Identification of Genetic Markers for Anaerobic Dichloromethane Metabolism

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Background/Objectives. Dichloromethane (DCM) is an ozone-depleting agent and pervasive groundwater contaminant used as an industrial solvent but also occurs naturally, with substantial emissions from marine systems, wetlands, volcanic activity and biomass combustion. To date, anaerobic catabolism of DCM has been reported in three bacterial strains obtained at distant geographical locations and from distinct environments, indicating that the natural capacity for anaerobic DCM catabolism is widespread. DCM is metabolized via the Wood-Ljungdahl pathway; however, the genetic basis for the initial dechlorination reactions(s), which is likely to offer specific biomarkers for DCM catabolic potential and/or activity, has yet to be identified.

Approach/Activities. Newly available genomes of the three anaerobic DCM degraders, *Dehalobacterium formicoaceticum* (Defo), '*Ca.* Dichloromethanomonas elyunquensis' (Diel), and Peptococcaceae bacterium DCMF were compared using a BLAST-P-based reciprocal best hit approach in an effort to identify shared functional genes that might be responsible for DCM catabolism. Shotgun proteogenomics was applied to Diel and Defo cultures to confirm the expression of the identified DCM-related catabolic gene cassettes. Over 18,000 metagenomes, representing a full range of environment types, were scanned for the presence and abundance of the identified gene cassette. Qualitative and quantitative PCR assays were developed and applied to samples from a DCM-contaminated site and samples representing environments whose metagenomes contained homologs of the DCM catabolic marker genes.

Results/Lessons Learned. Comparative genomics revealed that each of the three described DCM-degrading organisms contains a highly homologous *mec* cluster comprising eight to 10 genes. Each gene is highly similar across the anaerobic DCM degraders (>90% amino acid (AA) identity) while having very low similarity to any gene in any other sequenced microbial genome (<35% AA identity), with few exceptions. Functional annotation of this gene cassette revealed a two-component regulatory system, a corrinoid methyl-carrier protein, four methyltransferases, a corrinoid protein regeneration system, and a cation exchange membrane protein. Shotgun proteomics analyses performed with two DCM degraders revealed that several of the proteins encoded on this gene cluster were highly expressed during growth with DCM. A scan of all metagenomes available in the JGI IMG system revealed the presence of homologous gene clusters in a diverse set of environments including peat bogs, the deep subsurface, and several low oxygen marine systems such as the Eastern Pacific Oxygen Minimum Zone (OMZ). In addition, qPCR assays were developed which allowed for sensitive detection and enumeration of the core genes across a DCM gradient in a contaminated groundwater plume and the oxygen gradient in the Eastern Pacific OMZ. The results presented here offer novel insights into the potential mechanism and underlying conserved genetic basis for anaerobic DCM dechlorination which may offer specific tools for assessment and bioremediation monitoring at sites impacted with DCM.