Induction of PCB degradative pathway by plant terpenoids as growth substrates or inducers

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ABSTRACT

The eventual goal of this study is to elucidate roles of plant terpenoids (*e.g.*, cymene, limonene and others) as natural substrates in the cometabolic biodegradation of PCBs and to develop an effective PCB bioremediation technology. The aim of this study was to examine how plant terpenoids, as natural substrates or inducers would affect the biodegradation of PCB congeners. Various PCB degraders that could grow on biphenyl and several terpenoids were tested for their PCB degradation capabilities. The PCB congener degradation activities were first monitored through resting cell assay technique that could detect degradation products of the substrate. The congener removal was also confirmed by concommitant GC analysis. The PCB degraders, *Pseudononas* sp. P166 and *Caynebacterium* sp. T104 were found to grow on both biphenyl and terpenoids (*(S)*-(-) limonene, *p*-cymene and α-terpinene) whereas *Arthrobacter* B1B could not grow on the terpenoids as a sole carbon source. The strain B1B grown on biphenyl showed a good degradation activity for 4,4′-dichlorobiphenyl (DCBp) while strains P166 and T104 gave about 25% of B1B activity. Induction of degradation by cymene, limonene and terpine was hardly detected by the resting cell assay technique. This appeared to be due to relatively lower induction effect of these terpenoids compared with biphenyl. However, a subsequent GC analysis showed that the congener could be removed up to 30% by the resting cells of T104 grown on the terpenoids. This indicates that terpenoids, widely distributed in nature, could be utilized as both growth

and/or inducer substrate for PCB biodegradation.

INTRODUCTION

Several hundred million pounds of PCBs are known to be released into the environment until now, where they became persistent by adsorbing into organic compounds in soil and sediments due to their hydrophobicity, resulting in the accumulation in a variety of biota including humans. PCBs are strictly regulated because of their potential toxicity (e.g., mutagenicity and endocrine disruption). Microbial degradation, however, holds the greatest promise for an inexpensive way of decontaminating polluted sites with PCBs. Anaerobic degradation of PCB occurs through dechlorination of highly chlorinated congeners to less chlorinated ones (4). Then occurs aerobic degradation of less chlorinated congeners to chlorobenzoates. Biphenyl has been used to isolate and growth bacteria that partially transform PCB congeners and to enhance biodegradation in soil, however, not a normal constituent of soil (8).

Therefore, alternative natural substrates having structural similarity to biphenyl woud exist in nature(6). Plant flavonoids equal to biphenyl in promoting metabolism of PCBs were found (5). *L*-carvone and other *p*-menthene structure motif compounds (limonene, *p*-cymene and isoprene) induced *Arthrobacter* sp. B1B to cometabolize Aroclor 1242 (7). PCB biodegradation appears to be heavily dependent upon sterospecificty of the target congeners (1,2,3)

MATERIALS AND METHODS

PCB degraders used in this study were *Pseudomonas sp.* P166, *Arthrobacter sp.* B1B *and Rhodococcas sp.* T104. They grown on MSM containing biphenyl or terpenoids as sole carbon source and maintained on mineral salts agar plates with biphenyl crystals. Biodegradation was monitored by resting cell assay (7) using 4,4′-dichlorbiphenyl or 2,2′-dichlorbiphenyl as a substrate.

The degradation activity was also confirmed by GC (Finnigan).

RESULTS AND DISCUSSION

The PCB degraders, *Pseudononas* sp. P166 and *Caynebacterium* sp. T104 were

found to grow on both biphenyl and terpenoids ((*S*)-(-) limonene, *p*-cymene and α-terpinene) whereas *Arthrobacter* B1B could not grow on the terpenoids as a sole carbon source (Fig. 1). This indicates that a terpenoid substrate range of PCB degraders would be strain-specific. The strain B1B grown on biphenyl showed a good degradation activity for 4,4′- dichloro- biphenyl (DCBp) while strains P166 and T104 reached about 25% of B1B activity (Fig. 2). This means 4,4′-DCBp would be relatively a poor substrate for T104 and P166.

Induction of degradation pathway by cymene, limonene and terpine was hardly detected by the resting cell assay technique. This appeared to be due to relatively lower induction effect of these terpenoids compared with biphenyl. However, the concommitant GC analysis showed that the congener could be removed up to 30% by the resting cells of T104 grown on the terpenoids.

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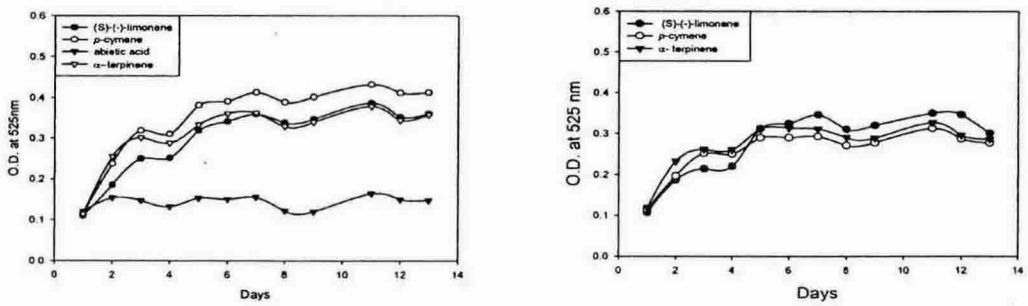

(a) (b)

Figure 1. Growth of *Pseudomonas* sp. P166 (a) and *Rhodococcus* sp. T104 (b) on various terpenoids (300 ppm).

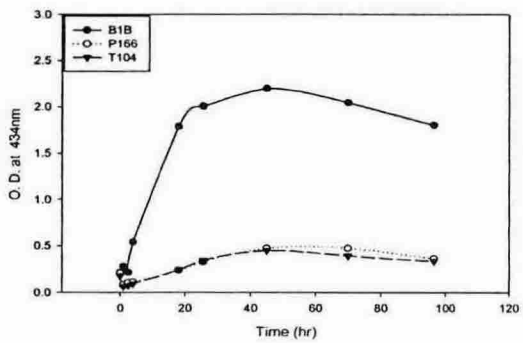

Figure 2.. Production of a ring cleavage product (2-hydroxy-6-oxo-phenylhexa-2,4-dienoic acid) by resting cells of PCB degraders grown on biphenyl.