Development and Use of Integrated Microarray-based Genomic Technologies for Assessing Microbial Community Composition and Dynamics

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To effectively monitor microbial populations involved in various important processes, a 50-mer-based oligonucleotide microarray was developed based on known genes and pathways involved in biodegradation, metal resistance and reduction, denitrification, nitrification, nitrogen fixation, methane oxidation, methanogenesis, carbon polymer decomposition, and sulfate reduction. This array contains approximately 2,000 unique and group-specific probes with <85% similarity to their nontarget sequences. Based on artificial probes, our results showed that at hybridization conditions of 50°C and 50% formamide, the 50-mer microarray hybridization can differentiate sequences having <88% similarity. Specificity tests with representative pure cultures indicated that the designed probes on the arrays appeared to be specific to their corresponding target genes. Detection limits were about 5–10 ng genomic DNA in the absence of background DNA, and 50–100 ng (~1.3×10^7 cells) in the presence of background DNA. Strong linear relationships between signal intensity and target DNA and RNA concentration were observed ($r^2 = 0.95$–0.99). Application of this microarray to naphthalene-amended enrichments and soil microcosms demonstrated that composition of the microflora varied depending on incubation conditions. While the naphthalene-degrading genes from *Rhodococcus*-type microorganisms were dominant in enrichments, the genes involved in naphthalene degradation from Gram-negative microorganisms such as *Ralstonia*, *Comamonas,* and *Burkholderia* were most abundant in the soil microcosms (as well as those for polyaromatic hydrocarbon and nitrotoluene degradation). Although naphthalene degradation is widely known and studied in *Pseudomonas*, *Pseudomonas* genes were not detected in either system. Real-time PCR analysis of four representative genes was consistent with microarray-based quantification ($r^2 = 0.95$). Currently, we are also applying this microarray to the study of several different microbial communities and processes at the NABIR-FRC in Oak Ridge, Tennessee. One project involves the monitoring of the development and dynamics of the microbial community of a fluidized bed reactor (FBR) used for reducing nitrate and the other project monitors' microbial community responses to stimulation of uranium-reducing populations via ethanol donor additions in situ and in a model system. Additionally, we are in the process of designing a phylogenetic array based on over 5,000 different 16S rRNA genes from microorganisms similar to those in FRC groundwater, and developing novel strategies for increasing microarray hybridization sensitivity. Finally, great improvements to our methods of probe design were made by the development of a new computer program, CommOligo. CommOligo designs unique and group-specific oligo probes for whole genomes, metagenomes, and groups of environmental sequences, and uses a new global alignment algorithm to design single or multiple probes for each gene or group. We are now using this program to design a more comprehensive functional gene array for environmental studies. Overall, our results indicate that the 50-mer-based microarray technology has potential as a specific and quantitative tool to reveal the composition of microbial communities and their dynamics important to processes within contaminated environments.

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