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Metabolomics, Lipidomics, and Kinetic Flux Profiling: Developing Tools for Monitoring the Physiology of Ecologically Relevant Microbial Communities

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Background/Objectives. Recent advances in liquid chromatography–mass spectrometry (LC–MS) based metabolomics and lipidomics have furthered understanding of metabolism in a variety of systems. Not only can such techniques be used to discover biomarkers for the physiological state of the system, they can also be used to probe the global metabolism of a sample by providing information on the concentration of thousands of molecules (i.e. the metabolome and lipidome) from a single sample. The utility of metabolomics methods can also be enhanced by monitoring the incorporation of stable isotope-labeled nutrients, either ^{15}N or ^{13}C, into the metabolome using kinetic flux profiling techniques (KFP) that are designed to capture the net passage of metabolites through pathways. The coupling of metabolite concentration and flux measurements can be used to determine both the amounts of metabolites within and relative rates of flux through many biochemical pathways in parallel, and the combination of these methods allow a global snapshot of cellular metabolism to be obtained. Despite the power of these techniques, biological and technical challenges hinder the use of metabolomics to interrogate microbial communities sampled from their natural habitat. Herein, our efforts to circumvent these challenges and apply these emerging systems biology techniques to natural microbial communities will be discussed.

Approach/Activities. Several analytical methods using ultra performance LC–high resolution MS (UPLC–HRMS) and UPLC–tandem mass spectrometry (UPLC-MS/HRMS) have been employed to ensure a broad coverage for the detection of metabolites from a variety of biological samples derived from many environments and all kingdoms of life. Using the metabolomics techniques, 90-180 known metabolites and *ca* 1500-5000 spectral features arising from water-soluble molecules with unknown structures. The lipidomics platform detects 400-600 known lipids with *ca* 5000-8000 spectral features from unknowns. These techniques have been optimized for the analysis non-mammalian samples by including compounds that are important to a diverse range of species (such as the metabolites involved in the cryptic sulfur cyclic in roseobacter and the sulfolipids that are found in aquatic environments. The combination of high resolution MS data to determine and molecular formulae and fragmentation data to help elucidate structure will be a powerful tool to characterize water- and lipid-soluble intercellular metabolites as well as extracellular carbon containing compounds.

Results/Lessons Learned. Several vignettes from our work studying microbial communities in soil and aquatics systems will be used to highlight the utility of metabolomics to understand the metabolism of viruses, bacteria, cyanobacteria, and algae. The information gained from these meta-metabolomics experiments is often enhanced when coupled with other systems biology tools, such as meta-transciptomics; and the integration of multi-omics techniques to study the metabolism of aquatic microbial consortia will also be discussed.