Mercury Methylation: Genetic and physiological determinants of methylmercury production in sulfate-reducing bacteria (ORNL Hg SFA, Microbial Genetics and Transformations)

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In mercury (Hg) contaminated soils and waters, anaerobic bacteria are responsible for converting Hg to toxic and bioaccumulative methylmercury (MeHg). MeHg production has primarily been confirmed in subsets of dissimilatory sulfate-reducing bacteria (DSRB) as well as dissimilatory iron-reducing bacteria (DIRB). In order to determine the diversity of this activity, 8 *Desulfovibrio* species have been tested for the first time and 4 were capable of methylation, bringing the total to 11 out of 23 tested. Phylogenetically, there is no obvious pattern to the capability, with the rates varying by up to 10-fold amongst the methylators. 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. No obvious gene patterns were found, although several candidate genes have been identified and are being tested.
The model organism to date for this work has been *Desulfovibrio desulfuricans* ND132. This strain was chosen for its high methylation rate. Methylation rates by ND132 are strongly dependent on the Hg concentration and medium chemistry, but under optimal conditions in the absence of sulfide N132 can methylate about 30% of 10 ng/ml $HgCl_2$ during batch culture growth. Some small thiol ligands enhance the amount of MeHg produced but these are different than the thiols that enhance methylation in *Geobacter* spp. ND132 is one of only two mercury methylating sulfate-reducers to have its genome sequenced (JGI). The genome contains 3478 candidate protein-encoding genes with 65.2% G+C and a total size of 3.8Mb, but was left in 1 scaffold and 6 contigs. We have undertaken completing the genome and to date have filled 4 of the 6 gaps which were due to both sequencing errors and hard GC stops. To elucidate the genes responsible for mercury methylation we have developed a facile genetic manipulation system via conjugation in ND132 to create a random Tn5 transposon mutant library, as well as a screening assay, and both have been adapted for high-throughput. The initial target is 5,000 mutants but up to 10,000 may be required. Currently, 3,352 mutant constructs have been created and >900 have been assayed for loss of methylation, with 47 showing decreased methylation to some degree. Decreased methylation in these 47 will be validated using stable isotopes ICP-MS and sequencing. If validated, these candidate genes will be deleted using targeted mutagenesis in order to confirm they are involved in

Hg methylation. Through this methodology the responsible genes and pathways for Hg methylation in ND132 may well at last be identified.