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Metaomics-Enabled Approaches for Identifying Biomarkers Directly from Mixed Microbial Communities

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Background/Objectives. Molecular biology tools targeting functional biomarker genes are increasingly used in bioremediation applications to interrogate sites, guide decision-making regarding implementation of biostimulation or bioaugmentation, and for site monitoring. These tools have historically been designed based on sequences from isolated species that can be cultured in the lab. However, most microbes in the environment are not amenable to isolation. Compounding this problem, the vast diversity of microbes present at field sites makes isolation of all relevant community members intractable and perhaps impossible, limiting available assays. Furthermore, a largely overlooked issue is that the accuracy of qPCR assays is often limited because of primer-template mismatches, which occur due to variations between the sequences of biomarker genes in laboratory model organisms and the sequences of the same genes found in the microorganisms that are active in the field. Metaomics tools, including metagenomics and metatranscriptomics, use next-generation sequencing to bypass the need to isolate species, allowing the direct analysis of field-relevant mixed microbial communities. Metagenomics and metatranscriptomics offer an unprecedented opportunity to rapidly obtain sequences of functional genes involved in bioremediation, guide development of next-generation molecular biology tools, and support precision bioremediation of traditional and emerging contaminants.

Approach/Activities. We are developing multiple approaches for identifying biomarkers directly from mixed communities via metaomics and bioinformatics using a field-derived mixed microbial community that degrades *o*-xylene under methanogenic conditions as a model system. Previous work has focused on the isolation and characterization of microbes capable of *o*-xylene degradation, but it is unclear if available assays accurately quantify *o*-xylene-degrading bacteria in the field. We analyzed the metagenome of this community to evaluate and improve *o*-xylene degradation biomarker assays (i.e., quantitative PCR assays). Additionally, we are comparing community metatranscriptomes under degrading and non-degrading conditions to identify new biomarkers.

Results/Lessons Learned. The metagenome of the methanogenic system contains abundant sequences encoding enzymes similar to benzene succinate synthase (*bssA*), which hypothetically catalyzes the first step in *o*-xylene degradation. However, the diversity of these *bssA*-like genes indicates that currently available assays would miss the most abundant members, giving an incomplete understanding of the system. Comparative metatranscriptomics approaches are expected to confirm the relevance of individual *bssA* sequences, as well as identify a suite of new putative biomarkers. New (RT)-qPCR assays have been developed for site monitoring. Meta-omics approaches are suitable for other recalcitrant and emerging contaminants and facilitate the rapid development of field-relevant molecular biology tools with improved accuracy over previous methods.