Mercury Methylation: Genetics and physiology of methylmercury production (ORNL Hg SFA, Microbial Genetics and Transformations)

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In mercury (Hg) contaminated areas, anaerobic bacteria convert Hg(II) to toxic methylmercury ($MeHg^{+}$); primarily the sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB). We identified 11 *Desulfovibrio* strains that constitutively produce MeHg, and 12 strains lacking this ability. Protein-normalized Hg methylation rates varied by ~1 order of magnitude. An updated 16S rRNA phylogeny indicates that Hg-methylators are widely distributed phylogenetically, but closely related species within recent branches show the same capability/inability for Hg methylation. These findings along with more sequenced methylators allows for more refined comparative genomics to predict high value gene targets for methylation and demethylation. We are exploring demethylation rates to evaluate whether non-methylating *Desulfovibrio* have superior demethylation capability. One of the weaker methylators, *D. africanus*, was characterized for methylation and differential gene expression over the growth curve due to extreme pleiomorphology at different growth phases. While no methylating genes were identified, the *feoAB* genes appear to positively correlate with Hg exposure and methylation.

We developed and refined several protocols correlating Hg methylation rates with growth phase, [Hg] and speciation. This is critical for comparisons across chemical conditions and strains. Methylation in our model organism *Desulfovibrio desulfuricans* ND132 is dependent on growth phase, [Hg] and medium chemistry. We found high bioavailability of Hg complexed with diverse thiols, suggesting that ND132 does not take up Hg as intact Hg-thiol complexes. We also found that Hg bioavailability under sulfidic conditions is enhanced by DOM, suggesting HgS nanoparticle bioavailability. This provides a mechanism for observed correlations between DOM and Hg methylation. Further, DOM aromaticity predicted HgS bioavailability, as it predicts nanoparticle growth rates. ND132 is 1 of 2 Hg-methylating SRB with a sequenced genome (JGI). The genome was left in 1 scaffold and 6 contigs and we have now developed a universal procedure to close genomes. To elucidate the genes responsible for Hg-methylation, a random Tn5 transposon library and screening assay were adapted in ND132. Currently, >6,600 mutants have been created. The screening assay was recently modified. Complete sample digestion with 3N HNO_3 (24 h) now overcomes a cell density effect on MeHg recovery (above) with direct ethylation at pH 3.9 with wild type controls at different cell densities allowing for greatly improved discrimination of mutants of interest. Since February 2012, 480 new mutants have been re-assayed to identify mutants using a ratio of methylation potential (MeHg pg.ml-1) and optical density (OD600). These genes will undergo targeted deleted to confirm their involvement in Hg methylation so that the Hg methylation genes and pathways may at last be identified.