Spatial Interactions of Autotrophs and Heterotrophs Elucidated Using Advanced Quantitative Imaging Techniques

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Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph consortia whose member genome sequences have been defined. Our project leverages unique capabilities including multi-omics measurements, advanced functional imaging, taxonomic profiling and metabolic and regulatory network modeling to elucidate underlying reaction mechanisms within complex microbial communities. Our research plan supports DOE goals to achieve a predictive understanding of microbially-mediated carbon and energy transformation.

Elucidating the molecular mechanisms by which individual microorganisms in microbial communities exchange metabolites is an important challenge for increasing our fundamental understanding of the roles of specific microbial interactions in key biogeochemical cycles. An additional scientific challenge is to understand the importance of spatial distance between community members because natural microbial communities are spatially heterogeneous and the means by which microbes interact across various spatial gradients is not well understood. Here we addressed the hypothesis that autotrophs serve as the sole nitrogen source for heterotrophs when only nitrate is available (see Lindemann et al. poster). A second hypothesis is that certain heterotrophs would spatially associate with the autotroph, due to the need for both carbon and nitrogen sharing. Thus, providing a soluble nitrogen source should disrupt the spatial linkages by removing obligatory interactions. To test these hypotheses we employed a suite of advanced imaging capabilities together with novel quantitative image analysis techniques. For the experiments, we leveraged a tractable model consortium in which a single photoautotroph (a cyanobacterium) provides the fixed carbon for a suite of naturally, co-isolated heterotrophs. We used this model system to decipher the spatial context of interactions occurring between members. In particular, the unicyanobacterial consortium, UCC-O, was a valuable resource in this respect because most of the members have been isolated and genome sequences are available, facilitating the use of genome-enabled approaches for deriving metabolic models.

Dual-channel confocal laser scanning microscopy (CLSM) was used to image the community level spatial organization and dynamics of autotrophs and heterotrophs. The morphology of cells in actively growing consortial biofilms was investigated by analyzing the CLSM micrographs using custom developed image analysis software that could quantify total biomass and the biomass distribution between the autotroph and heterotrophs[1]. Nanoscale secondary ion mass spectrometry (NanoSIMS), a powerful, high spatial resolution imaging approach, was then used

to visualize the metabolic activities of single cells. We created and applied a semi-automated image-processing pipeline for quantitative analysis of the NanoSIMS data[2]. This pipeline includes both elemental and morphological segmentation, thus producing a final segmented image that allows for discrimination between autotrophic and heterotrophic biomass. It also allows the detection of individual cyanobacterial filaments and heterotrophic cells, the quantification of isotopic incorporation of individual heterotrophic cells, and calculation of relevant population statistics. Fluorescence in situ hybridization (FISH) imaging with species-specific probes was used to spatially localize heterotroph species that were hypothesized to be dependent on metabolic linkages with the cyanobacterium based on genome predictions.

The combined use of these imaging tools was applied to analyze the community structure over time and to understand the uptake of ^{15}N provided as either nitrate or ammonium through the consortium. We found that the degree of ^{15}N incorporation by individual cells was highly variable when labeled with $^{15}NH_4+$, but much more even when biofilms were labeled with $^{15}NO_3-$. In the $^{15}NH_4+$-amended biofilms, the heterotrophic distribution of ^{15}N incorporation was highly skewed, with a large population showing moderate ^{15}N incorporation and a small number of cells displaying very high ^{15}N uptake. We also observed that one of the heterotrophs (HL-53) was always proximal to the cyanobacterial filaments, suggesting a new hypothesis that these cells form an epibiotic or parasitic relationship. We are currently performing additional experiments to test this. Our results show the feasibility of using imaging technologies for the quantitation and visualization of the spatial organization of individual cells within a community, their uptake of specific elements, and their orientation to other member species. These techniques will be employed in future experiments to gain further understanding of the spatial dependencies of microbial community interactions.

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