Halotolerance of *Planococcus halocryophilus* in Chloride and Perchlorate Brines

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Introduction

Remote sensing and in-situ measurements have detected a global distribution of chloride and perchlorate salts on the Martian surface [1,2]. These highly hygroscopic salts, such as calcium perchlorate, can absorb water from the thin Martian atmosphere and temporally form stable liquid solutions [3,4]. However, the habitability of these brines remains unclear; a knowledge gap we attempt to close with our research.

For this purpose we have been investigating the survivability of the bacterial strain *Planococcus halocryophilus* Or1, which has been isolated from the permafrost active layer in the Canadian High Arctic and can thrive under cold and salty conditions [5,6]. In an earlier study we described the enhanced microbial survivability of *P. halocryophilus* in subzero brines with eutectic salt concentrations [7].

In this study we have shown that the survival of *P. halocryophilus* cells in chloride brines increases greatly when lowering the temperature from 25°C to -15°C or -30°C. However, cell growth did not occur in any of the investigated brines, presumably due to the low water activities at these high salt concentrations.

Thus, in our follow-up experiments presented here we have determined for different temperatures the maximum concentrations of various chloride and perchlorate salts under which *P. halocryophilus* is still able to grow (for 4°C the results are preliminary). Cell growth was determined via CFU counts of biological duplicates. The results show that *P. halocryophilus* can tolerate significant amounts of all the salts studied. Such tolerance would be an essential adaptation for microbes to survive in Martian soils.

Growth Under Salt Stress Conditions

Background image: P. halocryophilus cell clusters (dark) on salt crystals (white/grey)

a) Higher salt contents in the growth media caused a **longer lag phase** in the growth curve with a more intense cell number reduction after inoculation (Fig. 1A)

b) At least in the case of $CaCl_2$ containing samples **lowering the temperature increased the salt tolerance** of *P. halocryophilus* (Fig. 1A)

c) Cells formed **clusters** under salt stress conditions (Fig. 1B)

d) After plating samples on agar plates **different types of cell colonies** occurred under salt stress conditions compared to salt-free (or low salt) conditions (Fig. 1C)

ZELMI-TUB 10.0kV 15.7mm x3.50k PDBSE(3D) 8.57um

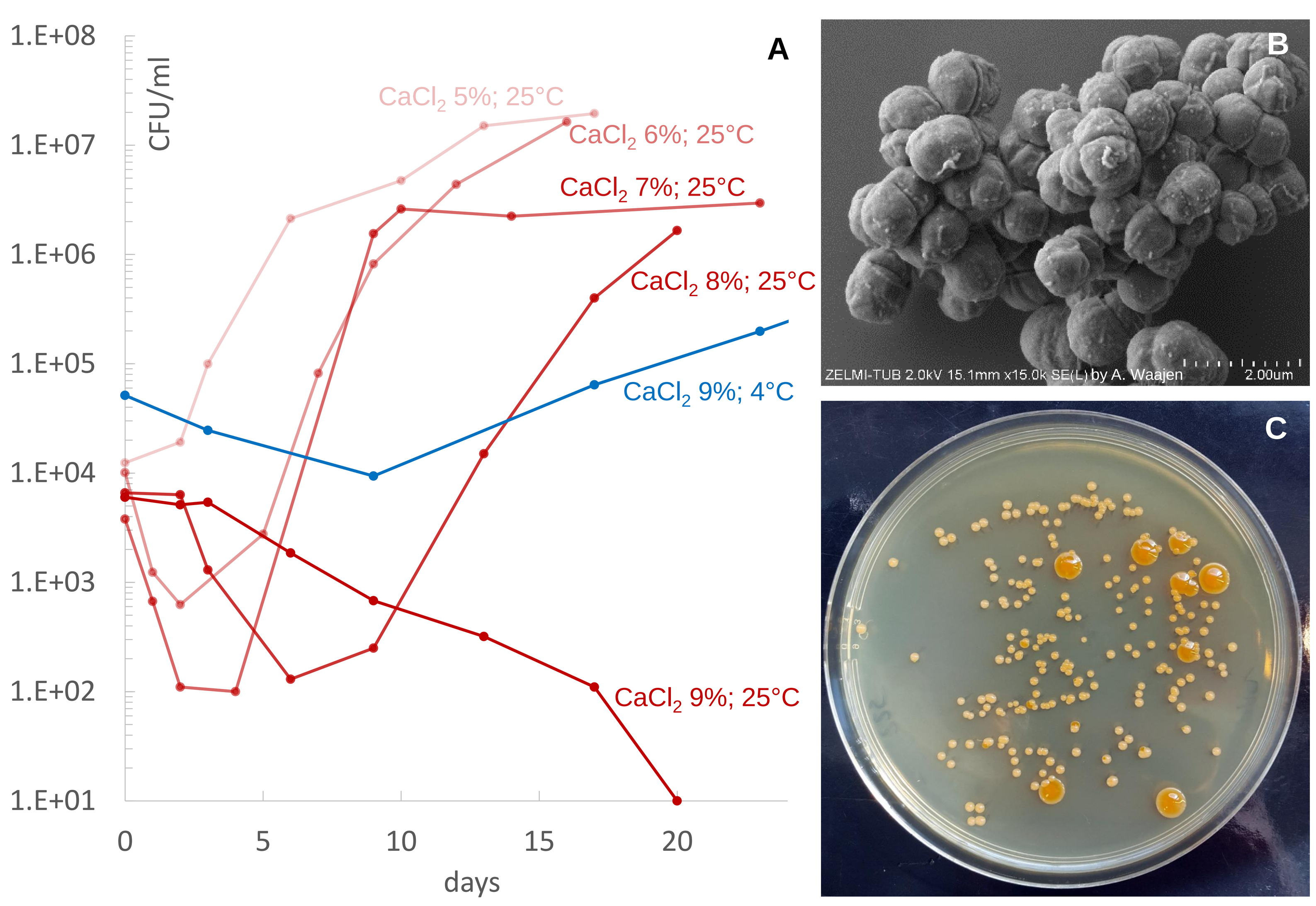

Figure 1: (A) Growth curves (CFU counts) of P. halocryophilus in $CaCl_2$ containing growth medium at 25°C and 4°C; (B) SEM image of cell clusters formed in 10% $NaClO_4$ samples; (C) Different cell colony types at salt free conditions (larger, orange) and salt stress conditions (smaller, pale).

Halotolerance at 25°C and 4°C

Background image: P. halocryophilus cell clusters in a 10% $NaClO_4$ sample

a) The salt that could be tolerated by *P. halocryophilus* in highest concentrations is NaCl, however **tolerance against $MgCl_2$ was remarkably** high as well (Fig. 1A): the Cl^- content in $MgCl_2$ samples was similar to those containing NaCl (Fig 1B) and ionic strength in $MgCl_2$ samples could even exceed the values for NaCl samples (Fig. 1C)

b) **$CaCl_2$ tolerance** was slightly reduced at 25°C but **could be increased by lowering the temperature** (the other salts had similar or lower salt tolerances compared to 25°C)

c) The **tolerance against perchlorate was** at least 2.5 fold lower than against chloride, but still **notably** high, e.g. 12 wt% $NaClO_4$ (1.1 mol/l)

d) *P. halocryophilus* could tolerate **water activities down to 0.9** (Fig. 1D)

5 µm

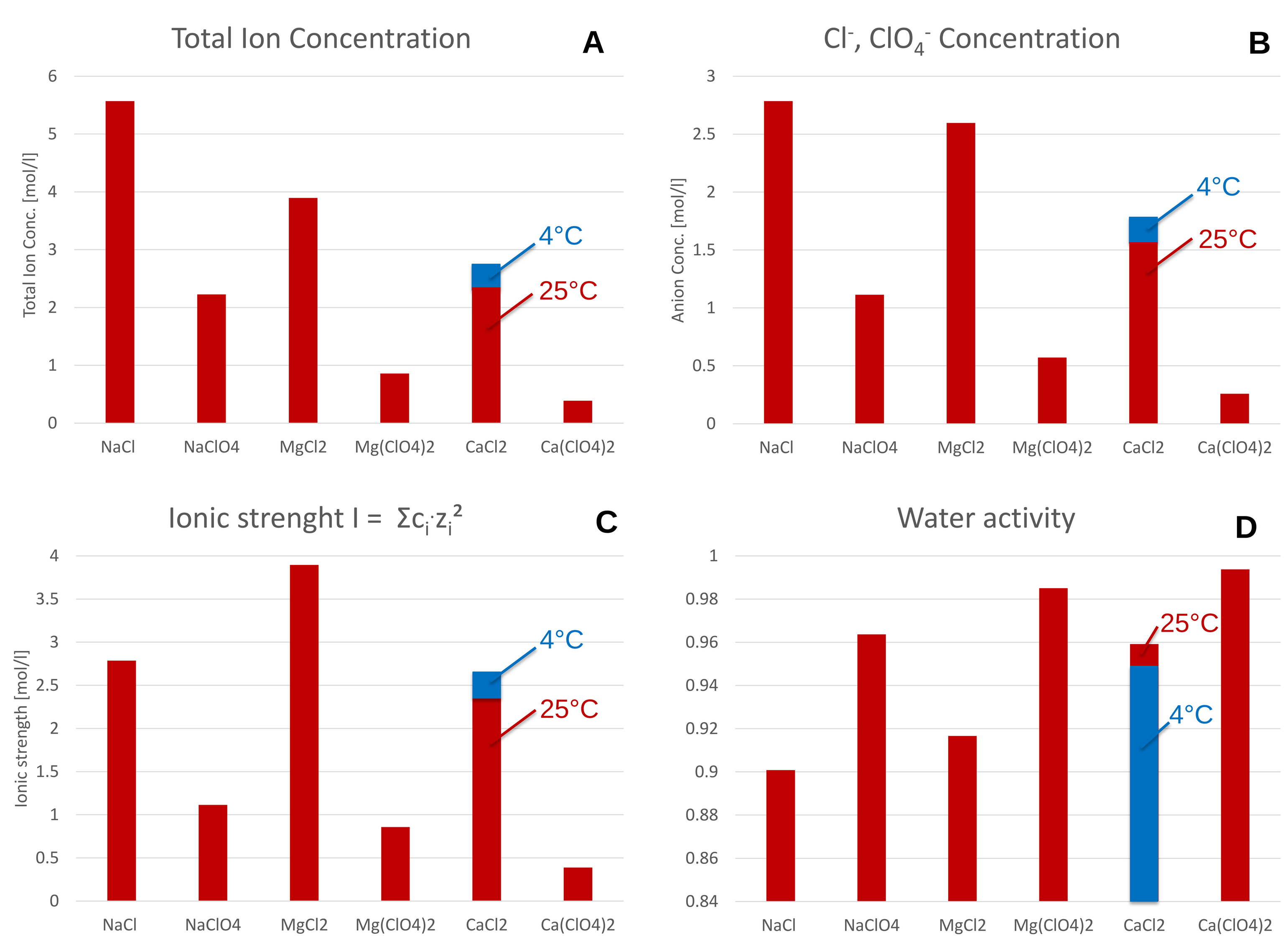

Figure 2: Halotolerance of P. halocryophilus. (A) Maximum total ion concentration; (B) Maximum anion (Cl^-, ClO_4^-) concentration; (C) Ionic strength at highest salt concentration; (D) Water activity at highest salt concentration.

Conclusions

- *P. halocryophilus* can tolerate and grow in a variety of highly concentrated solutions of salts that might exist on Mars.
- Cells under salt stress conditions form clusters that seem to protect at least the inner cells of the clusters from the osmotic stress.
- The cell survivability in concentrated brines can be enhanced by lowering the temperature [7]. In some cases also the halotolerance for growth can be increased by lowering the temperature.
- This positive effect of lower temperatures is important for the habitability of brines on Mars with its average surface temperature of -55°C (218 K).
- However, the determined maximum salt concentrations are too low to cause a notable freezing point depression of water.
- Thus, bacterial strains comparable to *P. halocryophilus* have a higher chance to grow in the Martian subsurface with more moderate temperature conditions.

References & Acknowledgements

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