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Application of *hgcAB* Biomarker Detection and Characterization in the Environment

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Methylmercury (MeHg) is a common toxic contaminant but the relationship between the bacteria that produce MeHg and its concentration in the environment is poorly understood. The genes *hgcAB* are essential for microbial mercury (Hg) methylation. We recently developed universal qualitative *hgcAB* as well as quantitative clade-specific *hgcA* PCR probes for the three dominant Hg-methylating clades: *Deltaproteobacteria*, *Firmicutes*, and methanogenic *Archaea*. Here we apply these tools to environmental samples. To link *hgcAB* abundance and diversity with MeHg production, we compared *hgcA* qPCR abundance estimates as well as PCR estimates of diversity to metagenomic shotgun and 16S rRNA amplicon sequencing.

As a preliminary study to ensure the broad applicability, we examined samples from eight Hg-contaminated sites ranging in total Hg (HgT; 0.03-14 mg Hg/kg soil) and MeHg (0.05-27 µg Hg/kg soil) concentrations.

Metagenome and amplicon sequencing revealed that *hgcAB* diversity was dominated by *Deltaproteobacteria* in all eight sites while *Firmicutes* and methanogenic *Archaea* were ~50% less abundant. Results are currently being validated by *hgcA* qPCR. 16S rRNA sequencing did not identify *hgcAB* bacteria well. Overall, PCR-based *hgcAB* methods for Hg-methylator diversity are tractable for studying the relationship between Hg-methylators and soil Hg concentrations at a much reduced cost as compared to metagenomics.

We applied our *hgcAB* and *hgcA* biomarker tools to geochemically variable samples to ensure broad applicability. First, using anaerobic estuarine sediment slurry incubations from salt-marsh soils amended with isotopically labeled complexes of inorganic Hg, we confirmed the presence of *hgcAB*⁺ bacteria, with *hgcA*+ *Deltaproteobacteria* being predominant. Second, using soil cores from the Spruce-Peatland Response Under Climate and Environmental Change (SPRUCE) experiment, we confirmed the presence of *hgcAB*⁺ bacteria in all samples. Sequencing of *hgcAB* amplicons is currently underway to ascertain the Hg-methylator diversity. Lastly, we are currently using our PCR and qPCR *hgcA(B)* biomarker tools to analyze ~50 filters obtained from the oxygen-minimum zone of the Atlantic Ocean during a longitudinal cruise study from Iceland to Brazil. During these studies we have found that optimization for DNA extraction is usually required and is dependent upon the organic matter component of each environmental sample.