Enhancing PCB Biodegradation in Soil with Chlorobenzoate-Degrading Inoculants

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1. Introduction

The high costs and hazards associated with disposal of PCB-contaminated soil have spurred interest in developing alternative technologies, such as bioremediation, that might be used for *in situ* or on-site treatment. Previous investigators demonstrated that amending soil with biphenyl stimulated PCB biodegradation by indigenous PCB-cometabolizing bacteria [1,2]. In these studies, PCB mineralization was considered to be controlled by the rate of PCB cometabolism to intermediate metabolites (i.e., chlorobenzoates). Commensal organisms were believed to be the key mediators of chlorobenzoate (CBA) mineralization. But, because CBA-degraders typically do not metabolize PCBs, they were thought to have no direct impact on the degradation of PCBs *per se*.

Since the tests described above were done, three developments have warranted a re-examination of the role of CBA-degraders in PCB degradation. First, the potential for CBAs to serve as precursors of chlorocatechols that inhibit PCB metabolism has been established [3,4]. Second, bacteria that degrade di- and tri-CBAs, which are likely to be primary PCB degradation products, have been isolated [5,6]. Thus, the effect of inoculation of these organisms that are appear to be uncommon in soils, on PCB degradation had not been evaluated. Third, chlorobiphenyl-mineralizing strains had been constructed in which the metabolic activities of the PCB cometabolizers and CBA-degraders were consolidated [7]; the performance of these constructed strains in soil systems, however, was unknown. In this study we inoculated soil microcosms with cultures of PCB cometabolizers, CBA-degraders, and chlorobiphenyl-mineralizing strains to determine whether or not improving the CBA-degradation potential of the microbial population might enhance PCB biodegradation.

2. Methods

Microcosm preparation. An Altamont silt loam (41 g organic matter kg^{-1}) was spiked with unlabelled Aroclor 1242 (60 mg kg^{-1}) and [^{14}C]Aroclor 1242 (196 kBq kg^{-1}). The soil was also amended with biphenyl and ammonium nitrate at rates of 4,000 and 300 mg kg^{-1}, respectively. Subsamples (100 gm) of soil were then transferred to 250 mL Erlenmeyer flasks and inoculated with a cell suspension (Table 1) or an equal volume of sterile buffer. The flasks were then sealed, and affixed to a system that continuously flushed the headspaces through a solution of NaOH.

Microcosm analysis. Every two days the alkali solution was replaced and the levels of $^{14}CO_2$ trapped determined by liquid scintillation counting. Soil samples were also taken periodically for enumerating inoculant populations by serial dilution plating. At the end of the 60 day incubation, PCBs were extracted from the soils with a hexane-acetone mixture. These extracts were analyzed by liquid scintillation counting and by capillary gas chromatography (GC) with electron capture detection. Non-extracted ^{14}C (i.e., sorbed to soil or assimilated into biomass) was determined by counting samples of extracted soil suspended in scintillation cocktail.

3. Results

The highest PCB mineralization levels were measured in soils inoculated with the CBA-degraders strain JB2 (26%) or strain P111 (23%) alone (Fig. 1). The apparent effectiveness of strains JB2 or P111 in enhancing PCB mineralization was diminished when these were co-inoculated with the PCB cometabolizer strain PB133 (Fig. 1). Intermediate PCB mineralization levels occurred in soils inoculated with strain PB133 (11%) or the chlorobiphenyl-mineralizing strains UCR1 (9%) or UCR2 (15%). Analysis of ^{14}C in the extracts also showed that PCB degradation was most extensive in soil inoculated with strains JB2 and P111 alone (Fig. 1). However, when CBA-degraders were co-inoculated with strain PB133, the amount of ^{14}C recovered in the extracts increased significantly. Analysis of PCBs in the extracts by GC showed similar trends.

Populations of strain JB2 or P111 increased from 10^8 to 10^{11} colony-forming units (CFU) kg^{-1} soil during the first 22 days, and then decreased slightly during the balance of the study. During the same period, slightly smaller populations of strains JB2 or P111 were enumerated in soil receiving the dual inocula compared to soil inoculated with strain JB2 or P111 alone. In the non-inoculated soil CBA-degraders were not detected ($<10^6$ CFU kg^{-1}) during the study. Biphenyl degrader populations in the non-inoculated soil increased from 10^8 CFU kg^{-1} on day 0 to 10^{10} CFU kg^{-1} on day 22. Populations of the biphenyl-degrading inoculants were somewhat higher and averaged about 10^{11} CFU kg^{-1}. The exceptions were the co-inoculated treatments in which numbers of biphenyl degraders were equal to or less than that determined in the non-inoculated soil. Additional tests showed the frequency of recovering biphenyl-degrading, CBA-utilizers from soil inoculated with strain JB2 increased over time.

4. Discussion

Results from this study indicated that chlorobenzoate metabolism in soils was a limiting factor in the mineralization of Aroclor 1242. The results also indicated that augmenting the CBA-degrader population enhanced not only mineralization, but also upstream PCB transformation activities. In this respect, the greatest enhancement in mineralization levels were measured in soils inoculated with CBA-degraders alone, organisms that did not metabolize PCBs.

The mechanism(s) by which inoculation with CBA-degraders enhanced PCB mineralization are as yet undetermined. This result was consistent with the hypothesized lack of broad-spectrum CBA degradation capabilities in the indigenous population. Thus, strains JB2 and P111 may have simply degraded CBAs or other metabolites that

accumulated in the absence of an active indigenous population. An additional possibility is that inoculation with CBA-degraders may have facilitated more extensive PCB degradation. Supporting the latter hypothesis was the fact that significantly lower amounts of ^{14}C and PCBs were recovered in extracts from soil inoculated with CBA-degraders. If the there was no stimulation of PCB degradation, PCB recoveries might have been expected to be similar in all treatments. Possible mechanisms by which CBA-degrader inoculants may have enhanced PCB degradation include removal of CBAs serving as inhibitor precursors, and dissemination of CBA degradation genes to the indigenous biphenyl degraders resulting in recombinant strains with improved PCB degrading abilities.

Since the dual inocula augmented both the PCB-cometabolizing and CBA-degrading capacity of the indigenous population, these may have been expected to be most effective in enhancing mineralization levels. However, the consistently reduced levels of PCB mineralization in soils treated with dual inocula compared to that obtained with inoculants of CBA-degraders alone demonstrated that the former were not optimal for stimulating PCB mineralization. The decreased efficiency of the dual inocula appeared to be the result of negative interactions between the organisms in the inoculant, possibly increased competition for growth substrate. In this case as neither strain P111 or JB2 could metabolize biphenyl the limiting substrate was most likely benzoate produced from the degradation of biphenyl by strain PB133. However, if growth of either strain JB2 or P111 were adversely affected in the dual-inocula treatments, it was not readily apparent from the plate count data.

The decreased mineralization levels achieved with dual inocula relative to CBA-degraders alone illustrated the problems of inoculating multiple organisms into soil, and thus the potential advantages of constructed organisms for delivering both PCB cometabolizing and CBA degrading activity. In this study, mineralization levels obtained with the constructed, chlorobiphenyl-mineralizing organisms (strains UCR1 and UCR2) were at best 2% greater than that achieved by strain PB133 alone, and less than that achieved by strain JB2 or P111 alone. The results could be attributed to the lack of broad PCB- or CBA-degradation spectrum by strains UCR1 and UCR2.

5. Conclusions

These studies demonstrated that PCB mineralization and transformation could be enhanced in soils by the introduction of organisms that do not directly attack PCBs. Further work is needed, however, to identify the mechanism(s) underlying this effect, and thus develop strategies by which it might be used for bioremediation of PCB-contaminated soil.

6. References

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Table 1. Bacterial cultures used in these studies and their phenotypes

Culture	Phenotype[a]				
	BA	CBA	PCBA	BP	CBP
Pseudomonas sp. strain PB133	+	-	-	+	-
Pseudomonas sp. strain UCR1	+	+	-	+	+
Pseudomonas sp. strain UCR2	+	+	+	+	+
Pseudomonas sp. strain P111	+	+	+	-	-
P. aeruginosa strain JB2	+	+	+	-	-

[a] + indicates utilization of the substrate as a carbon and energy source. For CBP this also implies chloride release from selected congeners.
Abbreviations: BA, benzoate; CBA, chlorobenzoates; PCBA, polychlorinated benzoates; BP, biphenyl; CBP, chlorobiphenyls.

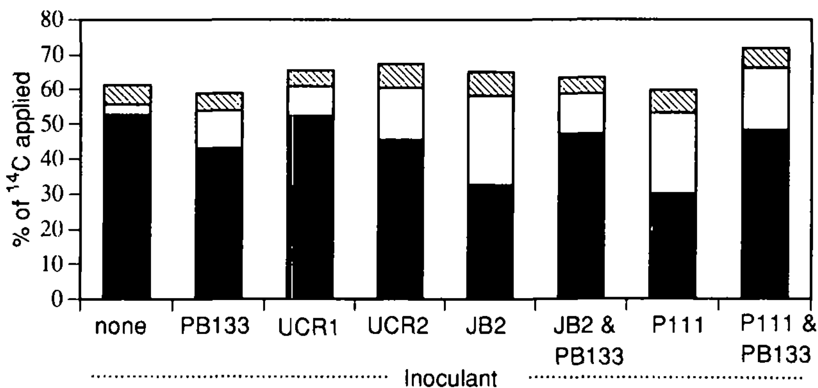

Fig. 1. Radioactivity partitioning in soil inoculated with test cultures after a 60 day incubation. Legend: ^{14}C in hexane-acetone extracts (■), $^{14}CO_2$ (□), and soil-associated ^{14}C following extraction (▧).