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Construction and Characterization of Two Recombinant Bacteria That Grow on *ortho*- and *para*-Substituted Chlorobiphenyls

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Cloning and expression of the aromatic ring dehalogenation genes in biphenyl-growing, polychlorinated biphenyl (PCB)-cometabolizing *Comamonas testosteroni* VP44 resulted in recombinant pathways allowing growth on *ortho*- and *para*-chlorobiphenyls (CBs) as a sole carbon source. The recombinant variants were constructed by transformation of strain VP44 with plasmids carrying specific genes for dehalogenation of chlorobenzoates (CBAs). Plasmid pE43 carries the *Pseudomonas aeruginosa* 142 *ohb* genes coding for the terminal oxygenase (ISP_{OHB}) of the *ortho*-halobenzoate 1,2-dioxygenase, whereas plasmid pPC3 contains the *Arthrobacter globiformis* KZT1 *fcb* genes, which catalyze the hydrolytic *para*-dechlorination of 4-CBA. The parental strain, VP44, grew only on low concentrations of 2- and 4-CB by using the products from the fission of the nonchlorinated ring of the CBs (pentadiene) and accumulated stoichiometric amounts of the corresponding CBAs. The recombinant strains VP44(pPC3) and VP44(pE43) grew on, and completely dechlorinated high concentrations (up to 10 mM), of 4-CBA and 4-CB and 2-CBA and 2-CB, respectively. Cell protein yield corresponded to complete oxidation of both biphenyl rings, thus confirming mineralization of the CBs. Hence, the use of CBA dehalogenase genes appears to be an effective strategy for construction of organisms that will grow on at least some congeners important for remediation of PCBs.

Sequential anaerobic-aerobic degradation schemes for PCB remediation have been proposed to take advantage of the anaerobes' better ability to attack the more highly chlorinated biphenyls and the aerobes' better ability to oxidize the less-chlorinated polychlorinated biphenyls (PCBs) (1). Anaerobic microbial communities often found in sediments reductively dechlorinate commercial mixtures of PCBs, typically accumulating the less-chlorinated *ortho*- and *ortho*- plus *para*-chlorinated congeners (6, 10). Although anaerobic removal of *ortho* chlorines has been reported, it has been described as a "rare and unique" activity (8, 48). Hence, the aerobic organisms that attack, and preferably grow on, the major congeners resulting from anaerobic dechlorination are important targets for a PCB bioremediation scheme.

Aerobic bacterial degradation of PCBs typically proceeds via the oxidative biphenyl pathway encoded by the *bph* genes. This cometabolic process does not allow bacteria to grow on PCBs, with only a few exceptions, and yields a chlorobenzoate (CBA) and (chloro)pentadiene as products (3–5, 9, 15, 21, 23). Yet the same bacteria normally possess oxidative pathways for nonchlorinated benzoate and pentadiene (9). On the other hand, a number of CBA-degrading bacteria that remove chlorine prior to oxidation of the aromatic ring, funneling the resulting nonchlorinated benzoate or catechol into central metabolic pathways, have been isolated (13, 14, 35, 42, 46, 50, 51). While no dehalogenation of chloropentadiene has been documented so far (9), several CBA dehalogenation genes have been cloned and characterized (18, 33, 37, 38, 39, 43, 44), and these are potentially useful for constructing organisms that would grow on PCBs.

The concept of complementation of degradative pathways for PCB cometabolism and CBA degradation in a single transgenic microbe was proposed long ago as a means for achieving complete PCB degradation (16). Indeed, several hybrid PCB-degrading strains have been engineered, primarily by in vivo two-directional conjugative mating of strains with the complementary metabolic activities (9, 19, 20, 26, 29). Among these, the hybrid strains *Pseudomonas putida* JHR and *Pseudomonas* sp. strain UCR2 have been reported to grow on environmentally important 2-, 2,4-, and 2,5-substituted PCB congeners; however, the growth was slow and required low substrate concentrations (19, 20). In contrast to the in vivo construction, the use of sequenced and well-characterized CBA dehalogenase genes for single-step in vitro engineering of PCB-growing organisms allows for targeting of specific PCB congeners and better prediction of expected intermediates, and it may enhance the rates of PCB degradation. The latter may be especially important for remediation of heavily polluted sites featuring PCB contamination at levels of several hundred or even thousands of parts per million (45).

Our major goal was to validate the concept of in vitro engineering for the construction of bacteria that would grow on PCBs by introducing dehalogenase genes into PCB-cometabolizing bacteria. Metabolism by *bph* pathway enzymes of the congeners targeted at the aerobic step of a two-phase PCB bioremediation scheme produces the respective *ortho*-, *para*-, and *ortho*- plus *para*-CBAs. Hence, we have cloned oxygenolytic *ortho*-dechlorination *ohb* (44) and hydrolytic *para*-dechlorination *fcb* (43) genes into PCB-cometabolizing *Comamonas testosteroni* VP44 (31). The data presented here demonstrate that introducing these dehalogenase genes resulted in highly efficient recombinant pathways, enabling the recombinant variants to grow on, dechlorinate, and completely mineralize *ortho*- and *para*-substituted monochlorobiphenyls.

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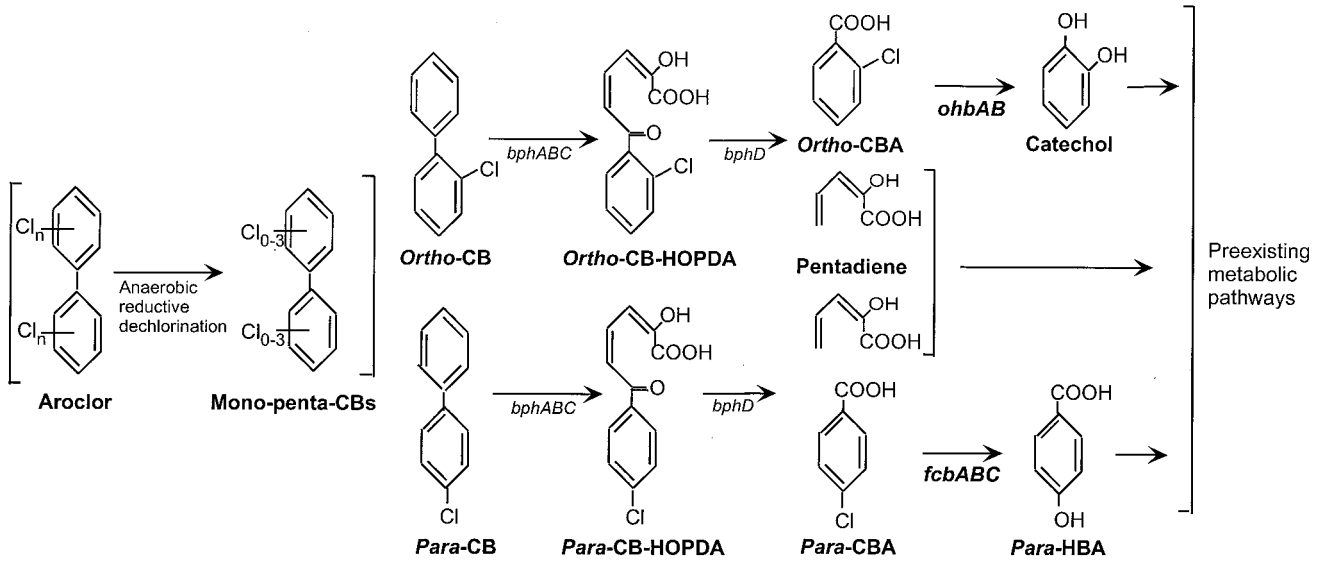

FIG. 1. Recombinant pathways for degradation of 2-CB (top) and 4-CB (bottom), constructed by upgrading preexisting pathways for oxidation of (chloro)biphenyl, 4-HBA, catechol, and pentadiene with the aromatic ring dehalogenase genes *ohbAB* and *fcbABC*, respectively. Also shown are anaerobic dechlorination of Aroclors, which results in accumulation of predominantly *ortho*- and *para*-substituted CBs (left) (6, 34), and the *bph*-controlled metabolism of the 2-CB and 4-CB via, respectively, 2-CB-HOPDA and 4-CB-HOPDA (16, 31), which results in the production of CBAs and pentadiene.

MATERIALS AND METHODS

Bacteria, media, and growth conditions. *Comamonas* sp. strain VP44 was isolated from the Pinheiros River, São Paulo, Brazil; it grew on biphenyl and cometabolized a wide range of PCB congeners (31). VP44 was maintained at 30°C on mineral medium K1 (50) with biphenyl (1 g/liter) added as a carbon source. *Escherichia coli* DH5αF′ (Bethesda Research Laboratories) and JM109 (49) were grown at 37°C in Luria-Bertani medium (28). CBAs (Sigma Chemical, St. Louis, Mo.) were used at concentrations of 1 to 10 mM. Chlorobiphenyls (CBs) (AccuStandard, New Haven, Conn.) were added from 5 M acetone stock solutions to final concentrations of 0.5 to 10 mM, and the acetone was allowed to evaporate prior to addition of the medium and bacterial inoculum.

Plasmids. Plasmid pE43 (44) carried the *Pseudomonas aeruginosa* 142 *ohb* genes cloned into the broad-host-range (*bhr*) vector pSP329, carrying a multiple cloning site and a *lacZ* α-complementation fragment cloned into the *Hae*II site of plasmid pTJS75, a derivative of R-factor RK2 (36). Plasmid pPC3 was constructed by cloning a 4,364-bp DNA fragment containing the structural *fcbABC* operon of *Arthrobacter globiformis* KZT1 into the *Hin*dIII site of the RK2-derived vector pRK415 under the control of the P_{lac} promoter (33). Plasmid DNA from *E. coli* cells was purified by using Wizard kits (Promega, Madison, Wis.), and an alkaline lysis procedure was used to recover plasmids from VP44 (25). Digestion with restriction endonucleases was performed by standard methods (25).

Electrotransformation. Competent cells of *E. coli* and strain VP44 were prepared (11) and transformed with plasmid DNA as described elsewhere (44). Transformants of VP44 were selected on L agar-tetracycline (10 μg/ml) and replica plated on K1 plates with CBAs and CBs as a growth substrate.

Growth assays. The batches in triplicate were inoculated from CBA- or biphenyl-grown stationary cultures and incubated on a rotary shaker at 200 rpm. Optical density (A_{600}), chloride release (7), protein yield (40), and CBA concentrations were measured at various times.

Resting cell assay. A resting cell assay was performed as previously described (31). The 2-, 4-, and 2,4-CBs (500 μM) were added from 200× acetone stock solutions. Accumulation of the *meta*-cleavage products 2-hydroxy-6-oxo-(2-chlorophenyl)hexa-2,4-dienoate (2-CB-HOPDA; λ = 394) and 2-hydroxy-6-oxo-(4-chlorophenyl)hexa-2,4-dienoate (4-CB-HOPDA; λ = 437) was measured spectrophotometrically.

Analyses. CBAs were analyzed by high-pressure liquid chromatography as described elsewhere (44). PCBs were quantified by gas chromatographic analysis using an electron capture detector (ECD) as described elsewhere (34). Samples were prepared as described previously (32), by using 2,5-3′,5′-CB (18 μM) as an internal standard.

PCR amplification and DNA sequencing. The 16S rRNA gene from strain VP44 was isolated by PCR using primers fD1 and rD1 (47). Nucleotide sequencing of the region corresponding to nucleotide bases 31 to 506 (17) of the gene was carried out at the Michigan State University DNA Sequencing Facility. Sequence editing was performed with the Sequencher version 3.0 software package (Madison, Wis.).

Nucleotide sequence accession number. The sequence of the 16S rRNA gene of strain VP44, bases 31 to 506, was deposited with GenBank under accession no. AF123317.

RESULTS

Characterization of the parental strain, VP44. The 5′-terminal 475-bp sequence of the 16S rRNA gene from strain VP44 was nearly identical to that of *C. testosteroni* RH 1104^T (24). The only discrepancy in the sequences of the two organisms occurred at position 497, where RH 1104^T contains a cytosine residue, while VP44 contains an adenosine residue. Hence, identification of strain VP44 as *C. testosteroni* (31) was confirmed.

Strain VP44 grew efficiently on biphenyl, with optimal growth at 5 to 10 mM; it also grew on benzoate, 4-hydroxybenzoate (4-HBA), catechol, and acetate. No growth was observed on 4-chlorocatechol or chloroacetate. Like many other biphenyl degraders (9), strain VP44 grew on low concentrations of 4-CB, and more notably, it was also capable of growth on 2-CB (up to 2 mM). However this growth was not accompanied by chloride release. Accumulation of the respective CBAs in the culture supernatant indicated that this limited growth must have derived from the oxidized nonchlorinated biphenyl ring, the pentadiene (Fig. 1). No growth was observed on 2,4-CB, possibly due to a higher toxicity of this dichlorinated congener.

Resting cells of *C. testosteroni* VP44 efficiently transformed 2-, 4-, and 2,4-CB into equimolar amounts of the 2-, 4-, and 2,4-CBA, respectively (Fig. 2) (data for 4- and 2,4-CB were essentially the same and are not shown). Only transient appearance of a *meta*-cleavage product (HOPDA) was detected during 4 to 6 h of incubation. When amended with the CBAs, the resting cells showed no consumption of the substrate.

Cloning and expression of the *ohb* and *fcb* genes in strain VP44. The recombinant *C. testosteroni* strain VP44(pE43) was constructed by introduction of the recombinant plasmid pE43. The plasmid carries structural *ohbAB* genes, which code for the

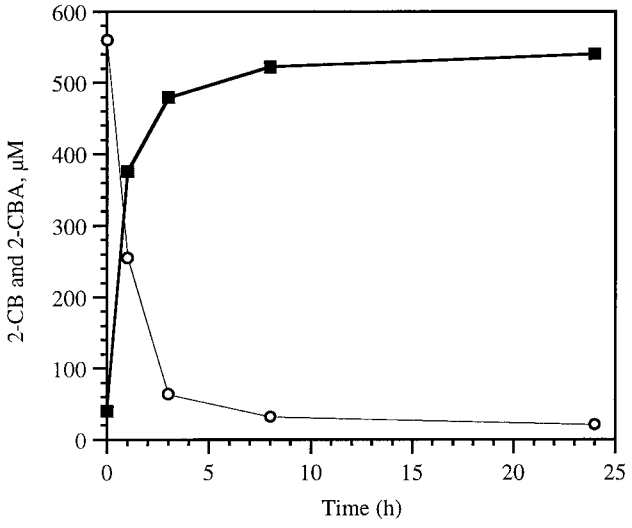

FIG. 2. Concentrations of 2-CB (○) and 2-CBA (■) measured in the presence of strain VP44 resting cells.

terminal component, iron-sulfur protein (ISP_{OHB}), of *P. aeruginosa* 142 *ortho*-halobenzoate 1,2-dioxygenase, and the putative transcriptional regulatory gene *ohbR* (44). Several transformants were selected on L agar-tetracycline and replica plated on K1 agar-tetracycline with 2-CBA as a sole carbon source and with L agar-tetracycline as a control. After approximately 8 weeks of incubation, fully grown colonies were formed on the 2.5 mM 2-CBA plates. The incubation period for initial growth was significantly shorter (up to 1 week) when a lower concentration of 2-CBA (1.25 mM) or a larger inoculum was used. This indicated that the initial growth of transformants is affected by the toxicity of 2-CBA. In subsequent transfers on K1 agar with 2-CBA, VP44(pE43) transformants reproducibly grew after 1 to 2 days of incubation. The recombinant strain VP44(pPC3) was similarly constructed by introduction of the recombinant plasmid pPC3. The plasmid carries the *A. globiformis* KZT1 *fcbABC* genes, which code for the enzymes involved in hydrolytic dechlorination of 4-CBA via formation of chlorobenzoyl-coenzyme A thioester (references 33 and 43; also unpublished sequencing data). In this case, replica-plated transformants formed fully grown colonies on 4-CBA (5 mM) plates after 1 week of incubation.

Ten colonies from each transformation were further analyzed. The recombinants were morphologically and phenotypically very similar to the parental strain and retained the ability to grow on biphenyl, benzoate, 4-HBA, and catechol. All recombinant clones maintained resistance to tetracycline and grew on CBAs even following repeated growth on nonselective media. Plasmid DNA isolated from the transformants was retransformed into *E. coli* and shown to retain the original restriction patterns. These data confirmed that plasmids pE43 and pPC3 were stably maintained in the strain VP44.

When grown on 2-CBA at concentrations of 5 and 10 mM, the recombinant strain VP44(pE43) showed a proportional increase in A_{600}, along with substrate disappearance (Fig. 3a), stoichiometric amounts of chloride released, and a proportional increase in protein yield (Table 1). The protein yield was comparable to that observed from growth on benzoate. No growth of strain VP44(pE43) was observed on 4-CBA. At concentrations of 2-CBA as high as 10 mM, strain VP44(pE43)

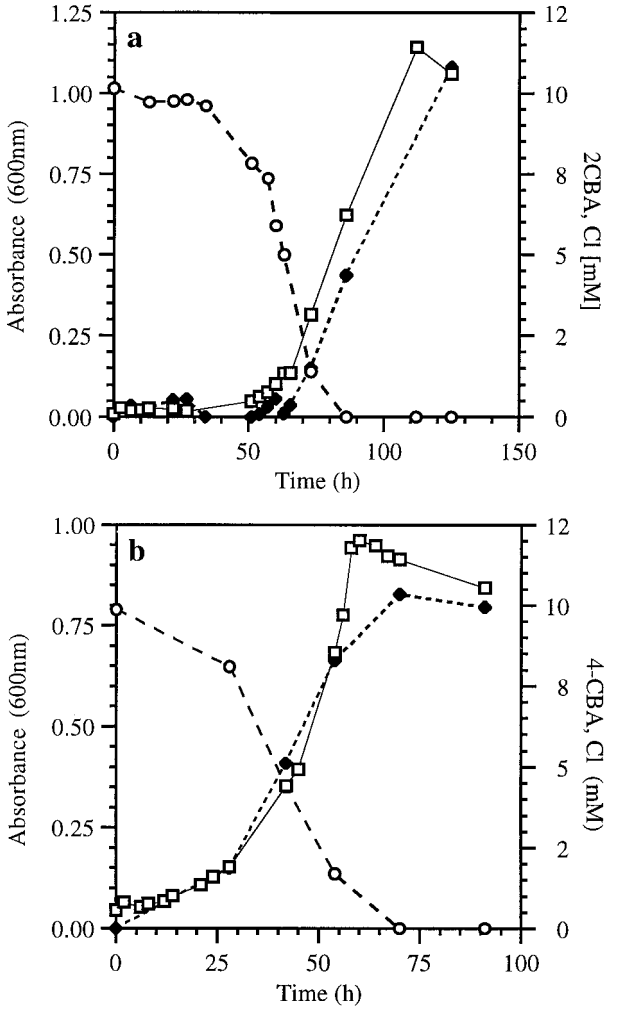

FIG. 3. Growth of recombinants on CBAs. (a) Growth of VP44(pE43) on 10 mM 2-CBA. (b) Growth of VP44(pPC3) on 10 mM 4-CBA. Shown are the optical density at 600 nm (□); disappearance of 2-CBA and 4-CBA (○), respectively; and accumulation of chloride (◆).

showed a dramatic increase in A_{600} after an initial lag period of about 50 h. The substrate was depleted after 80 h of incubation, at which point 10.4 mM chloride was measured in the medium (Fig. 3a; Table 1).

Growth of the recombinant strain VP44(pPC3) on 4-CBA (up to 10 mM) (Fig. 3b) was similar to that of VP44(pE43) on 2-CBA, except for a shorter lag period. As A_{600} increased over a 72-h period of incubation, the concentration of 4-CBA in the culture supernatant dropped below detection limits and the concentration of chloride rose to 10.1 mM (Fig. 3b; Table 1). The protein yield was proportional to the concentration of the substrate and was similar to that for benzoate-grown cultures (Table 1). Recombinant VP44(pPC3) did not utilize or dehalogenate 2-CBA (Table 1).

These results showed that the cloning and expression of the *ohb* and *fcb* genes in strain VP44 resulted in recombinant pathways for dechlorination of and growth on 2- and 4-CBA, respectively. During the degradation of CBAs, catechol, an immediate product of the *ortho*-dehalogenation reaction, and 4-HBA, a product of the hydrolytic *para*-dechlorination reac-

TABLE 1. Protein yield and chloride release by recombinant strains VP44(pE43) and VP44(pPC3) grown on CBAs and CBs

Substrate (mM)	44(pE43)		44(pPC3)	
	Protein (μg/ml) (±SD)	Chloride (mM) (±SD)	Protein (μg/ml) (±SD)	Chloride (mM) (±SD)
Benzoate (5)	181 (39)	0.4 (0.1)	228 (24)	0.7 (0.1)
2-CBA (5)	169 (15)	5.3 (0.4)	<10	<0.1
2-CBA (10)	373 (16)	10.1 (1.2)	<10	<0.1
4-CBA (5)	<10	<0.1	158 (9)	7.1 (0.2)
4-CBA (10)	<10	<0.1	257 (16)	10.4 (0.1)
Biphenyl (2)	154 (10)	0.5 (0.1)	188 (9)	0.1 (0.1)
2-CB (2)	177 (4)	1.9 (0.2)	69 (7)[b]	0.1 (0.1)
4-CB (2)	84 (6)[a]	0.7 (0.3)	160 (39)	1.8 (0.2)

[a] 4-CBA accumulated to 2.02 (±0.21) mM in the supernatant.
[b] 2-CBA accumulated to 1.88 (±0.13) mM in the supernatant.

tion, were never observed as intermediates, suggesting their rapid metabolism following the dechlorination step.

Mineralization of CBs by recombinant strains VP44(pE43) and VP44(pPC3). Strain VP44(pPC3) exhibited rapid growth on 4-CB, with only transient production of 4-CB-HOPDA and 4-CBA in the culture supernatant during early-log phase, and with concomitant release of stoichiometric amounts of inorganic chloride (Fig. 4a). The maximal accumulation of 4-CBA was about 5% of the original substrate concentration. Growth of strain VP44(pE43) on 2-CB was slightly slower, with a greater accumulation of 2-CBA (12.5% of the original substrate concentration), which persisted slightly longer (Fig. 4b). Transient production of CBAs in an early growth phase implied that dechlorination was the rate-limiting step for growth.

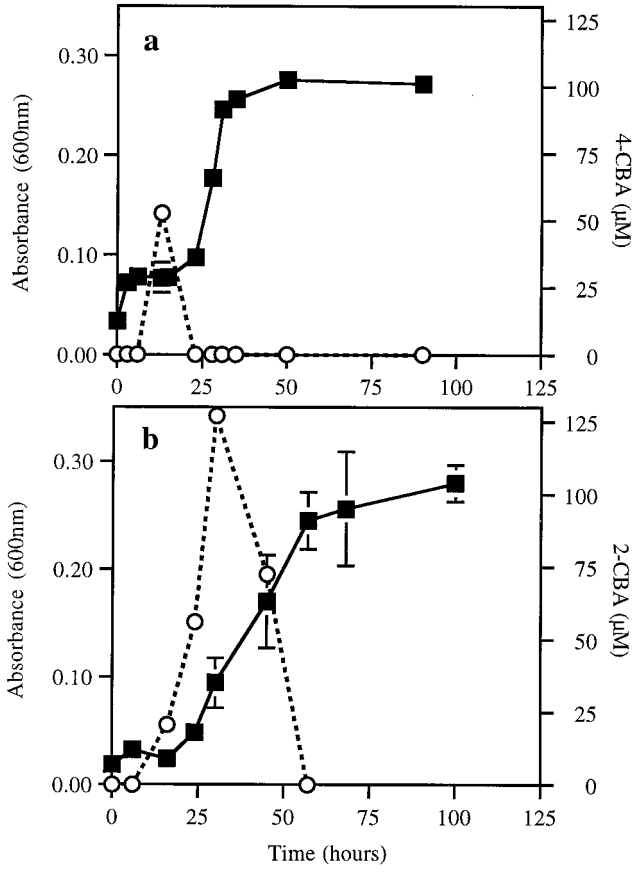

FIG. 4. Growth of recombinants on monochlorobiphenyls. (a) Optical density at 600 nm (■) and concentration of 4-CBA (○) during growth of VP44(pPC3) on 1 mM 4-CB. (b) Optical density at 600 nm (■) and concentration of 2-CBA (○) during growth of VP44(pE43) on 1 mM 2-CB. All data are means from three replicate cultures. Error bars, 1 standard deviation.

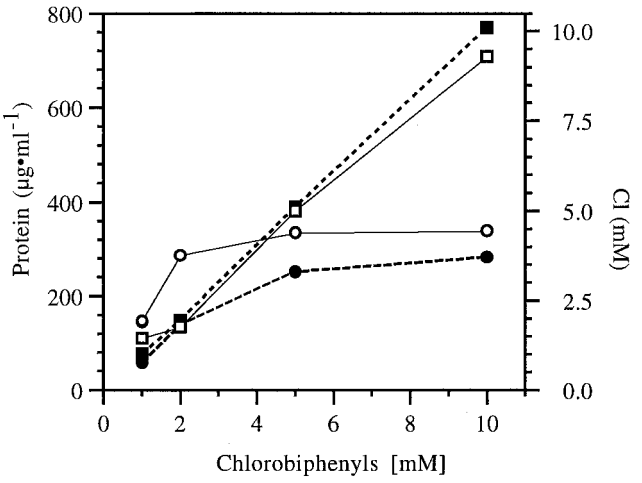

FIG. 5. Protein yield with increasing substrate concentration. Recombinant strain VP44(pE43) was grown on 2-CB (□) and recombinant strain VP44(pPC3) was grown on 4-CB (○), in liquid medium K1 containing 1 to 10 mM CB, and both were observed for protein production. Accumulation of chloride in the medium is shown for VP44(pE43) (■) and VP44(pPC3) (●).

Recombinant strains VP44(pE43) and VP44(pPC3) grew on 2-CB and 4-CB, respectively, at concentrations up to 10 mM (Fig. 5), at rates comparable to the growth rate of the parental strain, VP44, on biphenyl. When grown on 2-CB, strain VP44(pE43) yielded approximately the same amount of protein per millimole of substrate as that from growth on biphenyl and released stoichiometric amounts of chloride (Table 1). Protein production and chloride release (Fig. 5) were linear up to at least 10 mM 2-CB. Similarly, growth of the recombinant strain VP44(pPC3) on 4-CB was marked by stoichiometric chloride release, and the protein yield was comparable to that observed on biphenyl (Table 1). For strain VP44(pPC3), incomplete degradation was suggested by nonlinear production of protein and chloride release at concentrations of 4-CB higher than 5 mM (Fig. 5).

When the *ortho*-dechlorinating strain VP44(pE43) was grown on 4-CB, an inappropriate substrate for this recombinant, it produced, as expected, stoichiometric concentrations of 4-CBA, no chloride, and roughly half as much protein as when it was grown on 2-CB and biphenyl. Similar results were obtained during growth of the *para*-dechlorinating strain VP44(pPC3) on an inappropriate substrate, 2-CB (Table 1). This indicated that this growth occurred only from oxidation of a nonchlorinated ring of the biphenyl moiety, the pentadiene.

DISCUSSION

This study demonstrates an alternative approach for the construction of PCB-degrading bacteria, i.e., the use of genes encoding peripheral enzymatic activities for modification and funneling of xenobiotics into substrates for preexisting central metabolic pathways (Fig. 1). Previously, transgenic PCB-degrading strains were generated primarily in vivo by two-direc-

tional conjugative mating of strains with complementary pathways (19, 20, 26, 29). Those studies were based mainly on the frequent location of biodegradative genes on transmissible catabolic plasmids and/or their association with transposable elements. In vitro construction of PCB degraders was done previously by introduction of the *bph* operon into CBA-degrading bacteria (2, 12, 22, 27, 39). However, no previous report was published on the in vitro construction of a PCB-degrading pathway via the introduction of specific CBA dechlorination genes into naturally occurring biphenyl-degrading organisms. Among the potential advantages of the latter approach are better fitness of the naturally occurring biphenyl degraders for accessing PCBs and, in some cases, the presence of multiple *bph* genes encoding enzymes of differing substrate specificities which can increase the range of congeners metabolized.

The cloning and expression of the *ohb* and *fcb* operons for *ortho*- and *para*-dechlorination of CBAs, respectively, in *C. testosteroni* VP44 have resulted in highly efficient recombinant pathways for growth on and mineralization of high concentrations of the respective CBAs and CBs (Fig. 1). The rates of degradation of 2-CBA and 4-CBA by the recombinant strains VP44(pE43) and VP44(pPC3) were at least twofold higher than those reported for the parental strains *P. aeruginosa* 142 (35) and *A. globiformis* KZT1 (50), respectively. The previously constructed transconjugant *Pseudomonas* sp. strain JPL, which acquired the *iso*-functional *ohb* genes from *P. aeruginosa* strain JB2, was reported to grow on 3.2 mM 2-CBA (32), compared to 10 mM 2-CBA for VP44(pE43). Possibly, the better expression of the dehalogenation activity may be explained by multiple gene copies in the in vitro-constructed recombinants of strain VP44.

Degradation of 2-CBA by strain VP44(pE43) was fivefold more efficient than degradation by *P. putida* PB2440(pE43) that grew on 2 mM 2-CBA (44). We previously showed that the oxygenolytic *ortho*-dehalogenation activity in heterologous hosts apparently results from complementation of OhbAB, the terminal component of the strain 142 three-component *ortho*-halobenzoate 1,2-dioxygenase, with the reductase and ferredoxin components provided by host organisms (44). The differences in interactions of the *ohb* gene-coded enzyme with the components provided by the host could be a major factor causing the dramatic difference in *ohb* gene expression in these two bacteria, *C. testosteroni* and *P. putida*. Although CBA toxicity was apparent, the prolonged incubation period required for initial growth of recombinants on 2-CBA in both PB2440 (44) and VP44 is also consistent with a mutation leading to a better interaction between the terminal oxygenase ISP_{OHB} and a host's energy supply system.

As a result of an efficient expression of the dehalogenation genes, the recombinant strains VP44(pPC3) and VP44(pE43) were able to mineralize large amounts of the respective CBs. Our recombinants had higher growth rates on CBs than previously constructed PCB degraders with similar substrate specificities. Strain VP44(pPC3) grown on 2 mM 4-CB exhibited 90% chloride release, a doubling time of about 4 h, and a protein yield of 80 μg/μmol of 4-CB (final concentration, 160 μg/ml). Strain VP44(pE43) grown on 2 mM 2-CB exhibited 95% chloride release, a doubling time of about 7 h, and a protein yield of 88 μg/μmol of 2-CB (final concentration, 177 μg/ml). *Burkholderia cepacia* JHR22 was obtained by sequential matings and contained genetic backgrounds from three different degraders: the salicylate-growing *B. cepacia* WR401, the 3-CBA (chlorocatechol) pathway from strain B13, and the biphenyl pathway from *P. putida* JHR (19). It was reported to grow on several CBs, including 2-CB and 4-CB, and exhibited doubling times of approximately 16 and 10 h and chloride release levels of 80 and 50% when grown on 4 mM 2-CB and 4-CB, respectively (19). Complete degradation of 2.5 mM 2-CBA by the JHR22 occurred only after 5 days of incubation (41). Another strain, UCR2, isolated by multichemostat mating between a CBA degrader, *P. aeruginosa* JB2, and a biphenyl degrader, *Arthrobacter* sp. strain B1Barc (20), was reported to mineralize both 2-CB and 2,5-CB with doubling times of 20 and 48 h and dechlorination of 90 and 48.9%, respectively. Degradation of 500 ppm of 2-CB (ca. 2.6 mM) was completed in about 2 weeks (20). Our strain VP44(pE43) required only 80 h for depletion of 10 mM 2-CB.

The parental strain, VP44, was capable of growth on both 2-CB and 4-CB, at concentrations up to 2 mM, apparently via degradation of the nonchlorinated ring (pentadiene). In comparison, *P. putida* BN10 (29) inoculated at an optical density at least 10-fold higher was capable of oxidizing only about 0.5 mM 2-CB (calculated by production of 2-CBA), with a noticeable decrease in cell population. Under the same conditions, strain BN10 released about 3 mM 4-CBA from 4-CB, with an increase in cell density indicative of growth from pentadiene. Strain VP44 appears to be relatively tolerant to *ortho*-CB compared to other biphenyl degraders.

The ability of the recombinants to degrade much higher concentrations of the CBs, up to 10 mM, than the parental strain, VP44, indicated that the introduction of the dechlorination genes resulted in significantly improved rates of CB degradation. Conjugative mating of strain BN10 with the well-known 3-CBA (via the chlorocatechol *ortho*-pathway) degrader *Pseudomonas* sp. strain B13 produced 3-CB-degrading transconjugants of both the BN10 and the B13 type. Transconjugants such as BN210 and B131 rapidly (25 h of incubation) grew on 5 mM 3-CB with elimination of Cl^- (90%) via oxidation of 3-chlorocatechol. Unlike the recombinants constructed in our study, strains BN210 and B131 did not mineralize 2- and 4-CB. 3-CB, as well as other *meta*-chlorinated congeners, is not among the major PCBs targeted during the aerobic phase of anaerobic-aerobic PCB remediation (6, 34). The predominant products of anaerobic reductive PCB dechlorination are *ortho*-, *para*-, and *ortho*- plus *para*-substituted mono-, di-, and trichlorobiphenyls (6, 10, 34). The ability of VP44(pE43) to efficiently mineralize *ortho*-CB is especially significant. Up to 80 mol% of the PCBs present following anaerobic dechlorination of Aroclor 1242 consists of *ortho*- and *ortho*- plus *para*-chlorinated congeners; 2-CB alone may constitute as much as 40 mol% of the total PCBs in extensively dechlorinated sediments (6, 34).

Strain VP44 did not grow on CBs that contain halogen atoms in both rings of the biphenyl moiety, such as 2,2′- and 2,4′-CB, like many other naturally occurring biphenyl degraders that do not possess a pathway for oxidation of chlorinated pentadiene (3, 9). Strain VP44 also did not grow on chloroacetate, which is thought to be a key metabolite from oxidation of chlorinated pentadienes (9, 30). Introduction of the CBA dechlorination genes still should enable a host organism to grow on such congeners via dehalogenation and oxidation of the respective CBA. However, we were not able to achieve reproducible growth of the recombinant variants of VP44 on 2,2′- and 2,4′-CB in batch cultures, although growth was observed on solidified K1 medium. This is not unexpected in this strain, since VP44 does not efficiently oxidize 2,2′- and 2,4′-CB, yielding only 15 to 25% and 2 to 5% of the corresponding CBAs, respectively (our unpublished data). While our results provide a proof of concept for constructing organisms that will grow on PCBs using dehalogenase genes, we have shifted our

efforts towards using host strains that cooxidize more of the PCBs produced by anaerobic dechlorination.

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