CULTIVATING THE DEEP SUBSURFACE MICROBIOME. C. P. Casar[1], M. R. Osburn[1], T. M. Flynn[2], A. L. Masterson[1], B. R. Kruger[3], [1]Northwestern University (2145 Sheridan Rd, Evanston, IL *casar@u.northwestern.edu), [2]Argonne National Laboratory (Argonne, IL), [3]Desert Research Institute (Las Vegas, NV).

Introduction: Understanding the diverse physiology, ecology, and metabolic characteristics of microbial life in terrestrial subsurface environments is a critical step in the search for extraterrestrial examples. The subsurface offers protection from environmental stressors (i.e. extreme temperatures and radiation) that may exist on the surface of extraterrestrial bodies, and has the potential both for liquid water [1] and fuel for lithotrophic metabolisms [2]. Furthermore, biofilms that colonize fluid-filled fractures create microenvironments that aid survival under otherwise extreme conditions. These communities may represent a significant fraction of subsurface life [3]. To better understand these systems, we characterize rock-hosted biofilm communities from a deep, iron and sulfur-rich Mars analogue site.

The Sanford Underground Research Facility (SURF) is located at the former Homestake gold mine in Lead, South Dakota. The mine is currently accessible to a depth of 1,480 meters. Legacy boreholes intersecting fluid-filled fractures provide access to deep fluids that host lithotrophic microbial life [4]. Geochemical analyses show these fluids to be somewhat heterogeneous, with locally high concentrations of ammonia, nitrate, sulfide, sulfate, iron, and manganese. The geochemistry of these fluids ranges from oxidizing to highly reducing, with pH neutral to slightly alkaline and temperatures from 10-30 ˚C.

While planktonic microbial communities within borehole fluids have been previously described [4]; biofilm communities within these fractures have yet to be characterized. Here we describe a novel approach to culturing interstitial biofilms *in situ* using artificial substrates. In addition, we present the results of ongoing efforts to isolate and describe the metabolic capabilities of novel organisms in the laboratory, specifically those capable of respiring ferric oxide minerals.

Field Experiments: Sampling borehole fluids by filtering suspended cells captures only planktonic members of the microbial community, which may be only a small fraction of the deep subsurface microbiome [3]. We therefore designed flow-through samplers to target the attached communities and mitigate bias introduced by laboratory cultivation from fluid samples. Borehole fluids were directed into gas-tight glass cartridges filled with sterilized sand, Pyrex beads, and glass wool. Fluids exiting the cartridges flowed over a set of glass slides exposed to the mine atmosphere. Biofilms grown on these substrates can then be directly characterized using a broad array of analytical techniques including metagenomics, microscopy, C/N ratios, lipid extraction and profiling, and Raman spectroscopy. In doing so, we can link the community composition of the biofilms with associated mineral assemblages, microstructure, and biosignature composition as well as the geochemistry of the associated borehole fluids. A second array of cartridges filled with hematite, pyrite, and magnetite will be deployed in February 2017 to enrich for iron and sulfur-associated biofilms.

Preliminary observations indicate geochemical controls on biofilm formation, as black (likely reduced) biofilms within cartridges transitioned to red biofilms in the cartridge outflow under the oxygenated mine atmosphere. The thickest accumulations of biofilms were formed on glass wool cartridges, indicating surface textural controls on biofilm formation. Whole community $\delta^{13}C$ values of biofilms suggest both organic and inorganic carbon are incorporated into microbial biomass.

Lab Experiments: Many of the 16S rRNA gene sequences obtained from SURF have no closely-related relatives among organisms that have been previously isolated [4]. We attempt to shed light on the metabolic roles of these uncultivated organisms by using media designed to mimic *in situ* geochemical conditions. Abundant iron oxides are available at SURF; however, these insoluble substrates require specialized metabolic strategies. Batch cultivation experiments targeting anaerobic, heterotrophic and autotrophic iron reducers were inoculated with fluids collected from depths of 610 and 1,480 meters. Ferrihydrite, organic carbon, and hydrogen served as electron acceptors and donors. Isolate characterization includes microscopy and DNA sequencing to determine cell morphology, microbe-mineral associations, and taxonomy. Additional experiments will employ biofilms collected from field experiments as inocula.

Preliminary results indicate SURF microorganisms are capable of reducing ferric iron minerals. However, whether the iron is being actively or passively reduced remains unclear. Precipitates formed on glass walls of culture tubes are strikingly similar to those observed in cartridge experiments.

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