**Surviving the Stress: Unraveling Molecular Strategies in Psychrophiles Confronting Perchlorate-Rich Ice**

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**Introduction:** Perchlorate tolerance in microorganisms is of specific interest to the field of Astrobiology because of the suspected widespread occurrence of these oxy-ions in Martian soils and ice. Previous research indicates that certain extremophiles can endure environments with perchlorate, a compound known to primarily induce two types of stress: chaotropic and oxidative. To date, the tolerance of marine psychrophiles to perchlorate-amended has not been studied.

**Methodology:** The model marine psychrophilic bacterium, *Colwellia psychrerythraea str. 34H* (*Cp34H*), was grown in perchlorate-amended media (18%/weight) at two subzero temperatures (-1℃ & -5℃) and collected samples over time. Growth curves were measured using optical density at 600nm and proteomic samples were harvested via centrifugation.

*LC-MS/MS.*  Protein lysates were analyzed using data-dependent acquisition proteomics as described in Mudge et al. 2021**.** For each MS experiment, peptides were mixed with internal and external standards, and analyzed using a Thermo Fisher QExactive equipped with an inline EASY-nLC 1200 system.

*Data processing and analysis.* MS data was searched against the *Cp34H* protein sequences (Uniprot) with Comet and filtered to control false discovery rate using Peptide- and Protein-Prophet (FDR <0.01) (Eng et al., 2015, 2013, Nesvizhskii et al., 2003). Differential protein abundance was calculated in QPROT (Choi et al., 2015) using log fold change (LFC) as a metric signifying increased (+LFC) or decreased (-LFC) in abundance. Non-metric multidimensional scaling (NMDS) and weighted gene correlation network analysis (WGCNA) were completed on the dataset following Langfelder and Horvath, 2008. Functional enrichment analyses were completed for each significant WGCNA module.

**Results & Discussion:** Growth rates were suppressed in perchlorate medium at growth temperatures compared to control media, indicating that perchlorate ions further suppress growth at subzero temperatures for marine psychrophiles.

*WGCNA-derived protein modules.* Our WGCNA analysis of all significant proteins demonstrated that among the two experimental variables, the growth media (perchlorate vs control) accounted for the greatest variation within the defined protein modules. The proteins within the blue (n = 743) and turquoise (n = 1014) module yielded the highest correlation with growth media (0.974 and -0.973 respectively). Quantitative protein abundance analysis revealed that proteins in the blue module were increased in perchlorate-incubated cells, while the turquoise module proteins were decreased in the perchlorate treatment. Proteins within these modules are indicators of a metabolic response to perchlorates.

*Oxidative and chaotropic stress.* The top significant proteins with the highest LFC were involved cell membrane maintenance and adhesion. This includes PspA-IM30 (LFC +1.3) and glycosyl hydrolase (LFC +2.3) which are involved in maintaining the integrity and stability of membranes under these stresses. Here we present proteomic evidence of a marine psychrophile mitigating oxidative and chaotropic stress which has a destabilizing effect on glycan macromolecules.

*Transport and movement.* Decreases in TonB dependent receptor abundance in perchlorate treatments (LFC -2.9) implies a decrease in the ability to transport molecules across membranes. Additionally, perchlorates in these experiments decreased the abundance of flagellin (LFC -1.9), indicating that movement ceases with this particular stress, contrary to previous reports of *Cp*34H in amended brines (Mudge et al. 2021).

*Cellular adhesion as a biosignature*. The von Willebrand factor domain A protein (vWA) exhibited the most substantial increase in abundance (LFC +3.7). Known for its involvement in extracellular adhesion activities in eukaryotes and its role in coordinating the reception of divalent cations such as Mg2+ in prokaryotes. The combination of membrane enhancing and repairing proteins with the observed increase of this vWA protein suggests increased physical cellular adhesion when exposed to perchlorates. Biomarkers or microscopic observations of this physical reaction could represent a novel indicator of cell survival in response to perchlorate. These pathways are currently being explored in salt nodules from the Atacama Desert, a Mars analog here on earth.

**References:**

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