Genetic Approaches for Mercury Methylation Enzymology in *Desulfovibrio desulfuricans* ND132

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Methylmercury (MeHg+) is a potent neurotoxin which readily bioaccumulates in the food chain and poses significant risks to top predators in mercury contaminated regions. MeHg+ is primarily produced by anaerobic dissimilatory sulfate-reducing (DSRB) and dissimilatory Fe(III)-reducing bacteria, although interestingly only a subset of species within these groups are capable of mercury-methylation. The DSRB *Desulfovibrio desulfuricans* ND132 is a known methylator of mercury and is one of only a few strains of mercury-methylating DSRB to have its genome sequenced. The enzymology of mercury methylation remains elusive; hence, we propose to identify the genes and enzymes in ND132 necessary for the methylation activity. We have established genetic manipulation protocols in ND132 to target the deletion of candidate methylation genes and have initiated construction of a random transposon mutant library through conjugation. Thus far ca. 3000 mutants have been isolated and arrayed in a library that will include 10,000 mutants to ensure broad genome coverage. A high-throughput MeHg+ screening assay was developed and optimized to detect mutants altered in methylating capability and over 800 mutants have been screened to date. Additionally, biochemical assays and purification techniques are being applied to identify mercury methylation enzymes from cell extracts of ND132. Determining which genes or pathways are involved in MeHg+ production in ND132 may facilitate the development of new strategies to minimize or eliminate the production of MeHg+ in mercury contaminated environments.