Molecular Biological Tools: Where Will the Journey Take Us?

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Background/Objectives. Advances in understanding of the microbiology involved in the degradation and detoxification of groundwater contaminants enabled the successful implementation of bioremediation treatment at many sites. A crucial component of bioremediation involves monitoring of the microbiology and its activity related to contaminant detoxification. Initially limited to cultivation-based approaches, the advent of nucleic acid-based approaches to detect specific target sequences in environmental samples transformed our ability to monitor in situ microbial processes of interest. Microarray technology expanded this approach and enabled the simultaneous detection and semi-quantitative assessment of many genes to inform about the metabolic potential. A milestone was the development of quantitative real-time polymerase chain reaction (qPCR) technology, which allowed the sensitive detection and quantitative assessment of genes of interest in DNA extracted from environmental samples. qPCR technology has reached a high level of maturity and emerged as the tool of choice for the quantitative monitoring of genes (and transcripts) in environmental matrices. Congruent with technological advances, a number of process- and organism-specific biomarkers have been identified, allowing effective monitoring of the microbiology involved in contaminant detoxification. Additional tools including metagenomics, metatranscriptomics, metabolomics, and proteomics can now be applied to environmental samples (i.e., groundwater), and their combined application promises unprecedented information about the microbiology contributing to environmental cleanup. This talk will discuss contemporary and novel molecular biological tools (MBTs), and highlight opportunities for transitioning bioremediation from the current empirical practice to a scientific approach with predictable outcomes.

Approach/Activities. Reductionist approaches, such as cultivation and isolation, remain relevant to discover organisms, genes, and enzymes involved in contaminant degradation. As an example, recent efforts discovered *Dehalogenimonas etheniformans*, a bacterium that reductively dechlorinates vinyl chloride (VC) to benign ethene. *Dehalogenimonas* 16S rRNA genes were detected in 849 of 1,173 groundwater wells, and outnumbered *Dehalococcoides mccartyi* 16S rRNA genes in 65% of the samples surveyed. In addition to qPCR enumerating biomarker genes and transcripts, targeted proteomics provides quantitative information about protein biomarkers. Metagenomics generates information about the all genes present at a given site, which allows site-tailored qPCR assays and proteomics approaches to specifically target site-relevant biomarkers. As a novel approach, metabolomics is tested on groundwater samples to determine if small molecule (i.e., metabolite) analysis will reveal patterns that correlate with the activity of keystone dechlorinators.

Results/Lessons Learned. The increasing number of biomarker genes demands high-throughput qPCR, and new instrumentation can assay tens to hundreds of target genes (or transcripts) simultaneously in a parallel format. Targeted proteomics enumerates biomarker proteins, and it appears feasible to establish correlations between biomarker gene, transcript, and protein abundances with observed reductive dechlorination rates. The integrated application of a suite of MBTs will promote the transition from brute-force bioremediation approaches to refined treatment (i.e., precision bioremediation) with similar (or better) performance at substantially lower costs and lesser environmental impacts.