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Degradation of anaerobic reductive dechlorination products of Aroclor 1242 by four aerobic bacteria

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Abstract

We studied the aerobic degradation of eight PCB congeners which comprise from 70 to 85% of the anaerobic dechlorination products from Aroclor 1242, including 2-, 4-, 2,4-, 2,6-, 2,2′-, 2,4′-, 2,2′,4-, and 2,4,4′-chlorobiphenyl (CB), and the biodegradation of their mixtures designed to simulate anaerobic dechlorination profiles M and C. Strains *Comamonas testosteroni* VP44 and *Rhodococcus erythreus* NY05 preferentially oxidized a *para*-substituted ring, while *Rhodococcus* sp. RHA1, similar to well known strain *Burkholderia* sp. LB400, preferably attacked an *ortho*-chlorinated ring. Strains with *ortho*-directed attack extensively degraded 2,4′- and 2,4,4′-CB into 4-chlorobenzoate, while bacteria with *para*-directed attack transformed these congeners mostly into potentially problematic *meta*-cleavage products. The strains that preferentially oxidized an *ortho*-substituted ring readily degraded seven of the eight congeners supplied individually; only 2,6-CB was poorly degraded. Degradation of 2,2′- and 2,4,4′-CB was reduced when present in mixtures M and C. Higher efficiencies of degradation of the individual congeners and defined PCB mixtures M and C and greater production of chlorobenzoates were observed with bacteria that preferentially attack an *ortho*-substituted ring. PCB congeners 2,4′-, 2,2′,4-, and 2,4,4′-CB can be used to easily identify bacteria with *ortho*-directed attack which are advantageous for use in the aerobic stage of the two-phase (anaerobic/aerobic) PCB bioremediation scheme.

Introduction

The toxicity, persistence, carcinogenic potential of polychlorinated biphenyls (PCBs), and their tendency to bioaccumulate in food chains, make PCBs problematic environmental pollutants. Commercially produced PCB mixtures, such as Aroclors, typically contain between 60 and 80 different congeners with 1 to 10 chlorine atoms per biphenyl molecule (Frame et al. 1996). Aerobic microbial degradation of PCBs is usually limited to congeners containing 4–5 chlorines or less, and the degradation rates decrease as the number of the chlorine substituents increase (Furukawa 1982; Unterman 1996). The most promising approach for bioremediation of soils and sediments contaminated with PCBs is a sequential anaerobic-aerobic process (Abramowicz 1990). Anaerobic microbial communities found in sediments reductively dechlorinate Aroclors to lesser chlorinated chlorobiphenyls (CBs) which are expected to be more readily degraded by aerobic bacteria. Anaerobic dechlorination processes M and Q (*meta*- and *para*-dechlorination respectively) and their combination (process C) (Brown 1987a, b), all of which can remove both flanked and unflanked chlorines, could attack the broadest range of congeners of Aroclor 1242 and 1248 (Bedard & Quensen 1995) and thus are the most promising processes for bioremediation of these PCBs. Dechlorination patterns in the environment usually result from a combination of dechlorination processes that remove *meta* and/or *para* chlorines. *Ortho*- and *ortho*- plus *para*-substituted congeners including 2-, 4-, 2,4-, 2,6-, 2,2′-, 2,4′-, 2,2′,4-, and 2,4,4′-chlorobiphenyl comprise from 70 to 85 molar % of the anaerobic dechlorination products of Aroclor 1242 (Quensen & Tiedje 1998; Zwiernik et al. 1998) and are therefore primary targets for the aerobic stage of the two-phase PCB bioremediation scheme. Other dechlorination products such as 2,2′,6-, 2,3′,6- and 2,2′,4,4′-CB can accumulate during anaerobic dechlorination of more highly chlorinated Aroclors 1248, 1254, and 1260 (Bedard & Quensen 1995) but were not included in this study.

Information on the aerobic degradation of anaerobic dechlorination products of Aroclors is very limited. To our knowledge there are no data on the aerobic degradation of process M dechlorination products, and only *Alcaligenes eutrophus* strain H850 has been analyzed for the depletion of process C dechlorination products (Bedard et al. 1987b; Harkness et al. 1993). Other PCB-degrading microorganisms have been analyzed only for their degradation of Aroclors or defined PCB mixtures (Unterman 1996). The congener composition and their ratios in these mixtures differ from those found in dechlorinated Aroclors (Bedard & Quensen 1995; Bedard et al. 1986; Williams et al. 1997) and it is known that the rates of degradation of the individual congeners can vary significantly depending on their initial concentrations and on the composition of the PCB mixtures (Bedard et al. 1986; 1987b; Billingsley et al. 1997). The aerobic PCB degradation products formed by several bacterial strains have been identified (Bedard et al. 1987a; Furukawa 1982; Massé et al. 1989; Seeger et al. 1995), but very little is known about the stoichiometry involved (Bedard & Haberl 1990; Furukawa et al. 1979a). Information on dead end product formation is important in assessing strains for aerobic PCB bioremediation because of the potential to accumulate other toxic compounds. The primary goal of this investigation was to assess the biodegradability of the environmentally important PCB congeners resulting from process M or C dechlorination of Aroclor 1242 and identify the products of their aerobic transformation.

Previously PCB degrading aerobic bacteria were categorized into two groups based on their abilities to degrade defined PCB mixtures (Mondello et al. 1997; Williams et al. 1997). LB400-type strains (Mondello et al. 1997) or H850-like bacteria (Williams et al. 1997) exhibited a broad range of PCB degradation, but had relatively weak activity against di-*para*-substituted congeners. Strains with KF707-type specificity (Mondello et al. 1997) or MB1-like bacteria (Williams et al. 1997) degraded a narrower range of PCBs. They were more efficient with di-*para*-substituted congeners, but usually showed relatively weak activity against di-*ortho*-substituted congeners (Mondello et al. 1997; Williams et al. 1997). One strain with KF707-type specificity (*Rhodococcus erythreus* NY05) (Pellizari et al. 1996), one strain with LB400-type specificity (*Burkholderia* sp. LB400) (Mondello et al. 1997) and two bacteria with especially wide degradation spectra (*Rhodococcus* sp. RHA1 and *Comamonas testosteroni* VP44) were chosen for this investigation. The PCB degradative competences of strains VP44 and RHA1 were assessed with defined PCB mixtures; strain RHA1 was additionally tested with Kanechlor (Pellizari et al 1996; Seto et al. 1995). These two strains were reported to have high activities towards both di-*ortho*- and di-*para* substituted congeners (Pellizari et al. 1996; Seto et al. 1995) and were expected to be the most efficient in degradation of anaerobic Aroclor dechlorination products.

From the products formed, it was shown that two strains, *Comamonas testosteroni* VP44 and *R. erythreus* sp. NY05, preferentially attacked the *para*-substituted PCB rings, while the strain *Rhodococcus* sp. RHA1, like *Burkholderia* sp. LB400 (Bedard & Habert 1990), preferentially oxidized the *ortho*-chlorinated PCB rings. Higher degradation rates of PCBs and greater recoveries of chlorobenzoates (CBAs) were observed with bacteria that preferably attacked the *ortho*-substituted ring. The latter group of strains is advantageous for use in the aerobic stage of a two-phase PCB bioremediation scheme.

Materials and methods

Bacterial strains and culture conditions

Strains *R. erythreus* NY05 and *C. testosteroni* VP44 were previously isolated from soil in New York State, USA, and São Paulo State, Brazil, respectively (Pellizari et al. 1996). Strain *Rhodococcus* sp. RHA1 (Seto et al. 1995) was isolated in Japan from soil contaminated by γ-hexachlorocyclohexane. Strain *Burkholderia* sp. LB400 was isolated from a PCB contaminated site (Bopp 1986) and was obtained from the Culture Collection of the US Department of Agriculture. Bacteria were grown at 30 °C on K1 mineral medium (Zaitsev & Karasevich 1985) supplemented with biphenyl (1 g/l).

PCB congeners and their defined mixtures M and C

Mixtures M and C were made by mixing stock solutions of eight individual congeners according to their ratios found in anaerobic dechlorination patterns M and C (Zwiernik et al. 1998). Final concentrations of PCBs in mixture M (Mix M) were: 2-CB (80 μM); 4-CB (21 μM); 2,4-CB (14 μM); 2,6-CB (36 μM); 2,2′-CB (108 μM); 2,4′-CB (123 μM); 2,2′,4-CB (64 μM); and 2,4,4′-CB (54 μM). Final concentrations of PCBs in mixture C (Mix C) were: 2-CB (250 μM); 4-CB (25 μM); 2,4-CB (5 μM); 2,6-CB (50 μM); 2,2′-CB (150 μM); 2,4′-CB (10 μM); 2,2′,4-CB (5 μM); and 2,4,4′-CB (5 μM). Total concentration of PCBs in both Mix M and C was 500 μM. This concentration is within the optimal concentration range (100–250 μg/ml slurry which corresponds to 353 to 883 μM) for the anaerobic dechlorination of Aroclors (Abramowicz et al., 1993). In separate experiments, individual congeners were added to the following final concentrations: 2-CB (500 μM); 4-CB (500 μM); 2,4-CB (200 μM); 2,6-CB (100 μM); 2,2’-CB (100 μM); 2,4′-CB (100 μM); 2,2′,4-CB (50 μM); and 2,4,4′-CB (50 μM).

Resting cells assays

Cells were grown on biphenyl to an optical density of 1 to 1.5 at 600 nm, then washed twice in sterile K1 medium and resuspended in the same medium at an optical density of 2.0. Reactions were carried out at room temperature in 1-dram glass vials fitted with Teflon-lined screw caps and containing 990 μl of resting cells and 10 μl of PCB solution. Cells were incubated on a gyratory shaker at 150 rpm. To assess the dynamics of degradation and accumulation of transient intermediates, the entire contents of triplicate incubation vials were extracted and analyzed for PCB concentrations by GC/ECD and another five vials were analyzed by HPLC and UV-spectrometry for metabolites after 0, 1, 3, 8, and 24 h of incubation. PCB degradation was assessed by comparison with heat-killed (121 °C, 10 min) and cell-free controls incubated in parallel with live samples. Resting cell experiments were also performed using 500 μM of 2-CBA, 4-CBA, and 2,4-CBA to check the capabilities of selected strains for further transformation of chlorobenzoic acids.

PCB extraction and analysis

To quantify PCB degradation, the internal standard 2,3′,5,5′-CB (final concentration 18 μM) was added to each sample prior to extraction. Reaction mixtures were extracted three times with 1 ml of hexane and acetone (1/1, v/v). The combined extracts were analyzed on an HP 5890 gas chromatograph, (Hewlett-Packard Co., Palo Alto, Calif.) fitted with a Hewlett-Packard HP 5 column (15 m by 0.32 mm id, 25 μm film thickness, liquid phase 5% phenyl substituted methylpoly siloxane) and electron capture detector. For single congeners, the oven temperature program was 140 °C for 1 min, then 10 °C/min to 230 °C. For analysis of mixtures M and C, the oven temperature program was 140 °C for 1 min, then 3 °C/min to 188 °C. Inlet and detector temperatures were 220 and 325 °C, respectively. PCBs were quantified using four point calibration curves constructed using standards bracketing the concentrations expected in samples showing no degradation. Typical recoveries were 98 to 103% as judged by comparing analyses for control samples with the amounts added.

For identification of PCB degradation metabolites, 1 ml samples obtained after 3 or 24 h of incubation with the corresponding congener were acidified to pH 6 to extract phenolic compounds or pH 3 (Massé et al. 1989) and extracted three times with 1 ml of ethylacetate. Ethylacetate extracts were evaporated to dryness under a gentle stream of nitrogen gas, and the residue was derivatized by adding 5 μl of pyridine and 20 μl of N-O-bis-(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane (Supelco) and incubating at 60 °C for 30 min. The resultant samples were analyzed by gas chromatography-mass spectrometry. The mass spectra were recorded on a JEOL JMS AX505H mass spectrometer operated in the electron ionization mode and equipped with Hewlett-Packard HP5890J gas chromatograph. Separation was achieved on a J&W Scientific (Folsom, CA) DB-1 capillary column (30 m by 0.32 mm id, 25 μm film thickness, liquid phase dimethyl polysiloxane). The column temperature was increased from 140 °C to 320 °C at the rate of 5 °C/min.

Monitoring of 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA) and CBA formation

Formation of chlorobiphenyl *meta*-cleavage products, HOPDAs, was monitored by visible spectral scanning of resting cell assays mixtures with a Hewlett-Packard

HP 8452A spectrophotometer. Absorption was measured at the λ_{max} of the individual HOPDAs. Accumulation of CBAs was analyzed by HPLC with a Hewlett-Packard series 1050 chromatograph equipped with a multiple wavelength detector set at 230 nm. Separation was achieved on a reversed-phase LiChrosorb RP-18 column (Alltech) of internal diameter 4.6 mm and length 250 mm with an aqueous solvent system containing 600 ml of methanol and 1 ml of 85% *ortho*-phosphoric acid. The flow rate was 1.5 ml/min. CBAs used as standards were obtained from Aldrich.

Results

Transformation of the congeners with one non-chlorinated ring

2-, 4-, and 2,4-CB were easily degraded by all four strains. Equimolar accumulations of the corresponding chlorobenzoic acids were detected in all cases with the exception of the transformation of 2-CB by *Burkholderia* sp. LB400 and *Rhodococcus* sp. RHA1, which yielded only 67 to 73% of the expected 2-CBA (Table 1). The appearance of yellow color with λ_{max} 394 nm during incubation of 2-CB with RHA1 indicated incomplete transformation and accumulation of HOPDA which could account for the difference in concentrations between consumed 2-CB and accumulated 2-CBA (Figure 1A). Some fading of the yellow color and a shift towards shorter wavelengths was detected during the next two days of incubation indicating further transformation of the *meta*-cleavage product; however, no significant increase of 2-CBA concentration was noted ($<$5%).

The dynamics of accumulation of 2-CBA by *Burkholderia* sp. LB400 suggested that it could further degrade this acid. Furthermore, subsequent resting cell experiments using 2-, 4-, and 2,4-CBA as substrates showed that this strain degraded from 40 to 60% of 2-CBA (500 μM) during 24 h of incubation, while no disappearance of 4-CBA and 2,4-CBA was detected. No metabolites of 2-CBA degradation were found by HPLC and no growth of LB400 in liquid media supplemented with this chlorobenzoate was detected. The three other strains showed no further metabolism of 2-, 4- or 2,4-CBA.

2,6-CB proved to be the congener most resistant to microbial attack (Table 1). No more than 5% of it was depleted by strains *R. erythreus* NY05 and *C. testosteroni* VP44 with subsequent accumulation of trace amounts of 2,6-CBA, and only 8–9% was degraded by strains *Burkholderia* sp. LB 400 and *Rhodococcus* sp. RHA1 with only 4% of initial 2,6-CB converted to 2,6-CBA.

Transformation of congeners containing chlorine on both biphenyl rings

Two congeners with a ring containing one chlorine in the *para* position (2,4′ and 2,4,4′) were efficiently degraded by *Burkholderia* sp. LB400 and *Rhodococcus* sp. RHA1 (Table 2). Formation of 4-CBA as the major product from both of these congeners suggested that their *ortho*-substituted rings were preferentially oxidized. Transient appearance of intense yellow color with a λ_{max} of 434 nm occurred during the degradation of 2,4,4′-CB by *Burkholderia* sp. LB400. A strict correlation between low λ_{max} (390–400 nm) or high λ_{max} (430–440 nm) and the presence or absence of chlorine substituents at an *ortho*-position in the HOPDA, respectively, was observed by Seeger et al. (1995). Thus, these data also indicate that LB400 primarily oxidized the *ortho*-substituted ring (Seeger et al. 1995). Accumulation of low amounts of 2,4-CBA among the degradation products of 2,4,4′-CB indicated that LB400 is capable of some oxidation of the *para*-chlorinated ring of this congener.

Depletion from 82 to 98% of 2,4′- and 2,4,4′-CB was also observed with *R. erythreus* NY05 and with *C. testosteroni* VP44; however, amounts of chlorobenzoates formed did not exceed 12% of the expected (Table 2). Disappearance of these congeners was accompanied by formation of very intense yellow color (Figure 1B). We could not quantitate chlorinated HOPDAs because standards are not available and there are no data on their molar extinction coefficients; however, the differences between consumed 2,4′- and 2,4,4′-CB and produced CBAs, which were 88–95%, are likely attributable to the formation of HOPDAs. The trimethylsilated (TMS) metabolite of 2,4-CB was characterized by molecular ion at m/z 430, low abundance M^+-15 and M^+-35 ions and by a prominent ion M^+-117 arising from the loss of CH_3, Cl, and COOTMS from the molecular ion, respectively. The same fragmentation pattern was obtained for the metabolite of 2,4,4′-CB (molecular ion 464). The mass spectral features of these compounds are in good agreement with previously published mass spectra of the TMS derivatives of 3-chloro-2-hydroxy-6-oxo-(2-chlorophenyl)hexa-2,4-dienoate and 3-chloro-2-hydroxy-6-oxo-(2,4-dichlorophenyl)hexa-2,4-dienoate

Table 1. Transformation of the individual congeners containing chlorine on one biphenyl ring by resting cells of *R. erythreus* NY05, *C. testosteroni* VP44, *Rhodococcus* sp. RHA1 and *Burkholderia* sp. LB400

Congener	Initial conc. (μM)	Disappearance of PCB (%)[a,b]				PCB recovered as CBA (%)[a,b]			
		NY05	VP44	RHA1	LB400	NY05	VP44	RHA1	LB400
2-CB	500	92 ± 4	93 ± 5	99 ± 1	94 ± 4	2-CBA 90 ± 1	2-CBA 94 ± 3	2-CBA 66 ± 7	2-CBA 69 ± 6
4-CB	500	97 ± 3	95 ± 2	96 ± 3	98 ± 0	4-CBA 93 ± 2	4-CBA 96 ± 3	4-CBA 94 ± 2	4-CBA 98 ± 3
2,4-CB	200	93 ± 1	95 ± 1	100 ± 0	97 ± 1	2,4-CBA 99 ± 3	2,4-CBA 95 ± 2	2,4-CBA 94 ± 3	2,4-CBA 93 ± 5
2,6-CB	50	3 ± 2	5 ± 1	8 ± 1	9 ± 2	2,6-CBA <1	2,6-CBA <1	2,6-CBA 4 ± 3	2,6-CBA 4 ± 2

[a] Biphenyl grown cells were incubated for 24 h in K1 medium containing individual congeners.

[b] Data values are averages of triplicate samples from two separate resting cells assays ± standard deviation.

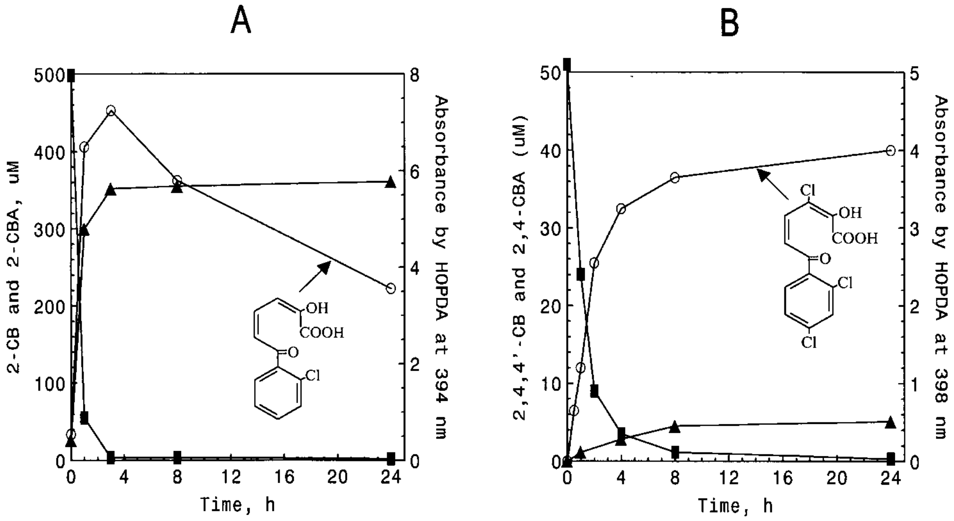

Figure 1. Degradation of 2-CB (A) and 2,4,4′CB (B) by resting cells of *Rhodoococcus* sp. RHA1 and *Rhodococcus eryhreus* NY05, respectively (■), and accumulation of the corresponding chlorobenzoates (▲) and HOPDAs (○). The resting cells were incubated in K1 mineral medium containing 500 μM of 2-CB or 50 μM of 2,4,4′-CB. Absorbances by HOPDAs were measured at their respective λ_{max}'s.

produced by some bacterial strains (Furukawa et al. 1979a, 1979b). Absorption maxima of these HOPDAs at λ_{max} 398 nm also indicated that they have the chlorine substituent at the *ortho*-position (Seeger et al. 1995) and therefore were formed by the oxidation of the *para*-chlorinated ring. Further transformation of the *meta*-cleavage products formed from 2,4′-, and 2,4,4′-CB should result in accumulation of 2-CBA and 2,4-CBA, respectively. Both strains yielded from 5 to 7% of 2,4-CBA from 2,4,4′-CB, but only NY05 produced about 2% of 2-CBA from 2,4′-CB. Recovery of about 5% of 2,4′-CB as 4-CBA by both strains showed that they could also oxidize the *ortho*-chlorinated ring (Table 2).

Efficient degradation (86–100%) of 2,2′- and 2,2′,4-CB containing chlorine in one *ortho*-position on each ring was observed with *Burkholderia* sp. LB400 and *Rhodococcus* sp. RHA1 (Table 2). Both strains transformed 2,2′,4-CB into the equimolar amounts of 2,4-CBA, and 57–76% of 2,2′-CB was recovered as 2-CBA. The strain RHA1 accumulated a yellow *meta*-cleavage product (λ_{max} = 394) from 2,2′-CB which could account for the difference in the depleted CB and accumulated 2-CBA. Non-equimolar accumulation of 2-CBA by strain LB400 could be due to the capability of this strain to further transform this compound as noted above. Activities of strains *R. erythreus* NY05 and *C. testosteroni* VP44 towards 2,2′- and 2,2′,4-CB were much lower (7–25%) (Table

2). Depleted 2,2′- and 2,2′,4-CB were transformed by these strains into equimolar amounts of 2-CBA and 2,4-CBA.

Stability of HOPDAs formed from 2,4′- and 2,4,4′-CB

No significant decrease of the HOPDAs formed from 2,4′- and 2,4,4′-CB by *R. erythreus* NY05 and *C. testosteroni* VP44 nor a corresponding increase of the chlorobenzoic acids were detected during another 72 h of incubation suggesting that the *meta*-cleavage products were quite stable. No further degradation of these HOPDAs was detected during a 12 h incubation with freshly prepared resting cells of NY05, VP44, RHA1 and LB400, indicating that inability of cells to degrade these compounds was not due to their starvation for some other factor.

Transformations of PCB mixtures typical of anaerobic dechlorination products

Three strains (NY05, RHA1 and LB400) were used in resting cell experiments with mix M and C. *Ortho*-directed strains RHA1 and LB400 were capable of depleting 65 and 78% of PCBs in mix M and 70 and 76% in mix C, respectively, and yielding 73 to 95% of degraded PCBs as chlorobenzoates (Table 3, 4). Lower efficiencies of degradation were observed with *para*-directed strain NY05 that depleted only 41% of PCBs from mix M and 61% from mix C with recovery of 90% of degraded PCBs as chlorobenzoates (Table 3, 4). The di-*ortho*- and especially di-*para*-substituted PCB congeners 2,2′-/2,6-CB and 2,4,4′-CB were the most persistent congeners in mixtures M and C.

Degradation of several congeners was affected when they were supplied in mixtures (Table 3, 4). In contrast to 81–86% of degradation of 2,4,4′-CB (50 μM) supplied individually, only 18–22% of this congener was degraded in mix M (54 μM of 2,4,4′-CB) by RHA1 and LB400. Interestingly, the *para*-directed strain NY05 that catalyzed complete degradation of individually supplied 2,4,4′-CB, also could not deplete more than 20% of the congener from mix M. More efficient degradation of 2,6-/2,2′-CB was observed in mix M than in mix C with both RHA1 and LB400 (Table 3, 4). 2,2′- and 2,6-CB have different ECD response factors and coelute as a single peak, but their relative degradation can be additionally evaluated by the amounts of 2- and 2,6-CBA formed. While expected amounts of 2,6-CBA were produced from mixtures, only 82 and 74% of expected 2-CBA was produced by strain RHA1 from mix M and C,

Table 2. Degradation of PCB congeners containing chlorine on both biphenyl rings by resting cells of *R. erythreus* NY05, *C. testosteroni* VP44, *Rhodococcus* sp. RHA1 and *Burkholderia* sp. LB400

Congener	Initial conc. (μM)	Disappearance of PCB (%)[a,b]				PCB recovered as CBA (%)[a,b]				Accumulation of HOPDA[c]			
		NY05	VP44	RHA1	LB400	NY05	VP44	RHA1	LB400	NY05	VP44	RHA1	LB400
2,4′-CB	100	83 ± 5	82 ± 5	97 ± 1	94 ± 3	4-CBA 8 ± 2 2-CBA 2 ± 1	4-CBA 4 ± 2	4-CBA 97 ± 3	4-CBA 87 ± 4	+++++ λ_{max} = 396	+++++ λ_{max} = 396	ND	ND
2,4,4′-CB	50	98 ± 1	94 ± 5	81 ± 6	86 ± 4	2,4-CBA 7 ± 3	2,4-CBA 5 ± 1	4-CBA 74 ± 6	4-CBA 63 ± 5 2,4-CBA 4 ± 1	+++++ λ_{max} = 398	+++++ λ_{max} = 398	ND	+ λ_{max} = 437
2,2′-CB	100	11 ± 3	25 ± 2	86 ± 6	100 ± 1	2-CBA 9 ± 3	2-CBA 22 ± 4	2-CBA 76 ± 5	2-CBA 57 ± 4	ND	ND	++ λ_{max} = 394	ND
2,2′,4-CB	50	7 ± 1	7 ± 3	100 ± 0	93 ± 2	2,4-CBA 6 ± 2	2,4-CBA 3 ± 1	2,4-CBA 97 ± 4	2,4-CBA 89 ± 3	ND	ND	ND	ND

[a] Biphenyl grown cells were incubated for 24 h in K1 medium containing individual congeners.

[b] Data values are averages of triplicate samples from two separate resting cells assays ± standard deviation.

[c] + to +++++ reflects relative intensity of absorption at the indicated wavelength; ND – not detected.

Table 3. Degradation of mix M and accumulation of chlorobenzoates by resting cells of *Rhodococcus erythreus* NY05, *Rhodococcus* sp. RHA1, and *Burkholderia* sp. LB400.^{a}

Strain	PCB congeners degraded from mix M^b (μM)							PCB recovered as CBA^b (μM)			
	2-CB 80 μM^c	4-CB 21 μM^c	2,2′-/2,6-CB 144 μM^c	2,4-CB 14 μM^c	2,4′-CB 123 μM^c	2,2′,4-CB 64 μM^c	2,4,4′-CB 54 μM^c	2-CBA	4-CBA	2,4-CBA	2,6-CBA
NY05	80 ± 0	21 ± 0	10 ± 4	14 ± 0	68 ± 6	3 ± 2	9 ± 1	121 ± 5	42 ± 3	20 ± 1	< 1
RHA1	80 ± 1	21 ± 0	58 ± 2	14 ± 0	117 ± 4	25 ± 3	10 ± 3	141 ± 6	132 ± 8	33 ± 2	3 ± 2
LB400	80 ± 0	21 ± 0	82 ± 6	13 ± 1	119 ± 4	59 ± 5	12 ± 3	121 ± 6	141 ± 8	49 ± 2	5 ± 2

[a] Biphenyl grown cells were incubated for 24 h in K1 medium containing mix M (500 μM).
[b] Data values are averages of triplicate samples from two separate resting cells experiment ± standard deviation.
[c] Initial concentration of the congener in mix M.

Table 4. Degradation of mix C and accumulation of chlorobenzoates by resting cells of *Rhodococcus erythreus* NY05, *Rhodococcus* sp. RHA1, and *Burkholderia* sp. LB400.^{a}

Strain	PCB congeners degraded from mix C^b (μM)							PCB recovered as CBA^b (μM)			
	2-CB 250 μM^c	4-CB 25 μM^c	2,2′-/2,6-CB 200 μM^c	2,4-CB 5 μM^c	2,4′-CB 10 μM^c	2,2′,4-CB 5 μM^c	2,4,4′-CB 5 μM^c	2-CBA	4-CBA	2,4-CBA	2,6-CBA
NY05	250 ± 0	25 ± 0	18 ± 5	5.0 ± 0	6.0 ± 0.8	0.4 ± 0.3	0	236 ± 4	27 ± 2	5 ± 2	< 1
RHA1	248 ± 2	22 ± 3	64 ± 1	4.0 ± 0.3	10 ± 0	2.4 ± 0.2	0.4 ± 0.1	279 ± 2	31 ± 6	4 ± 2	2 ± 1
LB400	250 ± 0	25 ± 0	86 ± 3	4.3 ± 0.4	8.4 ± 0.7	4.0 ± 0.7	1.5 ± 0.1	225 ± 10	41 ± 4	7 ± 3	4 ± 3

[a] Biphenyl grown cells were incubated for 24 h in K1 medium containing mix C (500 μM).
[b] Data values are averages of triplicate samples from two separate resting cells experiment ± standard deviation.
[c] Initial concentration of the congener in mix C.

respectively, suggesting that degradation of 2,2′-CB was affected in mixtures. The *Rhodococcus* strains NY05 and RHA1 that accumulated HOPDA during the transformation of several individual congeners, also produced HOPDAs from mixes M and C.

Discussion

Strains RHA1 and LB400 readily degraded seven of the eight congeners supplied individually; only 2,6-CB was poorly degraded, while strains VP44 and NY05 exhibited low activities toward di-*ortho*-substituted congeners which are poorly degradable by most bacterial strains (Bedard et al. 1986; Furukawa 1982). Contrary to conclusions that could be drawn from previous reports (Pellizari et al. 1996; Seto et al. 1995) our results show that the degradative competence of the strain *C. testosteroni* VP44 was similar to *R. erythreus* NY05, while the degradative competence of the strain *Rhodococcus* sp. RHA1 was similar to that of *Burkholderia* sp. LB400. Thus, strain VP44 that showed 92% degradation of 2,2′-CB in previous experiments with defined PCB mixtures could not deplete more than 25% of the congener in our assay. On the other hand, LB400 that is described as the strain with limited activity towards di-*para* chlorinated congeners depleted up to 86% of 2,4,4′-CB in our experiments. Lower than previously reported rates of degradation of 2,2′-CB by VP44 (Pellizari et al. 1996) and 2,6-CB by RHA1 and LB400 (Seto et al. 1995; Williams et al. 1997) are probably due to the 10–20 times higher concentrations of these congeners in our experiments. Product profiles obtained from congeners containing chlorine on both biphenyl rings indicated that strains NY05 and VP44 preferentially attack *para*-substituted biphenyl rings while RHA1, similar to LB400 (Bedard & Habert 1990), preferentially oxidized *ortho*-substituted rings.

Another difference between *ortho*- and *para*-directed strains is in the final products formed from 2,4′ and 2,4,4′-CB. An extensive transformation of these congeners into CBAs was observed with *ortho*-directed strains RHA1 and LB400, whereas *para*-directed strains transformed only 5–10% of these congeners into CBAs and accumulated HOPDAs as the major products. The *meta*-ring-fission product was also noted as the major metabolite produced by oxidation of the *para*-chlorinated ring of 2,4′- and 2,4,4′-CB by *Acinetobacter* sp. P6 and *Alcaligenes* sp. Y42 (Furukawa et al. 1979a, 1979b). Bedard and Haberl

(1990) showed that the four strains that preferentially oxidized the *para*-substituted ring of 2,4′-CB transformed less that 15% of this CB into CBAs and formed HOPDAs as the major metabolite while the two *ortho*-directed strains degraded this congener to 4-CBA in 90–100% yield. It is known that HOPDAs produced by oxidation of *para*-chlorinated rings of 2,4′-CB can be converted to chloroacetophenones by radiation with a spectral distribution similar to that of sunlight (Baxter & Sutherland 1984). In contrast to CBAs, chlorinated acetophenones are highly toxic, do not support growth of pure cultures, and tend to form toxic chlorophenols (Havel & Reineke 1993; Higson & Focht 1990). Thus, another advantage of the strains that attack the *ortho*-chlorinated ring of 2,4′- and 2,4,4′-CB is that they do not form persistent isomers of HOPDA but transform these congeners into equimolar amounts of CBAs. The latter can be subsequently degraded by indigenous microflora or by constructed bacteria with combined biphenyl and chlorobenzoate pathways (Brenner et al. 1994; Reineke 1998).

Similar to the results with individual congeners, strains LB400 and RHA1 were more efficient than *para*-directed strain NY05 in degradation of the defined mixtures M and C. Efficiency of degradation of 2,2′- and 2,4,4′-CB was affected when they were supplied in mixtures, possibly due to the presence of easily degradable congeners and higher total PCB concentrations (Table 3, 4). In the only comparable investigation, *Alcaligenes eutrophus* strain H850, also an *ortho*-directed strain (Bedard et al. 1987a), depleted 81% of 10 ppm Aroclor 1242 anaerobic dechlorination products (pattern C) extracted from the Hudson River sediments (Bedard et al. 1987b). Production of chlorobenzoates was not monitored in that work (Bedard et al. 1987b).

Thus, *ortho*-directed bacteria exhibited higher rates of degradation of dechlorinated Aroclor 1242 with greater production of CBAs than *para*-directed strains and are advantageous to use in the aerobic PCB bioremediation stage. Our results clearly demonstrate that information on depletion of PCBs in Aroclors or in defined mixtures supplied at low concentrations does not allow unequivocal identification of bacteria with desired specificity of the primary attack. Since degradation rate of the individual congener can vary depending on composition (Bedard et al. 1986, 1987b; Billingsley et al. 1997; Seto et al. 1995) and concentration of PCB mixtures (Bedard et al. 1986, 1987a), mixtures that simulate composition and concentration of anaerobic dechlorination products should be used in screening and any testing of proposed bioremediation processes. To easily discriminate the specificity of the primary PCB attack and select the *ortho*-directed bacteria, we suggest using a suite of *ortho* plus *para*-substituted congeners, specifically 2,4′-, 2,2′,4- and 2,4,4′-CB, and screening for accumulation of chlorobenzoates and *meta*-cleavage products. The advantage of this assay is that it is fast and does not depend on PCB concentrations. Selected *ortho*-directed strains should then be analyzed for degradation of defined PCB mixtures that simulate anaerobic dechlorination products of Aroclor 1242 or higher chlorinated Aroclors.

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