National Aeronautics and Space Administration

FY23 Strategic University Research Partnership (SURP)

Exploring abiotic constraints on microbial habitability in subsurface hypersaline brines

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I. Introduction and Motivation

The terrestrial subsurface boasts substantial heterogeneity in its environmental abiotic composition which is perhaps best exemplified by deep fracture fluid environments of Precambrian Shields. Our project will help constrain microbial habitability in Moab Khtosong brines and rock substrate by evaluating the biogeochemical support of the abiotic environment and investigating community metabolic networks under these conditions

- Microbial adaptations to high salinity and a biofilm vs planktonic lifestyle would always form and operate faster than changing geochemical conditions, and could provide ample adaptation strategies to climate, mineralogical, and salinity changes from late Noachian to modern Mars subsurface aquafers

- Microbial fluidic and later microbial mineral interfaces could show evidence of these adaptations over geologic time and we have spatially detected low biomass microorganism preservation in brines and solid evaporite minerals

- Due to the low biomass settings these detections, when possible, bode well for understanding the preservation potential of Mars analogue mineralogy (Fig. 1) specific lithologies in former aqueous settings, and extant life measurements in saline brines

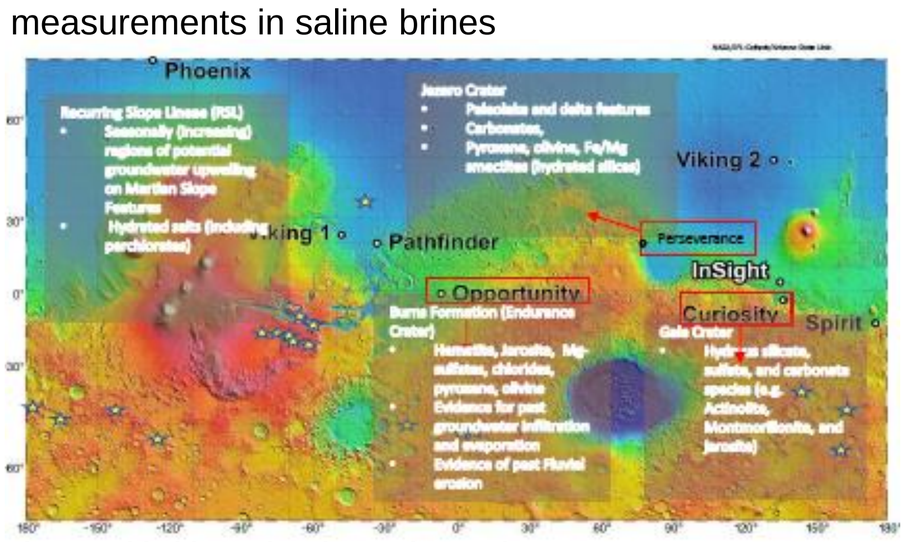

Fig. 1. Habitable ancient lacustrine environments on Mars. Features accessible on the Martian surface that are a product of groundwater upwelling allow us to model microbial maintenance energies that biology requires for sustainable inhabitation within subsurface brines.

IV. Significance/Benefits to JPL and NASA: This effort allows us to move from typical habitability measurements to direct measurements of biology for life detection. Future work and spinoff investigations will focus on microbial maintenance energy as a chemical biomarker and habitability index assessment

- We will model microbial maintenance/metabolic potential as it might exist at different depths or through time and at different subsurface depths Based on our laboratory experiments, we will utilize one of two models of reactive transport or no transport

- Taking the Martian Recurring Slope Lineae into account we can understand the fluid kinetics and subsurface to surface groundwater transport

II. Objectives

Biosignature detection for ancient/extinct signs of life relies on the preservation medium whereas extant/active life requires an agnostic approach to withstand the high burden of proof for biological validation (Perl et al. 2021).

- Spatial detections of microorganism preservation in brines and solid evaporite minerals has been observed Due to the low biomass settings these detections, when possible, bode well for understanding the preservation potential of Mars analogue mineralogy, specific lithologies in former aqueous settings, and extant life measurements in saline brines

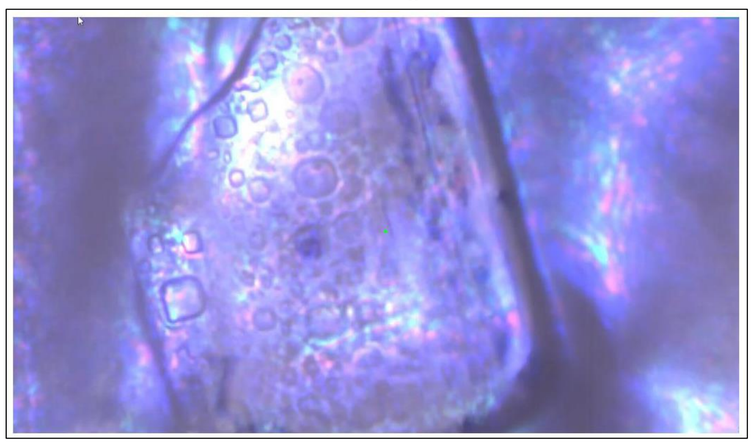

Fig. 2. Single cell Preservation per (3x) Fluid Inclusions. A significant milestone of this investigation has been the Raman-validated and spatially-confirmed presence of single cells per entombed fluids. Perl et al. (2020; 2023) has shown this type of preservation on the order of 10^3–10^8 in the Great Salt Lake, however this is one of the first occurrences of single cells

III. Approach and Results

For positive enrichments cellular abundances will be estimated, cells will be imaged microscopically (Fig. 2), DNA will be extracted from positive enrichments for taxonomy (Fig. 3), and expression activity will be evaluated via fluorescent molecular probing and transcriptomic analysis. For positive enrichments of the rock amended media, the rock surfaces will be imaged with fluorescent stains and SEM to determine the distribution of attached cells and how they relate to the underlying

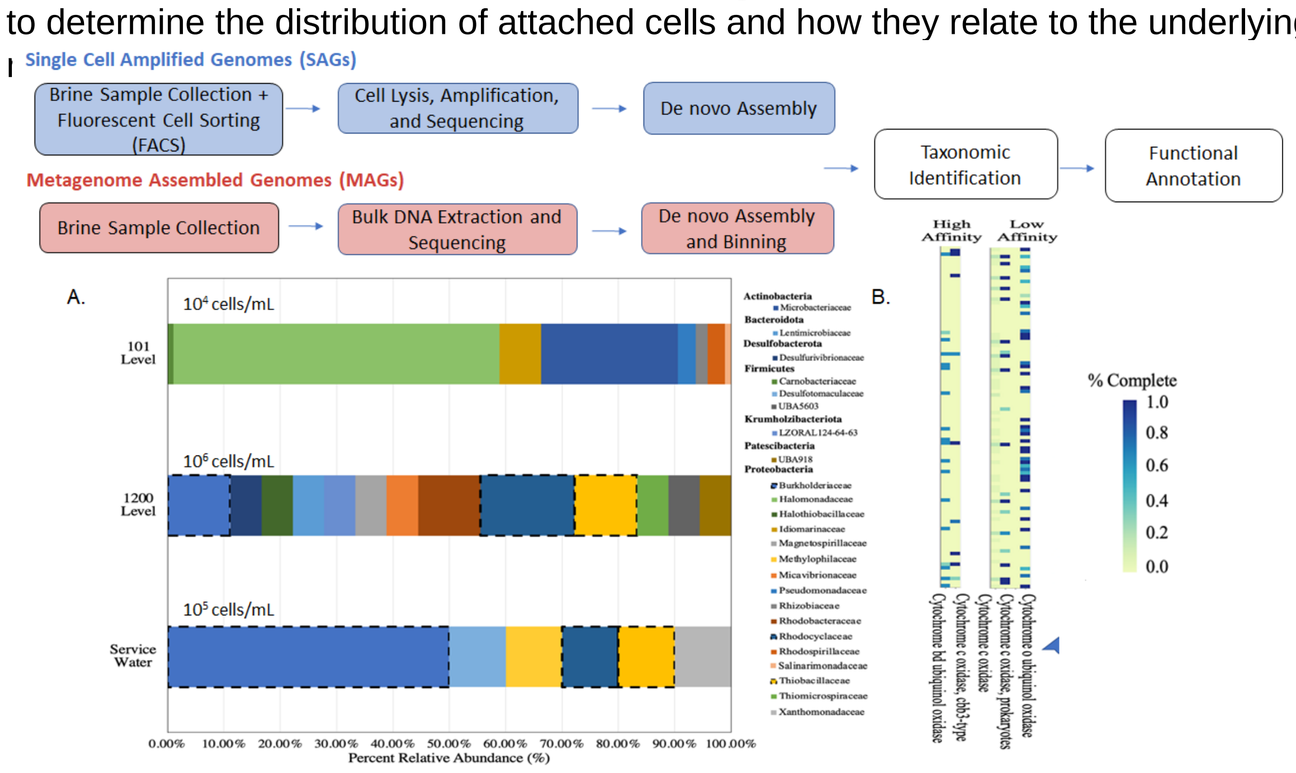

Fig. 3. (A) Percent relative abundance (%) of GTDB-Tk-identified families [10] in 101 level brine SAGs, 1200 level MAGs and service water MAGs. Shared taxa between the 1200 level and service water are shown with a dashed border. Family taxonomic names are distinguished by bolded phylum-level groups in the legend. (B) DRAM [11] summary metabolic module completion for 101 brine SAGs (1 SAG per row), including affinity categorization for cytochrome types (o vs. c/cbb-3 type) in electron transport chain (ETC) complexes.

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Publications:

1) Nisson, D.M., Kieft, T. L., Drake, H., Warr, O., Sherwood Lollar, B., Ogosawara , H., Perl, S.M., Friefeld , B.M., Onstott, T.C., (2023) Hydrogeochemical and Isotopic Signatures Elucidate Deep Subsurface Hypersaline Brine Formation through Radiolysis Driven Water Rock Interaction
2) Perl, S.M. Nisson, D.M., Onstott, T.C. (in-prep) Detections of Cellular Material in Low Biomass Mars Analog Brine Systems
3) Nisson, D. Kieft, T.L., Hernandez, J.C., Perl, S.M., Stepanauskas , R., Warr, O., Lollar, B.S., Yokochi , R., Chmiel , G., Caffee , M., Liebenberg, B., Onstott, T.C. (2021) Influence of Alpha Particle Radiolysis on the Formation and Microbial Metabolic Composition of a Deep Subsu rface Hypersaline Brine in the Witwatersrand Basin, South Africa. AGU Fall Meeting 2021
4) Perl, S.M., Nisson, D. M., Onstott, T.C. (2022) Detections and Validations of Single Cell Microorganism Preservation and Associated Biosignature Activity. Astrobiology Science Conference 2022, Atlanta, GA. in Detecting Life and Habitable Environments on Mars: A Synthesis of Applied, Analog, Experimental and Theoretical Approaches I Oral. Abstract # 203 02
5) Radiolysis-Driven Evolution of an Ancient Subsurface Habitable Brine in the Witwatersrand Basin, South Africa. DM Nisson, TL Kieft, H Drake, O Warr, BS Lollar, H Ogasawara, SM Perl Goldschmidt 2023 Conference

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