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BACTERIAL HALOTOLERANCE STRATEGIES: THE COMPATIBLE SOLUTES CASE

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We identified betaine as the main compatible solute accumulated by *Bacillus pumilus*, *Cobetia marina*, and *Salinibacter ruber* when exposed to salinity conditions similar to those of the liquid water ocean of Europa. Quantitative nuclear magnetic resonance spectra helped in the compatible solute characterization while different concentrations of NaCl, Na_2SO_4, and $MgSO_4$ were used to simulate the salinity of Europa's ocean.

Sodium chloride (NaCl) and other chlorides are common in water bodies on Earth. Surface geological features of the satellite Europa in combination with magnetometer studies state the basis for the existence of a deep aqueous ocean beneath an icy water crust, where sulfates either of magnesium or sodium ($MgSO_4$ or Na_2SO_4) are present in near-saturation concentrations (Vu et al. 2016).

As the average salt content on terrestrial oceans is around 3.5% NaCl, all organisms thriving at higher NaCl concentrations are considered halophiles. One of the strategies they used is the synthesis and/or accumulation of low-molecular weight organics with high-water solubility known as compatible solutes. These molecules do not interfere with the metabolism of their hosting organism but help in the stabilization of some enzymes, the maintenance of cellular turgor, and in protecting from extreme conditions, such as high salinity, high or low temperature, or desiccation (Avendao et al. 2015).

We characterized, by ^{1}H quantitative Nuclear Magnetic Resonance (qNMR), the compatible solutes accumulated by *Bacillus pumilus* (GenBankFJ867397), *Cobetia marina* (DSM5160) and *Salinibacter ruber* (DSM13855) when exposed to water activities (a_w) ranging from 0.99 to 0.85 due to specific concentrations of NaCl, Na_2SO_4, and $MgSO_4$. The chemical shift, in parts per million (ppm), and the multiplicity of each NMR signal were used as the identification parameters (Figure 1).

When the microbial culture media was modified with NaCl, *B. pumilus* accumulates betaine, glutamate, and glutamine. As the salinity concentration increased (lower a_w values) the presence of betaine was more evident. The case for *C. marina* was different as this halophilic bacterium accumulates betaine, but also ectoine and hydroxyectoine as the NaCl concentration increases. When its media were modified with sulfates ($MgSO_4$ or Na_2SO_4), only betaine was detected. The case for *S. ruber* was particular as this extreme halophile accumulates only betaine regardless the chemical nature or concentration of the added salt. These results strongly support the hypothesis that the stress imposed to organisms by salts different from NaCl is not the same (Ramírez et al. 2019). We envisage a relationship between the identity of the compatible solutes accumulated by a bacterium and the specific salinity condition of its environment. We acknowledge funding from the Agencia Espacial Mexicana-CONACyT Program (grant A3-S-65162).

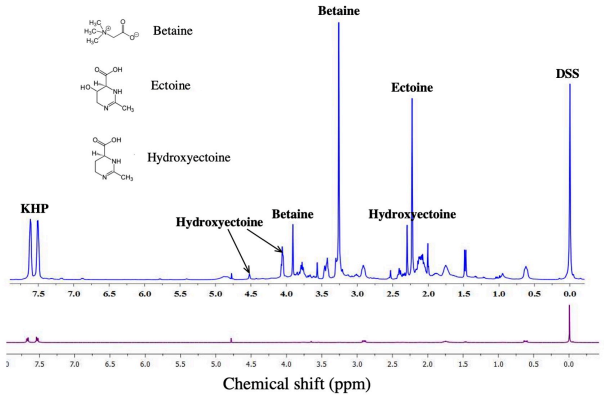

Fig. 1. Nuclear magnetic resonance spectra (400 MHz) of *C. marina* without (lower profile) and with 1.37 M NaCl (upper profile). Signals corresponding to betaine (3.8, 3.2 ppm), ectoine (2.0 ppm) and hydroxyectoine (4.5, 4.0, 2.3 ppm) are identified. KHP, used as an internal standard, is identified as the signal at 7.6 ppm.

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