Salinibater ruber as a model for the habitability of Europa's ocean.

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Introduction: Life on Earth can be found in environments that are considered extreme in comparison with the conditions to which mesophiles, or regular forms of life, develop [1]. The organisms that proliferate in the extreme environments are called extremophiles, and particular examples can be found in the three domains of life [2, 3]. Some extreme environments possess at least one physical or geochemical condition like those described for the planetary bodies of astrobiological importance in the Solar System [4]. This is the case for Europa, one of the satellites of Jupiter, whose most relevant characteristic is the presence of a liquid water ocean under its icy surface, enriched with sulfate compounds like $MgSO_4$ and Na_2SO_4 [5, 6]. This extraterrestrial saline environment gives us the opportunity to study the strategies used by halophilic bacteria in terms of compounds different to NaCl.

Salinibacter ruber is an extreme halophilic bacterium whose growth and adaptation to different concentrations of NaCl and $MgSO_4$ have been previously studied in our group [7]. The aim of this work is to evaluate the adaptation strategies of *S. ruber* when is subjected, at once, to $MgSO_4$ and $NaSO_4$, two of the main salty components of the ocean of Europa.

Materials and Methods: Growth kinetics were used to determine the optimal growth conditions at different concentrations of Na_2SO_4, and Na_2SO_4+$MgSO_4$. Growth rate and duplication time were used to evaluate the results. The presence of compatible solutes was evaluated by quantitative NMR, and the expression of proteins in different saline stress conditions was identified by electrophoresis and shotgun proteomics.

Results and discussion: Growth curves show a most favorable fit of *S. ruber* in Na_2SO_4 compared whit the growth in $MgSO_4$. These results may be correlated with the Hofmeister series, as the cation Na^+ is more cosmotropic than Mg^{2+}. This is also the case for the anion SO_4^{2-} when compared with Cl^- [8]. The optimal growth in Na_2SO_4 occurred at smaller concentrations than in $MgSO_4$ as Na_2SO_4 provides a higher ionic charge than $MgSO_4$. The best growth was observed at osmolarities of around 6.5 osm/L in both salts, regardless of the concentration.

The analysis of the protein expression at different NaCl, $MgSO_4$ and Na_2SO_4 concentrations shows particularities on the type and on the quantity of the proteins involved in the adaptation strategies of *S. ruber*. It has been found that *S. ruber* accumulates betaine as compatible solute when is subjected to saline stress on NaCl [7], so we will verify if other compatible solutes are also present when *S. ruber* is exposed to other salty conditions.

The present results show the capability of terrestrial organisms to subsist and adapt to salty environments different from NaCl. This faculty can be used to search with more detail, if they are also able to adapt to the salty conditions of the liquid water ocean of Europa, rich in sulfates. The study of the mechanisms of adaptation *of S. ruber* to sulfate salts allows the evaluation of the physical and chemical limits of some of the life forms we know, and eventually the study of the metabolic mechanisms that help them to survive, will give us clues on the possibility to find a living being in a scenario of astrobiological importance.

References:

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