucrose and trehalose. Freezing tolerance was also improved by the addition of compatible solutes and growth as biofilms. *A. ferrooxidans* and *S. thermosulfidooxidans* survived one week under simulated Martian surface conditions (6 hPa, -20°C, 0.13% O₂) in the form of dried biofilms. Thus, the conditions of the Martian surface (especially low temperature and low oxygen pressure) were favorable to the survival of cells. Although *A. ferrooxidans* was sensitive to UV-C radiation (F₁₀ = 29 J/m²) ample shielding is provided already by shallow layers of dust (especially containing Fe³⁺) or by upper cell layers in a biofilm. Comparing the average ionizing radiation dose on Mars with the tolerance of *A. ferrooxidans* (D₁₀ = 46 Gy), a population of these bacteria could remain viable in the shallow subsurface (0-3 m) of Mars for up to 10⁵ years. In sufficient depths, organisms would be protected from surface radiation and could persist even longer if intermittent maintenance metabolism and repair are possible.

The acidophilic iron-sulfur bacterium *A. ferrooxidans* can be considered a probable member of a potential Martian food web based on its metabolic capacities.