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Microbial Mercury Methylators in East Fork Poplar Creek: From the Field to the Laboratory

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Mercury methylation in the East Fork Poplar creek (EFPC) is primarily a result of microbial biotransformation both in sediments and in periphytic communities. Based on laboratory studies, mercury methylation is conducted by bacteria and archaea that harbor the *hgcAB* genes and that belong primarily to the Deltaproteobacteria, Firmicutes, and Methanomicrobia groups. Previous sequence studies of EFPC communities have identified the presence of potential methylators, but so far none has been characterized based on genomic data or isolated in culture from the site. To achieve a comprehensive understanding of the mercury biotransformations in EFPC, the primary organisms that catalyze methylation need to be identified and characterized. The extremely high diversity of the microbial communities where methylation occurs (up to thousands of species) makes that task difficult, as the methylators are a low fraction of the community (<1%).

To gain access to the microbial methylators in sediments and periphytic communities, we are developing fractionation techniques (e.g., gradient centrifugation) to separate bacteria and archaea from other biotic and abiotic components of the environmental samples. We are also applying flow cytometric fractionation of the complex EFPC microbial communities, based on various cellular characteristics including size and morphology, as well as labeling with antibodies raised against cultured bacteria and archaea that methylate mercury. Populations of cells enriched for methylators will be characterized by metagenomic sequencing and will also be used for laboratory enrichments to isolate and characterize novel Hg methylators.

In parallel, we are conducting laboratory experiments aimed at characterizing the interaction of known bacterial and archaeal methylators and how environmental factors impact methylation rates. Species related to those found in EFPC are being grown under steady state conditions in single cultures and in increasingly complex co-cultures to measure cell counts, Hg methylation rates, electron donors and acceptors, organic acids, H₂ and CO₂ concentrations, carbon balancing, and gene expression levels. The selected strains represent different anaerobic functional groups (iron and sulfate reduction, fermentation, syntrophy, methanogenesis), allowing us to directly characterize the phenotypic effects of multi-species interactions on Hg- methylation and cellular metabolism. Methylating organisms isolated from EFPC will ultimately be integrated in these studies and used to identify general and EFPC-specific metabolic and Hg biotransformation characteristics. Ultimately, the outcomes of these studies will further inform modeling of mercury biogeochemical processes in EFPC.