PERCHLORATE STRESS RESPONSES OF ALL THREE DOMAINS OF LIFE

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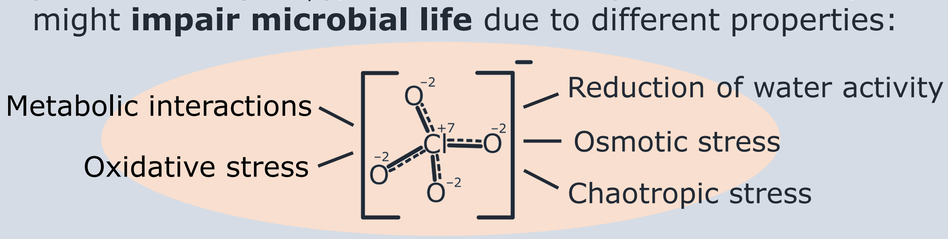
Motivation

Putative Martian microorganisms could have adapted to the dry, subzero environment of **present-day Mars** by resorting to **hygroscopic salts** that might ensure, at least temporarily, the formation of liquid brines by deliquescence[1].
Our investigations focus on highly deliquescent perchlorates (ClO_4^-), which are widespread on Mars[2], but might **impair microbial life** due to different properties:



The aim of our studies is to **identify perchlorate-specific stress responses** in order to draw conclusions on the microbial habitability of Mars and on potential biomarkers. For this purpose, we chose various **model organisms** from all three domains of life covering non- or meso-halophilic (*Escherichia coli*), halotolerant (*Debaryomyces hansenii, Planococcus halocryophilus*), and extremely halophilic (*Haloferax volcanii*) species.

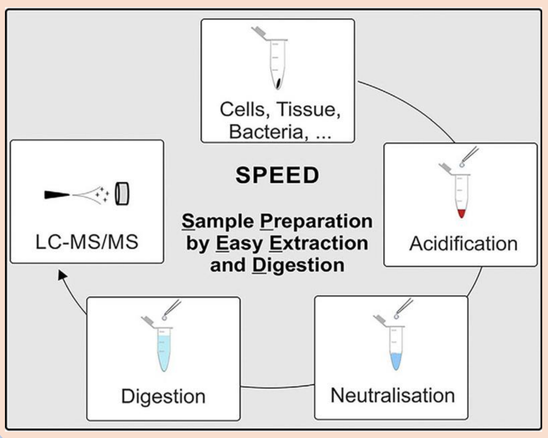
Methodology

Growth experiments

- Incubation of cells in the respective complex growth media containing certain concentrations of $NaClO_4$ or other solutes (e.g. NaCl, glycerol) for comparison puposes
- Stepwise adaptation to higher solute concentrations until maximum solute tolerances are reached
- Observing growth or death by measuring the optical density at 600 nm (OD_{600}) and counting colony forming units (CFU), as well as microscopical approaches

Proteomics

Characterization of stress responses via proteomics

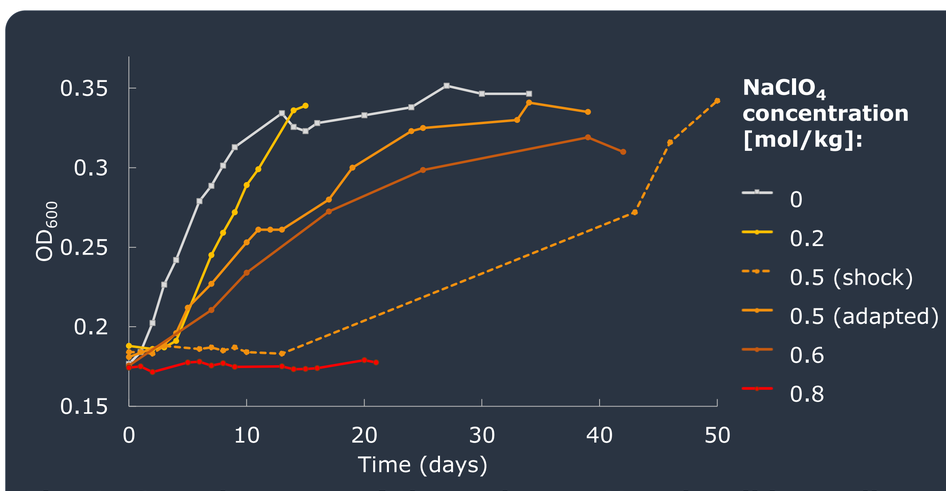


Doellinger, et al., 2020[3]

The SPEED protocol was recently developed by the Robert Koch Institute in Berlin[3].

It enables sample-type independent deep proteome profiling with high quantitative accuracy and precision.

Preliminary results



Archaea: Growth experiments with the halophilic *H. volcanii* demonstrated that $NaClO_4$ cannot completely substitute NaCl, which is essential for survival of the organism and can be tolerated up to saturation. However, growth medium supplemented with 1.7 mol/kg NaCl and 0.6 mol/kg $NaClO_4$ yielded growth when cells where long-term adapted to increasing perchlorate concentrations (Fig. 1).

Fig. 1: Growth curves of the archaeon *H. volcanii* in medium containing 1.7 mol/kg NaCl and varying $NaClO_4$ concentrations as indicated.
Growth was followed by optical density (OD_{600}) and confirmed via CFU counts (data not shown).

Bacteria: Growth experiments with *E. coli* in perchlorate-rich medium revealed formation of cell filaments (Fig 2A), while *P. halocryophilus* formed large cell clusters, which include both dead (red) and living cells (green, Fig. 2B)[4].

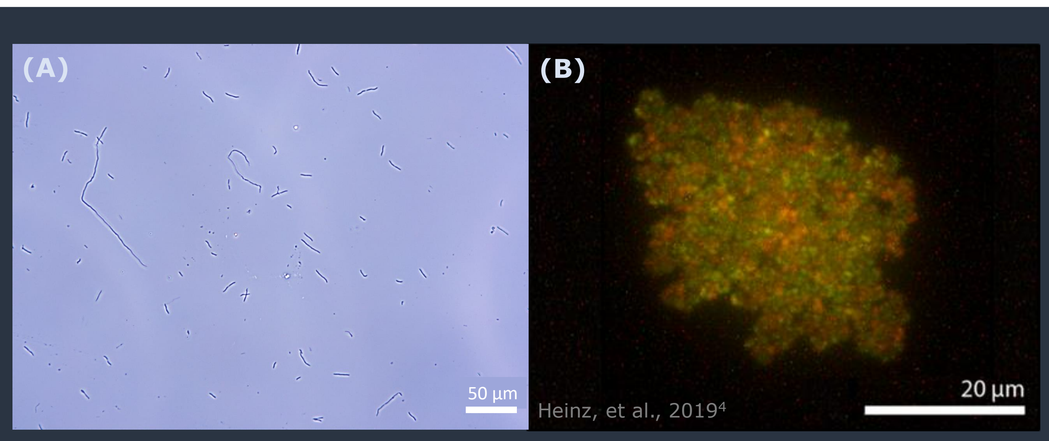
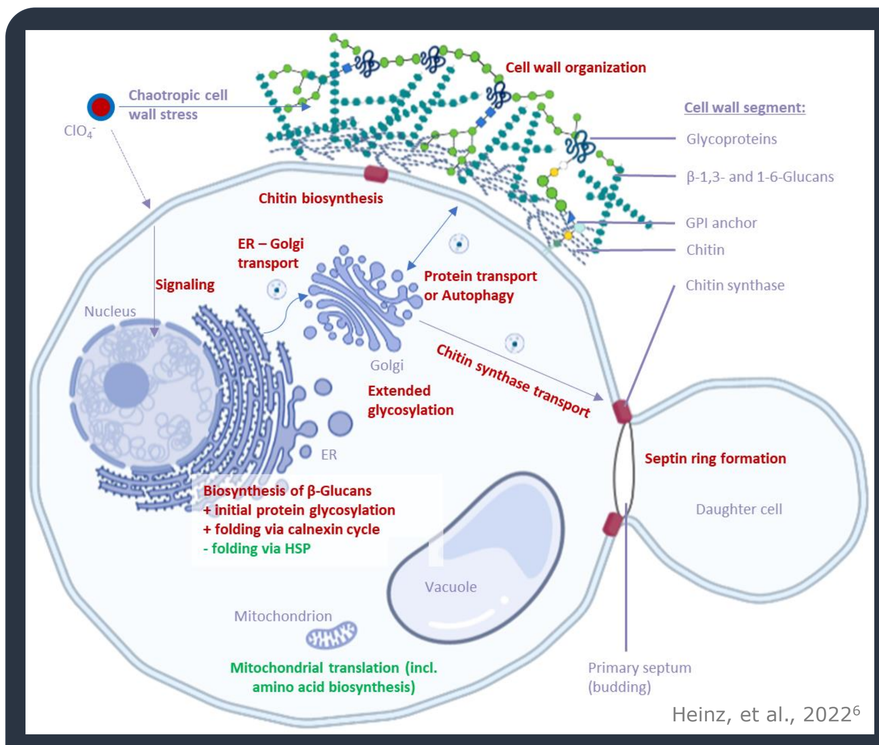


Fig. 2: Cell filamentation and clustering after perchlorate exposure.
(A) Cell filamentation after growth of *E. Coli* in perchlorate-rich medium, **(B)** Cell cluster of *P. halocryophilus* after perchlorate exposure and live/dead staining (green: intact cells; red: disrupted/dead cells).



Eukaryotes: The halotolerant yeast *D. hansenii* had the highest perchlorate tolerance reported to date (2.5 mol/kg $NaClO_4$)[5]. Protein analyses[6] disclosed that perchlorate generates chaotropic stress to the cell wall and other biomacromolecules such as proteins.

To counteract this type of stress, proteins were stabilized by glycosylation (incl. upregulation of β-glucan biosynthesis, protein glycosylation in the ER and Golgi, and the respective transport mechanisms), and glycosylated proteins were folded via calnexin cycle. The fungal cell wall was stabilized by biosynthesis of cell wall components such as chitin and glucans, and by cross linking of these components (Fig. 3)[6].

Fig. 3: Perchlorate-specific stress responses of the halotolerant yeast *D. hansenii*[6].
A mother cell and a budding daughter cell displaying the most relevant metabolic pathways with perchlorate-specific upregulations (red) and downregulations (green). Created with BioRender.com.

What's next?

- Using lethal **perchlorate-shock experiments** in addition to adaptation experiments
- Investigating the influence of **temperature** and **other ions** (e.g. Mg^{2+}, Ca^{2+}, ClO_3^-) on the stress responses
- Applying proteomic analyses also to *E. coli*, *H. volcanii*, and *P. halocryophilus*
- Extending experiments to **other organisms** (e.g. cyanobacteria, methanogens) and **environmental samples**
- Adding **additional analytical tools** such as metabolomics and lipidomics
- Identifying potential perchlorate-specific **biomarkers**

Implications for the habitability of Mars

- Hygroscopic **perchlorates increase the water availability** on Mars, while at the same time decreasing the cell survival by **chaotropic stress**, which destabilizes biomacromolecules, whereas oxidative stress is less prominent[5,6]
- Chaotropic stress leads to *(1)* reduced ClO_4^- tolerance compared to other solutes, e.g. NaCl (Fig. 1), *(2)* the formation of **cell clusters** and filaments (Fig. 2), and *(3)* the upregulation of **protein glycosylation** & cell envelope remodulations (Fig. 3).
- Likewise, putative organisms on Mars exposed to ClO_4^--rich brines might also form large cell aggregates and stabilize biomacromolecules and cell envelops by adaptations similar to those observed in our experiments
- **Perchlorate-specific biomarkers** might results from these adaptions, which is currently under investigation
- **In-situ resource utilization (ISRU)** technologies on Mars might rely on "chaotolerant" organisms / genes

References

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