Carbon Flow in Lithotrophic Acid Hot Springs Microbial Communities, Yellowstone National Park

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Project Goals: The objective of the Pacific Northwest National Laboratory Foundational Scientific Focus Area is to develop a predictive, genome-enabled understanding of how microbial interactions impart stability, robustness, and functional efficiency to microbial communities. To achieve this goal, research is being conducted on both natural mat communities and defined co-cultures designed to test hypotheses regarding processes naturally occurring in these communities. As part of this effort, we are elucidating carbon flow in the lithotrophic microbial communities in acid thermal springs in Yellowstone National Park, focusing particularly on the roles of autotrophy and heterotrophy in the communities.

Norris Geyser Basin is home to several acidic (pH ~3), sulfidic, hydrothermal (source temperature > 65 °C) springs, of which Beowulf, Grendel, and OSP Springs are examples. Microbial communities inhabit these springs that are based on chemolithotrophy as evidenced by the oxidation of sulfide and ferrous Fe and subsequent deposition of oxidation products. What remains unclear, however, is how carbon is initially introduced into the systems; whether by autotrophy, heterotrophy, or a combination route. We are exploring carbon cycling in chemotrophic springs in the Norris Geyser Basin to better understand both the initial carbon entry into the system and how fixed C is exchanged in the microbial community. Our immediate goals include (1) determining whether autotrophy plays a major role in carbon incorporation, focusing initially on *Metallosphaera yellowstonensis*, a community member whose genome includes genes from the 3-hydroxypropionate 4-hydroxybutyrate carbon fixation pathway, and (2) compiling a stable isotope inventory of an example spring (Beowulf, Figure 1) and the surrounding landscape to help identify additional carbon sources.

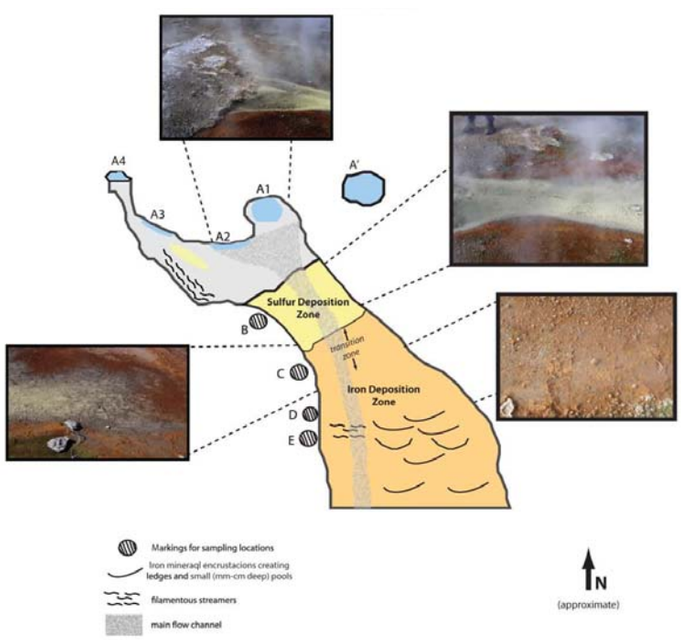

Figure 1. Beowulf spring in Norris Geyser Basin

By understanding how carbon is incorporated into and exchanged among microbial community members, we will gain insights into how microbial communities adapt to these extreme (high temperature, acidity, high sulfide and low biomass) environments.

Visual evidence reveals the two dominant metabolisms, shown in main Beowulf flow channel (Figure 1). Sulfide oxidation converts dissolved sulfide into elemental sulfur in zone B of the spring. The orange precipitates downstream of the sulfur deposition zone result from iron oxidizer activity.

M. yellowstonensis was isolated from Norris Geyser Basin and represents up to 20 % of the total

microbial nucleic acid associated with the iron oxide mats. *M. yellowstonensis* contains genes consistent with its presumed iron oxidizing metabolism.[1] Further, *M. yellowstonensis* has genes associated with the 3-hydroxypropionate, 4-hydroxybutyrate carbon fixation pathway. We sought to determine whether *M. yellowstonensis* actually does fix CO_2, and whether autotrophy occurs in the spring communities.

Pure cultures of *M. yellowstonensis* were incubated in mineral medium with or without yeast extract, and with un-labeled or ^{13}C-labeled CO_2 in the headspace. Ground pyrite was supplied as the electron donor, and O_2 was the electron acceptor. Cultures were incubated approximately two weeks, and the carbon isotope ratio of the acid-washed biomass was measured by isotope ratio mass spectrometry. When $^{13}CO_2$ was present, the isotope ratio of the biomass contained significantly more ^{13}C than when unlabeled CO_2 was present, demonstrating that *M. yellowstonensis* fixes CO_2. Much less ^{13}C was incorporated when yeast extract was present, suggesting that *M. yellowstonensis* grows heterotrophically in the presence of suitable organic substrates. Initial estimates of the $CO_2 \rightarrow$ biomass fractionation factor (~3.5 ‰) are consistent with the hydroxybutyrate fixation pathway[2] and very similar to that measured for *M. sedula* (3.1 ‰).[3]

Samples of iron-oxidizing microbial mat from two similar Norris Geyser Basin springs, Grendel and OSP, were excised and placed into bottles containing pyrite and the mineral medium used in the laboratory experiments. Unlabeled CO_2 or $^{13}CO_2$ was injected into the headspace, and the cultures incubated fourteen days at 65 -70 ^{o}C. Following incubation, the C isotope ratio of the acid-washed mat material was measured by isotope ratio mass spectrometry. Data clearly demonstrate $^{13}CO_2$ fixation by the microbial communities in both springs.

Our data thus far indicate that there may be additional carbon inputs to the iron mat community. The bulk carbon isotope ratios we have measured in the iron oxide mats range from ~=18 to ~-21 ‰. Based on the approximate fractionation factor measured in the laboratory, autotrophic fixation of dissolved inorganic carbon by *M. yellowstonensis* would yield fixed carbon with a predicted isotope ratio of ~-7.5 ‰. A simple metabolic model for the iron mats, in which carbon is fixed by *M. yellowstonensis* and then passed through a heterotrophic food chain (which typically results in little isotopic fractionation) is not consistent with the measured bulk carbon isotope ratio in iron oxide mat samples. Alternative hypotheses to explain the bulk carbon isotope ratio of the mats include (1) the presence of significant landscape carbon (e.g., plant detritis) in the mats, or (2) direct heterotrophy of landscape carbon by members of the iron mat community. The average carbon isotope ratio of organic material in the landscape (plant material, animal dung) is ~ -26 to -29 ‰, and that of dissolved organic carbon in Beowulf Spring is ~ -22 to -23 ‰. A mixture of passively present landscape carbon, or its heterotrophic uptake, in combination with carbon fixation by *M. yellowstonensis*, could explain the observed bulk isotope ratios. In the coming year, we will be working to associate carbon substrates with different community members by measuring isotope ratios of the different phylogenetic groups, and to further elucidate the flow of carbon through the iron mat community.

References

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