A Novel Biomarker for Monitoring Anaerobic In Situ Degradation of Benzene

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Background/Objectives. Biomarkers are powerful tools that can be used to garner evidence of in situ biodegradation of environmental contaminants. In the simplest terms, biomarkers are any molecules (e.g., DNA, RNA, proteins, metabolites, etc.) whose abundance changes in response to a biological or biochemical process. However, it can be very challenging to identify biomarker(s) that are diagnostic and broadly applicable to a target bioremediation process. Anaerobic benzene biodegradation is a poorly understood process thought to be catalyzed by more than one biochemical reaction (carboxylation, hydroxylation, and methylation have been proposed in literature). Many of the genes, enzymes and microorganisms involved have yet to be identified and thus biomarker availability is limited. Previously, our team has identified 16S rRNA gene biomarkers for two clades of anaerobic benzene-degrading bacteria (e.g., *Thermincola* and Desulfobacterota ORM2). A putative benzene carboxylation gene (*abcA*) has also been described in literature, which can reliably detect anaerobic benzene degradation potential under nitrate-reducing conditions. In this study, we present a novel biomarker that appears to be broadly diagnostic of benzene degradation under methanogenic and sulfate-reducing conditions.

Approach/Activities. The Edwards laboratory has maintained a methanogenic benzene-degrading enrichment culture (OR) for over 25 years, enriched from oil-contaminated sediments in Oklahoma. A large-scale bioaugmentation lineage of OR known as DGG-B™ is available commercially by SiREM. All cultures are grown in minimal medium amended with benzene. Between 2016 and 2018, metagenomic sequencing approaches were used to successfully reconstruct the complete (closed) genome sequence of the cultures' chief benzene degrader, Desulfobacterota ORM2. From this we created a proteomics database that was used to identify and quantify >300 ORM2 proteins synthesized during methanogenic benzene degradation. Comparative analyses of every anaerobic benzene-degrading enrichment culture described in literature (for which proteomics data was available) enabled us to pinpoint putative anaerobic benzene degradation genes. Primers for a quantitative PCR (qPCR) assay were designed and validated for a one functional gene of interest, an Fe-S oxidoreductase.

Results/Lessons Learned. High abundance proteins encoded by an uncharacterized ORM2 gene cluster were repeatedly detected in proteomic surveys of OR and DGG-B™ cultures. Our only clue into this gene cluster's function was a gene product that shared 46% amino acid identity to an enzyme (BamQ) involved in a downstream anaerobic degradation pathway for aromatic compounds. Comparative proteomic analyses revealed that homologous proteins (65–98% identity) encoded by syntenic gene clusters were produced in high abundance by other anaerobic benzene-degrading cultures, specifically ones enriched under methanogenic and sulfate-reducing conditions. Enzyme assays are now underway to confirm the biochemical function of this gene cluster. Our newly developed qPCR biomarker assay was able to accurately detect anaerobic benzene degradation potential in contaminated sediments and groundwater that was missed by other qPCR-based biomarker assays. This is exciting news for anyone interested in assessing the anaerobic benzene bioremediation potential of a

contaminated site. Moreover, this discovery puts us closer than ever at uncovering the elusive mechanism(s) of anaerobic benzene activation.