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Use of a Pentachlorophenol Degrading Bacterium to Bioremediate Highly Contaminated Soil

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ABSTRACT

A *Sphingomonas* species that mineralizes high concentrations of pentachlorophenol (PCP) was isolated from a PCP-contaminated EPA Superfund site. This bacterium, identified as *Sphingomonas* sp. strain RA2, is able to degrade PCP at concentrations of up to 300 μg/mL in liquid culture. This organism was tested for its ability to degrade high concentrations of PCP in a soil that did not contain organisms capable of degrading high concentrations of PCP. When inoculated into contaminated soil, *Sphingomonas* sp. RA2 mineralized PCP at concentrations of 300, 600, 900, and 1200 μg PCP/g of soil, but was unable to mineralize 1500 μg PCP/g of soil. Only very minimal loss of PCP was seen in uninoculated soils. The results of this study demonstrate that *Sphingomonas* sp. RA2 may be a useful organism for remediation of sites contaminated with high concentrations of PCP.

Index Entries: Biodegradation; bioremediation; *Sphingomonas*; pentachlorophenol; soil inoculation; bioaugmentation.

INTRODUCTION

If bioremediation is to become a viable method for cleaning up contaminated sites, microorganisms that can tolerate environmental stress and high concentrations of contaminants must be found. We have recently

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isolated an organism that can degrade high concentrations of pentachlorophenol (PCP) from a heavily contaminated soil at the Broderick Superfund site near Denver, CO (*1*). This bacterium, originally described as *Pseudomonas* sp. strain RA2 (*1*), was recently identified as a member of the new genus *Sphingomonas* (*2–4*). *Sphingomonas* sp. RA2 can use PCP as its sole carbon and energy source, and was able to mineralize PCP to CO_2 and Cl^- ions in pure culture (*1*). By optimizing growth conditions, the upper PCP toxicity limit for this organism in liquid media was increased from 160 μg/mL (parts per million or ppm) to 300 ppm.

The goals of the present study with *Sphingomonas* sp. RA2 were: (1) to determine if it can degrade PCP in unsterilized soil, and (2) to find the upper PCP toxicity limit for *Sphingomonas* sp. RA2 in the soil used in this study.

MATERIALS AND METHODS

A stock culture of *Sphingomonas* sp. RA2 was maintained in a mineral salts medium as previously described (*1*), except that the pH of the medium was 7.5 instead of 6.9, and 1.7 μg $FeSO_4 \cdot 7H_2O$/mL was added as a source of iron instead of Pfennig's solution (*5*). The stock had an initial PCP concentration of 300 ppm. Early-stationary-phase cells were added to PCP-contaminated soil at an inoculum level of 6×10^6 cells/g soil.

The soil used was a sandy loam that has been described in detail elsewhere as soil #2 (*6*). Sieved soil (2 mm) was placed in 250-mL biometer flasks and allowed to equilibrate overnight. On the following day, ^{14}C-PCP, unlabeled PCP, and deionized water were added to achieve the desired PCP concentration. After mixing the substrate and water into the soil, *Sphingomonas* sp. RA2 was added in enough liquid to obtain a final moisture level of 70% of field capacity (*3*). Controls were treated the same as the inoculated soils, except that they received sterile mineral salts solution instead of solution containing *Sphingomonas* sp. RA2. Evolved $^{14}CO_2$ was captured in 1.0 mL of 0.5*N* NaOH contained in the side arm of each biometer flask. The NaOH was added to 2.5 mL of scintillation cocktail and counted in a liquid scintillation counter.

RESULTS AND DISCUSSION

Figure 1 shows the mineralization of 300 ppm of PCP in soils inoculated with *Sphingomonas* sp. RA2. These results were very repeatable as indicated by the overlap of the two replicate curves of PCP mineralization shown in Fig. 1. Figure 1 also demonstrates that mineralization of PCP in inoculated soils was carried out by *Sphingomonas* sp. RA2 cells and not by indigenous organisms in this soil. Uninoculated control incubations lost $< 2\%$ of the added PCP after 3 mo of incubation (Fig. 1). The slight loss of PCP in control soils also rules out the possibility that significant abiotic

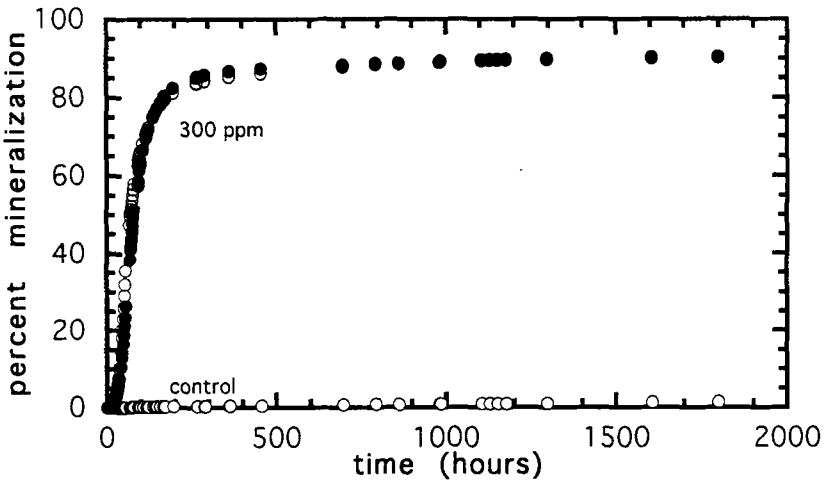

Fig. 1. Mineralization of 300 ppm of PCP by *Sphingomonas* sp. RA2 inoculated into replicate soil samples. The control soil received 300 ppm of PCP, but no inoculum.

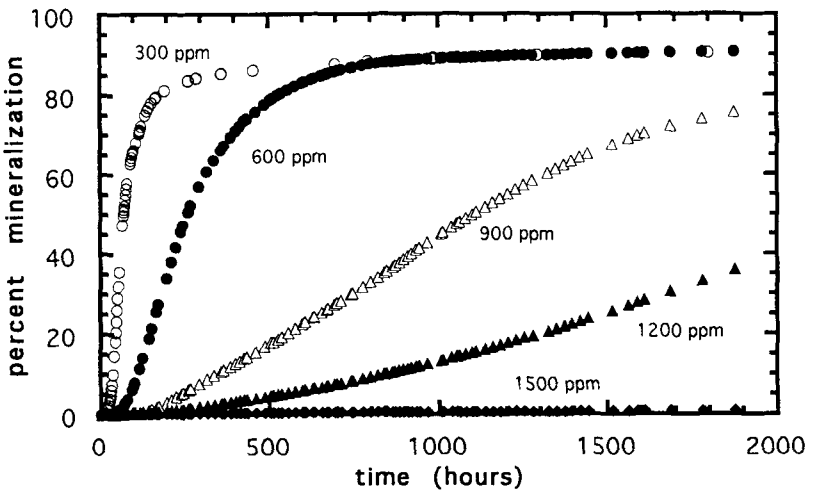

Fig. 2. Mineralization of different concentrations of PCP by cells of *Sphingomonas* sp. RA2 inoculated into soil.

mineralization of PCP occurred in the inoculated soils. In contrast to our results, Crawford and Mohn (*7*) introduced a *Flavobacterium* sp. into soil containing PCP at a concentration of 100 ppm, and saw roughly equal extents of mineralization in the control and inoculated soils after 30 d. Our results are similar to those of Edgehill and Finn (*8*), except that they showed enhanced PCP degradation at lower concentrations than those employed in the present study.

An experiment was then conducted to determine the highest concentration that *Sphingomonas* sp. RA2 could degrade in soil. Figure 2 shows mineralization of PCP in soil at concentrations ranging from 300 to 1500

ppm. At PCP concentrations of 300 and 600 ppm, *Sphingomonas* sp. RA2 mineralized just over 90% of ^{14}C-PCP to $^{14}CO_2$. This correlates well with the findings of Radehaus and Schmidt (*1*) that over 80% of PCP was removed from solution, whereas 14–17% was assimilated into biomass by pure cultures of *Sphingomonas* sp. RA2. At PCP concentrations of 900 and 1200 ppm in soil, mineralization had reached over 82 and 79%, respectively, after 4780 h of incubation (data not shown). The finding that *Sphingomonas* sp. RA2 can mineralize four times the amount of PCP in soil than in liquid may be due to the immobilization of cells in soil or the slower rate of diffusion of PCP in soil. Soils contaminated with 1500 ppm PCP released no $^{14}CO_2$ after over 2000 h of incubation. This indicates that PCP is toxic to *Sphingomonas* sp. RA2 somewhere between 1200 and 1500 ppm in soil.

Other studies that have involved inoculating PCP-degrading organisms into PCP contaminated soil have been successful to varying degrees, but were carried out at much lower PCP concentrations (*7,8*), or the inocula were grown on additional substrates and applied with immobilizing agents to reduce the toxicity of PCP (*9*). In the study that was most similar to our study (*10*), a *Rhodococcus* species was inoculated into a sandy soil containing 640 ppm PCP, but removed $<50\%$ of the PCP as CO_2 after 4 mo of incubation. In contrast, *Sphingomonas* sp. RA2 removed over 90% of PCP at a concentration of 600 ppm in <2 mo.

In conclusion, *Sphingomonas* sp. RA2 was extremely effective at removing high concentrations of PCP from the soil used in this study. In future research, we will explore the usefulness of this organism in other soils and bioremediation systems.

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