



GEOCHIP DEVELOPMENT AND APPLICATION

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Introduction. GeoChip 4, a new functional gene array, was developed to examine microbial communities. GeoChip 4 contains approximately 82,000 probes covering 141,995 coding sequences from 410 functional gene families related to microbial carbon (C), nitrogen (N), sulfur (S), and phosphorus (P) cycling, energy metabolism, antibiotic resistance, metal resistance/reduction, organic remediation, and bacteriophage. In addition, to assess pathogenic potential in diverse habitats, key virulence genes related to major virulence factors, such as adherence, colonization, motility, invasion, toxin, immune evasion, and iron uptake are included and probes for 46 functional genes involved in microbial responses to changes of temperature, osmolarity, oxidative status, and nutrient limitation as well as general stress responses were included to examine the response of microbial communities to environmental stresses.

The objective of this work was to produce an updated GeoChip to analyze the functional diversity, composition, structure, metabolic potential/activity, and dynamics of microbial communities.

Methods. Computational evaluation was used to determine probe specificity. GeoChip 4 was then applied to study several microbial communities, including a long-term warming site in Central Oklahoma, USA to examine the effects of long-term warming, community samples from soil, oil plume, and saliva to assess the occurrence of virulence genes in natural environments, and oil plume samples to assess the stress response of microbial communities to environmental disturbance.

Results. Experimental evaluation showed that less than 0.002% false positive rate was observed for both synthesized oligonucleotide and genomic DNA targets under 42°C. As little as 500 ng community DNA was sufficient for reliable and reproducible hybridizations. A strong correlation was observed between signal intensities and DNA concentrations.

Statistical analyses showed that microbial communities harboring virulence genes were responsive to environmental perturbations, which drove changes in abundance and distribution of virulence genes.

More ($p < 0.05$) stress response functional genes were detected in oil plume samples than non-plume samples. Functional structures correlated with environmental variables, suggesting that the indigenous microbial community experienced multiple stresses due to the oil plume, likely stemming from nutrient limitation caused by rapid growth of hydrocarbon-degrading *Oceanospirillales* populations.

Conclusions. These results indicate that GeoChip 4 is highly specific, sensitive and quantitative. In addition, , and the results indicated several significant mechanisms that microbial communities respond to long-term warming. To the best of our knowledge, GeoChip 4 is the most comprehensive functional gene array for microbial ecology studies.