Construction of a Novel Polychlorinated Biphenyl-Degrading Bacterium: Utilization of 3,4'-Dichlorobiphenyl by *Pseudomonas acidovorans* M3GY

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*Pseudomonas acidovorans* M3GY is a recombinant bacterium with the novel capacity to utilize a biphenyl congener chlorinated on both rings, 3,4'-dichlorobiphenyl (3,4'-DCBP), as a sole carbon and energy source. Strain M3GY was constructed with a continuous amalgamated culture apparatus (L. Kröckel and D. D. Focht, Appl. Environ. Microbiol. 53:2470–2475, 1987) with *P. acidovorans* CCI19, a chloroacetate and biphenyl degrader, and *Pseudomonas* sp. strain CB15(1), a biphenyl and 3-chlorobenzoate degrader. Genetic and phenotypic data showed the recipient parental strain to be *P. acidovorans* CCI and the donor parental strain to be *Pseudomonas* sp. strain CB15. In growth experiments with 3,4'-DCBP as a sole source of carbon, cultures of strain M3GY increased in absorbance of 0.07 to 0.39 in 29 days while reaching a protein concentration of 58 μg ml⁻¹ and 67% substrate dehalogenation. 4-Chlorobenzoate was identified from culture supernatants of strain M3GY by gas chromatography-infrared spectrometry-mass spectrometry; this would be consistent with the oxidation of the m-chlorinated ring through the standard biphenyl pathway. 4-Chlorobenzoate was converted to 4-chlorocatechol, which was metabolized through the meta-fission pathway. The construction of *P. acidovorans* M3GY, with the novel capability to utilize 3,4'-DCBP, thus involves the complete use of meta-fission pathways for sequential rupture of the biphenyl and chlorobenzoate rings.

Polychlorinated biphenyls (PCBs) are long-lived environmental pollutants. Their persistence is due to the inability of single indigenous soil microorganisms to utilize them as growth substrates. Bacteria capable of utilizing monochlorobiphenyls and dichlorobiphenyls (DCBs) as sole carbon and energy sources do so by oxidizing the unchlorinated ring (3, 5, 11, 12). There are also reports of chlorobiphenyl (CBP)-mineralizing strains which grow on CBPs with a concomitant release of inorganic chloride (1, 16, 23, 25). However, there are no definitive reports of any organism capable of utilizing a PCB bearing a chlorine on both rings, hereafter referred to as C1,C1'-PCB. Although claims of isolation of PCB-mineralizing bacteria have been made, these earlier studies must be viewed as equivocal because the cultures are not available, the description of the media was insufficient for the media to be reproduced by others, the purity of PCBs (absence of biphenyl [BP]) was not determined, and no accompanying data on growth curves and chloride liberation were given (9).

Aerobic bacterial catabolism of PCBs proceeds through a pathway involving the formation of chlorobenzoic acids (3). The metabolic fate of the remaining five carbons produced by hydrolysis of the ring fission product is not well documented, although these carbons are likely to be metabolized through chlorinated aliphatic intermediates. Furukawa and Chakrabarty (10) first recognized that a PCB-mineralizing bacterium must be able to express the genes necessary for catabolism of chlorobenzoic acids and BP. Only recently has the concept been put into practice in the construction of hybrid strains able to utilize monochlorobiphenyls (1, 16, 23). None of these hybrid strains, however, are able to utilize C1,C1'-PCBs. Clearly, the ability to degrade chlorinated aliphatic metabolites formed with the subsequent production of chlorobenzoates (CBAs) from C1,C1'-PCBs must also be considered in construction of a PCB-utilizing bacterium.

Construction of recombinant strains by complementation of their catabolic pathways for the purpose of biodegradation has become a common practice in recent years. The hybrid strains have the combined catabolic characteristics of their parents and possess new degrading abilities. It would be desirable to recruit all of the necessary genes from the various genomes to construct a bacterium capable of utilizing C1,C1'-PCBs as carbon and energy sources. Such an organism would be a significant advance toward aerobic bioremediation of PCB-contaminated soils. In this report we describe the construction, genetics, and catabolic abilities of a recombinant bacterium, *Pseudomonas acidovorans* M3GY, which expresses a novel catabolic ability to utilize 3,4'-DCBP as a sole carbon and energy source. To our knowledge this is the first substantiated report of a bacterium utilizing BP chlorinated on each ring in media containing no growth-supportive additives.

**MATERIALS AND METHODS**

**Media.** Cultures were grown in a mineral salts medium (MSM) consisting of 10 mM K₂HPO₄, 3 mM NaH₂PO₄, 10 mM (NH₄)₂SO₄, and 1 mM MgSO₄ in deionized water supplemented with, per liter, 10 ml of chloride-free trace element stock solution which contains the following (per liter): CaSO₄, 200 mg; FeSO₄·7H₂O, 200 mg; MnSO₄·H₂O, 20 mg; NaMoO₄·2H₂O, 10 mg; CuSO₄, 20 mg; CoSO₄·7H₂O, 10 mg; and H₂BO₃, 5 mg.

**Chemicals.** 3-CBP was purchased from Chemicals Procurement Laboratories Inc. (New York, N.Y.) and also from Alfa Products, Johnson Matthey Co. (Danvers, Mass.). 4-CBP was acquired from Pfaltz & Bauer (Waterbury, Conn.), and 3,4'-DCBP and 3,3'-DCBP were obtained from Accustandard.

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(New Haven, Conn.) and were determined to be greater than 99% pure. BP was not detected by gas chromatography (GC). Chloroacetate was purchased from Distillation Products (Rochester, N.Y.) and recrystallized twice from dichloromethane before use. trans-3-Chlorocrotonic acid (3-CCA) was obtained from Dixon Fine Chemicals (Sherwood Park, Alberta, Canada). 3-CBA, 4-CBA, BP, pentafluorobenzylbromide (PFBB), and tetrabutylammonium hydrogen sulfate were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). 4-Chlorocatechol (4-CC) was obtained from Helix Biotech Co. (Richmond, British Columbia, Canada).

**Strain construction.** The recombinant strain was constructed by the continuous amalgamated culture method (20) using two parental strains: P. acidovorans CC1 and *Pseudomonas* sp. strain CB15(1). Strain CC1 has been described previously as *Alcaligenes* sp. strain CC1, an obligately aerobic gram-negative cocoid rod that is both oxidase and catalase positive and nonmotile (19). However, using the flagellum staining method of Heinbrook et al. (13), we found that this strain has one to three polar flagella. It accumulates poly-β-hydroxybutyrate as an intracellular-carbon reserve, does not grow on t-arabinose, and does not grow on Luria broth at 41°C. It can grow on lactate, fructose, malonate, ethanol, and p-hydroxybenzoate but not on glucose, L-arabinose, or succrose. According to these taxonomic descriptions (8), we propose that strain CC1 be designated *P. acidovorans*. Strain CB15 is a recombinant organism produced by continuous amalgamated culture mating of *Acinetobacter* sp. strain P6, a BP β-oxidizer, with *Pseudomonas* sp. strain HF1, a 3-CBA oxidizer (1). The parental strains CC1 and CB15 used in the mating experiments were maintained in the upper chemostats at 0.5 g of 3-CCA liter⁻¹ and 0.5 g of 3-CBA liter⁻¹, respectively. The ability to utilize 3-CCA, the only commercially available β-chloro unsaturated acid, was considered a priori, as similar analogs might result from meta cleavage of C1,C1'-PCBs. The column contained ceramic beads coated with 50 mg of 3,3'-DCBP. Samples were taken from the lower chemostat after 2 days and were transferred into 3-CBA broth. Upon growth, cultures were sequentially inoculated into a series of culture media: 3-CBP, 4-CBP, 3-CCA, BP, and 3,4'-DCBP. Strain M3GY was ultimately cultured on solid media as previously described (1).

**GC-mass spectrometry (MS) and GC-infrared spectrometry (IR)-MS.** GC-MS and GC-IR-MS analyses were performed on a 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) fitted to a Hewlett-Packard 5896A mass spectrometer and 5965B infrared detector. The injector temperature was held at 180°C; the ion source and quadrupole were held at 100 and 200°C, respectively. The infrared transfer line was held at 270°C, and the light pipe was held at 250°C. A 30-m DB-5 capillary column (J&W Scientific, Folsom, Calif.) with a 0.25-µm film thickness was used. The initial temperature was 32°C; this was held for 3 min, then ramped at 5°C min⁻¹ to 180°C, and then ramped to 280°C at 15°C min⁻¹. Helium was used as a carrier gas at a flow rate of 1 ml min⁻¹.

**Derivation of metabolic intermediates.** Methylation was performed by addition of an ethereal solution of diazomethane to concentrated ether extracts until a yellow color persisted. Samples were then concentrated while placed on ice with a gentle stream of dry N₂. PFBB derivatives of carboxylic acids were made by incubating 3 ml of culture supernatant of 3,4'- DCBP-grown cells with 100 µl of 100-mg ml⁻¹ tetrabutylammonium hydrogen sulfate and 20 µl of PFBB. PFBB derivatives were recovered by three repeated extractions with 2 ml of ethyl acetate. After recovery, PFBB derivatives were dried with anhydrous Na₂SO₄ and then concentrated as described above.

**Growth assays.** Strain M3GY was tested for the ability to utilize test substrates by inoculation into 125-ml Erlenmeyer flasks containing substrate (0.5 or 0.3 g liter⁻¹) and 50 ml of MSM. Flasks were placed on a rotary-platform shaker at 28°C and shaken at 200 rpm. For less soluble substrates (3-CBP and 4-CBP), 8 g of glass beads was added. For growth assays with 3,4'-DCBP, glass beads were coated with 10 mg of substrate.

Growth was determined by monitoring increased optical density (ΔA₅₅₀) along with the release of inorganic chloride and protein concentration.

**Protein assays.** Protein concentration was measured in whole cells by the Biuret method (21). In cell extracts, protein concentration was measured by the method of Bradford (6). Bovine serum albumin was used as a protein standard.

**Determination of inorganic chloride.** Inorganic chloride was measured potentiometrically with a 94-17B chloride ion-selective electrode coupled with a 90-02 reference electrode (both from Orion Research Inc., Boston, Mass.). NaCl was dried to a constant weight at a 115°C and used as a CI⁻ standard.

**Growing-cell transformations.** Cultures of strain M3GY were grown in 1-liter flasks containing 0.5 liter of MSM supplemented with 0.3 g of 4-CBP liter⁻¹. Cultures were harvested at various time points by centrifugation at 15,300 x g at 2°C for 10 min. The supernatant was then analyzed for growth intermediates.

**Resting-cell transformations.** Cultures of strain M3GY were grown on 1 g of BP liter⁻¹ in 1 liter of MSM. The medium was contained in 2.8-liter Fernbach flasks, placed on rotary platform shakers at 28°C, and shaken at 200 rpm. Cells were harvested at late exponential log phase and centrifuged at 15,300 x g at 2°C for 10 min. The pellets were washed twice with 50 mM phosphate buffer, pH 7.5, and resuspended to a final A₅₂₅ of 10. The cell suspension was divided into aliquots of 3 ml and incubated with 3 mM substrate. Transformations were stopped at appropriate intervals by adjusting the pH of the cell suspension to 1 and then centrifuging it.

**Oxygen uptake.** Oxygen uptake was measured with an oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) as previously described (18).

**Preparation of cell extracts.** Cultures of strain M3GY were prepared as described above except that the pellet was resuspended in 10 ml of phosphate buffer. The cells were disrupted by sonication with an Ultrasonics W185 cell disruptor (Heat Systems-Ultrasonics, Inc., Plainview, Long Island, N.Y.). The crude cell extracts were clarified by centrifugation at 40,000 x g for 30 min at 2°C. Cell extracts were used without further purification.

**Assays for meta-pyrocatechase activity.** Cultures of strain M3GY were grown in 2.8-liter Fernbach flasks with 1 liter of MSM supplemented with 0.3 g of 4-CBP liter⁻¹. Enzyme assays were performed with cell extracts as previously described (27).

**Extraction of metabolic intermediates.** Acidified samples were centrifuged to remove cells. The supernatant was then saturated with NaCl or (NH₄)₂SO₄. The saturated samples were extracted with 3 volumes of ethyl acetate or ether. The extracts were pooled and dried over anhydrous Na₂SO₄. The extracts were then concentrated by rotary evaporation at 25°C to 100 µl and derivatized for GC-MS or GC-IR-MS analysis.

**Isolation of DNA.** Isolation of chromosomal and plasmid DNA was performed as described previously (7). Separation of plasmid and chromosomal DNA was done with an ethidium bromide-CsCl density gradient (22) in two repeated cycles if necessary.
DNA manipulation techniques. Digestion of DNA with restriction endonucleases (Bethesda Research Laboratories, Bethesda, Md.) and electrophoresis of agarose gels were performed as described previously (7). Linear DNA fragments were recovered from agarose gels by using a Geneclean Kit as described by the manufacturer (Bio 101, Inc., La Jolla, Calif.).

DNA hybridization experiments. After transferring DNA from the agarose gel to a nylon membrane (26), hybridization experiments were performed with a Genius nonradioactive labeling and detection kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), according to the manufacturer's instructions, under conditions of high stringency (68°C, 0.1% SSC [100% SSC is 0.15 M NaCl plus 0.015 M sodium citrate]).

RESULTS

The recombinant strain M3GY was morphologically and phenotypically very similar to the parental strain P. acidovorans CC1. It was a gram-negative rod motile by one to three polar flagella and utilized lactate, fructose, malonate, ethanol, glucose, and p-hydroxybenzoate but was unable to utilize L-arginine, L-arabinose, or sucrose. The substrate utilization pattern determined on Biolog GN microplates showed 98% similarity between strains M3GY and CC1 but only 64% similarity between recombinant strain M3GY and parental strain CB15. Genomic fingerprinting and hybridization of total DNA showed strong genetic homology between the recombinant strain and parental strain CC1, while only moderate homology was observed between the recombinant strain and strain CB15 (data not shown). Thus, considering substrate utilization and genetic homology, the recombinant strain was designated P. acidovorans M3GY.

When grown on 3-CCa, strains CC1 and M3GY were each found to have a single plasmid, designated pRB1 (7.7 kb) and pRB2 (10.2 kb), respectively. Restriction endonuclease digestion and hybridization revealed that plasmid pRB2 was created by insertion of a 2.5-kb fragment into plasmid pRB1. A phenotypical variant of M3GY, designated M3G-C1, was obtained by curing M3GY of plasmid pRB2 by repeated culturing on minimal medium plates supplemented with 0.5 g of 3-CBa liter⁻¹. This suggested that genes involved in the degradation of this compound are carried on this plasmid. However, strain M3G-C1 was still able to utilize chloroacetate. No hybridization signal was detected when chromosomal DNA

![FIG. 1. DNA-DNA hybridization of partial EcoRI restriction fragments from plasmids pRB1 and pRB2 and chromosomal DNA of P. acidovorans M3G-C1 probed with randomly primed digoxigenin-labeled plasmid pRB1. Lane 1, chromosomal DNA of M3G-C1; lane 2, plasmid pRB2; lane 3, chromosomal DNA of Escherichia coli HB101.](image1)

![FIG. 2. DNA-DNA hybridization of clc operon from randomly primed digoxigenin-labeled plasmid pTCB45 (28) to Acinetobacter sp. strain P6 (lane 1), Pseudomonas sp. strain HF1 (lane 2), plasmid pTCB45 (lane 3), chromosomal DNA from E. coli HB101 (lane 4), P. acidovorans CC1 (lane 5), and P. acidovorans M3GY (lane 6).](image2)

![FIG. 3. Growth curve of recombinant strain M3GY on 3,4'-DCBP. Release of inorganic chloride was not monitored because of sample size limitations. However, analysis of the culture supernatant at the conclusion of the experiment indicated 67% mineralization. O.D., optical density.](image3)
of cured strain M3G-C1 was probed with plasmid pRB1 (Fig. 1), which excluded integration of 3-CCa genes into a chromosome. As the growth of the cured strain on 3,4'-DCBP was comparable to that of strain M3GY, growth on 3,4'-DCBP was not related to the utilization of 3-CCa but may be related to utilization of chloroacetate.

When the trichemostat was run in the absence of strain CB15, a recombinant able to use 3,4'-DCBP could not be isolated during an equivalent time period. Moreover, when the genes of the cle operon from Pseudomonas sp. strain PS1 (28) were used as a probe under conditions of high stringency, the probe hybridized with total DNA of strains HF1, CB15, and M3GY but not that of strains P6 and CC1 (Fig. 2). Thus, strain M3GY, which is most similar to parental strain CC1, acquired the genes encoding the modified ortho-fission pathway from the other parental strain, CB15. Moreover, its ability to utilize 3,4'-DCBP was a result of genetic exchange between both parental strains.

Growth of strain M3GY on 3,4'-DCBP was measured by increased A_{525} and protein concentration (Fig. 3). Utilization of 3,4'-DCBP was accompanied by a fivefold increase in absorbance, from 0.07 to 0.39, over a 29-day period. The final protein concentration of the culture medium reached 58 μg ml⁻¹. Dechlorination measured during growth experiments indicated that 67% of the aryl-bound 3,4'-DCBP chlorines were converted to inorganic chloride. These data show that strain M3GY is able to utilize 3,4'-DCBP as a sole carbon and energy source. Moreover, neither parental strain was able to utilize or dehalogenate 3,4'-DCBP.

Resting-cell transformations of 3,4'-DCBP by strain M3GY were performed to determine which ring was initially oxidized during catabolism of 3,4'-DCBP. Although 3-CBa and 4-CBa are indistinguishable by GC-MS, the isomers have distinguishable infrared spectra. A metabolic intermediate of 3,4'-DCBP catabolism was detected at low levels after incubation for 2.5 h by GC-IR-MS analysis of resting-cell transformation extracts. The intermediate was not detected in the zero time control or after 1 h of incubation and had disappeared after 5 h of incubation. The mass spectrum of the intermediate was in excellent agreement with that of an authentic sample of methylated 4-CBa (Fig. 4). The infrared spectrum of this intermediate was superimposable over a spectrum of authentic 4-CBa methyl ester (Fig. 5) and was not in agreement with the infrared spectrum of 3-CBa. On the basis of these data, the

FIG. 4. Mass spectrum of the methylated intermediate of 3,4'-DCBP metabolism by strain M3GY identified by GC-IR-MS analysis as 4-CBa.

FIG. 5. Infrared spectrum of the metabolic intermediate collected from the same analysis described in the Fig. 4 legend.
intermediate was identified as 4-CBa. These data show that the initial oxygenation of 3,4'-DCBP occurred at the ring bearing the Cl in the m position, to eventually lead to 4-CBa via the meta-fission pathway. Although 3-CBa was not detected as an intermediate in any of our experiments, an alternative pathway involving oxidation of the ring bearing a Cl in the p position cannot be excluded.

Chloroacetate was detected as its PFBB derivative in the 3,4'-DCBP-grown culture medium by GC-MS (Fig. 6). The mass spectrum of the metabolic intermediate was identical to that of an authentic standard of chloroacetate.

4-CC and its meta-cleavage product were detected as coelution products in the culture supernatant of 4-CBP-grown cells by GC-MS analysis in the 20-eV electron impact mode (data not shown). Attempts to separate the two compounds by varying the temperature ramp and injecting them onto a 60-m capillary column were unsuccessful. Similar results were observed in metabolic studies of chlorocatechols (16a). The methylated meta-cleavage product (2-hydroxy-5-chloromuconic semialdehyde) showed a strong M+ at m/z 204 and a characteristic M + 2 peak at 206 in an abundance ratio typical of a chlorine-containing compound. The ion peak at 169 represented M – Cl and showed no ion at 171. The ions at m/z 145 and 147 were in the proper ratio for a chlorinated compound and represent M – COOCH3, typical of methyl esters. The M+ from 4-CC was evident as a strong peak at m/z 144 and M + 2 at m/z 146 in the characteristic 3:1 ratio indicating the presence of a chlorine. Another peak was present at m/z 127 and 129, representing the loss of OH, M – OH. The peaks at m/z 127 and 129 would represent a spontaneous rearrangement to an unsaturated cyclic ketone, as evidenced by the loss of 28 as a carbonyl functional group, giving peaks at m/z 99 and 101 in a 3:1 ratio.

The metabolism of 4-CBa was investigated in strain M3GY. Strain M3GY could not use 4-CBa as a sole carbon source; nevertheless, BP-grown resting cells dehalogenated 4-CBa (50% in 12 h), presumably through glycolate (Fig. 7). Thus, 4-CBa does not appear to induce its own catabolism and requires BP, 3,4'-DCBP, or 4-CBP for induction.

M3GY exhibited a functional meta-cleavage pathway for metabolism of 4-CC. 4-CBP-grown cell extracts converted 4-CC into 2-hydroxy-5-chloromuconic semialdehyde at a rate of 116 nmol min⁻¹ mg of protein⁻¹. An alternative pathway for hydrolytic dehalogenation of 4-CBa to 4-hydroxybenzoate and via the protocatechuate pathway could be excluded, as cells did not produce 4-hydroxybenzoate nor did they show immediate O2 uptake (within a 5-min incubation) on it or on protocatechuate beyond endogenous levels.

**DISCUSSION**

Recombinant *P. acidovorans* is the first pure culture with the capacity to utilize a Cl,Cl'-PCB as a sole carbon and energy source. Our data show that strain M3GY initially attacked 3,4'-DCBP on the ring having a chlorine in the m position to yield 4-CBa, which was converted to 4-CC. A productive meta-cleavage pathway of 4-CC is substantiated by 2,3-dioxygenase activity with subsequent production of 2-hydroxy-5-chloromuconic semialdehyde and eventual detection of chloroacetate. Metabolism of 4-CC through the meta-fission pathway would lead to the formation of pyruvate and chloroacetalddehyde, the latter of which could be oxidized to chloroacetate in accordance with the scheme shown in Fig. 7.

The ability of BP utilizers to grow on chloroacetate is rare, as only 1 of 45 BP utilizers was able to do so, whereas chloropropionates and chlorobutyrate were utilized by most isolates (14). The ability to dehalogenate chloroacetate may play an important role in the utilization of Cl,Cl'-PCBs because chloroacetate or chloropropionate will be formed—except with an m-chlorosubstituent—through the meta-fission pathway.

**FIG. 7.** Proposed pathway for metabolism of 3,4'-DCBP by strain M3GY. Intermediates denoted by asterisks have been identified by GC-MS or GC-IR-MS analysis. The remaining intermediates are proposed according to the meta-fission pathway.
pathway (Fig. 7). Thus, conversion of chloroacetate to chloroacetyl coenzyme A, in contrast to dehalogenation to glycolate, would clearly present problems with biosynthesis or the tricarboxylic acid cycle (e.g., cis-aconitase).

Although strain M3GY demonstrated ortho-fission activity in cell extracts of 4-CBP-grown cells and harbors the clc operon (Fig. 2), the role of catechol 1,2-dioxogenase and the ortho-fission pathway appears irrelevant to the degradation of 4-CC, as this compound is metabolized not via spontaneous removal of chlorine by cycloisomerization of 3-chloro-cis-cis-muconate (ortho-fission pathway) (24) but by formation of chloroacetate (meta-fission pathway). The presence of the clc operon may play a role in an alternate pathway for the metabolism of 3,4'-DCBP to 3-CB by oxidation of the p-chloro-substituted ring. Growth of strain M3GY on 3-CB through 3-CC would have to proceed through the ortho-fission pathway because the meta-fission pathway would generate a suicidal acyl halide. Moreover, 3-CB is readily utilized as a growth substrate, in contrast to 4-CB, and would not likely have been detected to provide direct evidence for an alternate pathway. The important genes transferred from strain CB15 most likely were those encoding the dioxygenation of 4-CB and the subsequent dehydrogenation of the carboxyl diol to 4-CC.

Although 4-CBa is a central metabolite in the catabolic pathway of 3,4'-DCBP, it could not be utilized as a growth substrate by strain M3GY. Similar phenomena have been observed with 3,4-dichlorobenzoate (2) and 3,5-dichlorobenzoate (15). In the former case, the product, which was utilized as a growth substrate, was formed only when cells were induced by growth on 4-CB. In the latter case, 3,5-
 dichlorobenzoate was readily oxidized but did not induce production of the dioldehydrogenase, which was induced only by growth on 3- or 4-CB.

Why the recombinant strain cannot utilize 3,3'-DCBP as a growth substrate even though this substrate was used for its selection remains unclear. Metabolism of 3,3'-DCBP in this organism may yield 3-CC, which is known to be an inhibitor of metapyrocatechases (4, 17). Such an inhibition was observed in the parental strain CB15, although it did not prevent mineralization of 3-CBP through 3-CC (1). In contrast, metabolism of 3,4'-DCBP in strain M3GY proceeds through 4-CC, thereby avoiding the formation of 3-CC. Alternatively, the inability of strain M3GY to grow on 3,3'-DCBP may be related to high substrate specificity. In preliminary experiments, 3-CB was not detected in 3,3'-DCBP resting-cell transformations. Thus, strain M3GY may be unable to convert the ring fission product of 3,3'-DCBP, 2-hydroxy-4-chloro-6-oxo-6-(3'-chlorophenyl)-hexa-2,4-dienoic acid, into products of the meta-fission pathway.

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