

Development of Molecular Tools for the Accurate Assessment of Mercury Methylation Potentials in any Environment

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Mercury methylation produces the neurotoxic, highly bioaccumulative methylmercury (MeHg) and requires two proteins (HgcA and HgcB). Gene detection and quantitation to determine at risk environments is critical. Universal degenerate PCR primers spanning *hgcAB* were developed to capture the organismal gene diversity in all Hg-methylators from the *Deltaproteobacteria*, *Firmicutes* and methanogenic *Archaea*. Hg-methylator quantitation was addressed by developing degenerate qPCR primers for *hgcA* for each Hg-methylating clade. To validate primer specificity, pure cultures (including methylators and non-methylators: 12 *Deltaproteobacteria*, 7 *Firmicutes*, and 6 methanogenic *Archaea*) were assayed. A single agarose gel band was observed for the majority of these organisms with known *hgcA*, validating this approach. Additionally, each clade-specific qPCR primer set only amplified *hgcA* within its clade. To mimic an environmental sample, gDNA from all clades is being combined in different ratios to assess qPCR primer specificity.

In the ecological context, we sought to combine *hgcA* abundance and diversity with MeHg generation rates and site geochemistry. We performed 16S rRNA and metagenomic sequencing on eight Hg-contaminated sites ranging from tidal marshes to Arctic permafrost. Custom algorithms were used to filter *hgcAB* from the metagenomes. For all sites, geochemical parameters (Fe, SO₄, S²⁻) as well as Hg(II) and MeHg were quantified. In the metagenomes, the *Deltaproteobacteria* dominated the *hgcAB* pool from all sites while *Firmicutes* and *Archaea* were each 50% less abundant. In parallel, *hgcAB* clone libraries were constructed for each site. The clone library approach allowed us to verify the identity of *hgcAB*+ organisms by mapping sequences back to the 16S data. Comparison of the qPCR with the metagenomes determined the accuracy of the qPCR primers. Development of degenerate qRT-PCR *hgcA* primers is underway. The development and validation of these highly specific and quantitative molecular tools will allow for the rapid and accurate risk management assessment in any environment.

Globally, we queried *hgcAB* diversity and distribution in all available microbial metagenomes. The genes were found in nearly all anaerobic, but not in aerobic, environments including the open ocean. Critically, *hgcAB* was absent in ~1500 human microbiomes, suggesting a low risk of endogenous MeHg production. New potential methylation habitats included invertebrate guts, thawing permafrost, coastal “dead zones”, soils, sediments, and extreme environments, suggesting multiple routes for MeHg entry into food webs. Several new taxonomic groups potentially capable of Hg-methylation emerged, including lineages having no cultured representatives. Here we are generating a new global view of Hg-methylation potential.