Isolation and Characterization of *Desulfitobacterium frappieri* sp. nov., an Anaerobic Bacterium Which Reductively Dechlorinates Pentachlorophenol to 3-Chlorophenol

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An anaerobic bacterium, strain PCP-1^T (T = type strain), which dechlorinates pentachlorophenol (PCP) to 3-chlorophenol, was isolated from a methanogenic consortium. This organism is a spore-forming rod-shaped bacterium that is nonmotile, asaccharolytic, and Gram stain negative but Gram type positive as determined by electron microscopic observations. Inorganic electron acceptors, such as sulfite, thiosulfate, and nitrate (but not sulfate), stimulate growth in the presence of pyruvate and yeast extract. The optimum pH and optimum temperature for growth are 7.5 and 38°C, respectively. The dechlorination pathway is: PCP → 2,3,4,5-tetrachlorophenol → 3,4,5-trichlorophenol → 3,5-dichlorophenol → 3-chlorophenol. This bacterium dechlorinates several different chlorophenols at *ortho*, *meta*, and *para* positions; exceptions to this are 2,3-dichlorophenol, 2,5-dichlorophenol, 3,4-dichlorophenol, and the monochlorophenols. The time course of PCP dechlorination suggests that two enzyme systems are involved in dehalogenation in strain PCP-1^T. One system is inducible for *ortho* dechlorination, and the second system is inducible for *meta* and *para* dechlorinations. A 16S rRNA analysis revealed that strain PCP-1^T exhibits 95% homology with *Desulfitobacterium dehalogenans* JW/IU-DC1, an anaerobic bacterium which can dehalogenate chlorophenols only in *ortho* positions. These results suggest that strain PCP-1^T is a member of a new species and belongs to the recently proposed genus *Desulfitobacterium*. Strain PCP-1^T differs from *D. dehalogenans* JW/IU-DC1 by its broader range of chlorophenol dechlorination. Strain PCP-1 is the type strain of the new species, *Desulfitobacterium frappieri*.

Only a few anaerobic bacteria that can reductively dechlorinate chlorophenols have been isolated in pure culture. *Desulfomonile tiedjei* DCB-1 is the best-described dechlorinating anaerobic bacterium to date (7). This organism is a strictly anaerobic, gram-negative, sulfate-reducing bacterium that obtains energy for growth from reductive dehalogenation of 3-chlorobenzoate with formate as an electron donor (8, 19). In the presence of 3-chlorobenzoate, this strain can also dechlorinate *meta*-substituted halobenzoates (9, 15) and chlorophenols (18). Madsen and Licht (16) isolated a *Clostridium*-like microorganism, strain DCB-2, that can dechlorinate 2,4,6-chlorophenol to 2,4-chlorophenol and finally to 4-chlorophenol; removal of *meta*-substituted chlorine has been observed only with 3,5-chlorophenol. *Desulfitobacterium dehalogenans* JW/IU-DC1, a gram-positive anaerobic bacterium, can dehalogenate a wide range of chlorophenols, including pentachlorophenol (PCP) and related compounds, at the *ortho* position (22, 23). This strain can use various alternative electron acceptors, including sulfur, sulfoxide anions (except sulfate), nitrate, and fumarate. Recently, a novel, gram-negative, facultatively anaerobic, rod-shaped bacterium which can grow anaerobically via reductive dehalogenation of 2-chlorophenol was isolated (5). This microaerophilic bacterium is a member of the delta subclass of the class *Proteobacteria* and is more closely related to the myxobacteria than to the sulfidogenic bacteria.

A methanogenic consortium that degraded PCP completely in medium containing glucose and formate as complementary carbon sources was enriched in our laboratory. This consortium dechlorinated PCP to phenol, which was then mineralized (12). Spore-forming bacteria were involved in the dechlorina-

tion of PCP to 3-chlorophenol (3-CP), and a syntrophic bacterium was responsible for the dechlorination of 3-CP (11). In this paper, we describe the isolation and characterization of anaerobic bacterial strain PCP-1^T (T = type strain), which was responsible for dechlorination of PCP to 3-CP in this consortium. Strain PCP-1^T is the only known anaerobic bacterium that is able to dechlorinate chlorophenols in *ortho*, *meta*, and *para* positions.

MATERIALS AND METHODS

Origin and cultivation of the PCP-dechlorinating consortium. The anaerobic consortium that dechlorinated PCP was obtained from a mixture of anaerobic sewage sludge and soil samples that had been contaminated with PCP (12). This consortium was maintained with agitation at 29°C in a 2-liter continuous-flow sludge reactor that was fed with mineral salt medium containing 35 µM PCP, 0.5 g of glucose per liter, and 1.1 g of sodium formate per liter (11, 12). This reactor was operated at a hydraulic retention time of 10 days. Under these conditions, the microorganisms in the reactor were free and not associated in granules.

Enrichment and isolation of the PCP-dechlorinating microorganism. Samples from the continuous-flow reactor were transferred into 70-ml serum bottles and incubated in a water bath at 80°C for 30 min. Then the cultures were incubated at 29°C, and PCP (3 mg/liter) was added 24 h later. The dechlorinating cultures were transferred periodically by using 10% (vol/vol) inocula into 70-ml serum bottles containing 25 ml of medium A. This medium was prepared as described previously (11, 12). It was composed of the minimal medium described by Boyd et al. (2) supplemented with 3 to 5 mg of PCP per liter, 1 g of glucose per liter, 2.26 g of sodium formate per liter, and 2% (vol/vol) adsorbent DEAE-Sephacel beads (Pharmacia Biotech). The DEAE-Sephacel was washed three times in mineral salt medium, sparged with N₂-H₂-CO₂ (80:10:10), and autoclaved for 15 min. After several months of enrichment in serum bottles, an aliquot was diluted serially in fresh medium, and 0.1 ml was inoculated onto Columbia agar medium ANA 1121 (Laboratoires Quelab, Montreal, Canada) that had been pre-reduced in an anaerobic jar (BBL GasPak system) for 48 h. The microbiological work was performed in an anaerobic chamber (Bactron II; Sheldon Manufacturing, Inc., Cornelius, Oreg.) under a gas mixture containing 80% N₂, 10% H₂, and 10% CO₂. After 10 to 15 days of incubation at 29°C under anaerobic conditions, several colonies were isolated; these colonies were resuspended in 2 ml of mineral salt medium and inoculated into medium A supplemented with 0.1% (wt/vol) yeast extract in a serum bottle. The resulting cultures were incubated at 29°C and were tested for PCP-dechlorinating activity. Some isolates were able to

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dechlorinate PCP to 3-CP. One of the strains isolated was purified by subculturing it on Columbia solid medium. The purity of this culture was checked by photonic microscopy after Gram staining and by examining the colonies on solid medium.

Culture conditions and characterization of PCP-1^T. Whether strain PCP-1^T could grow with different organic substrates or vitamins was determined by adding the following compounds to mineral salt medium containing 2% (vol/vol) DEAE-Sephacel beads: 20 and 55 mM pyruvate, 0.02 and 0.1% (wt/vol) yeast extract, 100 µg of hemin per liter, and 200 µg of 1,4-naphthoquinone per liter. Some biochemical properties of PCP-1^T were determined by using the anaerobic API 20E system (Bio-Mérieux, Montreal, Canada). Different electron acceptors were tested by adding 10 mM Na₂SO₄, 5 mM Na₂SO₃, 10 mM Na₂S₂O₃, or 10 mM KNO₃ to culture medium containing 55 mM pyruvate, 0.02% yeast extract, and 2% DEAE-Sephacel beads. In this experiment, cysteine-HCl (2 mM) was used as the reducing agent instead of Na₂S.

pH and temperature ranges for growth. The pH and temperature ranges for growth of strain PCP-1^T were determined by monitoring the increase in optical density at 660 nm in culture medium containing 55 mM pyruvate, 0.1% yeast extract, and 5 mM Na₂SO₃. Media having pH values ranging from 6.0 to 9.0 were prepared by varying the acid and base components of the buffer. The pH was kept constant by frequently adjusting it with sterile 2 N NaOH. The pH range for growth was determined at 37°C. The temperature range for growth was determined in pH 7.2 culture medium incubated at 15, 20, 29, 37, 40, and 45°C. Assays were performed in duplicate.

Sensitivity to oxygen. The sensitivity of bacterial growth and dechlorination of 2,4,6-trichlorophenol to oxygen was determined in 120-ml serum bottles containing 20 ml of culture medium which contained no reducing solution. The medium was prepared under a gas mixture containing 80% N₂, 10% H₂ and 10% CO₂. A volume of air corresponding to 0, 1, 2, 5, and 10% of the headspace volume was added to the gas mixture. Each bottle was inoculated with 1.0 ml of a PCP-1^T culture in the exponential growth phase. The bottles were incubated in a shaking incubator at 37°C for 8 days.

Analytical procedure. The different chlorophenols were analyzed routinely by high-pressure liquid chromatography (HPLC), using a reverse-phase NovaPak C₁₈ column (3.9 by 150 mm). The Waters system used comprised a model 510 pump connected to a model 481 Lambda-Max Lc spectrophotometer and a model Professional 350 Digital computer. Some chlorophenol degradation intermediates were identified with a gas chromatograph (Varian model 3500) connected to a mass spectrometer (Ion trap 800; Finnigan Mat). The HPLC and gas chromatography-mass spectrometry operating parameters used have been described previously (11, 12).

Electron microscopy. The morphology of PCP-1^T was determined with a Hitachi model 7100 electron microscope after negative staining with 1% phosphotungstate as described by Alain et al. (1). Ultrathin sections were also prepared. Bacteria were fixed by incubating them for 15 min at room temperature with 2.5% (vol/vol) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2). The cells were pelleted by centrifugation in an Eppendorf tube at 1,300 rpm (15,000 × g) for 3 min and washed three times with a 3% (wt/vol) sucrose solution in cacodylate buffer. The cell pellets were then postfixated with 2% (wt/vol) osmium tetroxide, dehydrated in acetone, and embedded in Spurr resin. Ultrathin sections were stained with 5% (wt/vol) uranyl acetate for 20 min and then with lead citrate for 5 min before they were examined.

Determination of G+C content. The guanine-plus-cytosine (G+C) content was determined after enzymatic digestions with nuclease P1 and alkaline phosphatase and HPLC separation of nucleosides as described by Mesbah et al. (17).

DNA manipulation. Extraction of total PCP-1^T DNA, PCR amplification of the 16S ribosomal gene, cloning and sequencing of the gene, and a sequence analysis were performed as described by Li et al. (14). Briefly, the 16S ribosomal gene was amplified by PCR by using universal eubacterial primers (4) and strain PCP-1^T genomic DNA. The resulting 1.6-kbp DNA fragment was then cloned in the Bluescript vector (Stratagene) and sequenced by the dideoxynucleotide method (21). The PCP-1^T sequence was compared with sequences obtained from databases by using the FASTA and BLAST programs. Phylogenetic analyses were performed by using different programs of the PHYLIP package (version 3.5) (10) and the most closely related species (see Table 1 and Fig. 5). The secondary structure of minimum free energy for an RNA molecule based on previously published values for stacking and loop-destabilizing energies was calculated by using the FOLD program (25) of the GCG sequence analysis package (Genetics Computer Group, Inc.).

Dechlorination of different chlorophenols. Experiments to determine whether various chlorophenols were dechlorinated were performed in serum bottles containing 25 ml of mineral salt medium supplemented with 20 mM pyruvate, 0.02% (wt/vol) yeast extract, and 2% (vol/vol) DEAE-Sephacel; 2.5 ml of an active culture was used as the inoculum in each experiment. The chlorophenols were added 24 h after inoculation at concentrations of 5 mg/liter (21 µM) for the tetrachlorophenols and 3 mg/liter (16 to 23 µM) for the other chlorophenols. In some experiments, 3 mg of PCP per liter (11 µM) was also added to the culture to induce dechlorinating activity. The cultures were incubated at 29°C. Triplicate preparations were analyzed by HPLC at zero time and on days 1, 2, 4, and 8.

Nucleotide sequence accession number. The GenBank accession number for the 16S rRNA sequence of *Desulfitobacterium frappieri* PCP-1^T is U40078.

RESULTS

Isolation and characterization of PCP-1^T. PCP-dehalogenating strain PCP-1^T was isolated after heat treatment and growth of enrichment cultures in serum bottles containing glucose, sodium formate and PCP. The strain was isolated by repeatedly preparing serial dilutions, inoculating preparations onto solid medium, and recovering the most dilute dechlorinating biomass. Strain PCP-1^T grew on anaerobic Columbia agar within 10 to 15 days when it was incubated at 29°C. The colonies of this organism were white, circular and convex with undulate margins, and 2.5 mm in diameter. Electron micrographs revealed that strain PCP-1^T cells are rod shaped and 2.2 to 4.5 µm long by 0.7 µm wide (Fig. 1). No flagellum was observed. A terminal endospore was present in some cells. The Gram stain reaction was negative, regardless of the growth phase. However, no outer membrane was observed in electron micrographs, suggesting that PCP-1^T is Gram type positive (Fig. 1B). The G+C content of the genomic DNA was 46% mol%.

The dechlorinating activities of isolated colonies were observed in liquid cultures only when 0.1% (wt/vol) yeast extract was added to medium A or when 20 mM pyruvate replaced glucose-formate as the secondary carbon source. As strain PCP-1^T could not grow on glucose and formate, it was necessary to have some other bacteria in the enrichment culture to transform these compounds into utilizable substrates. Pyruvate was the only defined organic substrate utilized in the presence of 0.02% yeast extract that provided carbon and energy to sustain fermentative growth of strain PCP-1^T. DEAE-Sephacel beads were important during cultivation and acted as an adsorbent to reduce the toxicity of some components of the medium, such as Na₂S (data not shown). However, the beads were not necessary when the Na₂S concentration in the medium was reduced by a factor of 10. A mixture of glucose and formate did not support growth. Hemin and 1,4-naphthoquinone were not necessary for growth and dechlorinating activity. The anaerobic API 20E system data showed that PCP-1^T was positive for indole formation; weakly positive for catalase activity; negative for hydrolysis of urea and esculin; negative for fermentation of glucose, mannitol, lactose, saccharose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose; and negative for gelatin liquefaction. Some other alternative electron acceptors, including sulfite, thiosulfite, and nitrate (but not sulfate) stimulated growth of PCP-1^T in the presence of pyruvate and yeast extract (Fig. 2), suggesting that PCP-1^T was able to reduce these inorganic substances. The pH range for growth of strain PCP-1^T was 6.0 to 9, and the optimum pH was 7.5. The temperature range for growth was 15 to 45°C, and the optimum temperature was around 38°C. Growth and dechlorination did not occur when a serum bottle contained 1% air in the gas phase. The generation time of strain PCP-1^T in medium containing 20 mM pyruvate, 0.02% yeast extract, and 5 mg of PCP per liter at pH 7.2 was approximately 3 h at 29°C.

Phylogeny. The 16S ribosomal gene of strain PCP-1^T was amplified by PCR and then cloned and sequenced. The PCP-1^T 16S rRNA gene is unusually long; this gene is 1,654 nucleotides long, which is about 100 nucleotides longer than most 16S rRNA genes. A search of the gene databases revealed that the 16S rRNA gene of *Desulfitobacterium dehalogenans* is very similar (level of similarity, 95%) to the 16S rRNA sequence of strain PCP-1^T. A 128-nucleotide insertion (nucleotides 63 to 190) is the main difference between the two sequences and is also responsible of the unusual length of the PCP-1^T 16S gene. This sequence is located in the secondary

FIG. 1. Electron micrographs of negatively stained strain PCP-1^T cells (A) and an ultrathin cross section of a strain PCP-1^T cell (B). (A) Bar = 1.0 μ m. (B) Bar = 0.1 μ m.

structure of helix 6-VI (*Escherichia coli* nomenclature) (20) (Fig. 3). An analysis of the putative secondary structure of this region of the PCP-1^T 16S rRNA revealed a long loop with 72% base pairing. In *Desulfotobacterium dehalogenans*, a small loop similar in size to the *E. coli* loop was predicted.

A phylogenetic analysis was performed with related species (Table 1 and Fig. 4). The species used were chosen on the basis of the results of phylogenetic analyses performed by Utkin et

al. (23) for *Desulfotobacterium dehalogenans* and by Collins et al. (6) for *Clostridium* and *Clostridium*-like species. The results of the phylogenetic analysis confirmed the close relationship between strain PCP-1^T and *Desulfotobacterium dehalogenans*. *Desulfotomaculum orientis* has been reported to have the 16S rRNA sequence that is most similar to the *Desulfotobacterium dehalogenans* sequence (23) (level of similarity, 93.4%), although more than 14% of the reported sequence is composed of undetermined or missing nucleotides. A comparison of the strain PCP-1^T, *Desulfotobacterium dehalogenans*, and *Desulfotomaculum orientis* 16S rRNA sequences revealed that strain PCP-1^T is more closely related to *Desulfotobacterium dehalogenans* (level of similarity, 96.8%) than to *Desulfotomaculum orientis* (level of similarity, 92.3%). These results suggest that strain PCP-1^T belongs to the genus *Desulfotobacterium*. Strain PCP-1 is the type strain of a new species named *Desulfotobacterium frappieri*.

Description of *Desulfotobacterium frappieri* sp. nov. *Desulfotobacterium frappieri* (frap.pi.e'ri M. L. gen. n. *frappieri*, of Frappier, in honor of Armand Frappier, a renowned Canadian microbiologist). Strain PCP-1 is the type strain of *Desulfotobacterium frappieri*. This strain has been deposited in the American Type Culture Collection.

Dechlorinating activity. The kinetics of dechlorination of PCP to 3-CP is shown in Fig. 5. PCP was dechlorinated to 3,4,5-trichlorophenol in less than 12 h. A lag of 36 h occurred before 3,4,5-trichlorophenol began to be dechlorinated to 3,5-dichlorophenol and 3-CP. A trace amount of 3,4-dichlorophenol was also found, and this trace probably originated from

FIG. 2. Growth of strain PCP-1^T in medium containing 55 mM pyruvate plus 0.1% (wt/vol) yeast extract (■) and in the same medium supplemented with the electron acceptors 10 mM sulfate (▲), 5 mM sulfite (◆), 10 mM thiosulfate (□), and 10 mM nitrate (●). Data are means of duplicate determinations. O.D. (600 nm), optical density at 600 nm.

FIG. 3. Secondary structure of helix 6-VI of *E. coli* 16S rRNA (20) and the proposed helices of *Desulfitobacterium dehalogenans* and *Desulfitobacterium frapperi*. The *E. coli* numbering is the numbering of Brosius et al. (3). Canonical pairs are connected by vertical lines, and G-U pairs are connected by colons. nt, nucleotide.

2,3,4,6-tetrachlorophenol, a known contaminant of PCP preparations. We examined the dechlorination of all possible chlorophenols by strain PCP-1^T (Table 2). Dechlorinations in *ortho*, *meta*, and *para* positions were observed. The following chlorophenols were not dehalogenated: 2-chlorophenol, 3-CP, 4-chlorophenol, 2,5-dichlorophenol, and 3,4-dichlorophenol. No dehalogenation of these mono- and dichlorophenols was observed even when PCP was added to the culture medium as a possible inducer.

DISCUSSION

We isolated a member of a new bacterial species that is responsible for dechlorination of PCP to 3-CP in a methanogenic consortium. On the basis of the results of a 16S rRNA sequence analysis, strain PCP-1^T is closely related to *Desulfitobacterium dehalogenans* JW/IU-DC1 (23). No other bacterial species are closely related to these two bacteria. Strain PCP-1^T and *Desulfitobacterium dehalogenans* share several characteris-

TABLE 1. Evolutionary distances between strain PCP-1^T and related taxa^a

Taxon	Evolutionary distance from:													
	<i>Acetogenium kiwi</i>	<i>Desulfotomaculum thermobenzoicum</i>	<i>Clostridium</i> -like strains	<i>Clostridium butyricum</i>	<i>Clostridium quercicolum</i>	<i>Sporomusa paucivorans</i>	<i>Megasphaera elsdenii</i>	<i>Clostridium thermoaceticum</i>	<i>Desulfotomaculum nigrificans</i>	<i>Desulfitobacterium dehalogenans</i>	Strain PCP-1 ^T	<i>Hellobacterium chlorum</i>	<i>Peptococcus niger</i>	<i>Quinella ovalis</i>
<i>Desulfotomaculum thermobenzoicum</i>	0.186													
<i>Clostridium</i> -like strains	0.230	0.227												
<i>Clostridium butyricum</i>	0.231	0.236	0.186											
<i>Clostridium quercicolum</i>	0.219	0.210	0.206	0.251										
<i>Sporomusa paucivorans</i>	0.216	0.214	0.206	0.238	0.102									
<i>Megasphaera elsdenii</i>	0.245	0.233	0.217	0.265	0.159	0.171								
<i>Clostridium thermoaceticum</i>	0.143	0.135	0.205	0.211	0.202	0.217	0.211							
<i>Desulfotomaculum nigrificans</i>	0.198	0.143	0.212	0.209	0.203	0.222	0.211	0.166						
<i>Desulfitobacterium dehalogenans</i>	0.196	0.175	0.187	0.198	0.189	0.198	0.218	0.163	0.161					
Strain PCP-1 ^T	0.231	0.231	0.192	0.209	0.194	0.193	0.231	0.174	0.178	0.032				
<i>Hellobacterium chlorum</i>	0.207	0.198	0.197	0.215	0.187	0.201	0.234	0.174	0.163	0.138	0.154			
<i>Peptococcus niger</i>	0.238	0.226	0.237	0.235	0.236	0.235	0.232	0.196	0.201	0.198	0.216	0.220		
<i>Quinella ovalis</i>	0.231	0.224	0.241	0.246	0.175	0.196	0.212	0.227	0.229	0.226	0.226	0.199	0.252	
<i>Escherichia coli</i>	0.289	0.300	0.278	0.267	0.333	0.331	0.313	0.281	0.303	0.279	0.289	0.283	0.310	0.326

^a The 1,421 unambiguously alignable nucleotide sites in the 15 sequences were used for pairwise comparisons in which the DNADIST program was used with the Kimura two-parameter option (13).

FIG. 4. Phylogenetic tree based on 16S ribosomal DNA sequences of strain PCP-1^T and related species. The organisms whose sequences were used for comparison (GenBank accession numbers are in parentheses) were *Desulfotobacterium frapperi* PCP-1^T (U40078), *Acetogenium kiwi* (L09160), *Clostridium butyricum* (X68176), *Clostridium quercicolum* (M59110), *Clostridium thermoaceticum* (M59121), *Desulfotobacterium dehalogenans* (L28946), *Desulfotomaculum nigrificans* (X62176), *Desulfotomaculum thermobenzoicum* (M59121), *Escherichia coli* (J01695), *Heliobacterium chlorum* (M11212), *Megasphaera elsdenii* (L15628), *Peptococcus niger* (X55797), *Quinella ovalis* (M62701), *Sporomusa paucivorans* (M59117), and the phenol-transforming organism *Clostridium* sp. strain 6 (U27710), which was recently described by Li et al. (14). The tree was derived by using the FITCH program of the PHYLIP package (version 3.5), the distances in Table 1, and the *E. coli* 16S ribosomal gene as an outgroup. A bootstrap analysis of the same sequences was performed by using the SEQBOOT program and 500 replicates. The distances were calculated from each replicate with the DNADIST program by using the Kimura two-parameter option. The FITCH program was used to derive the best tree for each replicate. The CONSENSE program was used to derive the consensus tree. A total of 500 replicates were examined in the bootstrap analysis, and the numbers at the forks indicate how many times a species or a group of species originated at each fork. Bar = 0.05 nucleotide substitution per site.

tics. They are both rod-shaped bacteria that are asaccharolytic and grow on media containing pyruvate and 0.1% yeast extract. Both organisms can use sulfite, thiosulfate, or nitrate, but not sulfate, as an inorganic electron acceptor. They are gram positive, although PCP-1^T stained gram negative. The G+C contents of their genomic DNAs are similar (46 mol% for PCP-1^T and 45 mol% for *Desulfotobacterium dehalogenans*). Finally, they can reductively dehalogenate several chlorophenols. However, *Desulfotobacterium dehalogenans* can dehalogenate only chlorophenols with chlorines in *ortho* positions, whereas strain PCP-1^T is not limited to dehalogenation at this position.

There are several differences between the two species. PCP-1^T forms spores, does not have flagella, and grows on 1.5% (wt/vol) agar plates. *Desulfotobacterium dehalogenans* apparently does not form spores, has flagella, and grows on solid media containing 0.6 to 0.9% agar but not in the presence of higher agar concentrations. The 16S rRNA gene sequences of these organisms exhibited 95% homology, and there was a 128-nucleotide insertion that was unique to PCP-1^T. These results suggest that PCP-1^T is a member of a species that is distinct from *Desulfotobacterium dehalogenans*. However, the

FIG. 5. Dechlorination of PCP by strain PCP-1^T in medium containing 20 mM pyruvate and 0.02% yeast extract. Symbols: ■, PCP; ●, 3,4,5-trichlorophenol; ▲, 3,5-dichlorophenol; ◆, 3,4-dichlorophenol; □, 3-CP; ○, PCP (autoclaved culture). Data are means from triplicate determinations.

similarities between the two bacteria suggest that PCP-1^T belongs to the recently proposed genus *Desulfotobacterium*.

Strain PCP-1^T is the first anaerobic microorganism isolated that can dechlorinate PCP to 3-CP. PCP and the tetrachlorophenols are first dechlorinated at the *ortho* positions, then dechlorinated at the *para* positions, and then dechlorinated at the *meta* positions. Also, *para* dechlorination occurs only if chlorine residues are present in both *meta* positions. Other anaerobic microorganisms, including *Desulfotobacterium dehalogenans* JW/IU-DC1 (22, 23), strain DCB-2 (16), and the recently described organism strain XZ-1 (24), can generally dechlorinate only at *ortho* positions. Strain DCB-2 can remove a *meta*-substituted chlorine only from 3,5-dichlorophenol. However, *Desulfomonile tiedjei* DCB-1 *meta* dehalogenates a variety of substituted halobenzoates (9) and chlorophenols (18). *Desulfotobacterium dehalogenans* can remove the halogen at the *ortho* position when the *para* position is substituted, as it is in chlorinated *p*-hydroxyphenylacetate, *p*-hydroxybenzoates, *p*-nitrophenols, and *p*-cresols (22).

The kinetics of dechlorination of PCP by strain PCP-1^T revealed that PCP was rapidly dechlorinated to 3,4,5-trichlo-

TABLE 2. Transformation of chlorophenols by strain PCP-1^T

Substrate ^a	Product(s) ^a	Position of dechlorination ^b
2,3,4,5-TeCP	3,4,5-TCP → 3,5-DCP → 3-CP	<i>o, p, m</i>
2,3,5,6-TeCP	2,3,5-TCP → 3,5-DCP → 3-CP	<i>o, o, m</i>
2,3,4-TCP	3,4-DCP	<i>o</i>
2,3,5-TCP	2,5-DCP	<i>m</i>
2,3,6-TCP	2,5-DCP	<i>o</i>
2,4,5-TCP	3,4-DCP	<i>o</i>
2,4,6-TCP	2,4-DCP → 4-CP	<i>o, o</i>
3,4,5-TCP	3,5-DCP → 3-CP	<i>p, m</i>
2,6-DCP	2-CP	<i>o</i>
2,4-DCP	4-CP	<i>o</i>
3,5-DCP	3-CP	<i>m</i>
2,3-DCP	No dechlorination ^c	
2,5-DCP	No dechlorination ^c	
3,4-DCP	No dechlorination ^c	
2-CP	No dechlorination ^c	
3-CP	No dechlorination ^c	
4-CP	No dechlorination ^c	

^a Abbreviations: TeCP, tetrachlorophenol; TCP, trichlorophenol; DCP, dichlorophenol; CP, chlorophenol.

^b *o*, *ortho*; *p*, *para*; *m*, *meta*.

^c No dechlorination occurred in the presence or in the absence of 3 mg of PCP per liter as an inducer.

rophenol and that there was a 36-h lag period before this compound was dechlorinated to 3,5-dichlorophenol and 3-CP. This result suggests that two different enzyme systems are involved in PCP dechlorination. The first system dechlorinates PCP rapidly and dechlorinates 2,3,4,5-tetrachlorophenol only at the *ortho* position to generate 3,4,5-trichlorophenol, while the second system dechlorinates 3,4,5-trichlorophenol at the *meta* and *para* positions to generate 3-CP. PCP and tetrachlorophenols may act as inducers of the *ortho* dechlorination, and 3,4,5-trichlorophenol may act as an inducer of dechlorination in the *para* and *meta* positions. For 3,4,5-trichlorophenol, a 36-h lag period is probably needed for the bacterial cells to induce and generate the dechlorination enzymes, whereas for PCP no lag period is needed. This hypothesis for induction of *ortho* and *meta-para* dechlorinations could explain the differences observed with 2,3,5-trichlorophenol dechlorination. 2,3,5,6-Tetrachlorophenol induced *ortho* dechlorination and the intermediate 2,3,5-trichlorophenol was dechlorinated to 3,5-dichlorophenol. However, when 2,3,5-trichlorophenol was tested separately, *meta* dechlorination was induced, and this chlorophenol was dechlorinated to 2,5-dichlorophenol. This hypothesis is currently being tested.

To our knowledge, PCP-1^T is the first anaerobic bacterium that is able to reductively dechlorinate PCP and different chlorophenols at *ortho*, *meta*, and *para* positions. Experiments are now being carried out to optimize the conditions for culturing PCP-1^T and to evaluate the potential for transforming other classes of halogenated compounds.

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