Mercury Methylation: Genetic Determinants of Methylmercury Production in the Sulfate-reducing Bacterium *Desulfovibrio desulfuricans* ND132 (Hg SFA at ORNL, Microbial Genetic Study Task)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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

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). We have focused our attention on the anaerobic DSRB *Desulfovibrio desulfuricans* ND132. This strain was chosen for its high methylation rate and phylogenetic similarity to the lost *Desulfovibrio desulfuricans* LS, for which methylation pathways were partially defined (Choi et al. 1994). Strain ND132 was isolated from estuarine mid-Chesapeake Bay bottom sediments where measured MeHg production rates are high. Methylmercury production by ND132 depends on Hg concentration and complexation in the culture medium. 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. We found that small thiol ligands did not alter the amount of MeHg produced by this organism. We propose to establish ND132 as a model organism for mercury methylation and have characterized its physiology and established various molecular approaches to determine potential genes involved in mercury methylation.

ND132 is an incomplete substrate oxidizer that utilizes a narrow range of electron donors and acceptors for respiratory and fermentative growth. It is an estuarine, mesophilic organism, with NaCl and pH optima of 1% and 7.8, respectively. ND132 is one of only two mercury methylating sulfate-reducing bacteria to have its genome sequenced, and we expect a finished genome from JGI within 3-6 months. The ND132 genome is comprised of 3478 candidate protein-encoding genes, 65.2% G+C, has a total size of 3.8 Mb and is currently at 45 contigs. To elucidate the genes responsible for mercury methylation, we isolated potential ND132 methyltransferase genes and transformed each into a nonmethylating strain of *Desulfovibrio*, and then measured MeHg production. This targeted approach has not yet identified a methylase; therefore, we have initiated the creation of a random Tn5 transposon mutant library with an attainable goal of >5000 mutants. Currently, we have over 1300 individual mutants ready for assay. Transposon mutants will be tested for lack of MeHg production, and a collection of nonmethylating mutants will be sequenced for identification of gene interruptions. Development of a high-throughput assay to identify nonmethylating clones is also under way.