SNOW ALGAE-BACTERIA CO-CULTURES EXHIBIT PATTERNED GROWTH (BIOVERMICULATION) UNDER FE-LIMITED CONDITIONS: IMPLICATIONS FOR BIOSIGNATURES ON ICY PLANETARY BODIES C. M. Phillips-Lander[1], Z. R. Harrold[1,2], J. Raymond[3], O. Tschauner[1], and E. M. Hausrath[1] [1]Department of Geoscience, University of Nevada-Las Vegas, Las Vegas, NV, USA (charity.lander@unlv.edu) [2]Desert Research Institute, Reno, NV, USA [3]School of Life Sciences, University of Nevada-Las Vegas, Las Vegas NV, USA

Introduction: The presence of life in icy and snowy habitats on Earth suggests icy planetary bodies like Europa and Enceladus and snowy or glaciated regions on rocky planetary bodies like Mars may be habitable [1-5]. However, these environments face significant challenges to life due to high UV fluxes, low temperatures, and liquid water and nutrient limitations, so evidence of life, particularly extant life, may be difficult to detect.

Microbial communities display patterned growth (biovermiculation), a biosignature of extant life [6] in extreme environments [7], which may be valuable tool for detecting life in these snowy and icy habitats. Biovermiculations produced by microorganisms have been identified at macroscopic scales in caves, soil crusts and creeks [7-10]. Scanning electron microscopy (SEM) indicates macroscopic biovermiculations are composed of dense networks of microorganisms and exopolymeric substances (EPS) [8]. To date, biovermiculations have not been demonstrated at microscopic scales and the factors that drive biovermiculation formation remain poorly understood, although Boston et al. [7] hypothesized nutrient limitation is a key factor in their formation.

Harrold et al. [14] demonstrated the use of Fe-containing minerals as a nutrient source by Fe-limited snow algae co-cultures, and previous studies have shown preferential microbial colonization of minerals to alleviate nutrient-limitation (Fe and P) [15-16]. The work presented here demonstrates biovermiculation pattern development by snow algae co-cultures under Fe-limited growth conditions.

Methods: We performed snow algae co-culture colonization experiments on polished thick- and thin- sections of andradite (A, $Ca_{3.0}(Fe_{0.6},Al_{0.4})_2(SiO_4)_3$, from Garnet Hill, CA, San Carlos olivine (F, $Mg_{0.9}Fe_{0.1}SiO_4$, Alfa Aesar), and quartz (Q, SiO_2, Ward Scientific #495886). We visually picked mineral chips using a Barska 20-40x binocular plain light microscope to ensure few inclusions were present; however, pyroxene inclusions were detected within andradite during electron microprobe analysis using a JEOL JXA-8900 at UNLV's Electron Microanalysis and Imaging Laboratory (EMIL).

Experimental Design Minerals were mounted in duplicate epoxy-embedded thin- and thick-sections. Epoxy mounts included each mineral individually, as well as an additional set of F, A, and Q embedded in a single epoxy thick/thin section (FAQ) to allow incubation together under identical conditions (Figure 1). We sterilized each section in 100% ethanol by sonicating three times (60, 30, and 30 sec), before air-drying in covered, sterile petri dishes.

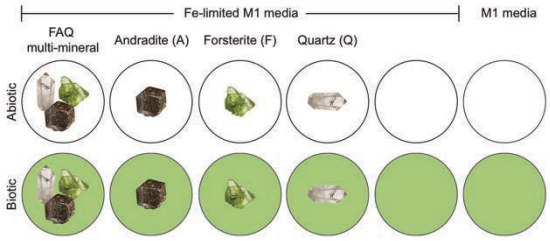

Figure 1: *Experimental design for thin- and thick-section experiments. M1 is full nutrient media to compare to Fe-limited conditions. Green color indicates biotic experiments. Experiments with no mineral added are represented by empty circles.*

Cultures We used *Chloromonas brevispina*-bacteria co-cultures, which were originally sourced from the Culture Collection of Algae, University of Texas, Austin (UTEX), B SNO96 culture, collected and isolated by Ronald Hoham, from Lac Laflamme, Quebec, Canada [17]. Cultures were maintained on M1 growth medium [18]. Fe-limited and full nutrient M1 media were autoclave sterilized, chilled to 4°C, and supplemented with filter-sterilized vitamin solution. For Fe-limited media, the Fe-EDTA solution was omitted and the trace element solution was replaced with a Fe-free trace element amendment. Both Fe-limited and full nutrient M1 media had an initial pH of approximately 5.3. We inoculated biotic experiments (Figure 1) with 1% v/v active *C. brevispina* co-culture in a laminar flow hood, mixed the inoculated medium, and then poured it over the sterilized sections in sterile petri dishes. The covered petri dishes were incubated under a lamp (2000-3000 lux) at 4°C for 30 days.

Secondary precipitates were evaluated on FAQ thin-sections using X-ray fluorescence and X-ray adsorption spectroscopy at the Advanced Photon Source, beamline 13IDE and the Advanced Light Source, beamline 10.3.2.

SEM We evaluated $\geq$10 fields of view for each thick-section at ~1-100 μm scales using a JEOL field-emission SEM (JSM-6700F) to compare unreacted and abiotically and biotically weathered thick-sections. We visually determined snow algae and bacteria attachment qualitatively (+/- relative scale) and quantitatively (cells cm^{-2}) using ImageJ. Colonization, defined by observations of cells that appear to be in the process of dividing and/or encased in EPS [15,16,19], was qualitatively determined.

Results and Discussion: *Microbial Attachment and Colonization* Cell counts on mineral surfaces were within error of each other for FAQ-A, FAQ-F, A, and F (Figure 2). Lower cell counts were observed in the FAQ-Q and Q experiments. We observed different trends in colonization between F and FAQ-F thick sections with no such differences observed for A or Q. Snow algae occurred as individual cells in FAQ-F (Figure 2 B.2) and as multi-cellular curvilinear clusters (biovermiculations) in F experiments (Figure 2 B.1). Bacteria occurred as small colonies associated with snow algae cell margins on F and as individual cells associated with etch pits in FAQ-F. In both FAQ-A and A experiments, snow algae occurred as individual cells associated with weathered pyroxene inclusions (Figure 2 A.1 and A.2). Bacteria (not shown due to image scale) occurred as colonies aligned along fractures and inclusions in andradite. Colonization on both Q and FAQ-Q was similar, such that snow algae occurred as small clusters of cells on quartz surfaces. Bacteria occurred as individual colonies and associated with snow algae surfaces and EPS.

Our study demonstrates both algal and bacterial community growth morphologies vary under different growth conditions, and we attribute this to differences in Fe-availability. FAQ-A and A surfaces were likely less Fe-limited than other treatments due to (1) larger andradite grains than forsterite grains and thus a higher surface area, (2) higher Fe contents in andradite (15.8 wt.%) than in forsterite (9.2 wt.%), and (3) similar dissolution rates of andradite and forsterite [20]. Microbes colonizing FAQ-F surfaces likely also not Fe-limited due to release of Fe from the FAQ-A surfaces, combined with Fe mined from F surfaces, evidenced as etch pits on F surfaces associated with bacteria.

Secondary precipitates were common on biologically weathered materials in F, A, and FAQ experiments, and were composed primarily of Fe-oxides associated with snow algae, bacteria, and EPS. The few precipitates detected on Q surfaces were primarily salts (including NaCl and minor $BaSO_4$), likely formed during freeze-drying of experiments for SEM analysis.

Biovermiculations occur in response to nutrient limitation. Small clusters of snow algae and curvilinear snow algae and bacterial growth morphologies associated with EPS occurred only on the F and Q mineral surfaces and in the FAQ-Q experiments, which is likely due to the absence of Fe. Therefore, these data support Boston et al.'s hypothesis [6] that biovermiculations form in response to nutrient limitations.

Biosignature Implications for Icy Planetary Bodies The formation of patterned microbial growth and EPS on mineral substrates by snow algae co-cultures suggests biovermiculation patterns are a potential biosignature in planetary environments where dust-covered snow and ice exist. However, these patterns may be restricted to environments where microbial cells form biofilms to overcome nutrient limitation.

References: [1] Sagan & Lederberg, 1976, *Icarus*, 28, 291-300. [2] Rothschild, 1990, *Icarus*, 88, 246-260. [3] Chyba & Phillips, 2001, *PNAS*, 98, 801-804. [4] Cockell & Raven, 2004, *Icarus*, 169, 300-31. [5] McKay et al., 2008, *Astrobio.*, 8, 909-919. [6] Boston & Schubert, 2015, *2nd Intl Planet. Caves Conf.* #9028. [7] Schubert et al., 2012, *Life Detect. in Extraterr. Samp.* #6048. [8] Jones et al., 2008, *J. of Cave & Karst Studies*, 70, 78-93. [9] Cornutt et al., 2009, *Bioastro. 2007: Mole., Micro., & ET Life, ASP Conf. Ser.* 420. [10] D'Angeli et al., 2017, *Hypogene Karst Regions & Caves of the World*, 161-168. [11] Hamilton & Havig, 2017, *Geobio. J.*, 15, 280-295. [12] Müller et al., 1998, *Polar Bio.,* 20, 14-32. [13] Tazaki et al., 1994, *Clay and Clay Min.*, 42, 402-408., [14] Harrold et al., in review, *Appl. Env. Micro*. [15] Phillips-Lander et al., 2014, *Geomicro. J.*, 31, 23-41., [16] Rogers & Bennet, 2004, *Chem. Geo.,* 203, 91-108. [17]Austin UoTa, 2017, https://utex.org/products/utex-b-sno-0096. [18] Hoham et al., 1979, *Phycologia*, 18, 55-70. [19] Bennett et al., 2001, *CMS Wksh. Lectures*, 14, 37. [20] Velbel, 1999, *Am. J. Sci*., 299, 679-696.

Acknowledgements: NASA grant # NNX14AN24A funded this work. We thank Minghua Ren, director of EMIL, and Sirine Fakra, LBNL, for their assistance. We thank Angela Garcia and Peter Sbraccia for their assistance in the laboratory and Mike Malaska for his helpful conversations.

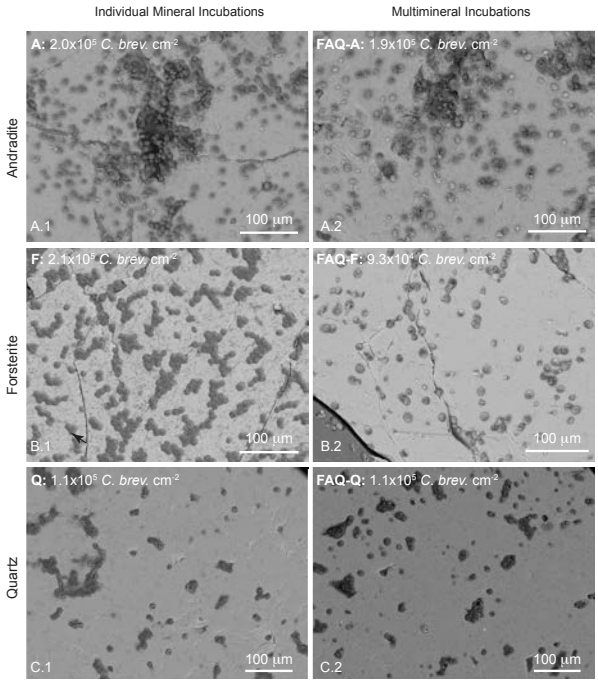

Figure 2: *Snow algae colonization of thick-sections. Snow algae are the dark grey spheres with associated EPS attached to mineral surfaces. Biovermiculations (curvilinear clusters of cells) occur in B.1 forsterite and quartz C.1, as well as FAQ-quartz C.2 experiments.*