Ecological Interactions Between Metals and Microbes Impacting Bioremediation

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The project objectives are to determine whether spatial heterogeneity in metal distribution is a major determinant of microbial community activity and diversity in contaminated soils; the effects of increased metal bioavailability and mobility upon microbial community activity and diversity; the role of metal-resistant bacteria in microbial communities that contain many metal-sensitive members (these microbes might function as either "bioprotectants" through their physiological activity, or reservoirs of transferable resistance genes); and the interactions among multiple toxicants upon microbial activity in mixed-waste sites. Distinct microbial communities were found in contaminated soils that varied in their concentrations of Pb, Cr, and aromatic compounds. Contamination with metals and hydrocarbons is correlated in these soils. So it is difficult to distinguish between their effects on community structure and activity. Microcosms were constructed in which either Pb^{+2} or CrO_4^{-2} was added at levels that produced acute modest or severe effects (50 or 90% reduction) on community activity. Either glucose or xylene was added as a C source, as substrates broadly used by microbes or restricted in their catabolism. Activity was monitored via carbon dioxide evolution, and total biomass changes by analyses of phospholipid phosphate. Heavy metal additions had more severe effects on xylene catabolism than on glucose. Temporal changes in bacterial community composition were determined by DGGE analysis of 16S rDNA genes. Addition of an organic energy source selected for a relatively small number of phylotypes. The addition of Pb or Cr(VI) modulated community response to the added organic substrate. Some phylotypes selected in the absence of metal additions were still selected at modest or severe metal stress, but there were also instances where different phylotypes were selected. Nucleotide sequencing of these phylotypes is in progress.

Xylene was used in these studies, as published reports maintain aromatic degradation was severely inhibited in metal-contaminated soils and metal-resistant aromatic degrading could not be cultured from these habitats. Real-time PCR was used to quantify copy numbers of aromatic dioxygenase genes in the microcosms. There were 10-fold increases in biphenyl dioxygenase and phenol hydroxylase genes in microcosms receiving xylene. However, 4 other dioxygenase genes were not detectable. Bacteria were isolated from microcosms that received xylene and Cr(VI). Initial attempts to isolate bacteria on media with both xylene and Cr(VI) were unsuccessful. Isolates were then obtained on complex media with Cr(VI)—all were *Arthrobacter* sp. unable to use xylene. When bacteria were selected on xylene as sole C source, 3 phylogenetic groups were isolated. Many of the *Arthrobacter* sp. selected in this way were resistant to high concentrations (>20 mM) of Cr(VI), but only if they were pregrown at 1 mM Cr(VI). The correlation between microbial activity, community structure, and metal level was analyzed on 150 mg of soil collected at spatial scales of <1, 5, 15, and 50 cm. There was no correlation between metal content and activity level. Soils <1 cm apart could differ in activity 10-fold and extractable Pb and Cr 7-fold. DGGE analyses indicated substantial differences at the <1 cm scale. The nucleotide sequence of phylotypes in samples with high metal concentrations is being investigated. The characteristics of bacteria isolated from these habitats were also investigated. *Arthrobacter* VN23-1 is Pb-resistant. Pb resistance can be transferred to a Pb-sensitive *Arthrobacter* strain by conjugation, although at a relatively low frequency. Pb resistance appears to be inducible, and the genetic trait is associated with Cd but not Zn resistance. A cloned gene has homology to P type ATPases and is related (but not identical) to a Pb-resistance determinant from the gram-negative bacterium *Ralstonia metallidurans*.