Use of Metagenomic and Metatranscriptomic Approaches to Elucidate Microbial Processes In Situ

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Background/Objectives. Molecular biology tools (e.g., quantitative PCR [qPCR] assays) targeting biomarker genes are commonly used to probe microbial communities for bioremediation applications. However, assay development is typically based initially on sequences from pure cultures. A growing body of work has demonstrated that laboratory cultures often may not include the microbial phylotypes that dominate in the field, and the microbes driving subsurface microbial processes may differ genetically in targeted genes compared to those microbes used for assay development. This fact leaves room for assay inaccuracy as it has been shown that a small number of mismatches between molecular assay primers and targeted genes can lead to under-predicting targeted gene quantities by multiple orders of magnitude. Currently, metaomic approaches (i.e., metagenomics and metatranscriptomics) provide an unprecedented opportunity to develop strategies for biomarker identification and qPCR assay development directly from field-relevant mixed microbial communities. These approaches hold the promise of expanding the menu of available assays for field site applications and increasing the accuracy of existing assays. In this work, we sought to identify biomarker genes and develop new qPCR assays directly from a field-derived mixed microbial community using multiple metaomic approaches.

Approach/Activities. To develop and compare meta-omics strategies for biomarker discovery and assay development, we used a methanogenic *o*-xylene-degrading enrichment culture as a model system. The current biomarker for anaerobic o-xylene degradation, benzylsuccinate synthase (*bssA*), was based on the known degradation pathway of an analogous compound, toluene. However, recent evidence indicates that although fumarate-adding enzymes are also involved in xylene degradation, the enzymes are distinct from typical benzylsuccinate synthases. Therefore, primers targeting *bssA* are not likely applicable to *o*-xylene degrading systems. To develop assays relevant to *o*-xylene degradation, we applied metagenomics and comparative metatranscriptomics. DNA and RNA were extracted from the culture during *o*-xylene degradation. RNA also was obtained after 3 days of feeding on glucose as the comparator. The bacterial community structure based on16S rRNA genes was determined, and a metagenome and two metatranscriptomes were bioinformatically processed.

Results/Lessons Learned. Although a clostridial-like *bssA* was present, the most abundant gene for a fumarate-adding enzyme was most similar to an *nmsA*, which encodes naphthyl-2-methylsuccinate synthase. RT-qPCR assays based on the metagenomic sequences obtained revealed that the *nmsA*-like gene was highly upregulated under o-xylene degrading conditions. With toluene, genes for other fumarate-adding enzymes were upregulated in addition to the *nmsA*-like gene. *In silico* protein structure modeling was also conducted to explore substrate binding in the predicted active site of the new fumarate-adding enzyme. Metagenomic sequencing allowed discovery of a new enzyme likely responsible for *o*-xylene degradation, which was previously undetectable by all published assay, thus highlighting the utility of non-targeted meta-omic approaches. However, the identification of additional new biomarkers was limited by gene annotation. Thus, comparative metatranscriptomics was conducted. Bioinformatic analysis revealed a suite of differentially expressed genes including several genes related to the beta-oxidation of benzylsuccinate, bbsBDE. Differential expression analysis of these additional putative biomarkers is on-going. Assays developed by this approach are expected to have a positive impact by supporting more accurate site characterization to resolve

microbially-mediated processses and the development of more accurate conceptual site models.