Poster #22-7

A Multi-Pronged Approach to Identifying the Biochemical Function of Hg Methylation Proteins in *Desulfovibrio desulfuricans* ND132

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BER Program: SBR
Project: ORNL Critical Interfaces Scientific Focus Area
Project Website: http://www.esd.ornl.gov/programs/rsfa/

Methylmercury (MeHg), an organic mercury (Hg) compound, is a potent toxin that bioaccumulates in food sources and is primarily produced by microorganisms in anaerobic organic-rich soils and sediments. A persistent challenge in studying environmental MeHg production is understanding how and why microbes produce the toxin. To date, little is understood about the physiological function of MeHg production by anaerobic microorganisms. It is postulated that the native function of the Hg methylation proteins (HgcAB) is not Hg methylation, but rather the methylation of an unknown metabolite. Mercury methylation has been linked to C1 carbon metabolism for acetyl-CoA and methionine biosynthesis, sometimes as part of the Wood- Ljundahl pathway; yet a specific biochemical pathway remains elusive. We are taking a systems biology approach to explore the physiological function of Hg methylation using *Desulfovibrio desulfuricans* ND132 as a model organism. For this study, we compared growth and metabolite profiles of various *D. desulfuricans* ND132 gene deletion strains related to carbon and Hg cycling with those of the wild-type strain. Mutant strains (e.g., Δ*hgcAB*, Δ*metH*, Δ*cobT*, Δ*hgcA*:T101A) that exhibited differences in Hg methylation capability compared with wild-types (e.g., 0–246%) were grown in defined media with various substrates (e.g., pyruvate, fumarate, lactate, sulfate, formate, acetate). Organic acid, anion, and metabolite concentrations were monitored throughout the growth of the cells to determine if changes in central metabolism coordinated to changes in MeHg generation between wild-type and mutant strains. Indeed, significant differences in substrate consumption, acetate production, and transcription of C1 metabolism genes were observed between the strains under fermentative and sulfate-reducing conditions. To further elucidate the native contribution of HgcAB to cellular metabolism, *D. desulfuricans* ND132 wild-type and Δ*hgcAB* cultures were grown together with various substrates to measure the epistatic fitness effects of deleting *hgcAB* under relevant environmental conditions. Identifying the conditions under which *hgcAB* provides a fitness benefit will aid in identifying other functions for this enzyme.