

Advancing accessible methods for Hg-methylating gene abundance and diversity in the environment

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The genes *hgcAB* are essential for microbial mercury (Hg) methylation but the relationship between the bacteria that produce methylmercury (MeHg) and factors determining its generation in the environment are poorly understood. Detection and estimation of their abundance, in conjunction with Hg concentration, bioavailability, and biogeochemistry, are critical in determining potential hot spots of MeHg generation. We recently developed universal qualitative PCR probes for *hgcAB* as well as the quantitative probes that select for *hgcA*⁺ organisms from the *Deltaproteobacteria*, *Firmicutes*, and methanogenic *Archaea*. Recent improvements have been made to increase primer sensitivity as well as overall workflow efficiency. These include alterations in protocols and the use of less degenerate primer combinations while maintaining broad diversity capture of *hgcAB*-positive organisms and allowing direct sequencing of *hgcAB* amplicons.

To further assess this approach, sediments from diverse locations (total Hg 0.03-14 mg Hg/kg soil; MeHg 0.05-27 µg Hg/kg soil) were used to compare *hgcA* abundance and *hgcAB* diversity to 16S rRNA sequencing directly from the samples and after clone library constructions, as well as to metagenomic shotgun sequencing. Metagenomics gives a comprehensive assessment since every gene in a sample is sequenced and gene counts yield gene abundance. 16S pyrosequencing did not identify *hgcAB* microorganisms well while *hgcA* qPCR abundance estimates agreed well with metagenomic estimates as did *hgcAB* diversity estimates.

Therefore, *hgcA(B)* PCR-based methods provide a reliant means to study relationships between Hg-methylators and geochemical factors. This cost-effective and simpler approach allows for more widespread use among laboratories and could be performed on-site or at mobile laboratories to provide rapid and accurate estimates of Hg-methylator abundance, thereby quickly informing risk assessment and management as well as for remediation strategies.