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Ecological Interactions Between Metals and Microbes That Impact Bioremediation

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RESULTS TO DATE: Bacterial Community Diversity at a Mixed Waste Contaminated Site The correlation between bacterial population structure and lead, chromium and organic compounds present along a 21.6 m transect was examined. There was a gradient of heavy metal (Cr and Pb) and petroleum hydrocarbon contamination in these soils. A 16S rDNA analysis method and fatty acid methyl esters derived from phospholipids (PLFA) analysis were used to compare microbial communities. Soil microbial DNA was extracted and community fingerprint patterns for each sample location were produced by DGGE separation of the V3 region of the 16S rRNA genes amplified by PCR. Visual analysis of DGGE patterns indicated that sample locations with high concentrations of total toluene (12,000 mg kg-1), xylenes (8,000 mg kg-1), methylene chloride (10,000 mg kg-1), lead (17,000 mg kg-1) and chromium (3,200 mg kg-1) have a different community composition from the community with lower metals (200 mg kg-1) and organics (1200 mg kg-1) content. Microbial biomass, indicated by total phospholipid-P, was greatest in soils with highest organic contamination. Cluster analysis of the similarity coefficients of common bands between samples also showed that the samples formed two groups. A similar delineation of communities was observed when the PLFA patterns were compared by PCA (principal component analysis). There were some differences in fingerprint patterns within these two main groups but they did not correspond to any of the soil factors analyzed (e.g., TOC, metals, pH or texture). There were two common bands from a total of 30 dominant DGGE bands in all the samples from across the transect. Initial nucleotide sequence analysis of the 16S rRNA gene indicated that these bands are most closely related to a Pseudomonas sp. and an uncultured hydrocarbon seep bacterium. Other genera identified by 16S rDNA analysis are Enterococcus, Actinomyces, Bacillus, Rhizobium and a couple with no close match. In summary, these analyses indicated there was a correlation between microbial community structure and high organic contamination and not any of the other factors. Microcosm experiments were conducted to test this theory under more controlled conditions. Using soils from this site, microcosms were set-up with either high, low, or no chromate and naphthalene (representative organic contaminant) additions. Changes in the microbial community structure were the same, based on DGGE analysis, in microcosms with either high Cr, low Cr or high Cr + naphthalene additions. The community structure in microcosms with low Cr + naphthalene, naphthalene alone or no addition (control) were the same but differed from the other group.

Chromium resistance of bacteria isolated from contaminated soil. To improve our understanding of the ecology of heavy metal resistance in bacteria, chromium resistant strains were isolated from contaminated soils. Soil was collected from an old tannery site contaminated with Cr in Cannelton, MI. The minimum inhibitory concentrations of metals for growth were used to determine metal tolerance of bacteria isolated from these soils. Chromium resistant bacteria could grow on concentrations as high as 50 mM CrO4-2. Many of these bacteria were also resistant to Pb, Cu, and Ni, but not Cd.

One of these strains, Arthrobacter Cr15, was studied in greater detail. Metal sensitive mutants of this strain were obtained by growth in the absence of Cr. The Cr resistance genes were determined to be on a mobile element by transferring the trait from the resistant to sensitive strain using conjugation experiments. Molecular genetic analysis revealed the presence of plasmids in both strains, however there was a deletion in the plasmid of the sensitive strain. Nucleotide sequence analysis of the deleted region showed presence of chromium resistance genes with amino acid similarity to ChrA and ChrB. Altogether the evidence suggests that horizontal gene exchange can contribute to the dissemination of this trait. The development of a metal-resistant population in a contaminated soil can result from: (i) vertical gene transfer (reproduction), (ii) horizontal gene transfer (including transposons and broad host range plasmids), and (iii) selection pressures on spontaneous mutants (due to the presence of metals). Transposable elements carrying mercury resistance genes have been linked to the distribution of this trait in nature. The presence of Cr resistance genes on a putative transposon suggests that horizontal gene

transfer may be an important factor for the development of metal resistant populations in contaminated sites. Future research will include hybridization experiments to determine the distribution of the same Cr resistant genes in other isolates from the same site and other Cr contaminated sites. ChrA and ChrB proteins in Gram negative microbes appear to function as efflux pumps in order to confer Cr resistance. Transport experiments in Arthrobacter Cr15 and its Cr-sensitive derivative did not show any difference in Cr accumulation between them, nor were there differences in Cr accumulation between the strains when assayed chemically. The Cr-resistance mechanism in this organism does not appear to require substantial energy expenditure, because growth yields were similar in cultures grown with or without Cr. In addition, it does not involve the reduction of Cr(VI) to Cr(III). The Cr resistance mechanism in Cr15 is capable of providing protection for Cr-sensitive microbes in a community. When Cr-sensitive microbes were inoculated into media in which Arthrobacter Cr15 had been pre-cultured, they were able to grow at higher Cr concentrations. Similar phenomena were noted for several other Cr-resistant microbes isolated from these soils.

Microbial activities in metal impacted soils. Two studies of microbial activity in response to organic nutrient inputs were conducted in soils impacted by Pb, Cr, or a combination of both metals.

The inhibitory effects of heavy metals upon microbial populations and the factors limiting microbial activity and growth were determined in long-term, highly chromium (Cr)- and lead (Pb)-contaminated soils. Total Cr and Pb concentrations were 260,000 and 10,000 mg kg-1 soil in Cr- and Pb-contaminated soils, respectively. 3H-leucine incorporation into macromolecules was measured in soil bacterial extracts to evaluate microbial responses to CrO42- or Pb2+. IC50 (heavy-metal concentration giving 50% reduction of microbial 3H-leucine incorporation compared to the control) was independent of soil contamination types, and was 4 mM and 0.02 mM for CrO42- and Pb2+, respectively. Stimulation of microbial activity and biomass by organic carbon was measured over 56 d. The adverse effects of Cr and Pb in soil systems were characterized in terms of the ratio of microbial biomass C to soil organic C, basal respiration per unit microbial biomass (qCO2), and the ratio of substrate-responsive respiration to microbial biomass C (substrate-responsive qCO2). Ratios of microbial biomass C to soil organic C were small with 0.42% in Cr-contaminated soil and 0.36% in Pb-contaminated soil. Values of qCO2 and substrate-responsive qCO2 were significantly higher in Pb- than in Cr-contaminated soils ($p < 0.01$). The results from the soil systems also indicate that Cr and Pb decreased microbial activities and led to the accumulation of soil organic C, and that Pb posed greater stress to soil microbes than Cr. However, microbial biomass and activity increased considerably after the addition of organic C (alfalfa and glucose), which implies that bioavailable organic C may still be the primary factor limiting microbial biomass and activity even in highly heavy-metal contaminated soils.

The impact of chromium (Cr) and lead (Pb) on the soil microbial community was examined in contaminated soils. The site had great heterogeneity with total Cr concentrations in six soil samples ranging from 64 to 1949 mg kg-1 soil while total Pb concentrations ranging from 235 to 9198 mg kg-1 soil. Microbial biomass and community structure was estimated from the analysis of phospholipids. The patterns of phospholipid fatty acids (PLFA) were considerably different among the six soil samples. Redundancy analysis suggested that the variation in PLFA was more dependent on the soil organic C than on the levels of Cr and Pb. The sensitivity of members of the microbial community to metal was determined by extracting bacteria from soil and measuring 3H-leucine incorporation as a function of metal concentration. All of the bacteria from six soil samples tested had IC50 values (heavy metal concentrations giving 50% reduction of microbial activity) of approximately 2.5 mM for CrO42- and 0.01 mM for Pb2+. In microcosm experiments, microbial biomass and the ratio of microbial biomass to soil organic C were not well correlated with the concentrations of heavy metals. Instead, specific rates of basal and substrate-responsive respiration differed significantly among the soil samples ($p < 0.05$). Soils with higher contamination of organic pollutants or heavy metals had higher specific microbial activities. Our results suggest that analysis of PLFA and measurement of metabolic activities are sensitive indicators in assessing the impact of heavy metals on soil microorganisms.

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