Ecological Interactions between Metals and Microbes that Impact Bioremediation

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Objectives

A. Determine the distribution of phylotypes and metal-resistance genes at the scale of spatial heterogeneity observed in microbial community activity.
B. Determine the effects of environmental factors on community responses to Cr(VI) contamination. These effects may be mediated by physiological responses, species selection, or gene transfer.
C. Determine the role of mobile genetic elements that confer Cr resistance. Microbes with these elements might function as either "bioprotectants" through their physiological activity, or reservoirs of transferable resistance genes.
D. Identify the novel physiological and genetic bases for bacterial resistance to Cr(VI) at concentrations of 15 to >50 mM.

Results

Chromium(VI) can be detoxified in soil if it is reduced to Cr(III), however, the Cr(VI)-reduction potential in anaerobic zones of terrestrial soils has not been extensively explored. Anaerobic microcosms were created with soil having (1) an organic energy source (glucose or protein); (2) a terminal electron acceptor [Fe (III) or NO_3^-]; and (3) no, low, medium, or high levels of Cr(VI) (concentrations that produced reductions of acute microbial activity of 0, 33, 50, or 75%, respectively). Microbial activity monitored by the evolution of CO_2 was stimulated by the addition of glucose in the microcosms without an apparent lag. However, higher doses of Cr(VI) had an inhibitory effect on the rate of CO_2 production. In addition, Cr(VI) was not detected in any microcosm 48 hours after setup, suggesting that detoxification of Cr(VI) is needed before the added carbon source can be used. The addition of protein to microcosms caused a different response by the community. The CO_2 concentration increased in two phases—an initial phase of slow CO_2 accumulation and a second phase of faster accumulation. The length of the initial slow phase increased with increasing concentration of added Cr(VI) and Cr(VI) was not detected when the fast phase commenced. Changes in bacterial community composition were monitored using PCR-DGGE of the 16S rDNA gene. In glucose-amended microcosms, the number of intense bands in DGGE profiles increased (1) with time across all treatments and (2) in treatments in which Cr(VI) was added. Although communities in microcosms receiving Cr differed from those in which no Cr was added, there was no effect by the amount of Cr added. In contrast, there were no significant differences in the bacterial community profiles in protein-amended microcosms with and without Cr additions. To confirm there was a Cr(VI)-tolerant population present, we isolated a total of 101 and 70 Cr^R strains, respectively, from glucose and protein microcosms amended with Fe(III) and medium Cr levels. Nearly all of the isolates were also able to grow at 0.5 mM Cr(VI), and a substantial number were able to grow in up to 10 mM Cr(VI). Real-time PCR was used to follow the *Geobacteraceae* populations in some of the Fe(III)0amended microcosms. In microcosms that received a carbon source but no Cr(VI), copy numbers of the *Geobacteraceae* 16S rRNA gene increased during maximum CO_2 production and then decreased back down to initial levels. In contrast, in microcosms amended with high concentrations of Cr, the *Geobacteraceae* 16S rRNA gene copy number remained constant throughout the entire experiment, suggesting that the iron-reducing bacteria in these soils were adversely affected by Cr(VI) addition. Our results show that Cr(VI) can be detoxified anaerobically in soils when appropriate organic substrates and electronic acceptors are provided.