

Mercury Methylation: Microbial Species and Communities Involved in Hg Transformations (ORNL Hg SFA, Microbial Genetics and Transformations)

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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. We have examined microbial communities involved in mercury methylation in streams receiving Hg from the Y12 plant at Oak Ridge in collaboration with the Field Task. Microcosm studies suggested active methylation downstream, with this activity being stimulated by the addition of sulfate or the carbon sources lactate, ethanol, methanol, or acetate. Both molybdate and cellobiose inhibited methylation activity. Water and sediment samples were analyzed for the microbial community complement phylogenetically via 454 pyrosequencing of the 16S rDNA gene V4 region. As expected, the addition of cellobiose stimulated the *Firmicute* population which has not been traditionally recognized as being able to participate in methylation while at the same time the delta-Proteobacteria population decreased, which has been repeatedly implicated in Hg-methylation. However, our recent discovery of the methylation genes led us to predict that both *Firmicutes* and methanogens are capable of this activity. Further inspection of the 16S rRNA sequences revealed that the *Firmicutes* stimulated did not possess the *hgcAB* genes and therefore explains the decreased methylation activity. However, this analysis did show that the relative abundance of methanogens that possess the *hgcAB* genes was increased. This suggests that, although the latter population increased in abundance, their methylation activity is low. This was in fact the case when our predictions were tested and revealed that all predictions for methylators and non-methylators were accurate, but that the methylating rate and yield for the *Firmicutes* and methanogens was far below that of the delta-Proteobacteria.

Efforts are now underway to optimize universal methylation gene primers and quantitative primers for accurate quantification of the methylation potential of any environment. We are now also determining; 1) the presence of the native function of these genes as well as 2) the remainder of the biochemical pathway that is/may be used to route carbon and electrons in the absence of mercury. Work is also ongoing to better understand the expression and abundance of these genes by determining and characterizing the promoters and regulators of these two genes.