Investigating the Dynamics of *hgcAB* and the Effect of Syntrophic Interactions on Hg-methylation

Caitlin Gionfriddo,[1] Regina Wilpiszeski,[1] Ally Soren,[3] Mircea Podar,[1] Josh Michener,[1] Brandy Barber,[1] Ann Wymore,[1] Grace Schwartz,[2] Scott Brooks,[2] Cynthia Gilmour,[3] Dwayne Elias[1]

[1] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830
[2] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830
[3] Smithsonian Environmental Research Center, Edgewater, MD 21037

Contact: (gionfriddocm@ornl.gov)

Project Lead Principle Investigator (PI): Eric M. Pierce

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Project Abstract: In the East Fork Poplar Creek (EFPC), anaerobic bacteria containing the *hgcAB* gene cluster, transform mercury (Hg) to monomethylmercury (MMHg). To improve our understanding of the MMHg generation potential, we are (1) determining the native function of *hgcAB* using genomics, biochemical pathway elucidation and fitness studies, (2) characterizing the EFPC microbial community, and (3) designing model microbial communities.

Identifying the native function of *hgcAB* is essential to predicting Hg methylation potential across a range of environmental systems. The possibilities include one-carbon metabolism for acetyl-CoA and methionine biosynthesis, metal resistance, or metalloid methylation. Here we explored *hgcA* expression patterns and regulation under different growth parameters using *Desulfovibrio desulfuricans* ND132. We utilized RT-qPCR and RNA-seq to observe changes in *hgcA* expression in conditions requiring postulated biochemical HgcAB functions (e.g. +/- formate, methionine, arsenate, mercury). Our results indicate that *hgcA* expression is significantly regulated across the growth stages under some conditions tested and may be regulated by *ars*R, typically known for regulating arsenate reduction and methylation genes. Deletion of *hgcAB* revealed differences in the transcriptome, proteome, metabolome, and metal speciation in growth media between wild-type and Δ*hgcAB* cultures as well as significant down-regulation of flagella and cilia, which has been confirmed by transmission electron microscopy. Significant differences in substrate consumption, acetate and biomass production, and expression of C1 metabolism proteins were observed between the strains under fermentative and sulfate- reducing conditions. The presence and abundance of Hg-methylators are often poor indicators for environmental MeHg concentrations. Therefore, understanding the molecular mechanisms of *hgcA* essential to expression and translation is needed to better predict environmental conditions that drive Hg-methylation potential in stream systems.

EFPC sediment and periphyton biofilm genomic analyses are the basis for designing model community experiments. Methylating and non-methylating species are being combined under the same geochemical conditions to determine whether the effect on Hg-methylation might be additive, subtractive or exponentially increased by complex multi-species interactions. We are actively monitoring for physiological changes (e.g., cell count, Hg-methylation rates, HgcAB expression levels) between experiment performed with single-species