EPSC Abstracts
Vol. 16, EPSC2022-465, 2022
https://doi.org/10.5194/epsc2022-465
Europlanet Science Congress 2022
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Metallosphaera sedula on a Mission – mimicking Mars in frames of the Tanpopo 4 mission

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With future long-term space exploration and life detection missions on Mars, understanding the microbial survival beyond Earth as well as the identification of past life traces on other planetary bodies becomes increasingly important. The series of the Tanpopo space mission experiments target a long-term exposure (one to three years) of microorganisms on the KIBO Module of the International Space Station (ISS) in the low Earth orbit (LEO) (Kawaguchi et al., 2020; Ott et al., 2020). In the search for possible past and/or present microbial life on Mars, metallophilic archaeal species are of a special interest due to their inherent extraordinary characteristics. Chemolithotrophic archaea (e.g., from the order *Sulfolobales*) employ a number of ancient metabolic pathways to extract energy from diverse inorganic electron donors and acceptors. *Metallosphaera sedula*, an iron- and sulfur-oxidizing chemolithotrophic archaeon, which flourishes under hot and acidic conditions (optimal growth at 74°C and pH 2.0), was cultivated on genuine extraterrestrial minerals (Milojevic et al., 2019; Milojevic et al., 2021) as well as synthetic Martian materials (Kölbl et al., 2017). In all cases, *M. sedula* cells were able to utilize given mineral materials as the sole energy source for cellular growth and proliferation. During the growth of *M. sedula* cells on these materials, a natural mineral impregnation and encrustation of microbial cells was achieved, followed by their preservation under the conditions of desiccation (Kölbl et al. 2020). Our studies indicate that this archaeon, when impregnated and encrusted with minerals, withstand long-term desiccation and can be even recovered from the dried samples to the liquid cultures (Kölbl et al., 2020). The achieved preservation of desiccated *M. sedula* cells facilitated our further survivability studies with this desiccated microorganism under simulated Mars-like environmental conditions and during the Tanpopo-4 space exposure experiment.

Consequently, *M. sedula* cells grown on various mineral materials were exposed at the Astrobiology Space Simulation facilities (DLR, Cologne) to a simulated Martian environment for one month, to ensure their suitability for a long-term experiment on the ISS within the Tanpopo-4 mission. Recovery of cells after exposure to a simulated Martian environment shows an insignificant impairment in cellular growth compared to ground control cells.

A set of desiccated *M. sedula* cells grown on Martian breccia NWA 7034 (Milojevic et al., 2021) has already been launched for 1 year outside the ISS to Mars-like conditions in frames of the Tanpopo-4 mission (2022-2023). Upon retrieval of cells from the ISS, we will evaluate survivability of this chemolithoautotroph protected by various mineral materials in Mars-like environment. Furthermore, high-resolution electron microscopy and (nano)-spectroscopy techniques will be applied to identify preservable biosignatures of chemolithotrophic life on Martian materials.

Resolving the interface of microbial interactions with Mars minerals under the influence of destructive Martian environmental constrains can bring us closer to identify traces of unicellular life for current and future Mars exploration missions. We will also reveal a role of mineral encapsulation as a natural shielding crust for cell protection during exposure to simulated Martian conditions.

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