Metabolite Characterization in Complex Microbial Communities

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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 communities are present in, and interact with, a wide variety of challenging ecosystems, such as soil. The members of these communities play vital roles in the cycling of carbon and nitrogen and for sustaining ecosystem productivity and overall health; however, the molecular bases for how community members interact with each other and their environment is yet to be fully elucidated. We have developed a suite of integrated molecular measurement capabilities for the comprehensive characterization of metabolites and other soil organic matter and for characterizing the mechanisms by which soil microbial communities interact with this matter. These capabilities include MetFish, and ultra-high throughput ion mobility spectrometry-mass spectrometry (IMS-MS) measurements combined with the associated physical-chemical property of collisional cross section (CCS).

MetFish is a suite of chemoselective probes that target metabolites and other small molecules containing amine, carboxyl, carbonyl, and hydroxyl functional groups, to enable their enrichment from complex and extreme ecosystems, such as soil and hypersaline systems. The MetFish workflow provides sensitive and specific quantification of metabolites, with limits of quantification at low nM levels in sample matrices containing up to 2 M total dissolved salts. We have applied MetFish in analyses of microbial consortia in hypersaline media, as well as soil and fracking fluid. For soil metabolomics, MetFish in combination with a high salt wash provided higher recovery (2-10 fold) of extracellular metabolites in comparison to water and low salt extractions, allowing for more precise quantification of extracellular metabolites while imparting minimal to no effects on microbial intracellular metabolite profiles.

We have also developed an automated solid phase extraction (SPE) coupled with IMS-MS metabolomics platform capable of performing both targeted and global measurements with high reproducibility (CV ≤ 3%), sensitivity and throughput (>8000 analyses per day). This combination of separation techniques allows in-depth metabolite characterization due to the fact that the SPE distinguishes each molecule based on chemical characteristics, IMS by structure, and MS by mass. To facilitate broad metabolite identification using the SPE-IMS-MS platform, we are creating a reference library of accurate mass and molecular CCS through experimental measurements and theoretical predictions based on chemical structures of the molecules. CCS is the key structural property measured by IMS-MS, and can be calculated accurately *in silico*, which helps overcome longstanding obstacles to metabolite identification in the absence of authentic chemical standards. We employ a custom quantum chemistry-based, supercomputer-driven software engine for calculating CCS in which chemical identifiers (i.e., InChIs) are converted into 2D structures, and then protonation/deprotonation states and adduct sites are predicted. Final geometry optimizations are performed using a density functional theory implemented in the NWChem quantum chemistry software developed at the DOE EMSL user facility. The generation of accurate predicted CCS values can facilitate the broad identification of detected molecules in combination with accurate mass and MS/MS spectra.

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