ISOLATION OF HALOTOLERANT SPECIES FROM ENVIRONMENTAL SAMPLES AND MICROBIAL STRESS RESPONSES AFTER PERCHLORATE EXPOSURE

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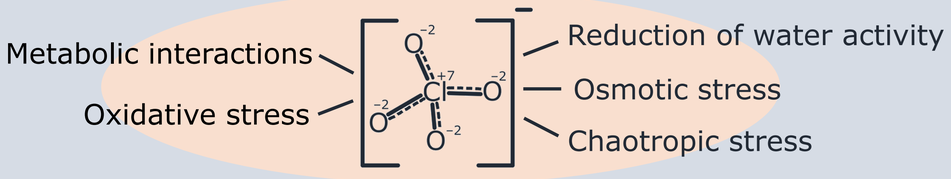
Motivation

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 as well as **strains isolated from hypersaline environments** such as the Don Juan Pond, Antarctica.

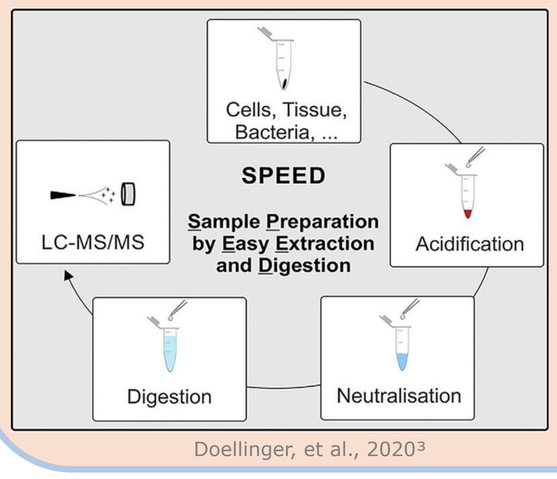
Methodology

Growth and isolation experiments

- Sampling hypersaline waters and sediments
- Incubation of aliquots from environmental samples in complex growth media containing $NaClO_4$ or $CaCl_2$
- Observing growth via optical density (OD) measurements, counting colony forming units (CFU), and through microscopical approaches
- Isolation of strains able to grow at elevated salt concentrations
- 16- or 18s rRNA sequencing of isolated species

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.

What's next?

- Analyzing **sequencing results** from isolated species
- **Full genome sequencing** in case of unknown species
- Applying proteomic analyses also to **other model organisms** as well as isolated species and compare them with *D. hansenii*
- Investigating the influence of **temperature and other ions** (e.g. Mg^{2+}, Ca^{2+}, ClO_3^-) on the stress responses
- Using **anaerobic cultivation** techniques
- Adding additional analytical tools such as **metabolomics** and **lipidomics**
- Identifying potential salt-specific **biomarkers**

Preliminary results

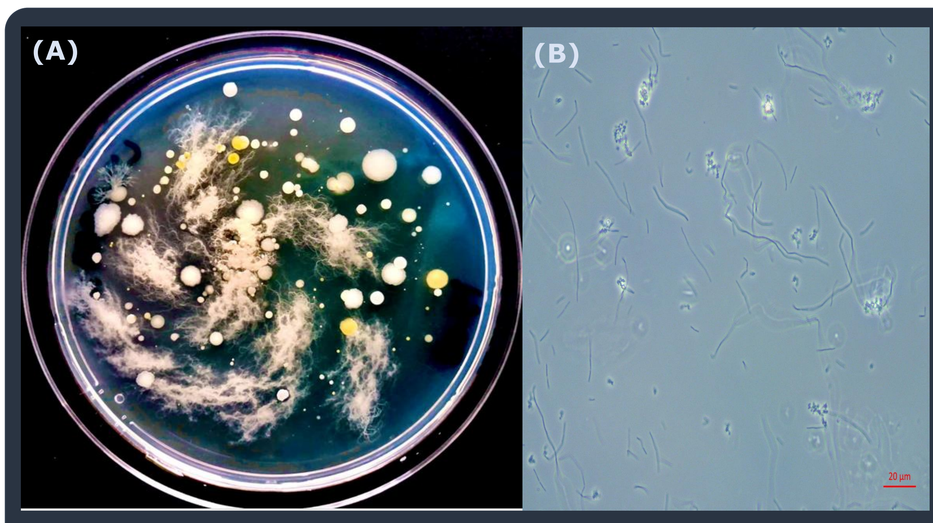


Fig. 1: Isolation of species from (hyper)saline environments.
(A) Species isolated from salt meadow samples (Luchwiesen, Germany) surviving in presence of 2 mol/kg $NaClO_4$, and **(B)** from the Don Juan Pond sediments growing in elongated cells when exposed to 1.7 mol/kg $CaCl_2$.

Isolation experiments:
Inoculation of liquid growth medium containing 2.0 mol/kg $NaClO_4$ with sample material from soil of the salt meadows 'Luchwiesen' in Storkow, Brandenburg, Germany, resulted in **survival** of various halotolerant species forming colonies when plated on salt-free agar plates (Fig. 1A).
Inoculation of growth medium containing 1.7 mol/kg $CaCl_2$ with sediment material from the $CaCl_2$-enriched Don Juan Pond, Antarctica resulted in **growth** of elongated cells (Fig. 1B) similar to *E. coli* exposed to perchlorate (Fig. 3A). Sequencing results are expected to arrive soon.

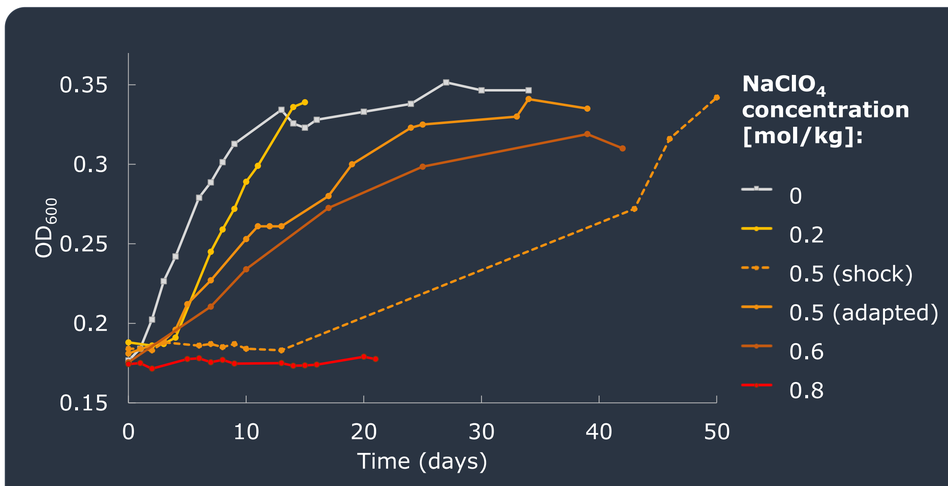


Fig. 2: Growth curves of the archaeon *Haloferax volcanii* in medium containing 1.7 mol/kg NaCl and varying $NaClO_4$ concentrations as indicated.
Growth was followed by optical density (OD_{600}) and confirmed via CFU counts (data not shown).

Archaea: Growth experiments with the halophilic *Haloferax 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. 2).

Bacteria: Growth experiments with *E. coli* in perchlorate-rich medium revealed formation of cell filaments (Fig 3A), while *P. halocryophilus* formed large cell clusters, which include both dead (red) and living cells (green, Fig. 3B)[4].

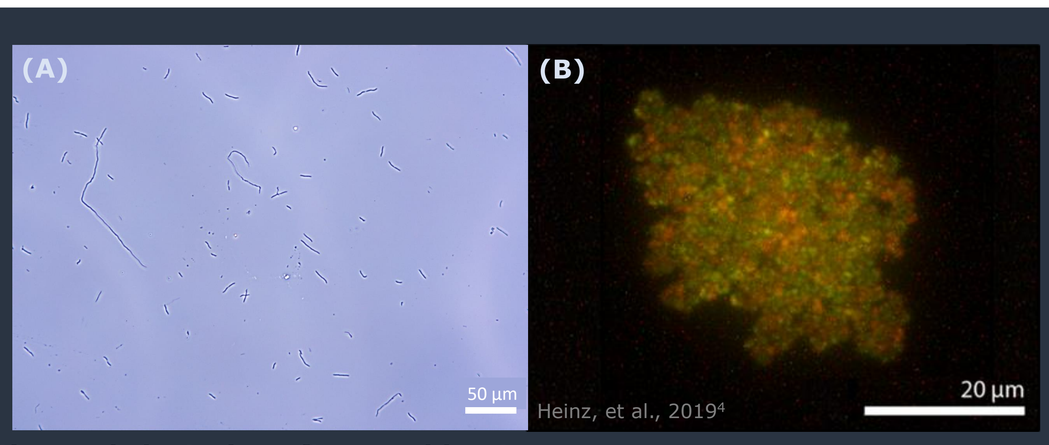


Fig. 3: Cell filamentation and clustering after perchlorate exposure.
(A) Cell filamentation after growth of *E. coli* in perchlorate-rich medium, **(B)** cell cluster of *Planococcus halocryophilus* after perchlorate exposure and live/dead staining (green: intact cells; red: disrupted/dead cells).

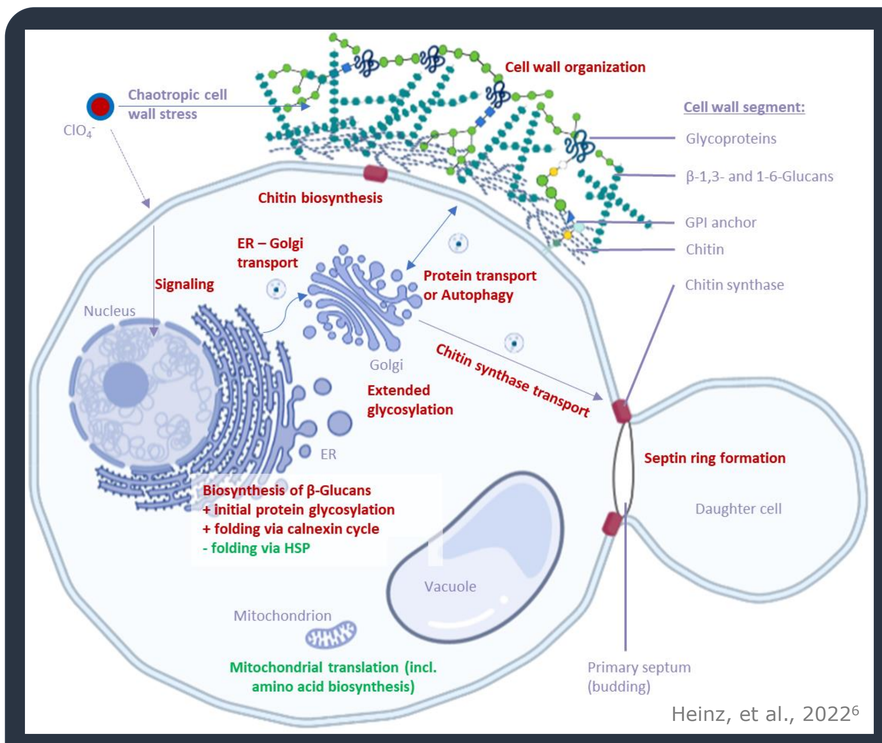


Fig. 4: Perchlorate-specific stress responses of the halotolerant yeast *D. hansenii*[6].
A mother cell and a budding daughter cell displaying the most relevant metabolic pathways with perchlorate-specific upregulations (red) and downregulations (green). Created with BioRender.com.

Eukaryotes: The halotolerant yeast *Debaryomyces hansenii* has 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, while oxidative stress seemed to play only a minor role. To counteract the chaotropic stress, proteins were stabilized by glycosylation (incl. upregulation of β-glucan bio-synthesis, 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. 4)[6].

Isolated species

Model organisms from all three domains of life

References

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