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The Role of Perchlorate on the Habitability of Mars

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Putative microbial life on Mars would encounter a severe scarcity of liquid water due to the prevailing freezing and hyperarid climate conditions. Interestingly, hygroscopic salts were discovered on Mars that enable water absorption from the atmosphere and lower the freezing point, potentially allowing the temporary occurrence of liquid briny water [1]. However, these brines contain high concentrations of salts, posing significant challenges for life, including high salinity, ionic strengths, and low water activity. Of particular interest are perchlorate (ClO₄⁻) salts, which are widespread on Mars [2] but rare in natural environments on Earth. Therefore, investigating the habitability of perchlorate brines and the effects of perchlorate ions on microbial cells through lab experiments is crucial. In this study, we summarize the results of our investigations into the perchlorate-specific stress responses of various organisms from all three domains of life (archaea, bacteria, and eukaryotes) and discuss their implications for the habitability of Mars.

As model organisms, we used *Planococcus halocryophilus* and *Escherichia coli* for bacteria, *Haloferax volcanii* for archaea, and *Debaryomyces hansenii* for eukaryotes. We employed various analytical tools, including growth experiments, fluorescence and scanning electron microscopy, and proteomics, to analyze the microbial stress responses. Microscopic analysis revealed that cells exposed to perchlorate stress often exhibited morphological changes, such as the formation of cell clusters in *P. halocryophilus* and *D. hansenii*, as well as cell filaments in *E. coli*. Growth experiments demonstrated that all tested organisms could adapt to higher perchlorate concentrations when repeatedly incubated with increasing amounts of perchlorate. Notably, the halotolerant yeast *D. hansenii* displayed the highest perchlorate tolerance at 2.5 mol/kg NaClO₄ (water activity a_w = 0.926), although its tolerance to NaCl was even higher at 4.0 mol/kg (a_w = 0.854) [3]. Proteomic investigations indicated that the chaotropicity of perchlorate (i.e., destabilization of biomacromolecules) likely contributed to *D. hansenii*'s decreased tolerance towards NaClO₄ compared to NaCl [4]. In response to the chaotropic stress, *D. hansenii* stabilized proteins through increased glycosylation and upregulated the biosynthesis and remodulation of the cell wall. Analyses of perchlorate-induced changes in the proteome of other investigated organisms are ongoing, with preliminary results showing only a limited overlap with the perchlorate-specific stress responses observed in *D. hansenii*. However, for both prokaryotes investigated thus far (*E. coli* and *H. volcanii*), the *de novo* inosine monophosphate (IMP) biosynthesis and purine metabolism appeared to play a crucial role in the metabolic perchlorate defense machinery, suggesting an increased requirement for nucleotide repair and stabilization. This might be due to chaotropic and oxidative stress resulting from perchlorate exposure.

The surprisingly high perchlorate tolerances of organisms like *D. hansenii* and the ability of various organisms to adapt to elevated perchlorate concentrations demonstrate that perchlorate in liquid solutions under ambient temperature is not as detrimental to life as often assumed.

References: [1] Martínez G.M. and Renno N.O. (2013) Space Science Reviews 175:29–51. [2] Clark B. C. and Kounaves S. P. (2016) International Journal of Astrobiology 15:311–318. [3] Heinz J. et al. (2021) Life 11:1194. [4] Heinz J. et al. (2022). Environ. Microbiol. 24:5051–5065.

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