Approaches to systematically examine carbon flux in microbial communities using 'omics' and stable isotope probing

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http://geomicrobiology.berkeley.edu/pages/amd.html

Project Goals: The primary goal is to develop integrated 'omics' methods, including stable isotope probing, for tracking carbon flows in microbial communities.

Earth's climate is regulated by biogeochemical carbon exchanges between the land, oceans and atmosphere that are chiefly driven by microorganisms, which produce and consume carbon dioxide (CO_2), methane, and organic matter and support the growth of higher organisms. Microbial communities are therefore indispensible to the study of carbon cycling and its impacts on the global climate system. However, approaches to examine carbon flux in communities systematically at the molecular level are inadequate. We are developing methods to track the flow of carbon into and through microbial communities using a well-characterized model system—acid mine drainage (AMD) biofilms.

We are using stable isotope probing (SIP)-proteomics and SIP-metabolomics to measure and characterize the incorporation of carbon compounds into proteins and metabolites from specific organisms. SIP-proteomics and SIP-metabolomics can determine ^{13}C or ^{15}N atom% of thousands of proteins and hundreds of metabolic features in the AMD community. Together with comprehensive community metagenomics, these novel approaches demonstrate which compounds are being consumed and which organisms are consuming them. Protein data from individual organisms, community metabolite profiles and measurements of carbon pools and fluxes will allow us to generate a model for carbon cycling in the community.

We have developed and validated a ^{15}N-based SIP-proteomic method using laboratory-grown AMD communities (Pan et al., 2011) and that has since been applied to further experiments (see below). We are now using ^{13}C-based SIP-proteomics to track the incorporation of $^{13}CO_2$ into microbial proteins. Newly developed methods for SIP-metabolomics with ^{13}C and ^{15}N constrain the biological origin and specific formulas of the metabolites generated by the AMD community, making metabolite identification more tractable. Together, these approaches will enable us to follow the movement of carbon through the community as it shifts from primary production in early growth stages to a mixture of heterotrophic and autotrophic metabolisms in the later stages of development.

The initial colonizer of the AMD biofilms, *Leptospirillum rubarum*, dominates early community development and carbon fixation in the system. *Leptospirillum ferrodiazotrophum* and other lower abundance community members appear later as

biofilms develop and diversify. Using community genomic data collected over a period of 7 years, we assembled the partial genome of a new bacterial species that expands the *Leptospirillum* clade: *Leptospirillum* Group IV. The new species shares 97% 16S rRNA sequence identity and 70% identity between shared proteins with its closest relative, *Leptospirillum ferrodiazotrophum*. The presence of nitrogen fixation and reverse TCA cycle proteins suggest an autotrophic metabolism similar to that of *Leptospirillum ferrodiazotrophum*, while hydrogenase proteins unique to *Leptospirillum* Group IV suggest an active role in the anaerobic setting.

Fungi often colonize late-developmental-stage biofilms at low relative abundance and may play an important role in recycling carbon in the community. We are employing genomics, transcriptomics, and proteomics to link functional activities encoded and expressed by fungi with biogeochemical processes within the ecosystem (Miller et al., in prep). We reconstructed the near-complete composite genome (27 Mbp) of the dominant fungal AMD community member, *Acidomyces richmondensis*. Approximately 900 unique fungal proteins were detected by proteomics in field samples, covering many important metabolic pathways. Genes involved in heterotrophic carbon cycling, including several glycosyl hydrolases involved in polysaccharide hydrolysis, were expressed at varying levels based on transcript abundances inferred from ESTs recovered from field samples.

We have shown that Archaea from the Thermoplasmatales lineage dominate submerged anaerobic biofilms in the AMD system and play an important role in nutrient cycling in the anaerobic and degradative portion of the carbon cycle (Justice et al., in prep). Members of the novel ARMAN nanoarchaeal lineage are especially abundant in the sunken biofilms, comprising at least 10% of the community based on FISH and metagenomic data analysis. Comparative community proteomic analyses show a persistence of bacterial proteins in sunken biofilms, but there is clear evidence of deamidation caused by acid exposure. Given the low representation of bacterial cells in sunken biofilms, we infer that deamidated proteins are derived from populations of lysed cells. Culture experiments demonstrated heterotrophic growth of A-plasma and *Ferroplasma* sp. on peptone, betaine, casein-derived peptides, and natural biofilm material, all coupled to iron reduction. The results demonstrate anaerobic archaeal growth via protein degradation, and possibly other organic carbon compounds. ^{15}N-based SIP-proteomics showed that archaea are the dominant species incorporating ^{15}N in sunken biofilms. The SIP-proteomic techniques also allowed us to profile the archaeal metabolic activities preferentially enriched for newly synthesized protein. These findings expand our understanding of the roles of Archaea in anaerobic nutrient cycling.

We are determining how a key parameter of global climate change, elevated temperature, regulates the flow of carbon through the microbial-based AMD ecosystem. Proteomic and metabolomic data highlight which community members and which modes of carbon cycling are affected by elevated temperature. This approach is establishing whether the carbon cycling pathways in the system are robust to the effects of climate change or, if not, where the cycling may break down.