Molecular Biology Level Investigations of the Mercury Methylating Genes hgcAB

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The discovery and verification of the mercury methylation genes, hgcAB, has been a seminal discovery by the ORNL Hg SFA. Their discovery now opens up new avenues of research. Efforts are now underway to determine the mechanism of action that allows for the biotransformation of Hg(II) to MeHg and to be able to detect and quantify the gene abundance and expression in the environment. To these ends, we have determined that replacement of cysteine residues in hgcA or hgcB that were predicted to be essential for activity are in fact required. These include the cysteines in the active site of hgcA as well as those now determined to be required for correct folding of the functional protein. Further, at least one of the two exposed S residues at the C-terminal end of hgcB are required for Hg-methylation to occur. In an effort to understand the mechanism of action and to determine the stoichiometry of hgcA to hgcB as well as to determine the plausible requirement of other protein(s), we are developing genetically tagged strains and antibodies for purification of the functional complex.

Detection of these genes in the environment is essential to understanding the relationships between geochemical parameters and the biological generation of MeHg. To this end, we have developed degenerate primers for the qualitative and quantitative assessment of hgcAB at the genetic and transcriptomic level for each of the Deltaproteobacteria, Firmicutes and Archaea. The primers have been optimized and tested against a variety of pure cultures of known methylators and known non-methylators as well as against a variety of environmental samples. Currently, we are correlating these molecular biology level results with the assessed geochemical and physicochemical parameters for each environment.