Lauren SEYLER Report rcvd. 9/18.18
Lewis and Clark Fund in Astrobiology

FROM THE FRYING PAN TO THE FIRE: HOW MICROBES SURVIVE RAPIDLY CHANGING CONDITIONS IN FLAT CONE SPRING, SENTINEL MEADOWS, YELLOWSTONE NATIONAL PARK –
Project Report

Sentinel Meadows is a low-lying meadow system that covers the northwestern portion of Lower Geyser Basin in Yellowstone National Park, Wyoming. The meadow is marked by siliceous sinter-forming cones, terraces, and aprons around active and inactive hot springs (4). Flat Cone Spring is a wide, shallow geyser cone on the north side of the meadows (44.57 °N, 110.86 °W) (Figure 1), discharging hydrothermal fluid approximately every 90 minutes, and surrounded by microbial growth: phototrophic silicified mats (chiefly Blastocatellales, Chlorobiales, Chloroflexales, and Cyanobacteria) and chemotrophic yellow and white filaments (Aquificales, Thermales, and unclassified bacteria) (16S rRNA gene sequence data generated by PI Hamilton, unpublished). These microbial communities experience drastic changes in environmental conditions when the hot spring flows. The temperature rises from 33 °C to 73 °C and anoxic fluid within the spring is suddenly exposed to air, rapidly altering the oxidation-reduction potential of the hydrothermal waters. Concentrations of sulfide, ferrous iron, and silica drop, while conductivity of the fluid rises by 60% (Hamilton and Havig, unpublished).

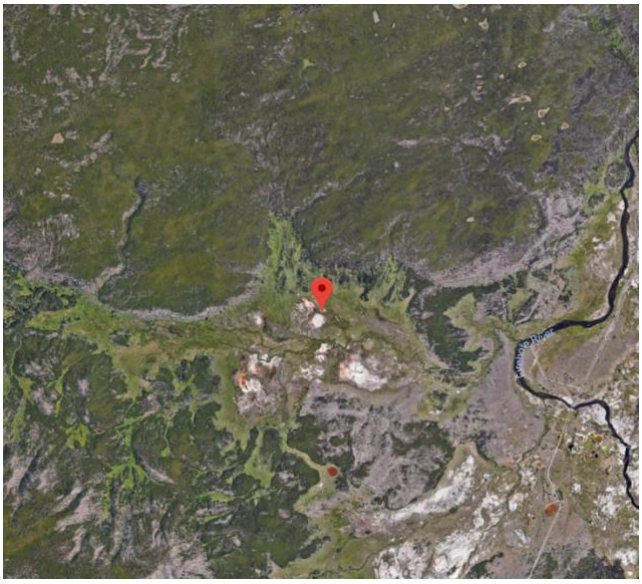

Figure 1. Satellite image of Sentinal Meadows and Flat Cone Spring (indicated in red). Image from Google Maps (http://maps.google.com).

It is uncertain how these filaments and mats are able to survive such sudden shifts in temperature and redox potential. Microbes that are able to live in extreme conditions of temperature and anoxia generally require a narrow set of parameters to survive. Moreover, the conditions experienced by the Flat Cone microbial communities change within seconds, and rapidly change back again. A deeper understanding of how the microbial communities survive these challenging conditions would not only expand our knowledge of life in extreme environments, but would also shed light on the adaptability of Earth's most ancient life forms. The aim of this project is to use metagenomics, metatranscriptomics, and metabolomics techniques to better understand how the microbial communities at Flat Cone survive the extreme changes in temperature, chemistry, and oxygen concentration that occur during eruption events.

Sampling for this project took place during a field trip with the Havig/Hamilton labs (University of Minnesota) between May 30, 2018 and June 9, 2018, under permit #7020 (issued to Havig and Hamilton by the Yellowstone research permit office). Sampling of Flat Cone Spring and other springs in Sentinal Meadows took place on day 2 of fieldwork, June 2.

Triplicate samples of phototrophic and chemotrophic microbial biomass (Figure 2) were collected for DNA, RNA, and metabolite extraction, prior to and during eruption of Flat Cone. Filaments/mats were collected with a sterile spatula, placed in cryotubes, and stored on dry ice. pH, temperature, conductivity, oxidation-reduction potential, and dissolved oxygen were also measured during each sampling event. Biofilm samples were frozen on dry ice and transported back to the University of Minnesota for storage and processing. Additionally, PI Hamilton established microcosms in glass serum vials using biofilm samples from nearby Steep Cone Spring, and the following day at Mitch's Last Stand Spring in Imperial Geyser Basin, two "on/off" systems similar to Flat Cone Spring. These microcosms were amended with ^{13}C bicarbonate and incubated under either light or dark (wrapped in foil) conditions in the spring's outflow channel for several hours and over multiple eruption periods. In addition to the samples I took at Flat Cone Spring, I will also perform analyses on these microcosms.

Figure 2. Chemotrophic microbial mats in outflow channel of Flat Cone Spring. Photo by PI Seyler.

Sample processing and analysis will be conducted as follows: DNA/RNA will be extracted at University of Minnesota, and metagenomic/transcriptomic sequence data will be generated using the Illumina NextSeq platform. Metagenomes will be assembled and binned *de novo* and metabolic pathways within genomes/bins will be reconstructed by comparison to databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG). Transcriptomic data will be mapped to binned genomes using the Functional Mapping and Analysis Pipeline (FMAP). Intracellular metabolites will be extracted at WHOI and analyzed via tandem liquid chromatography mass spectrometry (LC/MS/MS). Mass spec data will be analyzed using openMS, an open-source software C++ library for LC/MS data management and analyses, and compounds will be queried against the ChEBI and KEGG databases to aid in identifying metabolites and metabolic pathways. Putatively annotated compounds of interest will be ordered from Sigma Aldrich and run as standards to verify their presence in a sample.

As the amount of time required to upregulate or downregulate genes is relatively long compared to eruption times at Flat Cone Spring, we expect that the metatranscriptomes of the microbial community between and during flow events will remain relatively the same. Metabolites, however, have much shorter turnover times than mRNA (seconds vs minutes), and thus we expect that any differences in metabolic expression will be reflected primarily in the metabolome. Confirmation of the null hypothesis, i.e., that there is no differential metabolic expression during or between flow events at Flat Cone, would also be of great scientific interest. Whether the microbial community at Flat Cone survives extreme changes in temperature and redox conditions by switching metabolic strategies or by simply "riding it out," we can potentially learn a great deal about the limits of life on Earth by studying the survival strategies of these organisms.