

Genetic and Biochemical Approaches to Identify Mercury Methylation Enzymes in *Desulfovibrio desulfuricans* ND132

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BACKGROUND. The anaerobic sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 is a known methylator of mercury. The resulting compound, methylmercury, is a potent neurotoxin which bioaccumulates in the food chain and poses significant risks to top predators in mercury contaminated regions. The mechanism by which ND132 methylates mercury is currently unknown. Our goal is to identify the genes and enzymes in ND132 necessary for the methylation activity in order to create probes for monitoring this activity in the environment and to explore options for the prevention of mercury methylation.

METHODS. The first of two approaches applied established genetic tools for manipulation of ND132 and initiated construction of a random transposon mutant library through conjugation. A draft genome sequence is available for ND132 making the position of the insertion sites of key transposons easily identified. To screen the library for mutants altered in methylating capacity, a high-throughput assay was developed and established the standards for the assay. In the second approach, biochemical assays and purification techniques are being applied to identify mercury methylation enzyme(s) from cell extracts of ND132.

RESULTS. So far we have harvested ca. 2400 mutants in a library that will include 10,000 total mutants to ensure broad genome coverage and have screened 500 mutants. The methylmercury screening assay has been optimized for age of cell culture, culture atmosphere, time of incubation with mercury and incubation under anaerobic versus aerobic conditions. Additionally, conjugation has been shown to effectively transfer plasmids to ND132 for addition or deletion of genes, techniques essential to continue the analysis once a putative non-methylating transposon mutant or methylating enzyme is identified.

CONCLUSION. The discovery of the mercury methylating genes and pathway(s) will allow for new strategies to minimize or eliminate the production of this potent neurotoxin.