Bacterial presence in chaotropic perchlorates solutions at subzero temperatures: Implications to Mars

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Abstract

The environmental conditions dominating on Mars early in its geological history (4.5–3.5 billion years ago) are not precisely constrained, although climate models suggest that mean temperatures were generally below freezing. Under these conditions, the presence of liquid water has been linked to the decrease of the freezing point of the aqueous solutions by a variable enrichment in dissolved salts, forming high ionic solutions and brines. Here we evaluate the influence that the presence of microorganisms may exert on the physical properties of specific hygroscopic salts relevant to Mars, particularly perchlorates. Thus, we have cultivated bacteria in highly concentrated magnesium perchlorate solutions at sub-freezing temperatures. We studied their survivability as well as their interaction with the chaotropic perchlorates through a combination of techniques of molecular microbiology and aqueous geochemistry, recording the changes observed by Micro-Differential Scanning Calorimetry (µ-DSC) of the phases stability of the brine system. We found that this new methodology is accurate to determine how microorganisms can modify their environment and create a potentially more habitable niche.

1. Introduction

Geologic evidence indicative of flowing and ponding liquid water on the surface of ancient Mars appears abundantly across the Martian landscape, suggesting that liquid water was present in variable amounts and for long periods of time on and/or near the surface at different moments of Mars' history [1]. If the early Mars environments were "cold", the presence of liquid water can be explained because the freezing point of the aqueous solutions was decreased by a variable enrichment in dissolved salts [2] and high ionic solutions and brines has been suggested to be prevalent in a "cold and wet" Mars [3]. Here, we analyze the effects of the combination of cold and salty environmental conditions on microbial strains of *Rhodococcus* sp. JG3 and *Escherichia coli*, with the two fold aim of (1) better understanding whether halophile-psycrophile microorganisms can modify the freezing point depression in brines, and (2) extrapolating our results to constrain the limits of habitability on a "cold" Mars. Besides, we open the path to explore deeper the astrobiological implication of the role that ice binding proteins (IBPs), that control ice crystal formation [4], have in the adaptation and survivability of cell in these scenarios.

2. Materials and methods

The model used for our study was the psycrophile and halotolerant bacteria, *Rhodococcus sp.* JG3 in highly concentrated magnesium perchlorate solutions (20% wt and 50 % wt $Mg(ClO_4)_2$) at sub-freezing temperatures (263 and 253 K). Cultures of *Escherichia coli* was used in parallel as a non-psychrophilic strain. Our main technical approach involves the use of Differential Scanning Calorimetry (µ-DSC) in the bacterial cultures to record potential changes in the liquidus curve of magnesium perchlorate solutions above at both endmembers of the eutectic concentration (44 % wt). This technique measure the thermal properties of substances, and is the model method for the direct determination of enthalpy [5]. Besides, other techniques were applied to study the survivability of cells in perchlorates after 10 days of incubation in the mentioned salt and cold conditions. We evaluated the survivability rate by flow cytometry and confocal microscopy using the LIVE/DEAD™ BacLight™ Bacterial Viability Kit that stain differentially viable and non-viable cells. Moreover, due to the highly probable role of proteins in the adaptation of these bacteria to the experimental conditions above, protein extracts from the cultures after the 10 days of incubation were separate in a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to compare their protein pattern.

3. Results

After incubation time at 263 K, the major differences observed in the calorimetry measurements occurred in *Rhodococus* sp. JG3 cultures, where a decrease of almost 0.4 K in the liquidus curve of 20 wt% $Mg(ClO_4)_2$ solution was recorded compared to the sterile solution (Fig.1). Unexpectedly, a depression in the liquidus point was also detectable in *E. coli* cultures, but with lower differences to the sterile.

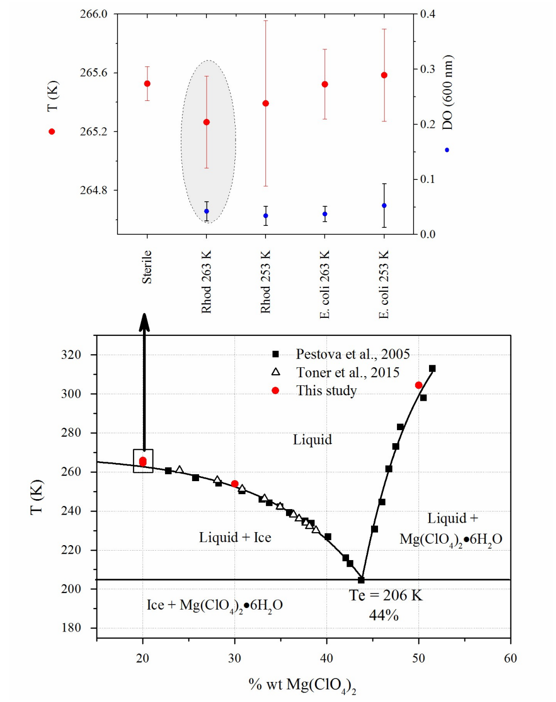

Figure 1. *Phase diagram of magnesium perchlorate, including our experimental data (red circles) and data from previous works (black squares [6] and white triangles [7]). The upper-inset zooms experimental data for the 20% wt solution (red circles) and final optical density (600 nm) of the cultures (blue circles).*

Flow cytometry analysis showed a reduced proportion of living cells in the two species analyzed (Fig.2). Although *E. coli* seems to show higher values of viable cells than *Rhodococcus* sp. JG3, these data came with the caveat of large standard deviations. In addition, a recovery of the cells of *Rhodococcus* sp. JG3 was observed after restoring the bacteria to optimal conditions.

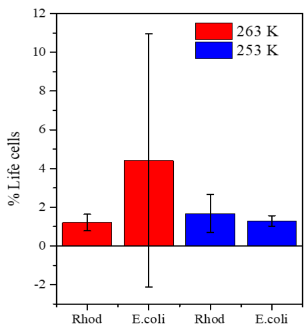

Figure 2. *Relative survival rate of cells (%) after 10 days of cultivation in 20% wt magnesium perchlorate at 263 and 253 K, as determined by flow cytometry.*

Furthermore, the protein patterns obtained by SDS-PAGE showed differences between the molecular machinery expressed in the cultures grown in optimal conditions and in stressed conditions.

4. Discussion and conclusions

Our study reveals that calorimetry is a suitable and promising method to analyze potential changes in the melting behavior of perchlorate solutions caused by the presence of bacterial cultures at subfreezing temperatures. Recent studies suggest that some chaotropes might enhance the growth opportunities for life in hostile environments [8] and here we show that at some extent extremophiles are able to modify the chaotropicity of the solutions in which they inhabit, generating more hospitable microenvironments in cold ecosystems. Although the majority of the cells in the analyzed cultures died after exposure to aforesaid conditions, still enough cells remained viable in the solutions and few surviving cells kept their ability to multiply when optimal environmental conditions were restored. Similar reversible responses of the chaotrophic effects on cells has been reported in fungi studies [9] and in thermophilic bacteria [10]. The differences in protein profiles founded between bacteria growing at optimal conditions and bacteria incubated in cold aqueous perchlorate solutions, provide clues about the potential role of IBPs as important players in the adaptability of life to cold environments. The molecules overexpressed as a consequence of these adaptation can be used as models for the search for biosignatures in planetary exploration. We conclude that, if life ever existed in Mars, microbial communities may have contributed to enhance the habitability of salty aqueous solutions at freezing temperatures, therefore amplifying their own survivability limits on a “cold and wet” planet.

5. References

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