PRELIMINARY FTIR OF BIOREDUCTION PRODUCTS FROM THE HALOTOLERANT AND PSYCHROTOLERANT SHEWANELLA STRAIN, BF02, AN IMPORTANT ASTRIBIOLOGICAL ANALOGUE MICROBE. E. C. Sklute[1], E. C. Taylor[2], J. A. Mikucki[2], M. D. Dyar[1,3], and P. A. Lee[4], [1]Planetary Science Institute, 1700 E. Fort Lowell Rd. Ste. 106, Tucson, AZ. 85719, ecsklute@psi.edu, [2]Dept. of Microbiology, University of Tennessee, 1311 Cumberland Ave. Knoxville, TN 37996, [3]Dept. of Astronomy, Mount Holyoke College, 50 College St., South Hadley, MA. 01075, [4]Hollings Marine Lab, 331 Fort Johnson Rd, Charleston, SC 29412.

Introduction: Antarctica's McMurdo Dry Valleys (MDV) have long been considered an important surface analogue for Mars [1-3]. The recent detection of aquifers in the deep (>100 m) subsurface of the MDV, below the permafrost [4], have expanded this potential analogue space to include subglacial lakes on Mars [5] and ice-covered ocean worlds like Europa [6]. One aquifer, covered by The Taylor Glacier, periodically releases some of this deep liquid at a surface feature known as Blood Falls (**Fig. 1**). The aquifer, which was likely "freeze-concentrated" as Taylor Glacier advanced, discharges liquid that is cold (~ -6°C), salty (8% salinity), ferrous (~0.4-3.4 mM) and rich in sulfate (50 mM). This brine also contains a viable, metabolically-activity chemosynthetic microbial community that has been shown to respire iron oxides using reduced sulfur compounds as electron donors [7].

The Blood Falls ecosystem provides a unique analogue because it has survived dramatic environmental change and contains microorganism with specific adaptations to this uniquely sequestered system [7]. Understanding how these microbial metabolisms may generate detectable biosignatures, specifically at surface discharge points, is invaluable for detecting life elsewhere. We are studying mineral signatures imprinted by microorganisms that are adapted to cold, sub-ice ecosystems on Earth, relating them to microbial genetics and evolved gases to help develop biomarkers of past and present life on other planets. Here we report the first Fourier Transform infrared (FTIR) spectroscopic measurements of reduction products from Blood Falls strain *Shewanella* sp. BF02.

Fig. 1. Blood Falls outflow fan in November 2018, shortly after a discharge event. Brines flow out clear and redden with time due to oxidation of iron-bearing phases.

Methods: Microcosms were designed to test for biological mineral alteration associated with growth of *Shewanella* sp. BF02 using a basal marine medium [8]. Peptone and yeast were replaced with SC Amino Acid Mix (13 mM) and lactate (10 mM). The carbonate buffered medium also contained 0.02 mM nitrate, 100 mM ferrihydrite, and 9.6 mM sulfate as potential electron acceptors, as well as 10 mM thiosulfate in some treatments (electron donor or acceptor). Microcosms were prepared anaerobically under N_2-headspace, but inoculated from a 2-week old culture grown aerobically on Marine Agar plates and 'rinsed' and resuspended in marine broth basal medium with no organic carbon sources and normalized to OD_{600} = 0.1. Microcosms were incubated in the dark at 10°C. Controls to differentiate abiotic from biologically mediated geochemical changes include 1) no cells (with and without thiosulfate), 2) autoclave-killed cells (without thiosulfate), and 3) live cells but no organic carbon (without thiosulfate).

Cell growth was monitored by counting colony forming units grown on Marine Agar plates after 3-5 days. The pH was monitored using an Accumet Pencilthin pH probe and a VWR Symphony handheld meter with an automatic temperature compensation probe. Reduction potential was measured immediately after sampling; 2.5 ml was transferred into a pre-gassed 15 ml falcon tube and measured with an Orion Sure Flow Combo Redox/ORP. After 25 days, one bottle per treatment was heat killed for 20 minutes at 40 °C before shipment on ice to the Dyar lab for FTIR analysis.

FTIR spectra were acquired from 360-4000 cm^{-1} on a Bruker Alpha FTIR spectrometer with a diamond attenuated total reflectance (ATR) attachment. Bottles were placed upside-down to accumulate mineral at the stopper. Approximately 1 mL of concentrated mineral was syringed from each bottle and allowed to dry on the ATR stage while spectra were continuously acquired. This procedure was performed both in and out of an anaerobic chamber.

Results and Discussion: Treatments inoculated with live cells were positive for growth, with the highest number of colony forming units counted in the Live Thiosulfate treatment ($1.0 \times 10^8 \pm 5.3 \times 10^6$ cell/ml (n = 3). Negative controls incubated without cells and with dead cells showed no growth.

Spectra did not noticeably vary when collected aerobically or anaerobically so only anaerobic spectra are

shown in **Fig. 2**. All control samples appeared reddish brown and spectrally resemble ferrihydrite, with the addition of a group of features ~990-1120 cm^{-1} likely due to schwertmannite [9]. Experimental cultures with live cells and an organic carbon source appeared black and showed substantial magnetism [**Fig. 3**]. Spectrally, they appear to be mixtures of magnetite and goethite with minor lepidocrocite. Features from ~1022-1122 cm^{-1} in these cultures no longer appear to match schwertmannite. The main difference between the cultures with and without thiosulfate is the depth and position of the feature ca. 600 cm^{-1}. It appears to be a mixture of the goethite absorption band at ~637 cm^{-1} and magnetite absorption ~551 cm^{-1}, and is shifted to higher wavenumbers for the thiosulfate bearing sample (607 vs. 577 cm^{-1}). It is unclear if there are additional minerals, like sulfides, that influence the spectrum, as these are hard to detect in the MIR. Raman will be used to search for these phases.

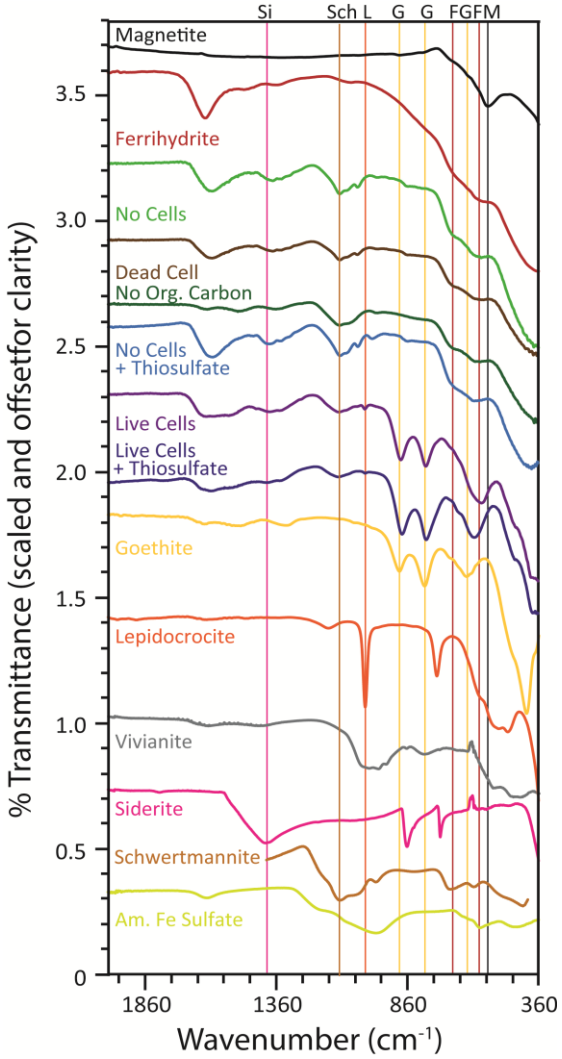

Fig. 2. FTIR ATR spectra of experimental and control cultures along with several reference minerals. Veridical lines are color coded by mineral from which the feature originates and labeled at top for clarity. Schwertmannite is the transmission spectrum of JB131 from [9].

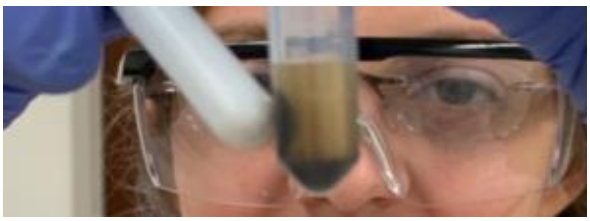

Fig. 3. Experimental cultures form a black material that is attracted to a magnet.

Table 1 lists pH and Eh values on day 0 and day 25 values of bottles sent for FTIR analysis. The pH did not change markedly over the course of the experiment or between treatments. Eh, however, is substantially lowered both by the addition of thiosulfate and the activity of live cells. Although the stable mineral at highly negative Eh and circumneutral pH should be pyrite [10], we observe both magnetite and goethite. These (oxyhydr)oxides could have formed earlier in incubation when Eh values were positive. However, magnetism was not observed until after day 13, after Eh decreased, indicating magnetite had not yet formed. These preliminary results imply that minerals that form outside their pH/Eh stability fields could be used as biomarkers to identify actively growing microbial cultures. Microbes in this system may be facilitating formation of phases that would not otherwise be stable.

Table 1. Average pH and Eh values for each microcosm.

Microcosm Condition	pH Day 0	pH Day 25	Eh Day 0	Eh Day 25
No Cell Control	6.91	6.58	204 mV	115 mV
Dead Cell Control	6.96	6.86	88 mV	136 mV
No Org. Carbon Control	7.01	7.09	94 mV	195 mV
No Cells + Thiosulfate	6.64	6.75	41 mV	33 mV
Live Cells	6.92	6.71	104 mV	-121 mV
Live Cells + Thiosulfate	6.98	6.97	33 mV	-249 mV

Future Directions: These experiments are being replicated to provide additional analyses of the end products, including mineralogy data (via Mössbauer, VNIR, and Raman spectroscopy) and volatile metabolites (via PTR-TOF-MS), along with concurrent transcriptomics and genomics changes.

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