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Microarrays Based on Oligonucleotide and Genome for Microbial Community Studies

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The microarray is a powerful genomic technology that is widely used to study biological processes. Although microarray technology has been used successfully to analyze global gene expression in pure cultures, adapting microarray hybridization for use in environmental studies presents great challenges in terms of specificity, sensitivity, and quantitation (Zhou and Thompson 2002). Although microarray-based genomic technology has attracted tremendous interests among microbial ecologists, it has only recently been extended to study microbial communities in the environment (Wu et al. 2001).

Based on the types of probes arrayed, microarrays used in environmental studies can be divided into three major classes: functional gene microarrays (FGA), Genome-Probing microarrays (GPM), and phylogenetic oligonucleotide microarrays (POAs). FGAs contain probes corresponding genes encoding key enzymes involved in various ecological and environmental processes, such as carbon fixation, nitrification, denitrification, sulfate reduction, and contaminant degradation. Both PCR-amplified DNA fragments (Wu et al. 2001) and oligonucleotides (oligos) derived from functional genes can be used to fabricate FGAs. To avoid confusion, the former is referred to as a PCR product-based FGAs whereas the later is referred to as an oligonucleotide-based FGAs. These types of arrays are useful in studying physiological status and functional activities of microbial communities in natural environments. GPAs are constructed using whole genomic DNA isolated from pure culture microorganisms and can be used to describe a microbial community in terms of its cultivable component.

In the presentation, for monitoring biodegradation potential and activity, we are going to show development of 1) a comprehensive 50-mer oligonucleotide microarrays containing probes (1,657) from all of the known genes (2,402) involved in biodegradation and metal resistance and 2) a genome-probing microarrays containing probes from lactic acid bacterial genomes. These microarrays were evaluated for bioremediation monitoring of contaminated soils and studying microbial community

dynamics during mixed culture fermentation. Our results demonstrated the developed microarrays offer rapid, powerful, new high throughput tool for monitoring potential and functional activity of microbial communities.

Probe specificity. Since much shorter sequences were used as probes, the 50-mer FGAs should have higher resolution than the PCR products-based FGAs. To experimentally determine the resolution power of the hybridization with the 50-mer FGAs, the effects of probe sequence similarity on hybridization signal intensity of 6 target genes from *Pseudomonas putida* Gpo1 and PpG7 were evaluated. The probes were artificially designed to be different by 2% (i.e., 1 bp length). All of the artificial probes have the same length, and mismatch was incorporated in random positions and the numbers of mismatches were increased from 1 to 12 corresponding to 2–24% differences to target sequences. Little hybridization was observed for probes showing 76–88% identity to the target DNA for all 6 genes, whereas the signal intensity increased substantially for probes showing more than 96% similarity to the target DNA. The SNRs varied from 0 to 0.5 for all of the genes when the probe sequence similarities were $< 88\%$, which was much smaller than the generally accepted threshold value of SNR = 3.0. Specificity evaluation with genomic DNA from pure cultures. Detection specificity of the microarray was tested using genomic DNA of four reference strains. Probes corresponding to all known genes reported in the reference strains had strong hybridization signals to their corresponding genomic DNA.

Detection Sensitivity. Determination of detection sensitivity of the 50-mer FGA-based hybridization. The detection sensitivity of the 50-mer FGA-based hybridization was determined using genomic DNA extracted from a pure culture of *T. aromatica* K172. Genomic DNA (1–1000 ng) from *T. aromatica* K172 was randomly labeled with Cy5. In environmental samples, the microorganisms of interest are present together with other diverse microorganisms. The existence of other non-target DNAs may affect the hybridization with target DNA and hence decrease detection sensitivity. To evaluate the detection sensitivity in the presence of heterogeneous non-target DNA, genomic DNA (10–1000 ng) from *T. aromatica* K172 were mixed with 1 μg of *S. oneidensis* MR-1 DNA, respectively, and randomly labeled with Cy5. At a hybridization temperature of 50°C in the presence of 50% formamide, the strongest hybridization signals were observed with 50 ng of *T. aromatica* K172 DNA for the target gene, 2-oxoglutarate ferredoxin-oxidoreductase beta subunit (gi19571178). Hybridization signals using 25 ng of genomic DNA, however, were barely detectable.

Detection of naphthalene-degrading genes in soil microcosms. To determine whether the devel oped microarrays could be used to detect microorganisms within the context of environmental applications, two microcosms were established with soil samples from TFD: one with naphthale ne vapor, and the other without naphthalene vapor as a control. The hybridization signals of 40 genes were significantly different (P = 0.05). Among them, 22 genes were highly different with more than 3 fold difference. Among them, *Ralstonia* sp. strain U2-type microorganisms containing

the plasmid might be one of major constituents for degrading naphthalene in the microcosm. However, in contrast to the enrichment experiment, the plasmid-encoded genes in *Rhodococcus* sp. NCIMB12038 were not detected with the microarray, thus indicating that the enrichment proces s could cause severe bias of the population compositions.

Various contaminated soils were analyzed using this microarray. Clustering analysis of hybridization revealed 5 main groups. Many genes related to naphthalene degradation were clust ered together and they were abundant in naphthalene-amended soil.

GPM for monitoring community dynamics during fermentation. A genome-probing microarray was constructed using 150 genomes of lactic acid bacteria. This GPM was used for community dynamics of Kimchi fermentation and gut microflora. Evaluation and application of this format of microarray will be comparatively presented with the oligonucleotide-based functional gene microarray.

References

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