Mercury Methylation: Microbial Communities Involved in Hg Transformations (ORNL Hg SFA, Microbial Genetics and Transformations)

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In microbial transformation and genetic studies, we examined microbial communities involved in mercury methylation in streams contaminated from past operations in the Y12 plant at Oak Ridge. In collaboration with the Field Task of the Hg SFA program, we investigated Hg methylating microbial communities in Hg contaminated streams and background sites. Initial microcosms suggest active methylation downstream, with this activity being stimulated by sulfate and inhibited by molybdate. Water and sediment samples were analyzed for the microbial community complement phylogenetically via 454 pyrosequencing of the 16S rDNA gene V4 region. We hypothesize that: 1) there is a greater diversity of genes related to pollutants at the contaminated sites; 2) a lower overall phylogenetic diversity is present at these sites, 3) some groups of microorganisms will correlate with areas contaminated with Hg and/or methylmercury (MeHg), 4) specifically, the number of *Deltaproteobacteria* (the group involved in methylation) will positively correlate with MeHg concentrations. Analysis of 60 samples revealed pronounced phylogenetic and functional differences related to seasonal trends. Geochemical principal component analysis of several sites showed that one area, Bear Creek, was substantially different due to the presence of U(VI) and nitrate and this was reflected in the microbial community that was mostly devoid of Proteobacteria. Virtually all of the microbial communities in the other five sites trended towards dissolved Hg. Further, such a correlation of the 454 data with geochemistry at the phylum and genus level showed that some Hg methylating bacteria such as *Geobacter* spp. do not correlate with either Hg or MeHg. However, both the Delta- and Epsilon- Proteobacteria as well as *Verrucomicrobia* all trended towards dissolved Hg, and *Desulfobulbus* spp. strongly trended towards MeHg. This is significant in that *Desulfobulbus propionicus* is a known Hg methylator. Methylation and demethylation are being investigated with the type strain under several different culturing regimes to coordinate differences in growth with these activities to point to biochemical methylation pathways. Enrichment and isolation using propionate and sulfate resulted pure cultures that did not methylate but these activities are being repeated. A second generation functional gene array with >1000 *mer* gene sequences is being deployed along with consensus *merA,B* qPCR primers while we work towards 6 meta –genomes and -proteomes.