Gene Markers for Monitoring Anaerobic Dichloromethane Biodegradation: Current Progress and Future Directions

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Background/Objectives: Dichloromethane (DCM) is a simple volatile halogenated solvent that is widely used in industrial applications and, correspondingly, is a widespread groundwater contaminant. Biomarkers for investigating potential or monitoring bioremediation of DCM in groundwater have remained unavailable due to the cryptic nature of the underlying biochemistry. Additionally, reported DCM biodegrading organisms are not closely related to one another, indicating that use of taxonomy as an indicator is not promising. Recently, the genetic basis for a dehalogenating DCM methyltransferase system was described and shown to be present and expressed by multiple anaerobic DCM degrading bacteria and in natural peat ecosystems. These genes offer a genetic foothold into anaerobic DCM degradation which may allow for monitoring in situ bioremediation of DCM at contaminated sites.

Approach/Activities: Two *mec* cassette genes, which encode putative DCM dehalogenating methyltransferases MecE and MecF, are sufficiently conserved and distinct from other non-DCM-related genes to allow for PCR primer design. qPCR primers for *mecE* and *mecF* were designed to capture the diversity of genome- and peat-ecosystem-derived gene alleles. These primers were applied to samples taken from a DCM-contaminated groundwater plume to calculate absolute *mecE* and *mecF* gene copy number and to calculate the proportion of gene copy numbers relative to absolute bacterial counts. DCM concentrations in the groundwater samples were measured directly to explore correlations between DCM concentration and DCM-methyltransferase gene copy numbers. Active expression of these genes was evaluated by examination of metatranscriptomics data.

Results/Lessons Learned: Copy numbers of *mecE* and *mecF* (encoding components of the putative DCM-methyltransferase) showed positive correlations with DCM concentration, reaching above 1e8 gene copies per mL in samples with two or more g/L DCM. At the highest DCM concentrations, up to 10% of bacteria harbored these genes, whereas at the plume periphery this number dropped down to one in a million. Examination of metatranscriptomes showed expression of almost entirely intact *mec* gene cassettes expressed at high levels (up to 50 TPM). These correlations between *mec* gene copy number, expression, and DCM concentration suggest that this might prove a simple and effective strategy for monitoring active DCM biodegradation or predicting capacity for DCM biodegradation. For transition to field applications, further development of this qPCR primer system should continue in two directions: 1) further in situ testing at contaminated sites to develop further insights into quantitative relationships between gene copy numbers, expression, and DCM dynamics, and 2) continual refinement of the *mec* gene primers based on newly emerging close homologs in public sequence databases.