Biodegradation **349:** 349–357, 2001.

Soil microbial population dynamics following bioaugmentation with a 3-chlorobenzoate-degrading bacterial culture

Bioaugmentation effects on soil microorganisms

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Accepted 15 September 2001

Key words: bioremediation, *Burkholderia*, *Comamonas testosteroni*, 3-chlorobenzoate

Abstract

Changes in microbial populations were evaluated following inoculation of contaminated soil with a 3-chlorobenzoate degrader. Madera sandy loam was amended with 0, 500, or 1000 μg 3-chlorobenzoate g^{-1} dry soil. Selected microcosms were inoculated with the degrader *Comamonas testosteroni* BR60. Culturable bacterial degraders were enumerated on minimal salts media containing 3-chlorobenzoate. Culturable heterotrophic bacteria were enumerated on R2A. Isolated degraders were grouped by enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction fingerprints and identified based on 16S ribosomal-DNA sequences. Bioaugmentation increased the rate of degradation at both levels of 3-chlorobenzoate. In both the 500 and 1000 μg 3-chlorobenzoate g^{-1} dry soil inoculated microcosms, degraders increased from the initial inoculum and decreased following degradation of 3-CB. Inoculation delayed the development of indigenous 3-chlorobenzoate degrading populations. It is unclear if inoculation altered the composition of indigenous degrader populations. In the uninoculated soil, degraders increased from undetectable levels to 6.6×10^7 colony-forming-units g^{-1} dry soil in the 500 μg 3-chlorobenzoate g^{-1} dry soil microcosms, but none were detected in the 1000 μg 3-chlorobenzoate g^{-1} dry soil microcosms. Degraders isolated from uninoculated soil were identified as one of two distinct *Burkholderia* species. In the uninoculated soil, numbers of culturable heterotrophic bacteria initially decreased following addition of 1000 μg 3-chlorobenzoate g^{-1} dry soil. Inoculation with *C. testosteroni* reduced this negative impact on culturable bacterial numbers. The results indicate that bioaugmentation may not only increase the rate of 3-chlorobenzoate degradation but also reduce the deleterious effects of 3-chlorbenzoate on indigenous soil microbial populations.

Abbreviations: 3-CB – 3-chlorobenzoate; ERIC – enterobacterial repetitive intergenic consensus sequence

Introduction

Contaminant-degrading microorganisms are sometimes added to polluted field sites in an attempt to enhance *in situ* bioremediation via bioaugmentation. Unfortunately, introduced degraders often do not survive in contaminated soil, and thus fail to increase degradation. Several studies have investigated the potential for bioaugmentation with contaminant-degrading microorganisms to enhance the remediation of soil pollutants. Numerous factors including nutrient limitations, predation, pH, and other environmental stresses are known to impact the ultimate success or failure of bioaugmentation as a remediation technology (Alexander 1999). Interaction and competition with indigenous soil microorganisms is believed to be another major determinant of an inoculant's ultimate fate; however, relatively little information is known about the changes in indigenous microbial populations following bioaugmentation.

A laboratory study was designed to determine the dynamics of bacterial populations in contaminated

soil resulting from addition of exogenous contaminant degraders. The compound 3-chlorobenzoate (3-CB) was selected as the model contaminant. Chlorobenzoates are common soil contaminants resulting from microbial cometabolism of polychlorinated biphenyls (Barriault & Sylvestre 1993). Degraders of 3-CB appear to be relatively widespread and diverse even in soils with no known exposure to 3-CB. Fulthorpe et al. (1998) successfully isolated 3-CB degraders from pristine ecosystems on five continents. In addition to being widely distributed, the 3-CB degraders were diverse, consisting of 48 different genotypes that tended to be unique to the site from which they were isolated.

Several 3-CB degraders have been isolated and extensively studied (Don et al. 1985; Ogawa & Miyashita 1995; Krooneman et al. 2000). Specifically, the 3-CB degrader *Alcaligenes* sp. BR60 (later renamed *Comamonas testosteroni* BR60 based on 16S sequence analysis) was isolated by Wyndham et al. (1988) from surface runoff waters near an industrial landfill. *C. testosteroni* BR60 was found to degrade 3-CB via the protocatechuate pathway encoded on a transposon contained on the 85 kb plasmid, pBRC60 (Nakatsu et al. 1997). *Comamonas testosteroni* BR60 has also been shown to degrade various other chlorine and methyl-substituted benzoates.

Laboratory studies have indicated the potential of *C. testosteroni* BR60 for bioaugmentation. Fulthorpe and Wyndham (1992) introduced *C. testosteroni* BR60 into lake water and sediment flow-through microcosms. The microcosms were exposed to various contaminants including 3-CB. The *C. testosteroni* BR60 inoculant initially survived better in 3-CB-contaminated microcosms as compared to uncontaminated microcosms. After 69 d, *C. testosteroni* BR60 was no longer detectable, but high populations of other 3-CB degraders became dominant. The plasmid pBRC60, containing the 3-CB catabolic transposon Tn5271, was transferred from the inoculant to indigenous bacteria, resulting in three phenotypically distinct clusters of 3-CB degraders. Rates of 3-CB uptake and respiration were correlated with numbers of Tn5271-containing bacteria. Likewise, Siciliano and Germida (1998) examined the potential for plant-bacterial associations including *C. testosteroni* BR60 to enhance the degradation of various chlorinated benzoic acids. Inoculation with *C. testosteroni* BR60 resulted in the death of most of the tested plants and failed to increase 3-CB degradation. However, when 3-CB was inoculated in the absence of a plant, *C. testosteroni* BR60 increased 3-CB degradation, reducing 3-CB levels to 74% of the control.

The specific objectives of this experiment were: 1) to assess the effects of *C. testosteroni* BR60 on the removal of 3-CB at two levels of contamination, 2) to determine the impact of *C. testosteroni* BR60 on both the development of indigenous degrader populations and adaptation of heterotrophic bacteria following amendment with 3-CB, and 3) to isolate and characterize indigenous degraders.

Materials and methods

Soil

Soil was collected from the surface horizon of a pristine, forested site in the Madera Canyon Recreational Area of the Cornado National Forest near Tucson, AZ. The soil was a sandy loam with a pH of 6.5 and organic matter content of 3.3%. Field-moist soil was passed through a 2-mm sieve and 100 g dry weight equivalent added to polypropylene jars. Sterile deionized water was added to bring the soil from the collected water content of 5.4% to 14.0% (w/w). Microcosms were incubated for 14 d at room temperature to allow for acclimation of indigenous microorganisms prior to imposing treatments.

Treatments and experimental design

A stock solution was prepared from 3-CB (99%)(Sigma Chemical Co. Inc., St. Louis, MO) similar to the method described by Di Giovanni et al. (1996) for 2,4-dichlorophenoxyacetic acid (2,4-D). Microcosms were amended with 0, 500, or 1000 μg 3-CB g^{-1} dry soil. *Comamonas testosteroni* BR60 was grown to late exponential phase in Medium A (Wyndham 1986) broth containing 500 μg 3-CB l^{-1}. Selected microcosms were inoculated with approximately 10^6 CFU of *C. testosteroni* BR60 g^{-1} dry soil. All treatments were conducted in triplicate. Soil was brought to 25% gravimetric water content (64% water holding capacity) and incubated at 27 °C. Microcosms were sampled at 0, 3, 7, 14, 21, and 28 d. After 16 d, 25 g (dry weight equivalent) of soil was removed from the microcosms that were initially amended with 500 μg 3-CB g^{-1} dry soil, added to new jars, and re-amended with an additional 500 μg 3-CB g^{-1} dry soil.

Quantification of 3-chlorobenzoate biodegradation

The 3-CB was extracted from soil by placing 1.2 g moist soil into 9.5 ml of extracting solution (6 μM Zwittergent detergent and 0.2% sodium hexametaphosphate) (Brendecke et al. 1993) and vortexed at setting 10 on a Vortex Genie^{TM} for 5 min. A 1.0-ml aliquot of the vortexed soil-extraction solution was placed in a 1.2-ml microcentrifuge tube and centrifuged at 16,000 $\times$ g for 10 min. Supernatants were passed through 0.45 μm pore-size polypropylene filters prior to HPLC analysis. The concentration of 3-CB was determined with a Waters Associates LC Module HPLC system and a Waters C18 column (3.9 $\times$ 150 mm) at a wavelength of 235 nm. Elution was isocratic, and the mobile phase was acetonitrile-acidified water (50:50, v/v) with a flow rate of 1 ml min^{-1}. Waters Millennium32 (version 3.05) software was utilized for peak integrations. Phosphoric acid was used to acidify the water to pH 2.6. Several Madera Canyon soil samples that were not contaminated with 3-CB were analyzed to confirm that no natural soil components eluted at the same time as 3-CB.

Enumeration of culturable bacteria

The soil extraction solution from the 3-CB quantification was serially diluted in 0.85% NaCl buffer. Total numbers of culturable 3-CB degraders were determined by spread-plating 0.1 ml of the appropriate dilution onto Medium A supplemented with 500 mg 3-CB l^{-1}. Indigenous 3-CB degraders were selectively enumerated on 3-CB minimal salts (MSM) plates prepared as described by Newby et al. (2000a) for 2,4-D indicator plates. The inoculant, *C. testosteroni* BR60, was capable of growth on Medium A but not the MSM indicator plates, thus allowing for the selective enumeration of indigenous 3-CB degraders. Culturable, heterotrophic bacteria were enumerated on R2A (Difco, Detroit, MI) containing 100 mg cycloheximide l^{-1}. All plate counts were conducted after incubation of plates at 27 °C for 6 d.

Isolation and characterization of dominant indigenous degraders

Approximately 200 degraders were isolated during the study. Bacteria were selected from plates at the highest (most dilute) dilution that produced a countable number of bacterial colonies. Selected bacteria were streaked to purity on the appropriate 3-CB medium. Isolates were then cultured in Medium A broth containing 500 mg 3-CB l^{-1}. A 1.2-ml aliquot of each culture was placed into 1.5-ml microcentrifuge tubes and centrifuged at 5220 $\times$ g for 5 min. Pellets were resuspended in 0.85% NaCl. The harvested cells were stored at -20 °C. Isolated degraders were grouped based on enterobacterial repetitive intergenic consensus (ERIC) PCR of the harvested cells as described by Versalovic et al. (1991). Selected bacteria from each ERIC profile were identified by 16S rDNA PCR using the forward primer 338F (5′-CTCCTACGGGAGGCAGCAG-3′) and reverse primer 784R (5′-GGACTACCAGGGTATCTAATCC-3′) (J. McQuaid, pers. comm.). The 100 μl reaction contained 1X PCR Buffer II (50 mM KCl, 10 mM Tris-HCl [pH 8.3]), 2.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0.2 μM of each primer, 2.5 U AmpliTaq Gold^{TM} polymerase (Perkin Elmer, Foster City, CA), and 5 μl of harvested bacterial cells. The reaction was conducted in a Perkin Elmer 9600 GeneAmp PCR System and consisted of an initial hot-start denaturation step for 10 min at 95 °C followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, and extension for 1 min at 72 °C prior to a final 5 min extension step at 72 °C. Positive PCR products were confirmed using agarose gel electrophoresis and ethidium bromide staining. The PCR products were purified with a QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA). The forward primer was used for dye termination PCR sequencing performed at the University of Arizona's Laboratory of Molecular Systematics and Evolution sequencing facility. Sequence analysis was performed with advanced BLAST 2.0 program (Altshul et al. 1990) found on the National Center for Biotechnology Information's World Wide Web site (http://www.ncbi.nlm.nih.gov). For phylogenetic analysis, unique 16S rDNA sequences were aligned with ClustalW, and an unrooted tree was generated by Jukes-Cantor distance correction and Neighbor-Joining construction using GCG/SeqLab (Wisconsin Package v. 10.1, Genetic Computer Group (GCG), Madison, WI). Unique degraders were also examined for the presence of plasmids using a modified miniscreen for large plasmids (Rodriguez & Tait 1983). Extracts were separated with gel electrophoresis and visualized with ethidium bromide staining.

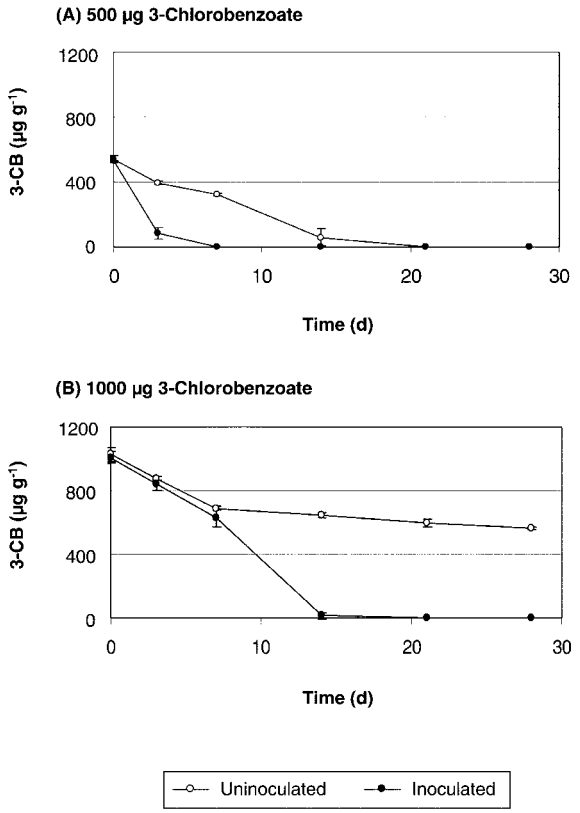

Figure 1. Concentrations of 3-chlorobenzoate (3-CB) in microcosms amended with (a) 500 and (b) 1000 μg 3-CB g^{-1} dry soil that were uninoculated or inoculated with *C. testosteroni* BR60. Error bars represent the standard deviation of three replicate microcosms.

Results

3-Chlorobenzoate biodegradation

Inoculation of the soil with *C. testosteroni* BR60 increased the rate of 3-CB degradation in both the 500 and 1000 μg 3-CB g^{-1} dry soil microcosms (Figure 1). In the 500 μg 3-CB g^{-1} dry soil microcosms, 3-CB was reduced to undetectable levels within 7 d in the *C. testosteroni* BR60 inoculated soil, but persisted until 21 d in the uninoculated soil. The inoculation effect was more pronounced in the 1000 μg 3-CB g^{-1} dry soil microcosms with 3-CB being undetectable within 14 to 21 d while 562.9 $\pm$ 5.7 μg 3-CB g^{-1} dry soil remained in the uninoculated microcosms after 28 d. In contrast, following re-amendment with an additional 500 μg 3-CB g^{-1} dry soil after 16 d of incubation, 3-CB degradation was more rapid in the uninoculated soil than the inoculated soil (Figure 2).

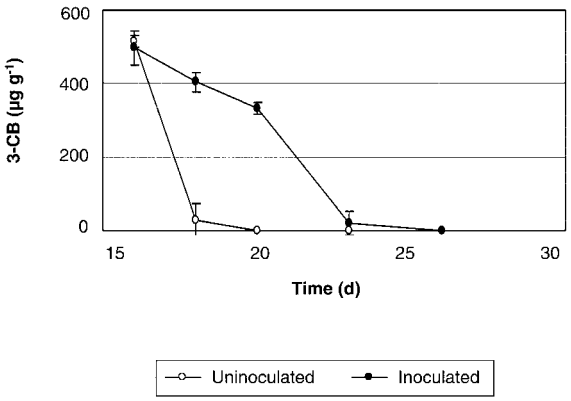

Figure 2. Concentrations of 3-chlorobenzoate (3-CB) in microcosms initially amended with 500 μg 3-CB g^{-1} dry soil and re-amended with 500 μg 3-CB g^{-1} dry soil after 16 d. Microcosms were either uninoculated or inoculated with *C. testosteroni* BR60. Error bars represent the standard deviation of three replicate microcosms.

Microbial community dynamics

In the inoculated treatments, the total number of culturable degraders detected on Medium A increased from the initial inoculum level of 10^6 CFU g^{-1} dry soil to approximately 10^8 CFU g^{-1} dry soil with the increase being more rapid in the 500 than the 1000 μg 3-CB g^{-1} dry soil microcosms (Figure 3). In contrast, no indigenous 3-CB degraders were detected (on MSM plates) in the inoculated microcosms throughout the experiment without 3-CB re-amendment (data not shown). All degraders isolated on Medium A from these inoculated microcosms were later confirmed to be the *C. testosteroni* BR60 inoculant. However, a population of indigenous degraders (approximately 10^5 CFU g^{-1} dry soil) did develop in the inoculated, 500 μg 3-CB g^{-1} dry soil microcosms following re-amendment with an additional 500 μg 3-CB g^{-1} dry soil (Table 1). In the uninoculated microcosms, culturable indigenous 3-CB degrader numbers increased in the uninoculated, 500 μg 3-CB g^{-1} dry soil microcosms from undetectable levels at 0 d to approximately 10^8 CFU g^{-1} dry soil by 14 d, and then decreased to less than 10^7 CFU g^{-1} dry soil by 28 d (Figure 3). Degrader numbers were statistically the same on the Medium A and MSM plates (data not shown). In contrast, no 3-CB degraders were detected in the uninoculated, 1000 μg 3-CB g^{-1} dry soil microcosms during the experiment.

The 3-CB contamination, at 1000 μg 3-CB g^{-1} dry soil, initially decreased the number of culturable heterotrophic bacteria in both the uninoculated and

Table 1. Numbers of indigenous and total 3-chlorobenzoate (3-CB) degraders in microcosms initially amended with 500 μg 3-CB g^{-1} dry soil and re-amended with an additional 500 μg 3-CB g^{-1} dry soil after 16 d. Microcosms were either uninoculated or inoculated with *C. testosteroni* BR60

	Sampling time			
	18 d		23 d	
Treatment	Indigenous	Total	Indigenous	Total
	3-CB degraders (CFU g^{-1} dry soil)			
Uninoculated	3.93 ($\pm$1.59) $\times$ 10^8	3.88 ($\pm$1.56) $\times$ 10^8	1.60 ($\pm$0.09) $\times$ 10^8	1.44 ($\pm$0.33) $\times$ 10^8
Inoculated	None detected	2.37 ($\pm$0.57) $\times$ 10^6	1.36 ($\pm$0.60) $\times$ 10^5	1.41 ($\pm$0.39) $\times$ 10^8

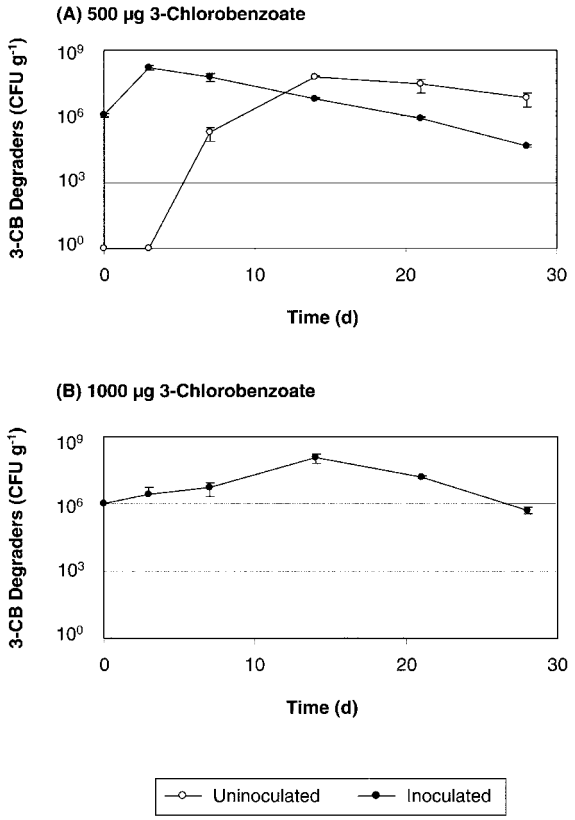

Figure 3. Numbers of 3-chlorobenzoate (3-CB) degraders in microcosms amended with (a) 500 and (b) 1000 μg 3-CB g^{-1} dry soil that were uninoculated or inoculated with *C. testosteroni* BR60. Error bars represent the standard deviation of three replicate microcosms.

inoculated microcosms (Figure 4c). However, inoculation with *C. testosteroni* BR60 appeared to either mask or partially alleviate the initial impact of 3-CB on the indigenous culturable heterotrophic soil bacteria. In the 1000 μg 3-CB g^{-1} dry soil at 3 d, numbers decreased to minimal levels of 1.0 $\times$ 10^8 CFU g^{-1} in the inoculated microcosms as compared to 5.6 $\times$ 10^7 CFU g^{-1} dry soil in the uninoculated microcosms.

Potentially, the high numbers of degraders that developed may have masked some of the bioaugmentation effects on culturable heterotrophic bacteria. After 3 d, numbers of culturable heterotrophic bacteria were higher in *C. testosteroni* BR60 inoculated microcosms than uninoculated microcosms at all 3-CB levels including the uncontaminated control (Figure 4); therefore, degrader numbers as detected on Medium A were subtracted from R2A plate counts to determine the number of culturable heterotrophic bacteria not capable of 3-CB degradation (Figure 5). When the results from the 500 μg 3-CB g^{-1} dry soil microcosms were examined in this way, culturable heterotrophic bacterial numbers were essentially the same in both the inoculated and uninoculated microcosms except at 7 d. In contrast, bacterial numbers in the 1000 μg 3-CB g^{-1} dry soil microcosms were higher in the inoculated soil than the uninoculated soil, throughout the duration of the study except at 7 d.

Dominant indigenous degraders

Approximately 200 degraders were isolated from both the uninoculated and *C. testosteroni* BR60 amended soils. Degraders were placed into three distinct groups based on ERIC-PCR fingerprints. Selected bacteria from each group were identified by 16S rDNA sequences. All degraders that were isolated from the inoculated soil were confirmed to be the inoculant, *C. testosteroni* BR60. The dominant degraders from the uninoculated, 500 μg 3-CB g^{-1} dry soil microcosms consisted of two distinct *Burkholderia* spp. and were designated as AZ102 and AZ163, comprising approximately 90 and 10% of degraders, respectively (data not shown). Phylogenetic analyses confirmed both indigenous degraders to be distinct from the *C. testosteroni* BR60 inoculant but similar to other chlorinated

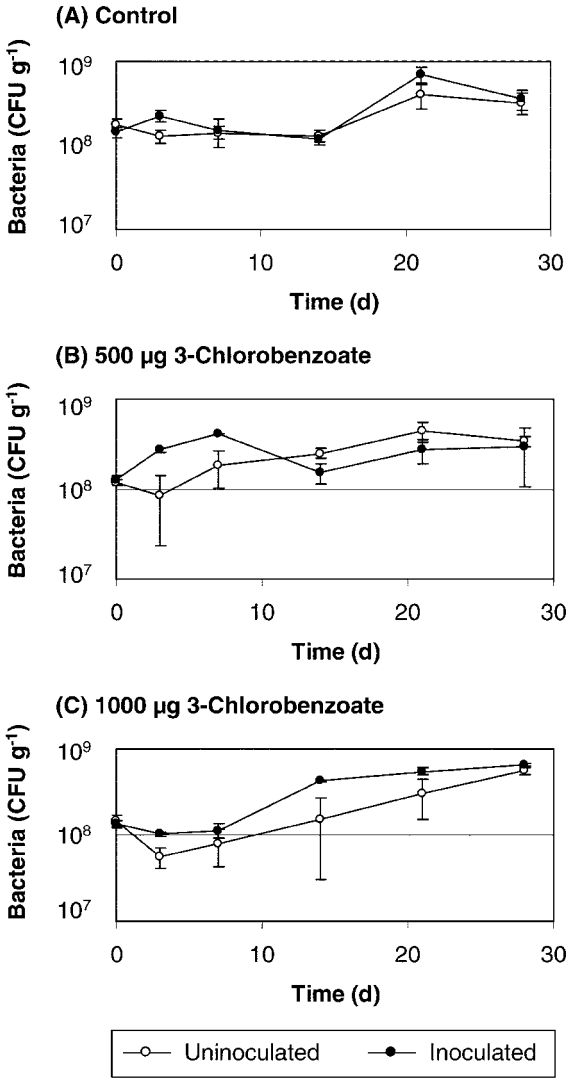

Figure 4. Numbers of culturable heterotrophic bacteria in microcosms amended with (a) 0, (b) 500, and (c) 1000 μg 3-chlorobenzoate g^{-1} dry soil that were uninoculated or inoculated with *C. testosteroni* BR60. Error bars represent the standard deviation of three replicate microcosms.

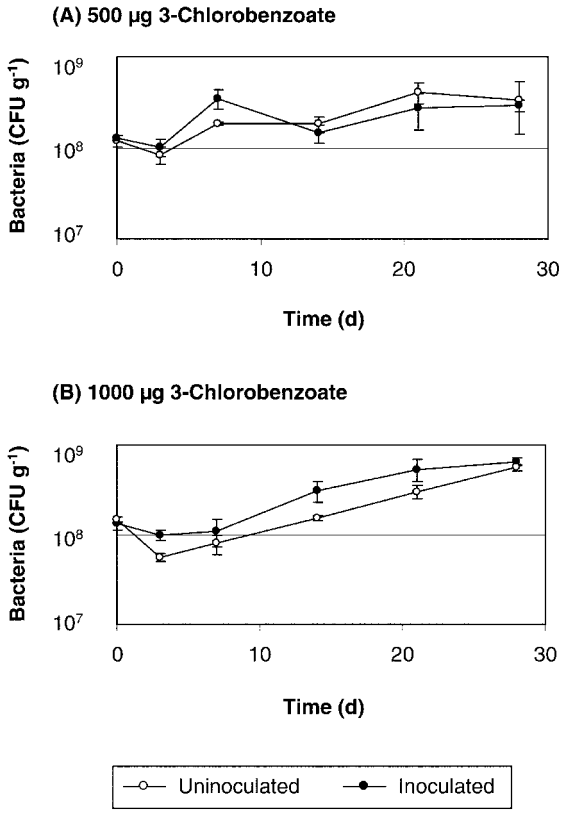

Figure 5. Numbers of culturable heterotrophic bacteria after subtraction of 3-chlorobenzoate (3-CB) degraders. Microcosms were amended with (a) 500 and (b) 1000 μg 3-CB g^{-1} dry soil and were uninoculated or inoculated with *C. testosteroni* BR60. Error bars represent the standard deviation of three replicate microcosms.

hydrocarbon degrading bacteria including a 2,4-D degrading bacterium isolated by Velicer et al. (unpubl., GenBank # AF184931) (Figure 6). All indigenous degraders isolated from microcosms re-amended with 500 μg 3-CB g^{-1} dry soil were identified as *Burkholderia* sp. AZ102. Both Burkholderia sp. AZ102 and AZ163 contained plasmids that were smaller than the 85 kb plasmid in the *C. testosteroni* BR60 inoculant (Figure 7).

Discussion

The *C. testosteroni* BR60 inoculant proliferated in the contaminated soil and increased the rate of 3-CB degradation at both levels of 3-CB contamination; however, there were clear differences in 3-CB biodegradation at the two different levels of contamination. Increased degradation in the 500 μg 3-CB g^{-1} dry soil amended microcosms appeared to be primarily the result of elimination of the acclimation period in the inoculated soil. A large indigenous population of 3-CB degraders developed in the uninoculated soil and, once established, degraded the 3-CB at a rate similar to that of the *C. testosteroni* BR60 inoculant. In contrast, no indigenous degraders were detected in the uninoculated, 1000 μg 3-CB g^{-1} dry soil microcosms during the study. While 3-CB was almost completely degraded in the inoculated soil by 14 d, approximately 500 μg 3-CB g^{-1} dry soil still remained in the un-

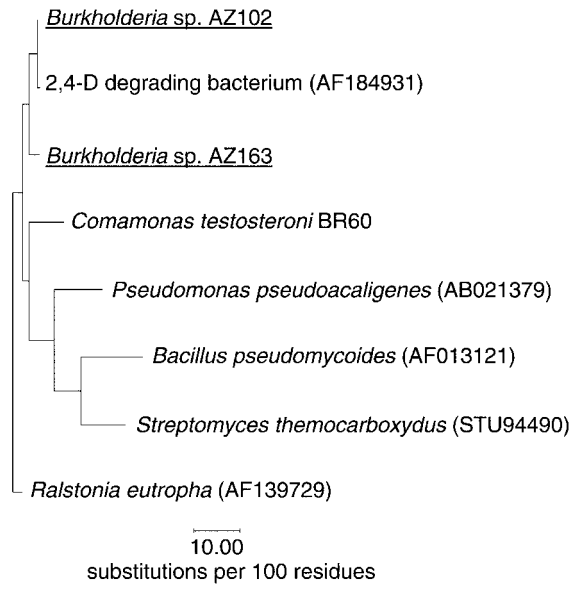

Figure 6. Phylogenetic tree generated from 16S rDNA sequences of indigenous 3-chlorobenozate degraders (underlined) and selected bacteria from GenBank.

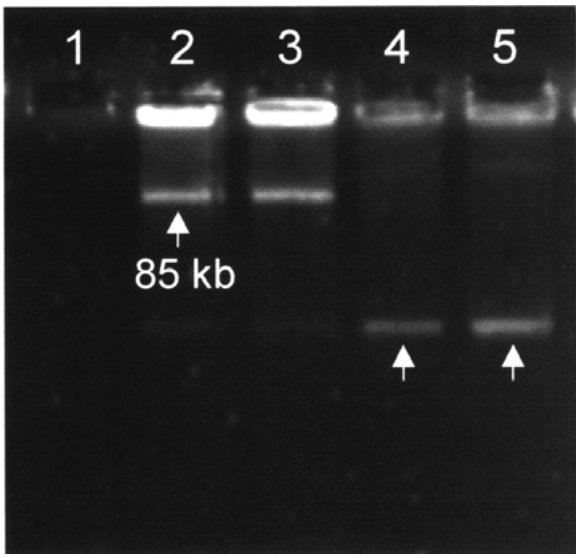

Figure 7. Plasmid screens of *C. testosteroni* BR60, prior to soil inoculation (lane 2) and after re-isolation from soil (lane 3), and the indigenous 3-chlorobenzoate degraders *Burkholderia* sp. AZ102 (lane 4) and *Burkholderia* sp. AZ163 (lane 5). The negative control was run in lane 1.

inoculated soil even after 56 d (data not shown). The higher concentration of 3-CB apparently inhibited the development of the indigenous 3-CB degrader population. As indicated by culturable heterotrophic bacterial numbers, addition of 1000 μg 3-CB g^{-1} dry soil decreased culturable bacteria by 60% between 0 and 3 d. The indigenous degraders may have been eliminated along with other bacteria during the initial exposure to 3-CB. It appears likely that the higher 3-CB level was toxic to the indigenous degraders since the 1000 μg 3-CB g^{-1} dry soil also slowed proliferation of the *C. testosteroni* BR60 inoculant with numbers increasing to 10^8 CFU g^{-1} dry soil by 3 d in the 500 μg 3-CB g^{-1} dry soil microcosms, but not until 14 d in the 1000 μg 3-CB g^{-1} dry soil microcosms.

Degradation was so rapid in the inoculated 500 μg 3-CB g^{-1} dry soil microcosms that there was little time for development of the indigenous degrader populations under 3-CB selective pressure; therefore, a portion of the soil from each the uninoculated and inoculated treatments was re-amended with 500 μg 3-CB g^{-1} dry soil at 16 d after the 3-CB was mostly degraded in both treatments. In contrast to the previous results, degradation proceeded more rapidly in the uninoculated soil than the inoculated soil. The most likely reason for this result is the higher number of degraders in the uninoculated, 500 μg 3-CB g^{-1} dry soil microcosms at 16 d as compared to the inoculated, 500 μg 3-CB g^{-1} dry soil microcosms. The 3-CB had been completely degraded in the inoculated soil for approximately 9 d prior to re-amendment with 3-CB while the uninoculated soil still contained 3-CB, albeit a low concentration, and thus a higher degrader population at the time of re-amendment. Following the re-amendment of the inoculated microcosm with 500 μg 3-CB g^{-1} dry soil, degrader numbers increased to approximately the same as those in the uninoculated soil.

Another striking difference between the results from the re-amended uninoculated and inoculated soils and the results from the initial study was the development of an indigenous degrader population (approximately 10^5 CFU g^{-1} dry soil) in the inoculated soil. The indigenous degraders were confirmed to be *Burkholderia* sp. AZ102 based on 16S rDNA sequences. No isolates of *Burkholderia* sp. AZ163 were found in the re-amended, inoculated soil. It is not known if a population of *Burkholderia* sp. AZ163 was not present or just too low to be detected. Since both *Burkholderia* sp. AZ102 and *Burkholderia* sp. AZ163 have similarly sized plasmids, it is possible

that the 3-CB degrading *Burkholderia* sp. AZ163 in the initial experiment resulted from transfer of degradative genes from *Burkholderia* sp. AZ102, the more numerous degrader. The transfer may have occurred once the population reached a level higher than that the 10^5 CFU g^{-1} dry soil present in the re-amended soil. This is only speculative, but Newby et al. (2000a & 2000b) also detected high levels of plasmid pJP4 transfer among *Burkholderia* spp. in Madera Canyon soil.

Inoculation with *C. testosteroni* BR60 reduced the negative effects of 3-CB on culturable heterotrophic bacterial numbers at the highest level of contamination, 1000 μg 3-CB g^{-1} dry soil. The differences between the inoculated and uninoculated microcosms cannot be explained solely on the basis of reduction in 3-CB toxicity. Even at 3 d when limited degradation had occurred, heterotrophic bacterial numbers were higher in the inoculated soil than the uninoculated soil. It is possible that partial 3-CB-degradation products were made available to indigenous degraders as a result of degradation by *C. testosteroni* BR60. Another possibility is a portion of the added *C. testosteroni* BR60 perished and provided a metabolizable nutrient pool for indigenous bacteria. Nevertheless, the differences in results between the 1000 and 500 μg 3-CB g^{-1} dry soil treatments suggest that this inoculation protection of indigenous bacteria was a real occurrence and not just an artifact of confounding effects. The inoculated, 500 μg 3-CB g^{-1} dry soil microcosms received the same amount of *C. testosteroni* BR60 initially, but there was not a corresponding difference between the numbers of culturable heterotrophic bacteria in the inoculated and uninoculated microcosms.

Conclusions

Addition of *C. testosteroni* BR60 increased degradation of 3-CB at both tested levels of contamination; however, inoculation with *C. testosteroni* BR60 delayed the development of the indigenous 3-CB degrader populations. Indigenous degraders were only detected in the inoculated soil after a re-amendment with additional 3-CB. It is unclear if inoculation with *C. testosteroni* BR60 affected the composition of indigenous degraders. Addition of *C. testosteroni* BR60 reduced the initial decrease in the number of culturable heterotrophic soil bacteria following amendment with 1000 μg 3-CB g^{-1} dry soil. This level of 3-CB also completely inhibited the development of indigenous degraders. The results indicate that bioaugmentation may be beneficial not only by increasing the rate of contaminant degradation but also by decreasing the deleterious effects of contaminants on indigenous soil microorganisms.

Acknowledgments

Special thanks are offered to the Coronado National Forest for permission to collect Madera Canyon soil and to Dr. R.C. Wyndham for providing a culture of *C. testosteroni* BR60. The authors would also like to thank Jessica Snider for assistance with this study. This work was supported by NIEHS Basic Superfund grant 5 P42 ESO4940-09.

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