Linking microbial identity and function in phototrophic mats and biofilms

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Project Goals: The LLNL Biofuel SFA investigates systems biology of complex microbial communities relevant to bioenergy production. To understand nutrient cycling and potential biofuel production in complex microbial communities we employ an integrated analysis of energy flow using multi-scale approaches including biogeochemical, stable isotope probing, metagenomic/transcriptomic, proteomic/metabolomic and computational analyses. Our ultimate goal is the development of multi-scale models that can predict ecological and biochemical relationships within multi-trophic microbial systems.

Cyanobacterial communities that form laminated mats are highly diverse microbial assemblages, and have been studied for decades as analogs for early earth life. Yet the partitioning of light and geochemical energy into biomass within these complex microbial systems is not clear. To gain a comprehensive understanding of these communities, we cultivate and compare members of hypersaline microbial mats for phenotypic characteristics, and use both MS proteomics and NanoSIMS stable isotope imaging to investigate mechanisms of energy flow.

To approach the relationship between microbial community composition and function in these mats, we have isolated a large number of the organisms and measured biogeochemical rates in both pure cultures and defined isolate mixtures. Isolates were tested for growth on two dozen different substrates, and whole genome metabolic reconstruction used to analyze the functional roles these isolates may play in the community. Experiments with isolate mixtures with presumably similar functional roles are being used to test the degree to which functional redundancy is important for resisting environmental stress. We have documented differences in substrate preference by heterotrophic bacteria, and salinity tolerance and photosynthesis versus irradiance relationships in cyanobacteria. We have found a positive growth-photosynthesis relationship between cyanobacteria and all heterotrophic bacteria tested to date. We are investigating these effects with a suite of ‘omics techniques and a culturing approach where the three dimensional structure of natural mats is recreated using artificial substrates.

It has become increasingly clear that many microbial primary producers can also play roles as organic consumers, but there are few studies that assess metabolic regulation of photoautotroph organic matter consumption. This is especially relevant in mats because cyanobacteria produce an extensive organic extracellular matrix, providing the community with a physical buffer and a rich source of nutrients. We examined a single cyanobacterium and associated heterotrophs isolated from a microbial mat. By applying stable isotope tracing at the single cell level, we can quantify cyanobacterial assimilation of complex extracellular organic C and N under different metabolic conditions (Figure 1) (Stuart et al 2015; Stuart et al in review). We investigated the metabolic foundations of organic matter reuse by comparing exoproteome composition and incorporation of ^{13}C-^{15}N labeled extracellular organic matter in a unicyanobacterial biofilm incubated under different light regimes. Reuse by cyanobacteria accounted for almost half of all uptake in the community, indicating they are successful competitors for organic C and N. Under

lighted conditions, we measured increased excretion of extracellular polymeric substances (EPS) and proteins involved in micronutrient transport, suggesting requirements for micronutrients may drive substrate uptake during daylight hours. When photosynthesis was chemically inhibited, cyanobacteria incorporated extracellular organic matter with a low C/N ratio. By contrast, in the dark, cyanobacteria incorporated high C/N extracellular material, decreased their excretion of EPS, and increased expression of degradative exoproteins, implying use of the extracellular domain for C storage. Simultaneously, in prolonged dark incubations, associated heterotrophic bacteria increased in abundance and upregulated their transport proteins, suggesting that cyanobacterial reuse may incidentally control heterotroph resource availability under normal day-night diel regimes. Light availability and resulting metabolic status of these primary producers may dictate both composition and turnover rates of extracellular organic matter.

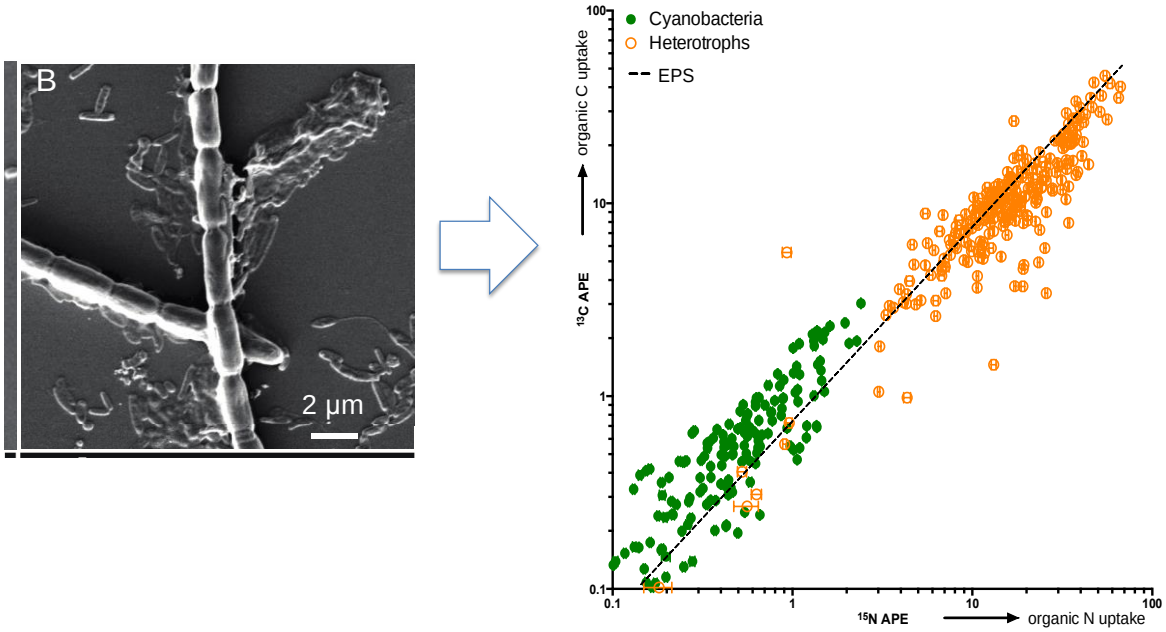

Figure 1: Stable isotope probing allows us to track extracellular organic matter incorporation in a mixed biofilm community. (Left) SEM image of cyanobacterial trichomes and associated microbes in a biofilm. (Right) ^{13}C and ^{15}N enrichment of cells analyzed via NanoSIMS. Each point represents ^{13}C and ^{15}N atom percent excess (APE) for a single trichome (solid) or bacterial cell (outlined). Dotted line indicates the ratio of ^{13}C-^{15}N labeled EPS-substrate, as determined by IRMS.

References

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This research was supported by the LLNL Biofuels Scientific Focus Area, funded by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under FWP SCW1039. Work at LLNL was performed under the auspices of the U.S. Department of Energy under Contract DE-AC52-07NA27344 and at Pacific Northwest National Lab supported by the OBER/GSP under the Pan-Omics project.