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

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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 and demethylating 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. 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, a correlation of the 454 data with geochemistry at the phylum and genus level showed that in areas with higher MeHg, the greatest number of sequences corresponded to the methylating *Geobacter* and *Desulfobulbus* spp. as well as with *Byssovorax* and *Desulfonema* spp. Methylation and demethylation are being investigated with *Desulfobulbus propionicus* under several different culturing regimes to coordinate differences in growth with these activities to point to biochemical methylation pathways.

Efforts to better understand the carbon and electron flux in three methylating and demethylating communities used sediments from intact cores to; a) determine the depth section of the cores with the greatest activity, and b) determine the carbon sources that would stimulate and/or inhibit these activities. Analysis with stable isotopes revealed minimal stimulation with lactate and ethanol while cellobiose inhibited both activities. Ongoing analysis includes a second generation functional gene array with >1000 *mer* gene sequences using DNA and reverse transcribed mRNA, 454 pyrosequencing to correlate differences in organism abundance with DNA and mRNA abundance so as to determine the the organisms and genes responsible for Hg methylation and MeHg demethylation in East fork Poplar Creek.