Integrated Nucleic Acid System for In-Field Monitoring of Microbial Community Dynamics and Metabolic Activity

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Molecular analysis of subsurface microbial communities requires some combination of sample collection, concentration, cell lysis, nucleic acid purification, polymerase chain reaction (PCR) amplification, and specific detection in order to address fundamental questions of microbial community dynamics, activity, and function in the environment. As a result of our prior NABIR grant, we developed a suite of integrated microparticle chemistries, Fe- and SO_4-reducer phylogenetic probes and suspension arrays, and combined sample purification/bead array fluidic devices for the automated and direct analysis of 16S rRNA and microbial community dynamics in environmental samples. However, changes in microbial community composition and/or abundance are still insufficient to detect or make conclusions regarding specific microbial activity. Thus, fieldable methods for the direct analysis of functional genes and messenger RNA (mRNA) in the environment are still required. The objective of this project is therefore twofold. First, we seek to verify the 16S rRNA methods, Fe- and SO_4-reducer array and instrumentation on sediment and groundwater samples taken from the Schiebe/Roden FRC site, and continue to expand the probe suite for microbial community dynamics as new sequences are obtained from DOE-relevant sites. Second, we propose to address the fundamental molecular biology and analytical chemistry associated with the direct analysis of functional genes and mRNA (hence, microbial activity) in environmental samples. These studies will investigate the behavior of oligonucleotide and cDNA capture and detection probes for direct mRNA purification and detection on microparticle surfaces; "tunable surface" chemistries to increase mRNA capture and detection efficiency; new mRNA reporter and detection chemistries required for the development of in-field monitoring methods and devices; and fluidic strategies for integrating complex biochemistry for direct detection of mRNA in sediments and groundwater, without using PCR.

Recognizing that not all field sites contain enough RNA for direct hybridization and detection, and as a prelude to cDNA versus mRNA detection, efforts in the first two quarters of the project have focused on investigating the behavior of DNA (e.g., PCR amplicon) versus RNA hybridization and applying both DNA and RNA methods and model array to DOE sites. The testable hypothesis is that hybridization specificity on the 85-bead array will differ between rRNA and equivalent PCR amplicons. At the spring meeting, we will present comparative DNA and RNA hybridization data for a validation set of Fe- and SO_4-reducing microorganisms, preliminary hybridization data for a time course microcosm study initiated from the Schiebe/Roden FRC field site, and tunable bead array rRNA profiles from Phil Long's UMTRA field site before and after biostimulation. The team's latest advances (and challenges) in autonomous, single-particle RNA purification, fragmentation, and labeling chemistry will likewise be presented, with a discussion of the implications for developing fully autonomous mRNA analysis systems.