Chemical Analysis of Carbon and Nitrogen Cycling Through the Extracellular Matrix Produced During the Formation of a Multi-Species Community

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Project Goals: The goal of the Metabolic and Spatial Interactions in Communities (MOSAIC) Foundational Scientific Focus Area is to understand the fundamental mechanisms by which microbial metabolic interactions and spatial organization impact carbon, nitrogen, and energy dynamics in microbial communities. Our studies focus on the coupling of carbon and nitrogen cycles in microbial communities, the role of environmental variables in governing the rates of these cycles, and the impact of environmental perturbations on microbial community dynamics. We employ tractable model consortia whose member genome sequences have been defined, advanced omics measurements, functional imaging, taxonomic profiling, and modeling to elucidate interaction mechanisms within complex microbial communities. Our research supports the DOE goals to achieve a predictive understanding of Earth's integrated biogeochemical processes.

Microbial autotroph-heterotroph interactions influence biogeochemical cycles on a global scale, but the molecular mechanisms underlying microbial community interactions and functional processes for which microorganisms cycle carbon, nitrogen and energy are largely unknown. We hypothesize that the extracellular polymeric substance (EPS) matrix is an exchangeable resource that can serve as a complex and dynamic pool of biologically-available carbon, nitrogen, and other nutrients during nutrient limitation or other types of environmental stress. We have investigated the EPS matrix using a tractable, autotroph-heterotroph consortia. These stable consortia cultures, containing one autotrophic cyanobacteria and multiple heterotrophic bacteria, have been previously isolated and temporal community member dynamics have been established.

To test our hypothesis, we developed an extraction protocol to isolate distinct operational fractions of EPS from the communities, and performed a temporal study of the EPS matrix to determine changes in the distribution of biological molecules within the EPS fractions during community maturation. The chemical components of the purified EPS fractions were evaluated using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy analysis to determine the relative abundances of lipids, protein, sugars, and nucleic acids in EPS fractions. Principle component analysis was used to resolve temporal- and EPS fraction- dependent changes in these biological molecules that were indicative of carbon and nitrogen cycling in the EPS matrix. Temporal changes were observed in the distributions of lipids observed in EPS, while the relative abundances of sugars remained stable over the study and possibly suggested that the extracellular carbon in nascent polysaccharides was being quickly consumed by heterotrophic community members. Interestingly, we observed low abundances of proteins and nucleic acids in all EPS fractions. The low abundance of extracellular proteins suggested that they were quickly degraded and consumed by the community, indicating that nitrogen cycling (as nitrate) through the community was also, in part, dependent on autotrophic metabolism. To identify the community members that are secreting EPS degrading enzymes, we are using activity-based probes that are selective towards functionally active glycosyl hydrolase and serine and cysteine protease activities, in conjunction with mass spectrometry-based proteomics, to directly identify and quantify the functionally-active extracellular EPS degrading enzymes. This work enhances our understanding for how a microbial community cycles carbon, nitrogen and energy through a community through the EPS matrix and establishes a foundation for understanding carbon and nitrogen cycles through other autotroph-heterotroph systems and microbial communities found in complex physical systems such as soils.

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