



## Halophilic life on Mars ?

Helga Stan-Lotter (1), Sergiu Fendrihan (2), Marion Dornmayr-Pfaffenhauer (1), Anita Holzinger (1), Tatjana K. Polacsek (3), Andrea Legat (1), Michael Grösbacher (1), and Andreas Weigl (1)

(1) University of Salzburg, Microbiology, Salzburg, Austria (helga.stan-lotter@sbg.ac.at), (2) Romanian Bioresource Center and Advanced Study Research Association, Bucharest, Romania, (3) Open University, Geomicrobiology Group, Milton Keynes, UK

**Background:** The search for extraterrestrial life has been declared as a goal for the 21th century by several space agencies. Potential candidates are microorganisms on or in the surface of moons and planets, such as Mars. Extremely halophilic archaea (haloarchaea) are of astrobiological interest since viable strains have been isolated from million years old salt deposits (1) and halite has been found in Martian meteorites and in surface pools. Therefore, haloarchaeal responses to simulated and real space conditions were explored. Immuno assays for a potential Life Marker Chip experiment were developed with antisera against the universal enzyme ATP synthase.

**Methods:** The focus of these studies was on the application of fluorescent probes since they provide strong signals, and detection devices are suitable for miniaturization. Viability of haloarchaeal strains (*Halococcus dombrowskii* and *Halobacterium salinarum* NRC-1) was probed with the LIVE/DEAD BacLight™ kit and the BacLight™ Bacterial Membrane Potential kit. Cyclobutane pyrimidine dimers (CPD) in the DNA, following exposure to simulated and real space conditions (UV irradiation from 200 - 400 nm; 18 months exposure on the International Space Station [ISS] within the ADAPT experiment by Dr. P. Rettberg), were detected with fluorescent Alexa-Fluor-488-coupled antibodies. Immuno assays with antisera against the A-ATPase subunits from *Halorubrum saccharovorum* were carried out with the highly sensitive Immun-Star™ WesternC™ chemiluminescent kit (Bio-Rad).

**Results:** Using the LIVE/DEAD BacLight™ kit, the D37 (dose of 37% survival) for *Hcc. dombrowskii* and *Hbt. salinarum* NRC-1, following exposure to UV (200-400 nm) was about 400 kJ/m<sup>2</sup>, when cells were embedded in halite and about 1 kJ/m<sup>2</sup>, when cells were in liquid cultures. Fluorescent staining indicated a slightly higher cellular activity than that which was derived from the determination of colony forming units. Assessment of viability with the BacLight™ Bacterial Membrane Potential kit gave strong signals with *Hcc. dombrowskii* and the control microorganism *E. coli*; as expected, the uncoupler CCCP diminished the membrane potential. Reaction times were generally longer with *Hcc. dombrowskii* than with *E. coli*. *Hcc. dombrowskii* from the ISS experiment showed > 80% viable cells when judged with the LIVE/DEAD kit. CPD formation was detectable in about 3-5 % of the total cells. It is not yet known if growing cells of *Hcc. dombrowskii* were recovered from the ISS. ATPase subunits were detected in crude membrane preparations, in whole haloarchaeal and bacterial cells, and even in spores (from *Geobacillus stearothermophilus*), suggesting the usefulness of the ATP synthase as a molecular target for life detection.

**Conclusions:** Fluorescent dyes provide strong signals, which are suitable for remote detection and are compatible with high ionic strength. The advantages of staining with fluorescent dyes are rapid results on membrane intactness, membrane potential, and the presence of certain biomolecules. But more data are needed for a better correlation to cellular viability.

(1) Stan-Lotter H, Pfaffenhauer M, Legat A, Busse H-J, Radax C, Gruber C (2002) *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permian alpine salt deposit. *Int System Evol Microbiol* 52, 1807-1814.