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 \textit{D. desulfuricans} ND132 wild-type and associated mutants (\( \Delta hgcAB \), \( \Delta hgcAB::hgcAB \)). 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 \( hgcAB \) gene product in the C1 metabolic cycle of \textit{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.