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ORIGINAL PAPER

Cometabolic degradation of polychlorinated biphenyls (PCBs) by axenic cultures of *Ralstonia* sp. strain SA-5 and *Pseudomonas* sp. strain SA-6 obtained from Nigerian contaminated soils

Sunday Adekunle Adebusoye · Matthew O. Ilori · Flynn W. Picardal · Olukayode O. Amund

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Abstract Substantial metabolism of 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-tetraCB) and 2,3′,4′,5-tetraCB by axenic cultures of *Ralstonia* sp. SA-5 and *Pseudomonas* sp. SA-6 was observed in the presence of biphenyl supplementation, although, the strains were unable to utilize tetrachlorobiphenyls as growth substrate. The former was more amenable to aerobic degradation (~70% degradation) than the latter (22–45% degradation). Recovery of 2,5-chlorobenzoic acid and chloride from 2,3′,4′,5-tetraCB assay is an indication of initial dioxygenase attack on the 3,4-dichlorophenyl ring. The PCB-degradative ability of both strains was also investigated by GC analysis of individual congeners in Aroclor 1242 (100 ppm) following 12-day incubation with washed benzoate-grown cells. Results revealed two different catabolic properties. Whereas strain SA-6 required biphenyl as inducer of the degradation activity, such induction was not required by strain SA-5. Nearly all the detectable congeners in the mixture were extensively degraded (% reduction in ECD area counts for individual congeners ranged from 50.0 to 100% and 14.2 to 100%, respectively, for SA-5 and SA-6). The two strains exhibited no noticeable specificity for congeners with varying numbers of chlorine substitution and positions. The degradative competence of these isolates most especially SA-5 makes them among the most versatile PCB-metabolizing organisms yet reported.

S. A. Adebusoye (✉) · M. O. Ilori · O. O. Amund
Department of Botany and Microbiology, Faculty of Science, University of Lagos, Akoka, Yaba, Lagos, Nigeria
e-mail: sadebusoye@yahoo.com

S. A. Adebusoye · F. W. Picardal
Environmental Science Research Center, School of Public and Environmental Affairs, Indiana University, Bloomington, IN 47405, USA

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Abbreviations

CBs	Monochlorobiphenyls
CBA	Chlorobenzoic acid
diCBs	Dichlorobiphenyls
ECD	Electron capture detector
hexaCBs	Hexachlorobiphenyls
HMN	Heptamethylnonane
HOPDA	2-Hydroxy 6-oxo-6-phenylhexa 2,4-dienoic acid
MS	Mineral salts
PCBs	Polychlorinated biphenyls
tetraCBs	Tetrachlorobiphenyls
triCBs	Trichlorobiphenyls

Introduction

Despite active research spanning more than three decades, extensive regulatory actions, and an effective ban on their production since the late 1970s, polychlorinated biphenyls (PCBs) remain a focus of research and environmental attention. Polychlorinated biphenyls are among the most persistent and toxic substances in the environment (Sawhney 1986; Erickson 2001). Their presence is a cause for great concern because they are suspected of having adverse effects on human and animal health. The high cost and public opposition to current physical and chemical remediation technologies have stimulated interest in the use of microorganisms for bioremediation of PCB-contaminated sites (Hooper et al. 1990; Shannon et al. 1994;

Unterman et al. 1998). Quite unfortunately, no effective bioremediation strategies have yet been developed for the restoration of sites contaminated with PCBs, but the progress made in recent years has been sufficiently encouraging to lead investigators to believe that bacterial strains with enhanced capacity to degrade these pollutants can be developed.

Polychlorinated biphenyl degradation is complicated because they are produced as complex mixtures (Abramowicz 1990; Erickson 1997), with individual congeners exhibiting varying effects on the scavenging organism. PCB-degrading microorganisms vary with respect to the congeners they metabolize. This may be due to differences in genes encoding their PCB degrading enzymes. Some organisms do not transform PCBs that contain more than three chlorines, whereas, other strains, such as *Burkholderia* sp. LB400 (now *Burkholderia xenovorans* LB400), transform up to hexachlorobiphenyls (hexaCBs) (Bopp 1986; Abramowicz 1990). Bedard et al. (1987) documented extensive degradation of PCB commercial mixtures of Aroclors and environmentally transformed PCBs by *Alcaligenes eutrophus* H850. According to them, as many as 49 of the congeners identified in Aroclor 1242 were degraded, albeit to different extents. Owing to their exceptional abilities to attack a wide range of PCB congeners, *Burkholderia xenovorans* LB400, *Alcaligenes eutrophus* H850 and *Rhodococcus* sp. RHA1 are three of the most promising strains for use in PCB biotreatment process, especially in the aerobic stage. Consequently, the catabolic functions of these organisms have been extensively studied (Bedard and Haberl 1990; Hurtubise et al. 1998; Arnett et al. 2000; Sakai et al. 2003; Takeda et al. 2004; Rodrigues et al. 2006). However, these organisms have limited substrate range in terms of ability to utilize pure congeners as growth substrate. Therefore, practical application of such microorganisms for cleanup of polluted systems would require repeated inoculation to maintain the allochtonous population (Rodrigues et al. 2006) otherwise; biphenyl supplementation would be required as growth substrate thereby adding to the cost of bioremediation.

Until recently, it was generally believed that only monochlorobiphenyls (CBs) function as a sole source of carbon and energy. Bacterial strains capable of growth on CBs and dichlorobiphenyls (diCBs) would be effective in degrading environmental mixtures of PCBs with the potential to offset the anticipated problems with the use of strain LB400 or H850. Interestingly, we have reported exceptional abilities of some bacterial strains obtained from tropical soils to grow with several congeners of CBs and diCBs (Adebusoye et al. 2004, 2007b) as well as several congeners of chlorobenzenes and natural compounds (Adebusoye et al. 2007a). In the current study, we report extensive degradation of Aroclor 1242 containing predominantly triCBs and tetrachlorobiphenyls (tetraCBs) by two of these bacterial strains namely, *Ralstonia* sp. SA-4 and *Pseudomonas* sp. SA-5, both exhibiting different metabolic potentials. To the best of our knowledge, there has not been any previous report on the degradation of PCB mixtures by bacteria that are indigenous to the African environment, despite the fact that most environments in this continent are widely polluted with this group of xenobiotics without appropriate regulatory acts to monitor their discharge into the environment.

Materials and methods

Chemicals and stock solutions

Analytical grades of high purity (99–100%) PCB congeners and mixtures were supplied by AccuStandard Inc. (New Haven, CT. USA). 2,5-chlorobenzoic acid (98% purity) and biphenyl (>99% purity) were procured from Sigma-Aldrich Corp. (St. Louis, MO. USA) while 2,2,4,4,6,8,8-heptamethylnonane (HMN; >99% purity) was obtained from Fisher Scientific Co. (Springfield, NJ. USA). Methanol, ethylacetate and acetonitrile which were all HPLC grade were also purchased from Fisher Scientific.

A stock solution of each of the congeners and Aroclor 1242 was prepared in HMN–a non-biodegradable carrier (Adebusoye et al. 2007a, b) to improve mass transfer into the growth medium.

Bacterial strains

Ralstonia sp. SA-5 and *Pseudomonas* sp. SA-6 were isolated by enrichment on Askarel electrical transformer fluid (a blend of PCBs and chlorinated benzenes) following several repeated transfers (Adebusoye et al. 2007a). Strain SA-6 originated from a soil contaminated with electrical transformer fluid while SA-5 was obtained from a dumpsite. Both sites have been heavily contaminated with PCBs and/or PCB-containing materials for several decades. The two isolates were identified by 16S rRNA gene analysis coupled with phenotypic profiling using API 20E test systems. The 16S rRNA gene sequences have been deposited in the GenBank database under accession numbers DQ854845 and DQ854841, respectively, for SA-5 and SA-6. The organisms have been preserved in nutrient broth amended with 0.05% yeast extract and 20% glycerine at –80°C. Purity was controlled by frequent plating on nutrient agar and/or mineral salts (MS) agar supplemented with 2.5 mM benzoate.

Media and inoculum

Degradation experiments were conducted in chloride-free MS medium described previously (Adebusoye et al. 2007b). Organisms were grown on MS medium supplemented with 2.5 mM benzoate at 25°C on an orbital shaker incubator (100 rpm; Labline Instruments Inc.). Cells were harvested by centrifugation after 24 h incubation, washed twice in sterile MS medium lacking benzoate, and resuspended in the same medium.

Cometabolism of PCBs

Organisms were grown in MS medium (6 ml) in Balch tubes with adequate headspace for aeration. To each tube, selected PCB congeners were added from the stock to a final concentration of 100 ppm. The tubes were further amended with equal proportion of biphenyl, crimp-sealed with Teflon-coated stoppers, and incubated horizontally on an orbital shaker table (100 rpm) at 25°C for 10–12 days. Separate set of tubes containing tetraCBs with cells, but without biphenyl supplementation as well as tubes containing both xenobiotic but lacking cells served as control. Degradation of Aroclor 1242 was set up in similar manner in two sets of tubes. One set contained 100 ppm of the mixture and biphenyl while the other set contained only the PCB mixture. All data are means of samples from triplicate tubes for each experiment. Data reported are results from reproducible experiments.

PCB and metabolite analyses

The entire content of the reaction tubes (6 ml) were extracted once with 5 ml of hexane. The extracts were analyzed on an HP 5890 Series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA, USA) fitted with an electron capture detector, a fused silica capillary column (J & W Scientific, Folsom, CA, USA; 30 m by 0.53 mm id) coated with a 2.5-μm bonded liquid phase of Durabond-5, and HP 3396 Series II Integrator. The carrier gas was helium, with a linear velocity of 30 cm/s, while the makeup gas was argon. Samples (1 μl) were chromatographed by using a temperature program which was held at 70°C for 1 min, then raised to 160°C at 30°C/min, then to 200°C at 2°C/min for 1 min, and finally raised to 250°C at 10°C/min for 14 min. The inlet and detector temperatures were 200 and 275°C, respectively. Percent degradation was calculated by determining the net decrease (in ECD area counts) in residual PCB concentration in experimental cultures, compared with that of the non-inoculated controls.

For identification of aqueous fractions, HPLC (Waters Corp., Milford Mass, USA) equipped with a UV, dual absorbance detector (Model 2487) was used. Separation was performed on a YMC-Pack ODS-AQ reversed-phase column (YMC Co. Ltd., Kyoto, Japan). Chloride was similarly measured via the same HPLC, but using a conductivity detector (Model 432) and IonPac AS17 analytical column.

Results and discussion

The abilities of *Ralstonia* sp. strain SA-5 and *Pseudomonas* sp. strain SA-6 to grow on broad and unusual spectrum of xenobiotic compounds including some congeners of diCBs, triCBs, di- and trichlorobenzenes as well as benzoate and naphthalene have been previously demonstrated (Adebusoye et al. 2004, 2007a, b). It was equally shown that growth of these strains were not sustainable on any tetraCBs tested. However, in the present study, we observed cometabolism of tetraCB congeners by axenic cultures of the organisms in the presence of biphenyl supplementation as the growth substrate. Results displayed in Table 1 showed that 2,3,4,5-tetraCB was degraded by 21.84 ± 5.26% and 45.10 ± 16.88% by SA-5 and SA-6, respectively, after a 10-day incubation period. No chloride was recovered from the culture fluids suggesting oxidative attack on the unsubstituted phenyl moiety. Contrary to popular held views (Furukawa et al. 1979; Abramowicz 1990; Sylvestre and Sondossi 1994), 2,3′,4′,5-tetraCB was

Table 1 Degradation of 2,3,4,5-tetraCB in the presence of biphenyl by *Ralstonia* sp. strain SA-5 and *Pseudomonas* sp. strain SA-6

Isolate	Incubation period (day)	Initial dioxygenase attack[a]	% Degradation	Primary catabolic product	Chloride released (day)[b]	% Mineralization[c]
SA-5	10	2,3 position	21.84 ± 5.26	2,3,4,5-CBA	0	N/D
SA-6	10	2,3 position	45.10 ± 16.88	2,3,4,5-CBA	0	N/D

[a] Determined on the basis of the identity of the primary metabolic product

[b] Chloride was not detected during analysis of the culture media, indicating that the bacterial dioxygenase exclusively attacked the non-chlorinated aromatic ring

[c] Not determined, since the CBA metabolite recovered was not quantified

Data values are averages of triplicate samples ± standard deviation. Both the congener and biphenyl substrates were supplied at an equal concentration of 100 ppm

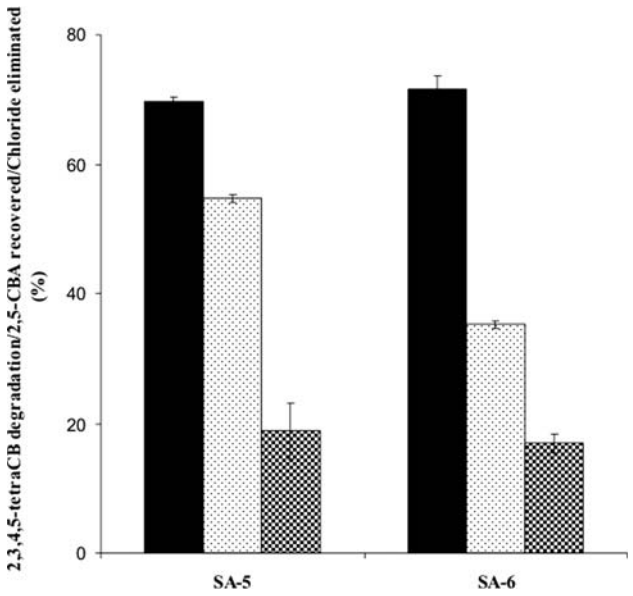

Fig. 1 Degradation of 2,3′,4′,5-tetraCB (■), 3,4-CBA recovered (□) and, chloride eliminated (▩) by bacterial strains. Percent degradation represents the net decrease (in ECD area counts) in experimental cultures, compared with that of the non-inoculated controls. Values presented are means of triplicate samples. Both the PCB substrate biphenyl were supplied in equal proportion at 100 ppm and incubated with each organism for 10 days

more amenable to aerobic degradation than 2,3,4,5-tetraCB substituted on one ring only. From the data summarized in Fig. 1, nearly 70% of the former was transformed by both organisms. Recovery of 2,5-dichlorobenzoic acid (2,5-CBA) from the culture media is obviously an indication of initial dioxygenase attack on the 3,4-dichlorophenyl ring. 2,3′,4′,5-tetraCB was cometabolically transformed to 2,5-CBA in near-stoichiometric amount (55%) only in tubes inoculated with SA-5 (Fig. 1). The quantities of chloride released from this isomer into the culture media generally fell below 20% of the expected for total mineralization of the 3,4-dichlorophenyl ring. Our inability to achieve a mass balance between the substrate transformed and the 2,5-CBA and chloride recovered could in part be attributed to incomplete transformation of the PCB substrate to CBA (Furukawa et al. 1979, 1983; Yagi and Sudo 1980), or that the CBA formed was further metabolized to intermediate chlorinated products (Adebusoye et al. 2007b). In the former, the results would suggest that at least some of the enzymes responsible for the various steps of the upper degradation pathway are far less tolerant of chlorines than the initial 2,3-dioxygenase. Early indication of transformation of 2,3′,4′,5-tetraCB was a colour change of the culture fluid to light yellow, particularly in tubes inoculated with SA-6. By interpretation, the PCB was metabolized through a *meta*-cleavage pathway similar to those found in other microorganisms (Furukawa et al. 1978; Bedard et al. 1986; Commandeur et al. 1996). It is not unlikely that this *meta* product–2-hydroxy 6-oxo-6-phenylhexa 2,4-dienoic acid (HOPDA) accumulated in the culture media in addition to a number of other chlorinated products that can also be produced in varying amounts (Furukawa et al. 1983; Sondossi et al. 2004).

The data generated during cometabolic transformation of Aroclor 1242 are tabulated in Table 2. Although unambiguous identification of the individual peaks with the exception of CBs was not possible with available instrumentation due to co-elution of some congeners, the degree of chlorine substitution was assigned based on the relative retention times of eluting peaks and comparison with retention times of selected mono- through hexachlorobiphenyl standards. In the control tubes without inoculation, no apparent decrease of the substrate was observed. This indicates that the depletion of the PCB from the assay media was due to the catabolic activities of the isolates rather than to non-specific losses such as compound volatility or adsorption to the glass tubes. The bacterial strains exhibited different catabolic potentials. The catabolic properties of *Ralstonia* sp. SA-5 present an interesting phenomenon. This organism transformed virtually all detectable congeners in Aroclor 1242 more extensively than previously reported strains (Bedard et al. 1987; Boyle et al. 1992) without the need of biphenyl as inducer of the enzymes of the upper pathway. Variance analysis showed that results obtained from congener analysis of tubes without biphenyl amendment did not differ significantly ($P < 0.05$) from those obtained in biphenyl supplemented cultures. In contrast, congener analysis of tubes inoculated with strain SA-6 showed that transformation of the Aroclor was significantly enhanced in the presence of biphenyl. With the exception of peak numbers 1, 2, 7, and 48, SA-6 depleted the congeners by a minimum of 15.5% and a maximum of 42.1% more in the presence of biphenyl than without biphenyl as co-substrate (Table 2). This is quite surprising to us having demonstrated recently that this organism had the widest substrate spectrum amongst five bacterial strains isolated including SA-5 in terms of utilization of pure PCB congeners as sole substrate for carbon and energy sources (Adebusoye et al. 2004, 2007a). However, this may be a function of different catabolic pathways employed in degradation of the PCB mixture. Since degradation of PCB mixtures, generally results in the formation of isomeric mixtures of CBAs (which differ in substitution and configuration from the original congeners) and other chlorinated catabolic products all of which could have severe consequences on the degrading organism. Perhaps, some of these products were more toxic to strain SA-6 then SA-5, resulting in suicide inactivation of requisites of the pathway.

Table 2 Comparison of degradation of Aroclor 1242 by the two bacterial cultures

		Percent degradation[a]			
		Ralstonia sp. SA-5		*Pseudomonas* sp. SA-6	
Peak no.	No. of chlorine	Without biphenyl	With biphenyl	Without biphenyl	With biphenyl
1	1	71.0(20.4)	70.5(12.5)	100 (0)	100 (0)
2	1	100(0)	100(0)	100 (0)	100 (0)
3	2	77.1(17.3)	86.0(4.0)	34.0 (8.0)	85.3 (1.4)
4	2	–[b]	–	27.1 (11.5)	89.6 (16.8)
5	2, 3	91.9(1.5)	89.4(4.2)	26.5 (12.6)	90.6 (6.5)
6	2, 3	93.71.3)	92.5(4.6)	33.1 (11.5)	88.6 (2.0)
7	2, 3	98.9(1.4)	89.7(7.3)	95.0 (2.5)	88.0 (1.5)
8	2, 3	89.6(2.8)	87.5(4.6)	37.1 12.3)	87.9 (3.3)
9	3	100(0)	82.7(24.5)	33.1(10.5)	81.8 (15.8)
11	3	98.1(14.3)	76.2(6.7)	31..4 (10.9)	77.4 (4.3)
12	3	91.0(2.5)	88.4(5.3)	37.5 (12.8)	84.6 (5.2)
14	3	90.0(2.6)	87.2(4.8)	28 (13.0)	87.8 (3.7)
15	3	91.3(1.8)	87.9(5.1)	35.8 (13.2)	86.8 (4.9)
16	3	100(0)	100(0)	33.6 (15.4)	87.4 (4.3)
17	3, 4	69.8(11.1)	71.1(15.6)	19.7 (9.3)	67.2 (5.8)
18	3	92.7(2.0)	89.4(4.7)	17.1 (10.3)	89.2 (3.6)
19	3, 4	92.2(2.1)	89.8(4.8)	17.3 (5.0)	89.8 (3.4)
20	3	90.3(2.4)	96.8(9.4)	15.5 (8.9)	85.7 (4.8)
21	3	94.8(1.2)	88.7(5.5)	44.5 (7.6)	86.8 (4.6)
22	3	89.6(2.5)	83.3(4.2)	38.6 (12.1)	80.4 (2.2)
23	3, 4	91.3(2.2)	85.5(6.1)	39.6 (11.4)	88.7 (4.0)
24	4	80.3(6.8)	81.0(12.2)	–	–
25	4	91.6(12.4)	83.1(10.2)	24.6 (5.1)	79.3 (11.5)
27	4	90.2(2.3)	85.7(4.6)	42.1 (8.2)	85.7 (4.4)
28	4	90.0(2.4)	85.3(4.5)	42.1 (16.5)	86.0 (4.2)
30	4	90.4(2.0)	86.0(5.1)	14.2 (9.9)	85.7 (5.0)
32	4	–	–	18.4 (6.7)	60.3 (10.4)
34	4	84.1(7.4)	86.1(4.3)	19.5 (3.2)	66.7 (11.5)
35	4	91.6(2.1)	87.3(4.7)	25.3 (7.6)	87.7 (4.3)
36	4	83.0(4.8)	86.1(9.1)	23.7 (9.7)	76.5 (13.2)
37	4	–	–	27.8 (8.2)	71.5 (7.1)
38	4	91.0(2.0)	86.1(4.8)	41.8 (11.5)	86.8 (4.4)
39	4	90.2(2.1)	87.3(6.5)	21.2 (8.4)	85.5 (4.8)
41	4	80.4(3.4)	82.0(11.1)	18.4 (7.6)	70.5 (6.8)
43	4, 5	91.3(2.0)	90.8(13.3)	18.3 (8.0)	86.9 (4.1)
45	4, 5	–	–	15.1 (3.1)	73.4 (15.3)
46	5	89.0(1.3)	91.3(4.5)	19.5 (3.0)	83.7 (1.8)
47	4, 5	100(0)	92.9(6.5)	31.2 (10.9)	78.8 (16.9)
48	5	50 (3.9)	86.8(3.6)	80.0 (0)	50.0 (13.9)
50	4, 5	–	–	34.3 (5.0)	50.1 (11.2)
52	5	91.1 (1.9)	85.5(4.6)	15.8 (9.6)	85.1 (4.2)
53	4, 5	62.3 (11.3)	79.3(6.4)	–	–
55	5	63.1 (15.1)	81.1(9.8)	–	–

Table 2 continued

Peak no.	No. of chlorine	Percent degradation[a] Ralstonia sp. SA-5 Without biphenyl	With biphenyl	*Pseudomonas* sp. SA-6 Without biphenyl	With biphenyl
56	5, 6	92.6 (6.8)	80.8(2.9)	21.8 (10.9)	79.5 (6.2)
57	6	91.3 (2.0)	85.6(3.2)	33.2 (12.7)	85.2 (4.4)

[a] Percent degradation represents the net decrease (in ECD area counts) in experimental cultures, compared with that of the non-inoculated controls

[b] Peaks that were not reproducible from one GC run to another

Values presented are means of triplicate samples. Values in parentheses represent standard deviations as percentages of the means. The PCB commercial mixture was supplied with or without biphenyl amendment at an equal concentration of 100 ppm and incubated with each organism for 12 days. Chlorine number assignment was based on retention time on a DB-5 megabore capillary column

Thorough evaluation of the congeners in Table 2 for strain SA-5 shows that transformation of individual congeners was slightly higher in tubes without biphenyl compared to those with biphenyl supplementation. This is an evidence for unique and efficient dioxygenase system in this isolate and also bringing to focus the increasing concern about the toxicity of biphenyl to microorganisms (Rodrigues et al. 2006). Although contrary to our observations, several workers have investigated the addition of biphenyl to significantly enhance the biodegradation of PCB commercial mixtures (Clark et al. 1979; Baxter et al. 1975; Brunner et al. 1985; Bedard et al. 1987; Kohler et al. 1988; Mondello 1989; Seto et al. 1995). Therefore, the degradation dynamics of SA-5 could have resulted from combined toxicity of Aroclor 1242 and biphenyl. Caution should, therefore, be exercised in the use of biphenyl for the sole purpose of enhancing PCB degradation potentials of microorganisms.

Relatively similar levels of degradation were observed for most of the congeners making up the PCB mixture, which further show that both SA-5 and SA-6 are capable of transforming a variety of congeners with varying degrees and positions of chlorine substitutions. Interestingly, peak numbers 56 and 57 assigned to more than five chlorine substitution were nearly eliminated (80–93%) at rates similar to those peaks assigned to 2–3 chlorine substituents, an indication that increasing number of chlorine substitution on the biphenyl nucleus has little or no effect on the patterns of degradation exhibited by both strains. The early work of Furukawa et al. (1978) and several others thereafter (Bedard et al. 1986; Abramowicz 1990; Seto et al. 1995; Yadav et al. 1995; Pieper 2005) acknowledged that microbial degradation of PCBs decreases with increasing number of chlorine substituents. However, our results reinforced the conclusion of Arnett et al. (2000) that the specificity of biphenyl dioxygenase was determined by the relative positions of the chlorine substituents on the aromatic nucleus rather than the number of chlorines on the rings.

In previous studies, significant degradation of most of the individual congeners in Aroclor 1242 by *Alcaligenes eutrophus* H850 and *Burkholderia* sp. LB400 and even *Phanerochaete chrysosporium* have been reported (Bedard et al. 1987; Boyle et al. 1992; Yadav et al. 1995). However, unlike our isolates, especially SA-5, the organisms required the addition of biphenyl for inducing the degradation activity, showed selective degradation of some congeners and were only able to degrade the mixture at relatively low concentration. Most importantly, the results of our study demonstrate a more extensive transformation of the Aroclor in a manner never reported before. To a significant extent, the spectrum of PCB congeners that can be transformed by an organism is determined by the specificity of the biphenyl 2,3-dioxygenase, the enzyme that catalyzes the first step in the upper pathway (Furukawa et al. 1979; Hernandez et al. 1995; Arnett et al. 2000; Pieper 2005). Consequently, the extensive degradation of Aroclor components reported herein without any noticeable specificity towards degradation of differentially substituted congeners may be a direct consequence of the broad specificity of the strains' biphenyl dioxygenases due to relax regiospecificity for the position where molecular oxygen is added to the aromatic ring and to the ability to catalyze the addition of molecular oxygen at *ortho*-chlorinated positions. This inference is further corroborated by the unique and rare ability of the organisms to grow on di-*ortho*-substituted diCB congeners (Adebusoye et al. 2007b) and di-*ortho*-substituted triCB isomers (Adebusoye et al. 2006) as sole sources of carbon and energy.

Conclusion

We have reported the metabolism of individual PCB congeners in Aroclor 1242. Therefore, under extremely favourable conditions and with special microorganisms, aerobic degradation of highly substituted PCB congeners

may be possible, although such organisms may be rare in the environment. Our results provide evidence for unique dioxygenase activity in both SA-5 and SA-6 strains. We have not identified any products of this metabolism, however, study is underway to determine end product distribution of the Aroclor transformation reactions in order to determine tolerance level of other enzymes of the upper pathway to chlorine substituents.

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