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ENVIRONMENTAL BIOTECHNOLOGY

Growth on dichlorobiphenyls with chlorine substitution on each ring by bacteria isolated from contaminated African soils

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Abstract Until recently, it was generally believed that the presence of more than one chlorine substituent prevented chlorinated biphenyls from serving as a sole source of carbon and energy for aerobic bacteria. In this study, we report the isolation of three aerobic strains, identified as *Enterobacter* sp. SA-2, *Ralstonia* sp. SA-4, and *Pseudomonas* sp. SA-6 from Nigerian polluted soils, that were able to grow on a wide range of dichlorobiphenyls (diCBs). In addition to growing on all monochlorobiphenyls (monoCBs), the strains were all able to utilize 2,2′-, 2,4′-, and 2,3-diCB as a sole source of carbon and energy. With the exception of strain SA-2, growth was also sustainable on 3,3′-, and 3,5-diCB. Washed benzoate-grown cells were typically able to degrade 68 to 100% of the diCB (100 ppm) within 188 h, concomitant with a cell number increase of up to three orders-of-magnitude and elimination of varying amounts of chloride. In many cases, stoichiometric production of a chlorobenzoate (CBA) as a product was observed. During growth on 2,2′-, and 2,4′-diCB, organisms exclusively attacked an *o*-chlorinated ring resulting in the production of 2-CBA and 4-CBA, respectively. A gradual decline in the concentration of the latter was observed, which suggested that the product was being degraded further. In the case of 2,3-diCB, the unsubstituted ring was preferentially metabolized. Initial diCB degradation rates were greatest for 2,4′-diCB (11.2±0.91 to 30.3±7.8 nmol/min per 10^9 cells) and lowest for 2,2′-diCB (0.37±0.12 to 2.7±1.2 nmol/min per 10^9 cells).

Keywords Polychlorinated biphenyls · PCBs · Biodegradation

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Introduction

Polychlorinated biphenyls (PCBs) are toxic, long-lived, and widely distributed environmental pollutants (Safe 1992; Bruhlmann and Chen 1999). Despite their recalcitrant character, PCBs are known to undergo microbial transformation under both aerobic and anaerobic conditions by different metabolic pathways (Maltseva et al. 2000; Abraham et al. 2002). Anaerobic reductive dechlorination preferentially removes *meta* and *para* chlorines from highly chlorinated congeners resulting in accumulation of predominantly *ortho*- or *ortho*- and *para*-substituted congeners (Wiegel and Wu 2000; Bedard 2001; Dai et al. 2002). Unfortunately, *ortho*-substitution often also limits aerobic biodegradation (Furukawa et al. 1979; Abramowicz 1990; Maltseva et al. 2000; Dai et al. 2002). This may be as a result of inhibition of the enzyme responsible for biphenyl ring *meta*-cleavage, 2,3-dihydroxybiphenyl 1,2-dioxygenase, by *ortho*-chlorinated PCB metabolites. Such metabolites with high affinity for the *meta*-cleavage enzyme may lead to its suicide inactivation (Dai et al. 2002). Isolation of bacterial strains that can aerobically utilize dichlorobiphenyls (diCBs), particularly *ortho*-substituted diCBs, as sole carbon and energy sources may therefore be useful in

preventing their accumulation during either stimulated PCB bioremediation or natural attenuation in the environment.

Bacteria capable of either utilizing mono- and diCBs as growth substrates or degrading them as co-metabolic substrates often do so by oxidizing the unsubstituted aromatic ring (Ahmed and Focht 1973; Kim and Picardal 2000; Abramowicz 1990). Few studies have focused on the potential use of axenic cultures for the degradation of diCBs as a sole carbon and energy source. Hickey et al. (1992) reported the degradation of 2,5-diCB by *Pseudomonas* sp. strain UCR2 isolated from a multi-chemostat mating experiment between a chlorobenzoate (CBA)-degrader, *Pseudomonas aeruginosa* strain JB2, and a chlorobiphenyl degrader, *Arthrobacter* sp. strain B1Barc. Although growth was slow, the authors reported 48% mineralization of the 2,5-diCB substrate. Potrawfke et al. (1998) demonstrated utilization of 2,4′- and 2,3-diCB as growth substrates by *Burkholderia* sp. LB 400. While 2,3-diCB was mineralized via the central intermediate 3-CBA through the chlorocatechol pathway, 4-CBA produced from 2,4′-diCB was excreted in stoichiometric amounts. More recently, Kim and Picardal (2001) demonstrated growth on 2,2′- and 2,4′-diCB by two isolates from PCB-contaminated sewage sludge, strains SK-3 and SK-4. In all these studies, the organisms were able to utilize only a very limited range of substrates as sole carbon and energy sources, e.g., all three monoCBs and only one to two diCBs (Potrawfke et al. 1998; Kim and Picardal 2001). In addition, all isolates were obtained from contaminated soils obtained from temperate climates, and little is known about PCB-degrading isolates from tropical regions.

In this paper, we provide the first report of isolates from tropical, African soils that are able to utilize diCBs as a sole carbon and energy source. In addition to utilizing *o*-substituted congeners, these isolates are able to grow on a substantially wider diCB substrate range than previously reported species. Included is a strain of *Enterobacter*, a genus not usually associated with biodegradation of chlorinated organic compounds.

Materials and methods

Enrichment, isolation, and classification of PCB-degrading bacterial strains

The bacterial strains utilized in this study were isolated by liquid culture enrichment techniques on Askarel (a commercial blend of PCBs and polychlorinated benzenes) fluid [0.1% v/v in a mineral salts (MS) medium; Kim and Picardal 2001] and purified on solid medium as previously described (Yagi and Sudo 1980; Kim and Picardal 2000). The soil inoculum used for enrichments was obtained from an industrial dumpsite at Ojota, Lagos, Nigeria and PCB-contaminated soil at the National Electric power Authority (NEPA) Transformer Workshop, Ijora, Lagos, Nigeria. The Ijora soil has been heavily contaminated with PCB-containing, electrical transformer fluids for many decades. Isolates were classified on the basis of 16S rRNA gene analysis (Marchesi et al. 1998; Grindle et al. 2003), coupled with phenotypic characterization using the API 20 E test systems (bioMerieux Vitek, Hazelwood, MO, USA). For analyses of 16S rRNA genes, genomic DNAs were isolated from overnight cultures of isolates in MS medium supplemented with 2.5 mM benzoate using ultra clean DNA prep kit (MoBio Laboratories, Solana Beach, CA). Two eubacterial PCR primers, forward primer 63f and reverse primer 1387r, were used to amplify approximately 1,300 bp of the 16S rRNA gene (Marchesi et al. 1998). The resulting PCR-generated fragments were gel-purified and cloned into the PCR 2.1-TOPO plasmid vector (Invitrogen Life Technologies, Carlsbad, CA). Recombinant plasmids were *Eco*RI-digested to confirm the presence of rDNA inserts. Recombinant plasmids with correct rDNA inserts were subsequently sequenced on an ABI 3700 sequencer (AP Biotech) and were probed against the GenBank database with the BLAST algorithm.

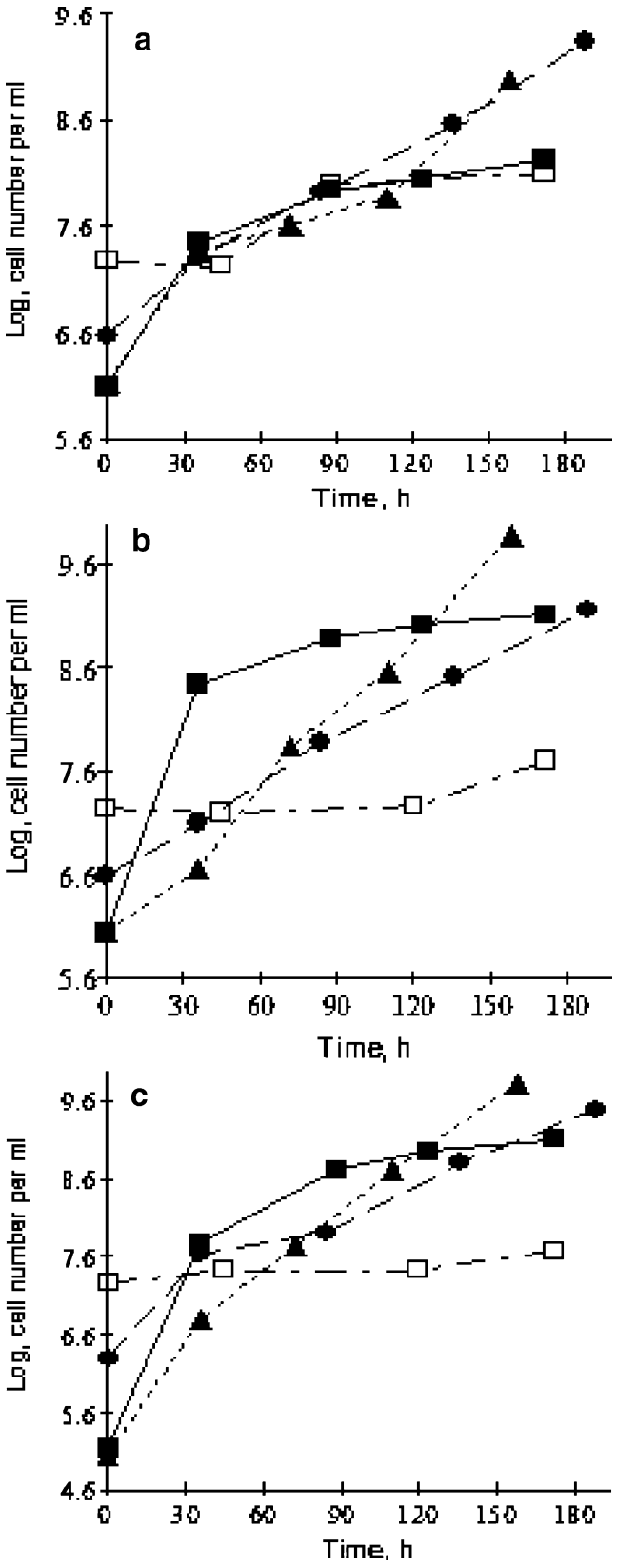
Enrichments and degradation experiments were conducted in a defined chloride-free MS medium consisting of 5 mM phosphate buffer (K_2HPO_4, KH_2PO_4; pH 7.3), 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$, and 0.076 g $Ca(NO_3)_2{\cdot}4H_2O$ in 1 l of deionized water supplemented with 0.1 ml each of trace minerals and vitamin solutions per liter (Kim and Picardal 2000). Stock solutions of relatively insoluble carbon sources, e.g., PCB congeners, biphenyl, or naphthalene, were prepared in heptamethylnonane (HMN) to improve mass transfer into the growth medium as previously described (Kim and Picardal 2000, 2001).

Chemicals

All PCB congeners were obtained from AccuStandard (New Haven, CI, USA) and had a purity of 99–100%. Standard solutions of PCBs (more than 95% purity), for the construction of standard curves, were purchased from Ultra Scientific (North Kingstown, RI, USA). Chlorobenzoic acids (CBAs) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Askarel fluid was generously provided by NEPA, Ijora, Lagos, Nigeria.

Degradation and growth studies

Growth rates and depletion of PCB congeners were determined in anaerobic culture (Balch) tubes containing 6 ml of MS medium, enough air headspace (21 ml) to maintain aerobic conditions over the course of the study,



◀ **Fig. 1** Growth dynamics of isolates SA-2 (**a**), SA-4 (**b**), and SA-6 (**c**) on diCB isomers. *Open square* Cell numbers in control tubes containing the HMN carrier but lacking diCBs, *shaded square* 2,2′-diCB, *shaded triangle* 2,4′-diCB, *shaded circle* 2,3-diCB. Error bars were eliminated to improve clarity. Typical standard deviations observed fell within 20 to 40% of the mean values plotted

and the selected carbon source. PCB congeners were added in 20-μl aliquots from the appropriate HMN stock solutions to a final concentration of 100 ppm. Tubes were inoculated with washed cells of cultures pregrown on 2.5 mM benzoate and were crimp-sealed with Teflon-coated stoppers to prevent losses from volatilization and/or sorption. Sealed tubes were incubated horizontally at room temperature (25°C) on a shaker table (100 rpm).

In our systems, growth was defined as concomitant degradation of the substrate, production of chloride and other metabolites, and increases in cell numbers greater than those experienced in control tubes lacking substrate. Co-metabolic substrate degradation was ruled out since substrates tested were of high purity, did not contain biphenyl, and biphenyl was not used as a substrate for inoculum growth. Substrate utilization was determined by sacrificing a whole set of three replicate tubes at each time point by the addition of 5 ml of hexane. Since complete tubes were sacrificed at each time point, slight differences in degradation rates between tubes sometimes resulted in sizeable error bars when displaying the data in Figs. 2, 3, and 4. After mixing for 12 h on a tube rotator, the hexane extract was removed for analysis of PCB congeners and the aqueous phase was frozen for subsequent analysis of chloride and chlorobenzoate products. Cell density was monitored in additional replicate tubes using an acridine orange direct counting method (Kepner et al. 1994). Mean initial degradation rates were calculated over the first 36 h and expressed as nanomole per minute per 10^9 cells.

Analytical procedures

Residual PCB congeners were analyzed by injection of hexane extracts (1 μl) into an HP 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with an electron capture detector and a DB-5 megabore capillary column (J & W Scientific, Folson, CA). Aqueous fractions were analyzed by high-performance liquid chromatography (HPLC; Waters, Milford, MA, USA) using a YMC-Pack ODS-AQ column (YMC, Kyoto, Japan). CBAs were identified by comparison of retention times with reference compounds by absorbance at 238 nm. Chloride was similarly measured via the same HPLC but using a conductivity detector and IonPac AS17 analytical column.

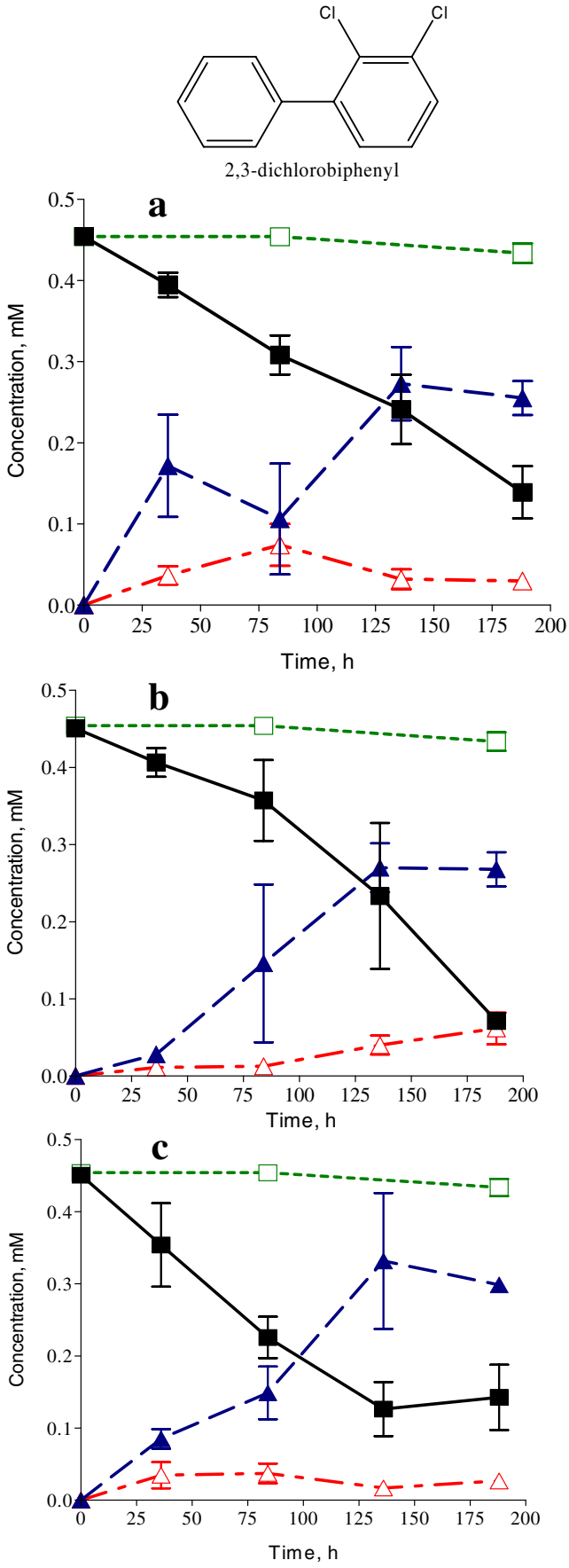


Fig. 2 Degradation of 2,3-diCB (*shaded square*) by benzoate-grown ▶ cells of strain SA-2 (**a**), SA-4 (**b**), and SA-6 (**c**) with concomitant chloride release (*open triangle*) and 2,3-CBA production (*shaded triangle*). 2,3-diCB concentration in control tubes lacking cells (*open square*) remained unchanged during the course of experiment. *Data points* represent the mean of three replicate tubes, while *error bars* represent standard deviation. Large error bars were due to differential response of cells to substrates in replicate tubes

Results

Isolation, characterization, and substrate utilization pattern of bacterial strains

Three isolates, SA-2, SA-4, and SA-6, were obtained that were able to utilize a relatively wide range of mono- and diCBs as sole sources of carbon and energy. The strains were all Gram-negative, catalase-positive, small motile rods. Molecular analysis, coupled with API 20 E test kit examination, was used to identify the isolates as *Enterobacter* sp. SA-2, *Ralstonia* sp. SA-4 and *Pseudomonas* sp. SA-6. All isolates were able to grow on benzoate, naphthalene, biphenyl, all monoCBs, 2,2′-, 2,3-, and 2,4′-diCB as sole carbon and energy sources. With the exception of strain SA-2, growth was also sustainable on 3,3′- and 3,5-diCB. However, all isolates were unable to grow on any of the following congeners tested: 2,3′-, 2,4-, 2,6-, 4,4′-diCB, 2,3,4-, and 2,4,5-trichlorobiphenyl. Similarly, the isolates were unable to grow on any of the chlorobenzoic acids tested (2-, 3-, 4-, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-chlorobenzoic acid). Incomplete aerobic PCB biodegradation often produces CBAs as products, and PCB-degraders generally have limited or no ability to degrade CBAs as growth substrates (Kim and Picardal 2001).

Growth on dichlorobiphenyl substituted on one ring

The growth curve of isolates on 2,3-diCB is illustrated in Fig. 1. Over the first 80 h, SA-2, SA-4, and SA-6 grew with mean generation times of 19, 20, and 35 h, respectively. We observed a slight to moderate increase in cell numbers in control tubes containing the HMN carrier but lacking 2,3-diCB. Over the course of the experiment, this increase was relatively small for SA-4 and SA-6 (Fig. 1b,c). In the case of SA-2 (Fig. 1a), however, we observed an almost tenfold increase in cell numbers in tubes containing HMN alone, likely due to continued cell division by the robust inoculum or utilization of endogenous substrates. In all cases, however, the increase in cell numbers was at least 1.5 orders-of-magnitude greater in tubes containing 2,3-diCB than in tubes containing the HMN carrier alone, clearly demonstrating growth on the congener.

Patterns of 2,3-diCB degradation were similar for the three isolates (Fig. 2). By the end of the experiments, less

than 30% of the initial 2,3-diCB was recovered in all cases, although the overall extent of degradation was greatest in SA-4 cultures (Fig. 2b). The near stoichiometric conversion to 2,3-chlorobenzoic acid and minimal Cl^- production (Table 1; Fig. 2) indicate that growth was primarily a result of metabolism of the nonchlorinated ring of 2,3-diCB.

Growth on dichlorobiphenyls substituted on both rings

A population increase of two to three orders-of-magnitude was observed during cultivation of organisms on 2,2′-diCB (Fig. 1). Over the first 80 h, mean generation times for SA-2, SA-4, and SA-6 growing on this congener were 14, 9, and 15 h, respectively. The three isolates all effectively degraded 2,2′-diCB, but product distribution varied between isolates (Fig. 3). SA-4 and SA-6 stoichiometrically transformed one ring of 2,2′-diCB to 2-CBA, concomitant with approximately equimolar elimination of chloride (Table 1 and Fig. 3). This suggested that both SA-4 and SA-6 were likely able to mineralize one of the *ortho*-substituted rings.

Although SA-2 degraded a similar amount of 2,2′-diCB over the course of the experiment, significantly less 2-CBA and Cl^- were produced. This suggested that neither aromatic ring was fully dechlorinated or that 2,2′-diCB degradation was otherwise incomplete. Less extensive degradation of 2,2′-diCB by SA-2 is further evidenced by more limited growth of this bacterium (Fig. 1a) as compared to SA-4 and SA-6 (Fig. 1b,c). In spite of approximately 26% mineralization of the substrate, the measured cell density of SA-2 increased from 1.2×10^6 to 1.7×10^8. Although a portion of this increase in cell numbers may be the result of continued cell division during use of endogenous substrate, there was an approximately tenfold greater increase in SA-2 cell numbers in tubes containing 2,2′-diCB than that observed for SA-2 in control tubes containing only HMN. This strongly suggests that cells utilized a portion of the degraded 2,2′-diCB as a carbon source even though chloride release was not stoichiometric. As in all cases, co-metabolic degradation was ruled out since washed cells were used, benzoate is not known to induce biphenyl degradation enzymes, and congeners were more than 99% pure.

As seen in Fig. 4, all isolates degraded 2,4′-diCB fairly rapidly, with somewhat higher growth yields than the other congeners tested (Fig. 1). Over the first 80 h, mean generation times for SA-2, SA-4, and SA-6 during growth on 2,4′-diCB were 15, 12, and 23 h, respectively. Although 2,4′-diCB depletion rates were similar for the three isolates, the stoichiometries of Cl^- and CBA production were quite varied (Fig. 4 and Table 1). In all cases, there was no production of 2-CBA in these organisms, thus, suggesting exclusive attack of the *ortho*-substituted ring. 2,4′-diCB was rapidly utilized in cultures of SA-2 and the pattern of metabolite production was unique (Fig. 4a). Although nearly 70% of the congener was transformed within 40 h, Cl^- release lagged substantially and was very limited until the final analysis was done at 158 h. 4-CBA production was also substantially less than would be expected if initial dioxygenase attack on the *ortho*-substituted ring had been followed by complete ring cleavage to produce 4-CBA. Although SA-2 was unable to grow on 4-CBA when this compound was presented alone, it is possible that incomplete 4-CBA metabolism during growth on 2,4′-diCB accounted for the limited 4-CBA accumulation and the slightly higher than expected Cl^- production.

Dynamics of 4-CBA production by both strains SA-4 and SA-6 indicated that the chlorobenzoic acid may not be the final product of 2,4′-diCB metabolism. A mean concentration of 0.28 mM 4-CBA was produced by SA-4 by 110 h, which would almost equal the amount expected if the transformed 2,4′-diCB was being stoichiometrically converted to 4-CBA (Fig. 4b). The 4-CBA concentration decreased to 0.03 mM by the end of the incubation, showing

Table 1 Aerobic degradation of dichlorobiphenyls isolates SA-2, SA-4, and SA-6

Congener	Initial conc. (mM)	Incubation period (h)	Disappearance of PCB (%)[a,b,c]			PCB recovered as CBA (%)[a,b]			Chloride eliminated (mM)[a,b]		
			SA-2	SA-4	SA-6	SA-2	SA-4	SA-6	SA-2	SA-4	SA-6
2,2′-diCB	0.38±0.0	172	92±8.0	95±4.8	86±5.6	2-CBA (45±4.3)	2-CBA (98±18)	2-CBA (93±38)	0.09±0.0	0.48±0.2	0.48±0.1
2,4′-diCB	0.39±0.0	158	100±0.0	97±1.0	97±4.2	4-CBA (45±13)	4-CBA 7.1±4.0	4-CBA (47±22)	0.45±0.0	0.19±0.1	0.19±0.0
2,3-diCB	0.45±0.0	188	69±12	84±3.8	69±17	2,3-CBA (82±12)	2,3-CBA (71±10)	2,3-CBA (96±1.4)	0.03±0.0	0.06±0.0	0.03±0.0

[a] Cells were pregrown with benzoate and washed before inoculated into MS medium supplemented with individual congeners.
[b] Data values are averages of triplicate samples sacrificed at each time point±SD.
[c] Percent degradation values were evaluated with reference to the amount recovered from non-inoculated tubes.

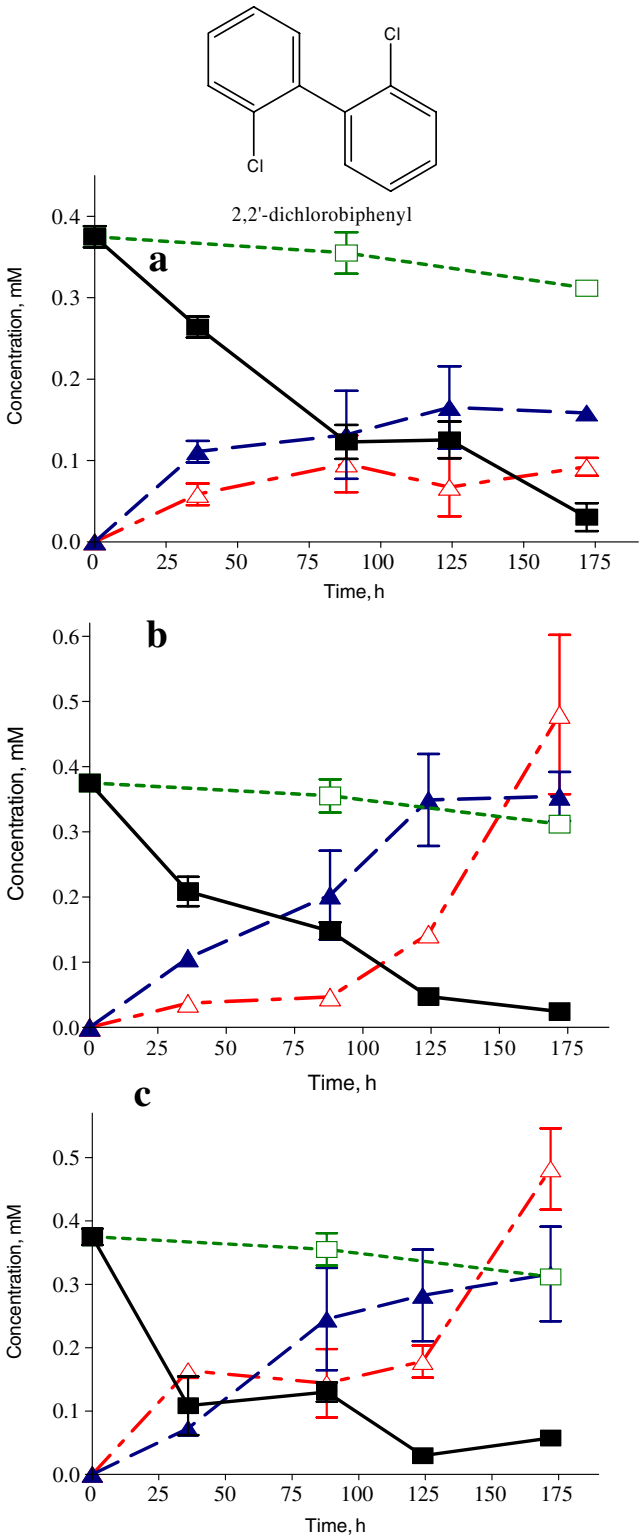


Fig. 3 Degradation of 2,2′-diCB (*shaded square*) by benzoate-grown cells of strain SA-2 (**a**), SA-4 (**b**), and SA-6 (**c**) showing chloride release (*open triangle*), and 2-CBA production (*shaded triangle*). 2,2′-diCB concentration in control tubes lacking cells (*open square*) remained relatively unchanged during the course of experiment. *Data points* represent the mean of three replicate tubes, while *error bars* represent standard deviation. Large error bars were due to differential response of cells to substrates in replicate tubes

that this compound was being metabolized further. A similar pattern was observed in cultures of SA-6 (Fig. 4c). 4-CBA concentrations peaked at approximately 0.36 mM and then decreased to 0.18 mM. In incubations with SA-4 and SA-6, this delayed transformation of 4-CBA did not result in any noticeable increase in Cl^- concentration, and overall Cl^- release clearly showed that full dechlorination of either ring did not occur. As with SA-2, 4-CBA may be partially co-metabolized during growth on 2,4′-CB, although it cannot serve as a growth substrate when presented alone.

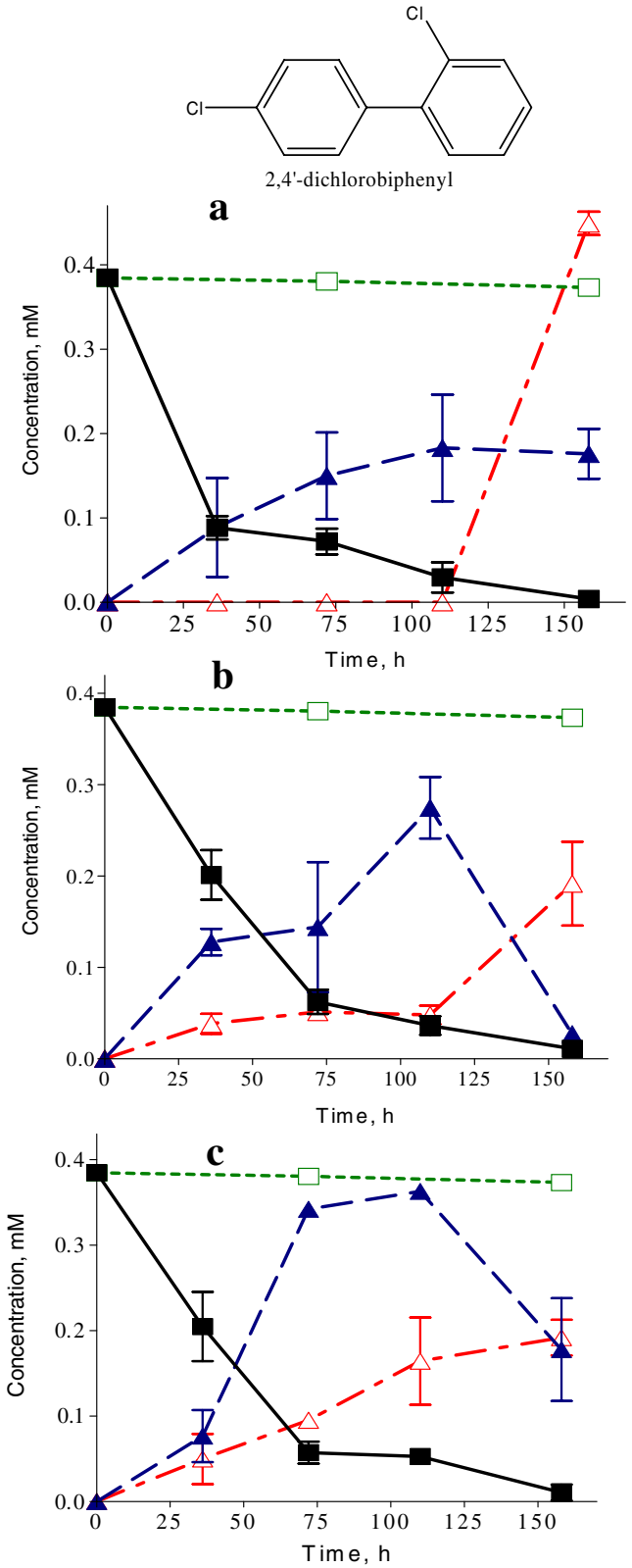
Initial rates of diCB degradation

Average initial diCB degradation rates for each of the three isolates are shown in Table 2. A comparison of the data shows that 2,4′-diCB was degraded more rapidly (11.2 to 30.3 nmol/min per 10^9 cells) than either 2,3 di-CB or 2,2′-diCB (<3.0 nmol/min per 10^9 cells). The higher initial degradation rate of 2,4′-diCB is consistent with the greater overall disappearance of this congener (Table 1) and somewhat higher growth yields (Fig. 1). 2,3-diCB was degraded at nearly identical initial rates (2.1 to 2.5 nmol/min per 10^9 cells) by all isolates. Although 2,2′-diCB was degraded at a similar rate (2.7 nmol/min per 10^9 cells) by SA-2, initial transformation rates of this congener by SA-4 and SA-6 were much lower at 0.47 and 0.37 nmol/min per 10^9 cells, respectively.

Discussion

Most known chlorobiphenyl-degrading bacteria were isolated by enrichment on biphenyl or monochlorobiphenyls, which may well explain their metabolic similarities. Using Askarel fluid as an enrichment substrate, however, we were able to isolate organisms with novel and broad PCB catabolic properties. Although the growth of bacteria on 2,2′-, and 2,4′-diCB was reported previously (Potrawfke et al. 1998; Kim and Picardal 2001), this is the first report of bacteria able to utilize both of these congeners and 2,3-diCB as sole sources of carbon and energy. Indeed, the isolation of all three bacteria from the same PCB-contaminated soil suggests that diCB-utilizing bacteria may be more widely distributed than previously believed, that novel or more extensive degradation pathways may be evolving in response to the selective pressure of long-term PCB exposure, or that the tropical soils used as our inoculum contained unique metabolic capabilities.

The contaminated soils were found to contain at least three PCB-degrading strains: *Enterobacter* sp. SA-2, *Ralstonia* sp. SA-4, and *Pseudomonas* sp. SA-6. Extensive research on the degradation of PCBs over the last few decades has resulted in the documentation of a diverse



◀ **Fig. 4** Degradation of 2,4′-diCB (*shaded square*) by benzoate-grown cells of strain SA-2 (**a**), SA-4 (**b**), and SA-6 (**c**) showing chloride release (*open triangle*), and 4-CBA production (*shaded triangle*). 2,4′-diCB concentration in control tubes lacking cells (*open square*) remained unchanged during the course of experiment. *Data points* represent the mean of three replicate tubes, while *error bars* represent standard deviation. Large error bars were due to differential response of cells to substrates in replicate tubes

group of bacteria, e.g., *Achromobacter* sp. (Ahmed and Focht 1973), *Alcaligenes*, sp., *Acinetobacter* sp. (Bedard et al. 1987), *Corynebacterium* sp. (Furukawa et al. 1978), *Rhodococcus* sp. *Burkholderia* sp. (Bopp 1986; Kim and Picardal 2001), and *Pseudomonas* sp. (Sondossi et al. 1992; Hickey et al. 1992), that can metabolize PCBs or, in the case of congeners containing one or two chlorines, use them as a sole source of carbon and energy. The ability of an enteric bacterium, i.e., *Enterobacter* sp. SA-2, to degrade recalcitrant *ortho*-substituted dichlorobiphenyls as a sole carbon and energy source appears to be an uncommon finding, as there have been no previous reports demonstrating PCB degradation by enteric bacterial strains. Although *Enterobacter aerogenes* was among the organisms isolated from a sewage with reported ability to degrade PCBs (Boyle et al. 1992), no further data was given on the organism's ability to utilize specific congeners or co-metabolize defined PCB mixtures. Earlier, Walia et al. (1988) described isolation of an *Enterobacter* sp. from PCB-contaminated soil. The organism was isolated after clearance of biphenyl from sprayed plates, but no definitive evidence of PCB biodegradation was provided.

Researchers studying the ecology of enteric bacteria have posited that enteric bacteria may often encounter aromatic and xenobiotic compounds (Juhasz and Naidu 2000; Diaz et al. 2001). Even though PCBs may not be expected to be usual substrates for enteric bacteria, there is evidence that competition for nutrients results in selection of substrates and shift in nutrient preference (Torsvik and Ovreas 2002). Since SA-2 was isolated from a site with over 60 years of pollution history, the presence of aromatic compounds may have resulted in the adaptation of this strain and development of the ability to utilize specific PCB congeners as sole sources of carbon and energy.

It is noteworthy that SA-2, SA-4, and SA-6 have the ability to utilize dichlorobiphenyls chlorinated on both rings more extensively than those substituted only on one ring. 2,2′- and 2,4′-diCB were degraded more extensively

Table 2 Rates of microbial utilization of diCBs (nmol/min per 10^9 cells)

Isolate	2,3-diCB	2,2′-diCB	2,4′-diCB
SA-2	2.14±1.0	2.7±1.2	11.2±0.91
SA-4	2.5±1.8	0.47±0.13	30.3±7.8
SA-6	2.1±2.1	0.37±0.12	26.0±10.0

Rates shown are the mean rates over the first 36 h for three replicate samples.

than 2,3-diCB by all three isolates (Table 1). Previously, Potrawfke et al. (1998) described utilization of 2,4′- and 2,3′-diCB by cultures of *Burkholderia* sp. LB400. More recently, two isolates from PCB-contaminated sewage sludge, SK-3 and SK-4, were also shown to grow on 2,4′- and 2,2′-diCB (Kim and Picardal 2001). It is interesting to note that our isolates show extensive dissimilation of PCB congeners substituted at the *ortho* position of one or both rings, a substitution pattern that may confer resistance to aerobic degradation via 2,3 hydroxylation. Since *ortho*-substituted congeners such as 2,2′-, 2,3-, and 2,4′-diCB are often found as major degradation products of anaerobic dechlorination (Bedard 2001), the presence of *ortho*-degrading, aerobic cultures may prevent accumulation of such congeners in natural systems and offer hope for the development of sequential anaerobic–aerobic bioremediation systems.

The degradation of dichlorobiphenyls observed in the present study may proceed via a *meta*-cleavage pathway similar to that previously described for other bacteria growing on chlorobiphenyls (Ahmed and Focht 1973; Parsons et al. 1988). This was evidenced by frequent or occasional transient production of yellow intermediates, presumably 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoates or HOPDAs especially during growth on 2,2′- and 2,4′-diCB. All the strains likely metabolized the non-chlorinated ring of 2,3-diCB in preference to the chlorinated ring, as indicated by insignificant amounts of chloride production coupled with near stoichiometric production of 2,3-CBA (Table 1). The product profiles obtained from 2,4′-diCB metabolism indicated that the strains preferentially attacked the *ortho*-substituted ring in a manner relatively similar to *Rhodococcus* sp. RHA1 (Maltseva et al. 2000), bacteria strain SK-4 (Kim and Picardal 2001) and *Burkholderia* sp. LB400 (Potrawfke et al. 1998). In several experiments, e.g., degradation of 2,2′-diCB by SA-2, non-stoichiometric recovery of chlorine as either Cl^- or CBA (Table 1) suggests the accumulation of unknown, chlorinated metabolites. Indeed, incomplete degradation of PCBs is frequently observed with the formation and accumulation of different intermediates (Bruhlmann and Chen 1999; Seeger et al. 1995). A general comparison of Cl^- production by our isolates with those in other studies is not possible since Cl^- production data, with relatively few exceptions (Potrawfke et al. 1998; Kim and Picardal 2001), are typically not reported in the literature. A possible explanation for the non-stoichiometric production of CBAs or Cl^- could be the partial accumulation of the *meta*-cleavage product as a result of either competitive inhibition of HOPDA hydrolase by chlorinated HOPDAs, as previously reported by Seah et al. (2001), or by varying enzyme specificity for different chlorinated HOPDAs (Seah et al. 2000). In addition, a number of other dead end products other than HOPDAs can also be produced in varying amounts (Sondossi et al. 2004). Further work is planned to better elucidate degradation pathways and end product distribution.

The isolation of these bacteria from PCB-contaminated, Nigerian soils increases our knowledge about prokaryotic diversity in poorly studied geographical habitats. As recently described by Floyd et al. (2005) in a review of environmental bacteria at the American Type Culture Collection, only 2.8% of total environmental holdings was acquired from the African continent. Our knowledge of environmental prokaryotes is clearly based on the disproportionate number of microorganisms isolated in North America, Europe, and Japan. It is reasonable to hypothesize that additional bacteria with novel metabolic capabilities maybe present amidst the diversity of microbial populations in tropical soils in other regions.

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