PRESERVATION OF DYNAMIC BIOLOGICAL PROCESSES FROM EXTANT HALOPHILIC LIFE: IN-SITU LESSONS LEARNED FROM PLANETARY ANALOGUE BRINES AND EVAPORITES.

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Introduction: Extant life can thrive in brine environments due to the availability of energy and nutrient sources that halophilic life requires to maintain metabolic processes and nutrient cycling. The dynamic nature of extant life due to consumption of nutrients, cell motility, phototaxis and chemotaxis, can present significant opportunities for preservation of biological processes in modern brine settings. While the robustness of this preservation can indeed occur over geologic timescales, it is the initial/geologically modern timescale that can provide the highest level of biogenicity.

The purpose of this paper is three-fold. First, we will discuss the modern preservation of halophilic microorganisms and how their biochemical products can impact the mineral record. Secondarily, we will review methodologies for in-situ and laboratory measurements that complement Martian and Europa mission-relevant studies and sample analyses. We conclude with an assessment of how to decipher between biologically-impacted brine and evaporitic mineralogy and abiotic mineral substrates with the intent to categorize biogenicity in the modern setting with long-term preservation in mind.

Terrestrial and Planetary Closed-basin Lake Systems & Brine Environments: Typically, the salinity of closed-basin lake systems on Earth can be ~10x of their oceanic environment counterparts. Due to a lack of circulation and major non-saline water inputs (taking into account rainfall and snowmelt), these sites are ideal targets for geochemical and geobiological planetary analogues [1] for Mars. Due to the lack of plate tectonics, as well as these sites having deltaic and other aqueous features preserved in the rock and mineral record, this allows for a greater in-situ assessment of local habitability. Brines and saline minerals have been given recent interest due to the search for extant life [2,3] in potential settings such as Martian Recurring Slope Lineae (RSL) where pockets of potentially free moving brine solutions sublimate [3] during the Martian summers and freeze in place during the colder months.

Modern Biogenic Preservation within Brine and Evaporite Minerals: The kinetic pathways from unsaturated brines into evaporite minerals allow can allow for chemical constraints to be place on potential microenvironments that are generated within fluid inclusions formed from the mineral precipitation process [1,5]. These inclusions allow for both steady-state preservation as well as in-vivo monitoring of halophile cells in these entombed settings (Fig 1). Chemotactic responses can easily be recorded post-preservation with respect to elemental chemistry of the original brine and lithology of the mineral and brine [6].

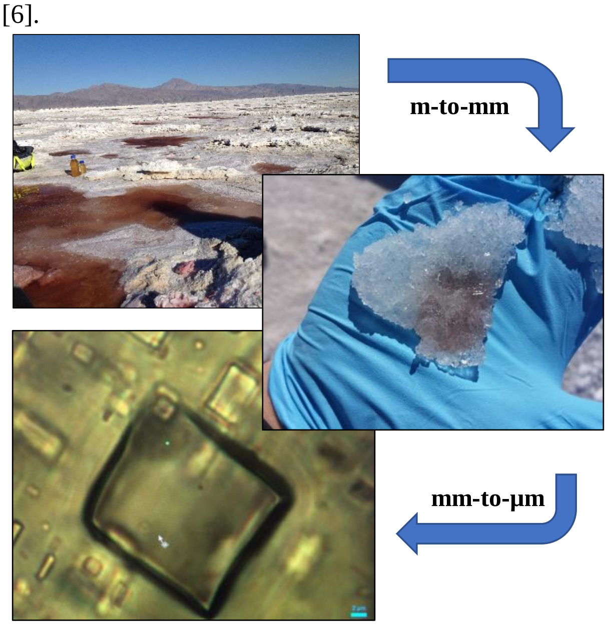

Fig. 1. Typical kinetic pathway from unsaturated brine to evaporite mineral assemblages. Largely constrained by elemental chemistry, these pathways can be used as kinetic endmembers for mineral preservation and potential brine nutrient sources for halophilic microorganisms. Geologically modern brines are the initial biogenic preservation points where in-situ detections of biochemical products can easily be measured. (A) Surface brine pool, (B) Precipitated NaCl. (C) Array of fluid inclusions with entombed halophiles. Scale bar is 2µm.

After solar flux exposure occurs in/on the authigenic evaporite and brine solution, the photobiological responses in the form of pigment generation is typically the first set of adaptation responses to such stresses. Survival strategies for terrestrial halophilic life typically respond to UV-A and UV-B solar flux. The impact without these adaptations can potentially lyse cells and disrupt nutrient cycling within fluid inclusions. Cellular adaptations to combat solar fluxes and high salt [7] on Earth have provided halophiles with the expressed genes to both filter Na as well as produce carotenoids that can be used as positive detections for biology [5]

Mission-relevant Sample Analyses & Biological Validation In-Situ: Current mission payloads do not yet have the ability to spatially resolve micron-scale features within ancient hydrated minerals left behind after late Noachian water-rock interactions on Mars. However, due to the nature of the halophile photobiological response, the generation of carotenoid pigment biomarkers, while occurring on the micron-scale (Fig. 2), can be observed on the meter-scale (Fig. 1a).

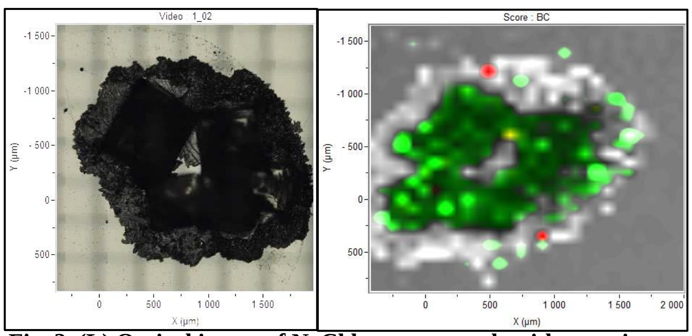

Fig. 2. (L) Optical image of NaCl hooper crystals with superimposed Raman PCA map on (R). The entombment process not only allows for halophilic microorganisms to be preserved but gives a non-permeable sub-crystalline fluid environment where cumulative carotenoid pigment chemistries are also preserved. Green sections in Fig. 2R show Raman wavelengths that correspond to β-carotene ($C_{40}H_{56}$) and Bacterioruberin ($C_{50}H_{76}O_4$) superimposed via PCA map [5,8]

Should these features be observed on surface outcrop by the SHERLOC investigation [9] on the *Perseverance* rover these sample would be ideal for future Mars Sample Return studies. Moreover, these features can be well preserved in partially frozen brine inclusions where psychrophilic microorganisms (that have halo-tolerant genes expressed) that can remain motile [10] at temperatures $\leq -15°C$. Needing water to remain in a fluidic state, cell motility in brines would be another ideal biosignature that can be recorded in the form on non-Brownian motion [11] along with potential chemotactic responses. While terrestrial nucleic acids can be preserved in modern settings [1,5,6] these biomolecules are not robust over geologic time. While these provided the best indications of species-level and taxonomic information [12], lipid biomarkers preserved in salt deposits can yield evidence of ancient life in the mineral record [13].

Modern brines in the Context of Geologic Time: Deciphering between potentially older/ancient biogenic pigment features within modern (liquid) brines and cryobrines presents a challenge with respect to duration of preservation and longevity of evidence. Recent work has shown [14] that even in warmer brine solutions, constraints on habitability and fluid duration can be made. These temperature endmembers can provide insight to the ecological stresses (T, salinity, a_w, others) that halophilic microorganisms need to adapt to in order to survive and create the very biomarker evidence that we are seeking in future in-situ campaigns for extant life [2] and for Mars Sample Return studies [15]

Categorizing Abiotic Brine Substrates and Biotic Features: While modern and ancient brines provide a significant preservation medium for dynamic extant life as well as signs of extinct/ancient microbial life, being able to decipher between abiotic fluids and biogenic features in these preservation mediums [12] is critical for validating biogenicity. For Mars, RSL brines would need to be sampled and concentrated in order to increase the probability of biogenic yield [6] for even the simplest biomolecules. For Europa, ice-melt from the irradiated surface ices would need to be heated slowly in order to reduce the potential for osmotic shock to the cellular membranes and brought onboard for a similar brine concentration. Bulk assessment (non-spatial) of pigment biomarkers as well other complex molecules would still have potential issues of salt inhibition and proper abiotic brine baselines would need to established to ensure any biogenic peak is both indigenous and repeatable.

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