"III Congreso Latinoamericano de Astrobiología (2021)"
Revista Mexicana de Astronomía y Astrofísica Serie de Conferencias (RMxAC), **55**, 101–101 (2023)

https://doi.org/10.22201/ia.14052059p.2023.55.37

PROTEOMICS OF *SALINIBACTER RUBER* IN THE CONTEXT OF THE EUROPA'S OCEAN SALINITY

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***Salinibacter ruber* thrives at salinity concentrations similar to those reported for the Europa's ocean. Proteomics analyses based on the shut-gun strategy and *i*TRAQ isobaric labeling will help us to identify and quantify the specific proteins used by this extreme halophile when placed in a sulfate-rich environment that simulates the Europa's ocean.**

Europa is the fourth-largest satellite of Jupiter. Superficial spectral evidence from the Near Infrared Mapping Spectrometer on board the Galileo orbiter showed highly distorted water ice absorption bands, incompatible with pure-H_2O ice material, but indicative of the presence of hydrated minerals enriched in magnesium and sodium sulfates. Other no-water-ice compounds like O_2, H_2O_2, CO_2, and SO_2 have also been detected. If oxidants can be delivered to the internal liquid water reservoirs, they can be a source of free energy available for biology. Sodium and potassium have also been measured from ground-based observations (Hibbitts et al. 2019).

Organisms adapted to live in environments containing high concentrations of sodium chloride (NaCl) are called halophiles. Life is extremely diverse in terrestrial oceans at temperatures around 275 K. Microbial metabolic activity has been found around 253 K and even at 233 K in Antarctic glacial ice. These discoveries notably place the idea of Europas habitability more likely. Another important contribution is the fact that the eutectic of water-sulfuric acid is 211 K, and chloride salts can have an eutectic as low as 223-230 K. The temperature at the base of the stagnant lid on Europa can vary from 230 to 250 K, making it possible to hypothesized that the interphase formed by the europan lowest ice shell and the internal ocean allow for some kind of biological activity. We have experimental evidence that *Salinibacter ruber* (DSM13855), an extreme halophile, grows under salinity conditions like those reported for the ocean of Europa (Ramírez et al. 2019). The growth rate and duplication time values indicate that *S. ruber* thrives quiet well in specific concentrations of NaCl, $MgSO_4$, Na_2SO_4 and equimolar combinations of $MgSO_4$ and Na_2SO_4. Sodium dodecyl-sulfate polyacrylamide (SDS-PAGE) gel electrophoresis helped us to detect proteins patterns associated with specific salinity conditions. We found that structures like the aspartate-tRNA ligase, the elongation factor thermo-unstable (EF-Tu), and the chaperonin GroEL-ES complex (Figure 1) are preferentially expressed by *S. ruber*. In prokaryotes these structures are responsible for maintaining the integrity of cellular proteins in response to environmental changes. Interestingly, the GroEL-ES complex aids in the folding and conformation maintenance of certain proteins meaning that *S. ruber* is using this protein upregulation to recover from the stress imposed by the salinity conditions that it is subjected to.

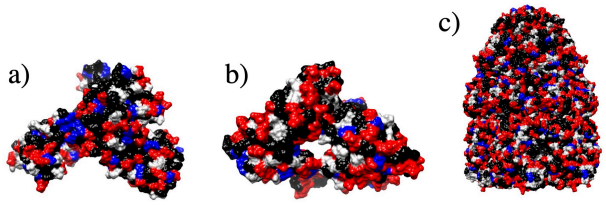

Fig. 1. *In silico* models of an aspartate-tRNA ligase (a), an elongation factor thermo-unstable (b), and a chaperonine GroEL-ES complex (c).

We are currently performing proteomic analyses of the *S. ruber* peptide content, based on the shut-gun strategy and the use of *i*TRAQ isobaric labeling, to identify and quantify with better resolution and accuracy the specific proteins used by this extreme halophile in its efforts to survive to the sulfate-rich environment that we are proposing as a laboratory analogue of the liquid water ocean existing under the frozen surface of the satellite Europa, one of the worlds in the Solar System with the most interest to astrobiological studies. We acknowledge funding from the CONACyT grant 377887.

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