

Mercury Methylation: Genes and Communities Involved in Hg Transformations (Hg SFA at ORNL, Microbial Genetic Study Task)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

D.A. Elias (PI), T.A. Vishnivetskaya, J.D. Mosher, M.M Drake, S.D. Brown, C.C. Brandt, S.C. Brooks—ORNL; C.C. Gilmour, *Smithsonian Environmental Research Center*; A.M. Kucken, J.D. Wall—*U. of Missouri*; A.V. Palumbo, ORNL

In microbial transformation and genetic studies, we have examined the genes and microbial communities involved in mercury methylation in streams contaminated as a result of 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 by sampling and characterizing Hg-contaminated streams and back-ground sites. Water and sediment samples from transects of a methylating area of the stream were characterized geochemically. They were analyzed for the community complement of microorganisms using both a functional gene array (FGA) and phylogenetically via 454 amplification and sequencing of the V4 region of 16S genes from the total community. 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), and (4) specifically, the number of *Deltaproteobacteria* (the group involved in methylation) will positively correlate with MeHg concentrations. Analysis of 60 samples as above revealed pronounced phylogenetic and functional differences that appear to be 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* as determined by RDA. 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 proprionicus* is a known Hg methylator. Hence, enrichment and isolation performed using propionate and sulfate has resulted in a pure culture that morphologically resembles this type strain. The metabolic and Hg methylation/demethylation characteristics are currently being assessed. We are also designing a second generation FGA with many known *mer* gene sequences, along with consensus qPCR primers, for testing the presence of *merA* and *merB* in field samples.