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Using antibodies against ATPase and microarray immunoassays for the search for potential extraterrestrial life in saline environments on Mars.

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For the search for extraterrestrial life it is proposed to use receptors such as labelled antibodies for the detection of organic biomarkers. One of these organic molecules to be tested is the universal enzyme ATP synthase which is present in highly conserved forms in all organisms on earth. Therefore it is necessary to evaluate antibodies against ATPase respectively ATP synthase and their subunits. As it is known, that there are halite deposits on Mars the experiments in this study have been carried out with regard to halophile microorganisms and saline environments.

Standard F1F0 ATPase from Escherichia coli LE 392 and Bacillus megaterium as well as haloarchaeal A-ATPase from Halorubrum saccharovorum and Halobacterium salinarum NRC-1 were used.
The cultivated cells, except Bacillus, were broken by passage through a French Pressure Cell. Separation of enzyme subunits was performed by polyacrylamide gel electrophoresis. Western Blotting with antisera produced in rabbit against A-ATPase subunits A (85 kD) and subunits B (60 kD) from Halorubrrum saccharovorum (1) showed positive reactions with the membrane fraction, which should be enriched with ATPase from Halorubrum saccharovorum, Halobacterium salinarum NRC-1 and Escherichia coli LE 392.

Particular attention was given to the question if ATPase subunits can be detected in whole cells. Therefore whole cell preparations of all cells and spore suspensions from Geobacillus stearothermophilus were tested against the antiserum as well as against protein-A-purified antibody against A-ATPase subunit A from Halorubrum saccharovorum. A positive immuno reaction of all cell preparations with the antiserum as well as with the purified antibody was detected. The spores of Geobacillus stearothermophilus reacted positively with the antiserum against subunit A of the A-ATPase from Hrr. saccharovorum.
A commercial antibody Rabbit Anti-V-ATPase subunit A polyclonal antibody from the GenScript Corporation reacted positively with membrane fraction and whole cell preparation of Halobacterium salinarum NRC-1, Escherichia coli LE392 as well as the whole cell fraction of Halorubrum saccharovorum and Bacillus megaterium.

Further experiments with antibodies against ATPase are proposed to be done with procedures that are more adjusted to the search for extraterrestrial life. Therefore tests with a microarray system (Rivas et al., 2008) were done at the Centro de Astrobiología in Madrid. Cellular extracts of environmental samples from a sea salt from Piranske (Slovenia) and a rock salt from Himalaya (Pakistan) were tested with a „supermix“ of 300 antibodies, additionally including an antibody against the subunit A of the A-ATPase from Halorubrum sacharovorum. Positive immuno reactions with antibodies against halophile cells as well as antibodies against exopolysaccharides could be shown.

(1)Gruber C, Stan-Lotter H (1997) Western blot of stained proteins from dried polyacrylamide gels. Anal Biochem 253, 125-127.
(2)Rivas LA, Garcia-Villadangos M, Moreno-Paz M, Cruz-Gil P, Gómez-Elvira J, Parro V (2008) A 200-antibody microarray biochip for environmental monitoring: searching for universal microbial biomarkers through immuno-profiling. Anal Chem 80, 7970-7979