DETECTION AND PRESERVATION OF EXTREMOPHILE BIOSIGNATURES IN LITHIUM-BEARING EVAPORITES FOR LIFE DETECTION. B. M. Javier[1,2], S. M. Perl[2-4], C. Basu[5], W. Fischer[6], A. J. Celestian[3,7]. [1]Department of Biology, California Institute of Technology, Pasadena, CA 91125 (bjavier@caltech.edu), [2]NASA Jet Propulsion Laboratory, California Institute of Technology, JPL Origins and Habitability Lab, 4800 Oak Grove Drive, Pasadena, CA 91109 (scott.m.perl@jpl.nasa.gov), [3]Mineral Sciences, Los Angeles Natural History Museum, Los Angeles, CA 90007, [4]Blue Marble Space Institute of Science, 600 1st Avenue, Seattle, Washington 98104, [5]Department of Biology, California State University, Northridge, 18111 Nordhoff Street, Los Angeles, CA 91330, USA, [6]Department of Geology and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, [7]Department of Earth Sciences, University of Southern California, Zumberge Hall of Science, Los Angeles, CA 90089.

Introduction: In the search for signs of extant life in the Martian subsurface, it is essential to understand what features of microbial communities could be universal and stable over geologic time, how biomarkers can be preserved in the surrounding mineral record, and how to detect and validate these biosignatures. It is imperative to understand how molecules that are uniquely the result of biological processes, such as metabolism, cellular respiration, or adaptive genes that are the response to stress from the surrounding environment, can be preserved in the surrounding evaporites. Deciphering how biology interrupts abiotic geochemical dynamics and how changes to mineral crystal structure, chemical composition, and physical properties can also be used as evidence of extant life.

Motivation: The preservation of biogenic molecules in evaporites [1] is relevant for life detection efforts on planetary bodies that once contained bodies of liquid water, such as ancient Mars or in the modern subsurface [2] as well as icy moons containing oceans of liquid water, such as the subsurface Ocean Worlds.

Brines and evaporites from predominantly NaCl chemistries have been well characterized on Earth—however the introduction of other cations, such as Li, and the resulting reaction byproducts [3] have not been thoroughly explored. If variations in lithium mineralization exhibit patterns resulting from unique biogenic processes observed in life on Earth, it could be assessed as having excellent preservation potential [4]. These changes could potentially be detected as variations to the evaporite crystal structure as well as fluctuations to the phase diagrams for lithium-containing minerals and brines. Variations in the Li-isotope ratios from fluid inclusions [5] integrated within the crystal structure could contain biogenic molecules, including intact cells.

The results from this study are essential for life validation since we will be able to discern lithium geochemistry that is the result of abiotic processes compared to the kinetics of lithium brines and salt crystals that are influenced by the presence of microbes and their biogenic byproducts. We will also gain a better understanding of how life adapts to be able to thrive in these extreme lithium-containing brine environments.

Methods: NRC-1 was cultured in media containing various levels of Li-ions in them. This was to test the ability for the haloarchaea to adapt to Li-induced stresses.

Li-Brine Generation. Halophilic broth was spiked with LiCl to simulate the levels of lithium found at field sites that serve as good extraterrestrial analogs for ancient Mars. The lithium concentrations from these field sites were taken from previously published data characterizing the geochemistry of these sites [6] (Table 1).

Table 1: Li-Brine Concentrations and Emulated Planetary Brine Ecologies.

Mars Analog Field Site	LiCl Concentration (nM)
Control	0
Searles Lake	1.27
Atacama	35.38
Salton Sea	4.72

Raman Analysis of Li-Brines. Raman spectra for these culture conditions were taken at fixed timepoints. 5μL of each culture media (brine including viable NRC-1) were placed on a slide and allowed to evaporate, creating Li-containing evaporite crystals with fluid inclusions containing intact cells. These fluid inclusions are the sites that were targeted for spectra-acquisition. The Raman data was taken at 532nm wavelength, 50% laser power, with a 50X LWD objective.

Growth Curves within Li-Brine: Once the cultures containing LiCl were spiked with NRC-1, the optical density (OD-600) was measured using a fluorometer at fixed intervals to determine the opacity of the culture. From this information, were created growth curves to visualize the effect the introduction of lithium as an environmental stressor has on the growth of these organisms (Fig. 1).

We observed a delay in the initiation of the growth phase for the NRC-1 cultured with LiCl. However, these microbes did enter the log phase several days after the negative control did. What was interesting was the culture that fared the best—the conditions simulating the Atacama brine pools, with the highest lithium levels at 35.38nM. This shows that halophiles not normally

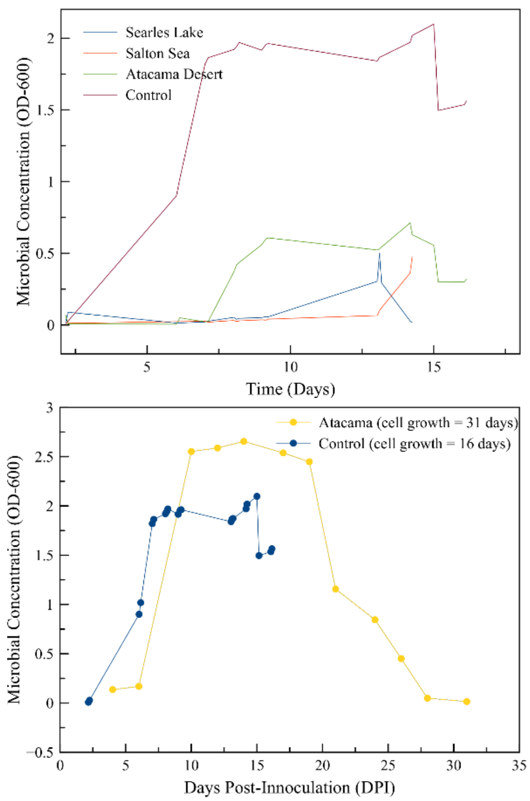

Fig. 1: Stunted Growth Curves from Halophilic Cultures. (Top) Three laboratory brines were generated using Li concentrations from planetary analog field sites in the Atacama Desert, the Salton Sea, and Searles Lake (Mojave Desert). **(Bottom)** Updated Atacama halophile Li-brine at 31 days growth.

found in lithium-dominated environments can tolerate Li-conditions, and at higher concentrations of lithium, adaptive genes may become transcriptionally active and allow the organisms to thrive.

Raman Analyses of Li-brines with Entombed Halophilic Microorganisms: Raman done on fluid inclusions containing entombed NRC1 (Fig. 2a) demonstrated the ability to detect organic biogenic molecules, including carotenoids (Fig. 2b).

Martian Meteorites containing Li-bearing minerals: Searching for signs of life in hypersaline environments is likely one of the best options we have for biosignature detection on planetary bodies that once had or have liquid water on them using our current technology. We can augment the knowledge of biosignature preservation in salts containing other ions. Since lithium was found in varying levels in the analog sites of interest, it could be expected that lithium would also be found in varying levels on other planets and moons. It has been previously found that there were detectable levels of lithium in Martian meteorites [7].

Li-Clays as Targets for Astrobiological Samples: We are also interested in clays containing lithium. Like halite, clays have also been shown to capture organics from microbial communities interacting with them [8]. Clays are often deposited when the finest grains of sediment settle in an evaporating closed basin system, making them especially useful for gaining information for studying the capture of biosignatures in 1) an environment that once had liquid water, making it habitable by most definitions, and 2) a system that was rapidly changing and had microbial communities that had to adapt to the harsh and rapid change of conditions of an evaporating basin.

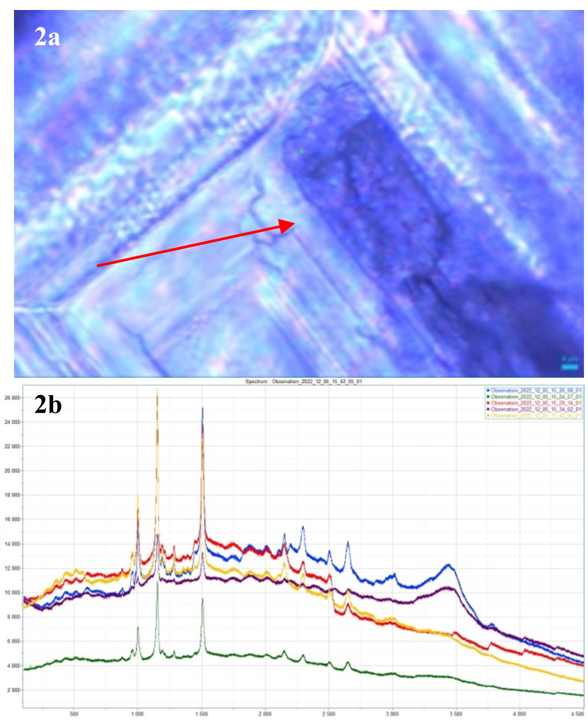

Fig. 2: Raman Spectra of Halophilic Communities within Li-brines. Raman spectra was acquired by targeting these fluid inclusions within the salt crystals (red arrow, 2a). Raman analysis of evaporites from our lithium-dominated brines show the ability for these brines to preserve biogenic molecules (2b) that can be used as a biosignature.

References: [1] Perl, S.M. (2021) et al., Astrobiology, doi: 10.1089/AST.2020.2318. [2] Sapers et al, (this conference) [3] Bradley, D. (2013) et al., US Geological Survey, [4] R.E. (2011) et al., Astrobiology, doi: 10.1089/AST.2010.0506. [5] Baxter, B.K. and Perl, S.M. (2020), Great Salt Lake Biology. doi: 10.1007/978-3-030-40352-2_16. [6] Zhang, Y. (2019) et al., Minerals Engineering, doi: 10.1016/j.mineng.2019.105868. [7] Magna, T. (2015) et al., Geochim Cosmochim Acta, doi: 10.1016/j.gca.2015.04.029. [8] Playter, T. (2017) et al., Chem Geol, doi: 10.1016/J.CHEMGEO.2017.04.007.