Poster #192

The Biochemistry of Mercury Methylation in Anaerobic Bacteria

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Microbial mercury methylation is an enzyme-catalyzed process carried out by certain anaerobic bacteria and archaea. The two-gene cluster *hgcAB* is essential for mercury methylation and encodes a cobalamin-dependent protein, HgcA, and a ferredoxin, HgcB, which are predicted to facilitate methyl transfer and cofactor reduction, respectively. Determining the specific roles and interactions of HgcA and HgcB with other cellular components offers insights into the biochemical pathways associated with bacterial mercury methylation. The results will help reveal key processes that control the fate and transformation of mercury in freshwater ecosystems and will provide essential data to inform metabolic and reactive transport models that can be used to predict mercury cycling from the molecular to the field scale.

The goal of this work is to identify molecular mechanisms and interactions of HgcA and HgcB to elucidate the biochemistry of mercury methylation. Heterologous expression of HgcB in *E. coli* as an N-terminal maltose-binding protein fusion in *E. coli* BL21(DE3) resulted in purified HgcB, which is 65% replete with [4Fe-4S] clusters. UV-Vis spectroscopic data demonstrates electron transfer to HgcB *in vitro* and the interaction of HgcB with Hg(II) has been characterized. Furthermore, *Desulfovibrio desulfuricans* ND132 strains were engineered for tandem affinity purification of HgcA and HgcB with 3xFLAG/TEV/StrepII tags in order to overcome limitations resulting from low abundance of the native proteins in cells. ND132 strains expressing tagged HgcA or HgcB retain mercury methylation activity at >50% of the wild-type strain. Preliminary results from tandem affinity purification and immunoblotting indicate that native HgcB can be purified to homogeneity pending verification by mass spectrometry. Ongoing work aims to develop a protocol to isolate native HgcA at sufficient yields for spectroscopic characterization. Covalent crosslinking will be used to reveal protein-protein interactions, which will in combination with structural bioinformatics help delineate the roles of HgcA and HgcB in the context of microbial carbon metabolism. A comprehensive understanding of the various geochemical and biochemical factors culminating in the production of MeHg will facilitate the development of effective strategies to limit production and bioaccumulation of methylmercury in the environment.