Metagenomic and Metatranscriptomic Analysis of Organohalide-Respiring Microbial Communities in PCB-Contaminated Sediment Microcosms

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Background/Objectives. Organohalide-respiring bacteria (OHRB; e.g., *Dehalococcoides mccartyi*) are well-known transformers of halogenated organics such as chlorinated ethenes (CEs) in groundwater environments. OHRB are also known to dehalogenate polychlorinated biphenyls (PCBs) sediments. However, our knowledge of CE-dechlorinating OHRBs is more advanced than for PCB-dechlorinating OHRBs. For example, the few reductive dehalogenase genes (*rdhA*) that have been implicated in PCB dehalogenation (e.g., *pcbA1*, *pcbA4*, and *pcbA5*) encode bifunctional tetrachloroethene (PCE)/PCB dehalogenases. It is possible that more diverse *rdhA* participate in PCB dehalogenation. OHRB also rely on microbial community interactions to sustain their growth in subsurface environments. However, it is not clear how OHRB-supporting microbial communities respond to different PCB contamination levels. The objectives of this work were to develop anaerobic PCB dehalogenating microcosms using sediment from a PCB-contaminated site, and to perform metagenomic and metatranscriptomics analyses to gain insights into the OHRB present and functional in these microcosms and interactions between OHRB and supporting microbial community members.

Approach/Activities. After 200 days of incubation, DNA and RNA were extracted from sediment microcosms with high PCB concentrations (HPCBM; 28.04 ± 2.89 µg/mL) that were showing evidence of OHRB growth and concomitant PCB dehalogenation, and from sediment microcosms with lower PCB concentrations (LPCBM; 4.28 ± 1.05 µg/mL) where OHRB growth and PCB dehalogenation were not evident. DNA and RNA were subjected to high-throughput shotgun sequencing to obtain metagenomes and metatranscriptomes, respectively. Separate assembly of HPCBM and LPCBM metagenomes yielded 927 metagenome-assembled genomes (MAGs) with 143 MAGs determined to be high quality, including two MAGs classified as *Dehalococcoides mccartyi* (Dhc). Both metagenome and metatranscriptome reads were mapped to the MAGs using Kallisto. Differential expression analysis was used to identify significant differences between HPCBM and LPCBM metatranscriptomes.

Results/Lessons Learned. Phylogenetic analysis of reductive dehalogenase gene (*rdhA*) sequences within the Dhc MAGs revealed that previously identified bifunctional PCE/PCB dehalogenase genes *pcbA1* and *pcbA4/5*-like *rdhA,* were absent, while several candidate PCB dehalogenase genes and potentially novel *rdhA* sequences were present. Of the 26 *rdhA* identified in Dhc MAGs, only two had been previously sequenced from PCB contaminated sites, and only one, apparently novel, *rdhA* was significantly expressed in HPCBM. This expressed *rdhA* was 49.6% identical to a *rdhA* from *D. mccartyi* in a dioxin-dechlorinating enrichment culture. These results suggest that PCB dehalogenase gene diversity extends beyond the currently known bifunctional PCE/PCB dehalogenase genes. The differential expression analysis also revealed significant differential expression in HPCBM of *cbiA*, the gene which encodes the final enzymatic step in prokaryotic corrinoid ring production. Transcripts of *cbiA* were from the phyla Euryarchaeota, Chloroflexi, Firmicutes, and Proteobacteria. More research is needed to identify novel *rdhA* targets that could be useful for bioremediation and natural attenuation strategies involving PCBs. Using genome-resolved metagenomics and metatranscriptomics analyses of PCB-contaminated sediment microcosms helped to identify the novel *rdhA* and overcame limitations of specific primer-based PCR analysis that relies on already identified *rdhAs*. provide new and useful insights into the relevant OHRB involved and the workings of microbial communities that support bioremediation of PCBs.