Coupled Use of DNA Microarrays, Voltammetry and X-Ray Studies for Profiling Changes in Microbial Community Structure and Metal Speciation in Response to Metal Contamination

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The objectives of this project are to develop and apply DNA array technology to profile and monitor microbial communities through time in process-level microcosms and naturally contaminated sediments treated with increasing Cr, Pb and/or Zn concentrations, and correlate microbial community structure to changes in metal speciation and mobility. The central hypotheses relate to: (1) the sensitivity and specificity of tunable surface microarrays relative to standard hybridization chemistry; (2) the biochemical process associated with metal speciation in artificial mesocosms and natural environments; and (3) changes in microbial community composition in response to increasing metal contamination.

Natural sediments (ca. 10^9 cells g^{-1}) were obtained from three sites in Lake DePue, Ill., which contains substantial zinc, copper, cadmium, lead and arsenic. Three sites were characterized and used to establish microcosms, ranging from 50 ppm to 30% Zn. Methanogenic archaea and sulfate-reducing bacteria were quantified by MPN enrichments, and methanogens, iron-reducers and heterotrophic anaerobes isolated using acetate as an electron donor. Microbial biomass was significantly higher at the more contaminated site. Isolates are currently being evaluated for growth and Zn metabolism at different Zn concentrations, with the intent to compare biologically-governed Zn speciation in monoculture to Zn speciation observed in Lake DePue sediments.

Mesocosms designed to select for metal-reducing bacteria or methanogens were established and monitored for pH, acetate, metal concentrations (Zn and Mn) and headspace gasses (carbon dioxide, methane and hydrogen), with weekly samples taken for DAPI counts and DNA analysis. Differences in acetate consumption and methane generation were observed between sediment microcosms. X-ray absorption spectroscopy of bioreactor solids was used to investigate changes in Zn and Mn speciation due to Zn and Mn amendments. Total nucleic acids are currently being extracted from mesocosm sediments for analysis by T-RLFP to compare changes in microbial community structure with Zn addition. Predominant, novel or unexpected T-RFLP signatures will be cloned and sequenced to identify species-specific probes for a ‘metal reducer’ microarray.

Microarray research during year one focused on specific technical challenges associated with the *direct* detection (i.e., no PCR amplification) of full-length rRNA targets relative to PCR-amplified, functional gene targets. Surface chemistry and probe attachment methods, target labeling and detection strategies, and novel solution conditions were evaluated to achieve specific and reproducible hybridization of *Geobacter chapelleii* 16S rRNA to universal and species-specific 16S rRNA probes. The specific detection and allelic discrimination of PCR-amplified, functional gene targets from an E. coli model system was very successful, regardless of probe attachment chemistry, labeling or detection strategy. However, the specific, reproducible hybridization of full-length 16S rRNA or rDNA targets to a 2-dimensional (versus 3-dimensional membrane or gel-pad) microarray remains a significant technical challenge. Sandwich hybridization and chaperone systems have been developed and compared to direct chemical labeling reporter systems. Short rRNA fragments (ca. 300 bp) hybridized with greater signal intensity than full-length (1500 bp) products, although both size fragments generate anemic signals relative to similar functional gene target assays. We postulate that the high degree of secondary structure, large target fragments, steric constraints at a two-dimensional surface and limited range of ionic strengths amenable to DNA:DNA hybridizations may limit the efficacy of DNA probes in a 16S rRNA microarray. To address these possible limitations of DNA probes for community-level 16S rRNA analysis, we are evaluating peptide nucleic acid probes under low-salt, high temperature buffer conditions to overcome steric constraints associated with large, 16S rRNA targets.