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Molecular, Genomic and Physiological Studies of Mercury Methylation

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The Microbiology team within the ORNL CI-SFA has made significant strides in understanding the Hg-methylation genes *hgcAB* from an ecological as well as from a biochemical standpoint. We have developed universal probes to better understand the diversity of organisms and environments harboring these genes along with clade-specific probes for quantification. The probes were tested against >30 pure cultures including 13 *Deltaproteobacteria*, nine *Firmicutes*, and nine methanogenic *Archaea* genomes. A distinct PCR product was confirmed for all *hgcAB* strains tested via Sanger sequencing. The clade-specific qPCR primers amplified *hgcA* and were highly specific for each clade. Recent improvements to the Firmicute qPCR protocol have allowed for a lower sensitivity, and we are currently testing reduced degeneracy (96 to 32-fold) in the universal probes to maintain diversity. To further validate these probes, we compared them to 16S rRNA sequences and both *hgcAB* and 16S rRNA sequences from metagenomes from eight mercury-contaminated sites. In both the metagenome and ORNL probe amplicon sequencing, *Deltaproteobacteria* dominated the Hg- methylator pool, and clade-specific qPCR probes were highly similar to the metagenomes, which showed that methylators and demethylators were abundant at low methylmercury (MeHg). Demethylators (estimated by *merB* abundance) but not sulfate-reducers (estimated by *dsrC* abundance) or methanogens (estimated by *mcrA* abundance) were abundant at high total Hg. These results suggest that high MeHg accumulation inhibits both methylators and demethylators. While *hgcAB* is predictive for methylating Hg, the abundance and widespread diversity suggests that *hgcAB* may provide a physiological function beyond Hg-methylation. Originally annotated as a carbon monoxide dehydrogenase (CODH) it has high sequence homology to the corrinoid iron-sulfur protein (CFeSp), and both act as methyl group carriers to generate acetyl-CoA. Since chloroform inhibited both CODH activity and Hg-methylation, we hypothesize that *hgcAB* codes for a membrane protein complex to form acetate from CO_2 for biosynthesis. We assayed organic acid metabolite and amino acid production from *D. desulfuricans* ND132 wild-type and associated mutants (Δ*hgc*AB, Δ*hgc*AB*::hgc*AB). All cultures were batch grown with pyruvate and fumarate. No differences in basic physiology (e.g., growth rate, cell yield, CO_2 or succinate production) were observed. Acetate production was ~2X higher in the wild type, supporting a role for the *hgc*AB gene product in the C1 metabolic cycle of *D. desulfuricans* ND132. The results of these studies will allow for more accurate identification and quantification of the Hg-methylators and will be essential in developing accurate and robust predictive models of Hg methylation potential.