Dechlorination and *para*-Hydroxylation of Polychlorinated Phenols by *Rhodococcus chlorophenolicus*

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In this paper we show that a polychlorophenol degrader *Rhodococcus chlorophenolicus* PCP-I initially attacked polychlorinated phenols (pentachlorophenol, 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol, and 2,3,5- and 2,3,6-trichlorophenol) by tetra- or trichlorohydroquinone-producing *para*-hydroxylation. The novel hydroxyl group was set in position 4, whether or not a substrate had chlorine substituent in this position. The hydroxyl was in each case derived from water molecules, as was shown by following the incorporation of oxygen from $H_2^{18}O$ into the reaction products. Nevertheless, the *para*-hydroxylation reaction required the presence of molecular oxygen, whereas further metabolism of the reaction product, tetrachlorohydroquinone, proceeded also in anaerobiosis. All polychlorinated phenols were readily transformed at 41°C, but none were transformed at 44°C. In contrast to this, tetrachlorohydroquinone was metabolized at a high rate at 50°C, but was not metabolized at 55°C. Polychlorinated phenols were specific inducers of the *para*-hydroxylating enzymes; *para*-hydroxylated reaction products did not induce these enzymes. On the other hand, the degradation of tri- and tetrachlorohydroquinone was induced by any of the chlorophenols and also by hydroquinones.

Degradation of polychlorinated phenols by pure bacterial cultures has been demonstrated by several research groups (2a, 4–6, 13, 23, 27, 28, 30), but the enzymatic reactions have not been characterized. The degradation routes of mono- and dichlorinated phenols, benzoates, and benzenes are known in more detail. Shelton and Tiedje demonstrated reductive dehalogenation of 3-chlorobenzoic acid by an obligate anaerobe (26). There have been many reports on aerobic bacterial degradation of mono- and dichlorinated phenols, benzoates, and benzenes through chlorocatechol; in these, dehalogenation followed the ring cleavage (3, 9, 15, 20). All work so far published on the destruction of benzenoid compounds supports the view that the benzene ring is recalcitrant against aerobic cleavage unless it carries at least two labilizing hydroxyl groups. If this is true also for PCP, the replacement of at least one chlorine substituent by a hydroxyl should precede aerobic ring cleavage.

We isolated a novel actinomycete *Rhodococcus chlorophenolicus* PCP-I (1) from a PCP-degrading mixed bacterial culture (2, 24, 29). *R. chlorophenolicus* was shown to degrade 11 different chlorophenols, most efficiently PCP, 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP (2a). It also degrades several polychlorinated guaiacols (8) and TeCH (2a). In this report we describe the initial attack on polychlorinated phenols, the reaction products, requirements for oxygen and temperature, inducers, and the origin of the incorporated oxygen.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: PCP, pentachlorophenol; 2345-TeCP, 2346-TeCP, and 2356-TeCP, 2,3,4,5-, 2,3,4,6- and 2,3,5,6-tetrachlorophenol, respectively; 235-TCP and 236-TCP, 2,3,5- and 2,3,6-trichlorophenol, respectively; TeCH, tetrachlorohydroquinone (2,3,5,6-tetrachloro-1,4-dihydroxybenzene); TCH, trichlorohydroquinone (2,3,5-trichloro-1,4-dihydroxybenzene); TeCC, tetrachlorocatechol (3,4,5,6-tetrachloro-1,2-dihydroxybenzene); 345-TCC and 346-TCC, 3,4,5- and 3,4,6-trichlorocatechol, respectively (3,4,5- and 3,4,6-trichloro-1,2-dihydroxybenzene); TeCR, tetrachlororesorcinol (2,4,5,6-tetrachloro-1,3-dihydroxybenzene); GLC, gas-liquid chromatography.

Bacterial strain and culture conditions. *R. chlorophenolicus* PCP-I (DSM 43826), used in all experiments, originated from a PCP-degrading mixed culture (2, 24, 29); it was shown to degrade or mineralize several chlorophenols, chloroguaiacols, and TeCH (2a, 8). The taxonomic properties of *R. chlorophenolicus* have been reported in detail (1). The inoculum was grown in liquid culture on rhamnose, and for most experiments it was preincubated in the presence of PCP as previously described (2a). Culture flasks were incubated in a gyratory shaker at 28°C in the dark, unless otherwise mentioned. Bacterial density was 5×10^8 to 8×10^8 ml^{-1} in all the studies.

Substrates and model compounds. PCP was from E. Merck AG (Darmstadt, Federal Republic of Germany); 2345-TeCP, 2356-TeCP, and the TCPs were from Ega-Chemie (Steinheim, Federal Republic of Germany); and TeCH was from Eastman Kodak Co. (Rochester, N.Y.). Commercially available 2346-TeCP (Fluka, Buchs, Switzerland) was purified by vacuum distillation. TCH, TeCC, TeCR, 345-TCC, and 346-TCC were synthesized by J. Knuutinen (Department of Chemistry, University of Jyväskylä). Chlorocatechols were prepared as described by J. Knuutinen (Ph.D. thesis, University of Jyväskylä, Jyväskylä, Finland, 1984). The same procedure was applied for the synthesis of TCH from 2,3,5-trichloro-4-hydroxybenzaldehyde (16). TeCR was prepared by chlorination of 1,3-dihydroxybenzene (22). The purity (>99% by GLC with flame ionization detection) and the structures of the model compounds were confirmed by quartz capillary gas chromatography,
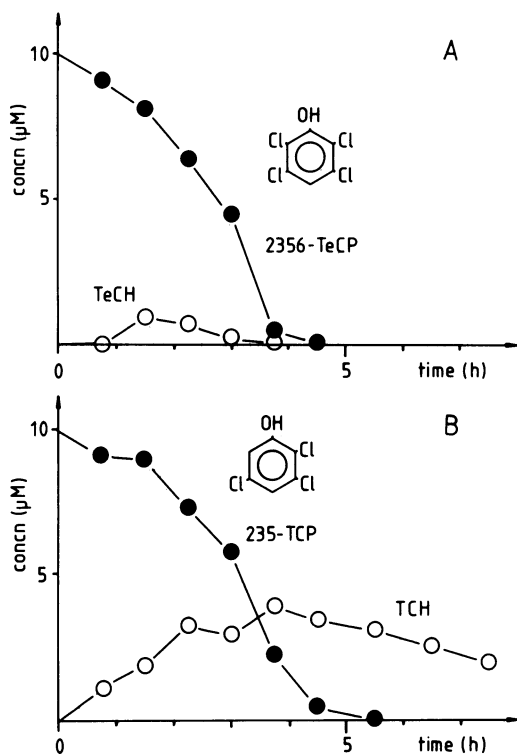
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FIG. 1. Production of metabolic intermediates from 2356-TeCP and 235-TCP by *R. chlorophenolicus*.

nuclear magnetic resonance spectroscopy, and mass spectrometry. The initial concentrations of the chlorophenols and chlorohydroquinones in the cultures were 10 μM throughout this work. TeCH was always introduced into cultures with ascorbic acid (0.1%, wt/vol), which slowed down nonbiological oxidation.

Adjustment of O_2 concentrations. Empty, 25-ml culture ampoules were evacuated with a vacuum pump and filled to atmospheric pressure with N_2 (<0.0001% O_2) passed through an oxygen-scavenging filter (Oxy-filter; Mikro-Laboratoriet, Højbjerg, Danmark). The procedure was repeated twice, and after a further evacuation a total of 15 ml of gas (O_2 or N_2 or both) was injected. The PCP-induced liquid culture of *R. chlorophenolicus* was deoxygenated by washing with helium (purity, 99.995%). The concentration of oxygen in the culture was monitored for by an oxygen electrode (model 97-08; Orion Research, Cambridge, Mass.), and maintained below 0.05 ppm; 10 ml of this deoxygenated culture was then injected into the culture ampoules containing the O_2-N_2 gas mixture. Each experiment was started by adding 50 μl of substrate solution (2 mM in 0.01 M NaOH).

Experiments with $^{18}O_2$ and $H_2^{18}O$. Culture ampoules were evacuated as described above. 2.0 ml of $^{16}O_2$ or $^{18}O_2$ (isotopic purity, 99%; Amersham International plc, Buckinghamshire, England), 18.0 ml of N_2 (<0.0001% O_2), and 5 ml of deoxygenated liquid culture of *R. chlorophenolicus* were then injected into the 25-ml ampoules. Alternatively, 2.0 ml of $^{16}O_2$, 18.0 ml of N_2, 4 ml of the bacterial culture, and 1 ml of $H_2^{16}O$ or $H_2^{18}O$ (isotopic purity, 96%; Amersham International) was injected into each evacuated ampoule. The reaction was started by adding a chlorophenol. The production of metabolites was followed in ^{16}O cultures; once the

maximum concentration was reached, the metabolites from all of the parallel cultures were extracted, ethylated, and analyzed as described below.

Analysis. The concentration of chlorophenolic compounds in the cultures was assayed by taking 0.5-ml samples, which were acetylated, extracted into hexane, and analyzed by gas chromatography as reported elsewhere (2a). A CP Sil 19 CB capillary column (Crompack International B.V., Middelburg, The Netherlands) was used. Metabolites emerging were identified by gas chromatography-mass spectrometry; 5 to 50 ml of culture was acidified with 0.1 volume of 5 M HCl, phenolic compounds were extracted into diethyl ether, water was removed from the extract with dry $MgSO_4$, and the solution was concentrated to a volume of 100 μl and ethylated with diazoethane as described for diazomethane by Schlenk and Gellerman (25). The derivatized metabolites were analyzed with a Hewlett-Packard HP 5880 gas chromatograph equipped with a SE-30 capillary column (Orion Analytica, Espoo, Finland) and an HP 5970 A mass selective detector.

RESULTS

Degradation intermediates from chlorophenols. *R. chlorophenolicus* removed 10 μM PCP, 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP completely from the cultures in 6 h. While the degradation proceeded, two major metabolites appeared in the medium. *R. chlorophenolicus* formed from PCP and 2356-TeCP a compound identical to TeCH by GLC and from 2345-TeCP, 2346-TeCP, 235-TCP, and 236-TCP a compound identical to TCH. Figure 1 illustrates the appearance of TeCH and TCH in the metabolism of 2356-TeCP and 235-TCP, respectively. TeCH accumulated transiently with a maximum concentration of 1 μM from 2356-TeCP (Fig. 1A). The maximum concentration of TeCH from PCP was about 0.2 μM. The highest concentration of TCH was observed during the metabolism of 235-TCP and 236-TCP (4 μM; Fig 1B). The concentration of TCH measured in the 2345-TeCP- and 2346-TeCP-degrading cultures was around 1 μM.

Figure 2 shows the mass spectra of the ethylated metabolites from 2356-TeCP and 235-TCP and of authentic TeCH and TCH. GLC retention times of the acetyl and ethyl derivatives of the tri- and tetrachlorinated dihydroxybenzenes (excluding trichlororesorcinols, which were not available) with two different capillary columns were determined. Table 1 lists the retention times of the model compounds and of the metabolites from 2356-TeCP and 235-TCP. The metabolites were identified as TeCH and TCH on following grounds. The metabolite from 2356-TeCP cochromatographed in GLC with the authentic TeCH, and its mobility was different from those of the other tetrachlorodihydroxybenzenes (TeCC and TeCR; Table 1); the metabolite from 235-TCP cochromatographed in two different columns with the authentic TCH, and its mobility was different from those of trichlorocatechols (Table 1); and the mass spectra of the metabolites from 2356-TeCP and 235-TCP were almost identical with the mass spectra of the authentic TeCH and TCH (Fig. 2).

Oxygen donor for the *para*-hydroxylation of chlorophenols. To trace the donor of oxygen, the reaction was allowed to proceed in ^{18}O-labeled water ($H_2^{18}O$-$H_2^{16}O$, 20:80, vol/vol) or under an atmosphere of $^{18}O_2$. The incorporation of label into a chlorophenol with chlorine in position 4 (2345-TeCP) and into another with no chlorine in this position (235-TCP) was determined by mass spectrometry.
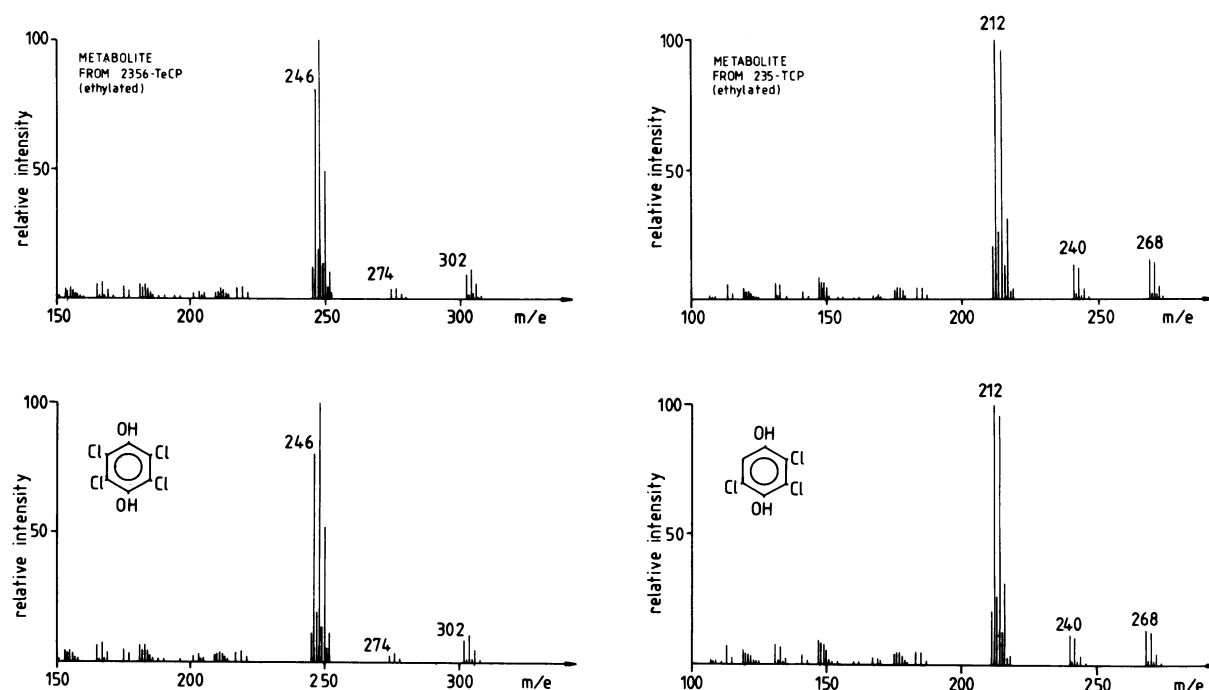
FIG. 2. Mass spectra of the ethylated metabolites from 2356-TeCP and 235-TCP and of the ethylated authentic TeCH and TCH.

Figure 3 shows the mass spectrum of TCH (molecular ions, m−28 ions, and m−56 ions) produced from 235-TCP in the absence of ^{18}O, in the presence of $^{18}O_2$, and in the presence of $H_2{}^{18}O$. TCH formed under an $^{18}O_2$ atmosphere had a mass spectrum practically identical to that formed in the absence of ^{18}O (Fig. 3A and B). In contrast, the presence of $H_2{}^{18}O$ in the reaction mixture led to a shift of base peak from 212 to 214 *m/e*, to the appearance of a peak at 220 *m/e*, and to other changes in the spectrum of the TCH produced (Fig. 3C). Table 2 lists the intensities of m−56 mass fragments of the metabolites from 235-TCP and 2345-TeCP in the experiments with ^{18}O and also the theoretical intensities of the mass fragments calculated on basis of the known abundance of different chlorine and oxygen isotopes. Comparison of the calculated to the experimentally obtained numbers shows that the hydroxyl group in the *para* position of the metabolites was derived from water and not from molecular oxygen (Table 2).

Dependence of *para*-hydroxylation on aerobiosis. We assayed for the degradation of PCP, 2346-TeCP, and 235-TCP by the PCP-induced bacteria under reduced and zero oxygen pressure; none of the chlorophenols tested was transformed under anaerobic conditions. Oxygen at 1% (vol/vol) in the gas phase of the culture ampoules resulted in slow degradation, and 5% O_2 allowed the maximal rate of hydroxylation (Fig. 4). In contrast, the degradation of TeCH by *R. chlorophenolicus* was not dependent on the presence of molecular oxygen (Fig. 4). This suggests that the dependence on an aerobic atmosphere was specific to the hydroxylation reaction. When O_2 was replaced with an alternative electron acceptor, KNO_3 (4 g liter^{-1}), *R. chlorophenolicus* neither grew on rhamnose nor degraded any of the chlorophenols.

Transformation of chlorophenols at different temperatures. We previously showed that the temperature optimum for growth of *R. chlorophenolicus* was 28°C, but it also grew at 18 and at 37°C (1). We followed the degradation of

chlorophenols by *R. chlorophenolicus* at different temperatures and determined the initial rates of degradation.

Figure 5 shows the temperature dependence of the rates of degradation of four different chlorophenols and of TeCH. The rate of degradation of all chlorophenols tested (PCP, 2356-TeCP, 2346-TeCP, and 235-TCP) increased with temperature up to 37°C. At 37°C the rate of degradation of the chlorophenols varied between 3.8 and 9.7 nmol h^{-1} per ml of culture (7 × 10^8 cells ml^{-1}). One feature in common for all the chlorophenols was the high rate of degradation at 41°C but the zero rate at 44°C (Fig. 5). PCP and 2346-TeCP differed from 2356-TeCP and 235-TCP in that they were degraded faster at 41°C than at 37°C (Fig. 5). The rate of disappearance of chlorophenols in sterile medium was less than 0.1 nmol h^{-1} ml^{-1} at 37°C. At all temperatures tested

TABLE 1. Mobilities of various tetra- and trichlorinated dihydroxybenzenes in GLC[a]

| Compound | Retention time (min) | | | |
| | Column I | | Column II | |
	Acetylated	Ethylated	Acetylated	Ethylated
Metabolite from:				
235-TCP	9.16	8.41	8.22	6.97
2356-TeCP	10.58	9.57	9.32	7.73
Authentic compounds				
TCH	9.16	8.41	8.22	6.97
345-TCC	9.45	8.38	8.76	6.97
346-TCC	8.63	6.71	7.89	5.01
TeCH	10.58	9.57	9.32	7.73
TeCC	10.88	9.33	9.93	7.46
TeCR	—[b]	12.33	—[b]	10.75

[a] Column I was CP Sil 9 CB, and column II was CP Sil 19 CB (Crompack). The temperature program was 1 min at 100°C, increasing by 8°C min^{-1} to 250°C.

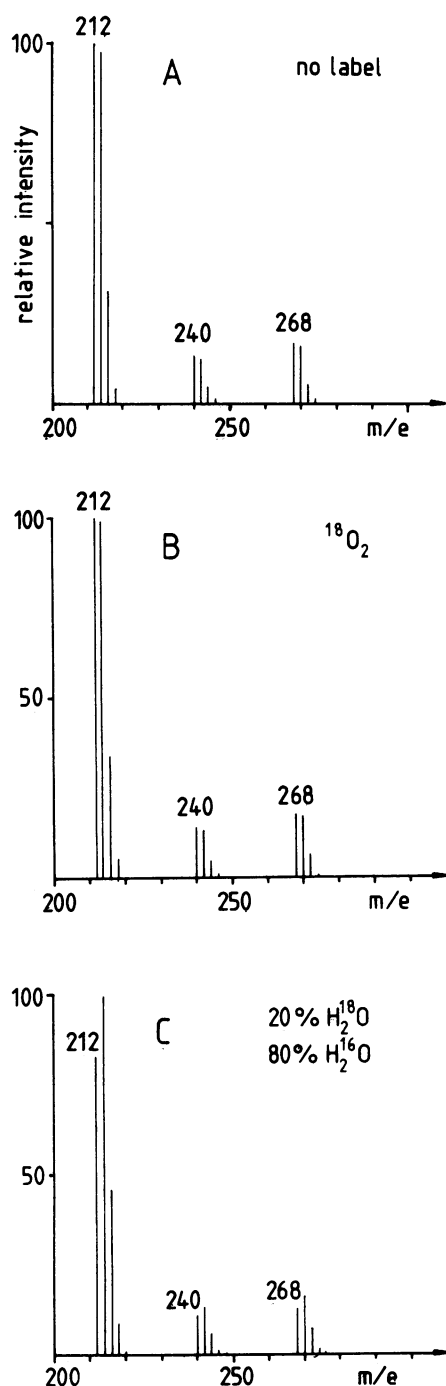[b] TeCR did not acetylate with the procedure used.

FIG. 3. Diagnostic mass fragments of the metabolite (TCH) formed from 235-TCP under an $^{16}O_2$ (A) or $^{18}O_2$ (B) atmosphere or in ^{18}O-labeled water (C) by *R. chlorophenolicus*.

TABLE 2. Incorporation of ^{16}O and ^{18}O from molecular oxygen and water in TCH-producing *para*-hydroxylation of 235-TCP and 2345-TeCP

Conditions	Relative intensities of m−56 mass fragments of TCH				
	212 m/e	214 m/e	216 m/e	218 m/e	220 m/e
No added ^{18}O					
Calculated[a]	100.0	97.5	31.7	3.4	0.0
Observed					
235-TCP	100.0	96.0	31.6	3.8	0.0
2345-TeCP	100.0	97.3	30.2	3.5	0.0
$^{18}O_2$-$^{16}O_2$, (99:1, vol/vol)					
Calculated[b]	1.0	100.0	96.8	31.4	3.4
Observed					
235-TCP	100.0	98.7	33.9	4.7	0.0
$H_2^{18}O$-$H_2^{16}O$ (20:80, vol/vol)					
Calculated[b]	81.6	100.0	45.8	9.3	0.7
Observed					
235-TCP	87.5	100.0	48.0	10.2	0.9
2345-TeCP	77.1	100.0	47.8	10.6	1.9

[a] Theoretical intensities were calculated from the abundance of ^{35}Cl and ^{37}Cl.
[b] Theoretical intensities if ^{18}O and ^{16}O molecules were used as oxygen donors in the same ratio in which they were introduced into the culture.

are expressed in an inducible manner (2a). The synthesis of the enzymes for the degradation of PCP, 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP was initiated by each compound and by PCP (2a). In an experiment described below we tested for the capability of different chlorophenols, TCH, and TeCH to set on the synthesis of the enzyme(s) for the *para*-hydroxylation of different chlorophenols. *R. chlorophenolicus* was preincubated (2a) either with chlorophenol or with chlorohydroquinone; chloramphenicol (60 μg ml^{-1}) was then added to prevent enzyme synthesis, and the degradation of all polychlorophenols, TCH, and TeCH was tested for.

The results presented in Table 3 show that each one of the chlorophenols induced the synthesis of the enzymes for the degradation of all of the chlorophenols. In contrast to this, the products of *para*-hydroxylation, TCH and TeCH, did not induce the production of a *para*-hydroxylating enzyme(s). The synthesis of the enzymes for the degradation of TCH and TeCH was initiated by the addition of any chlorophenol and also by the hydroquinones. The bacteria with no previous contact to any of these substrates did not degrade the compounds tested when chloramphenicol was present. With
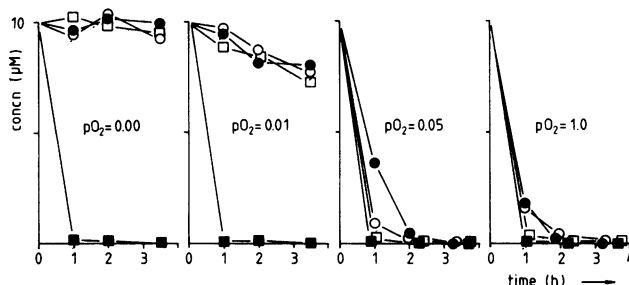
TeCH was metabolized faster than the chlorophenols, with an optimum at 50°C of 39.6 nmol h^{-1} per 7 × 10^8 cells. The rate of disappearance of TeCH in sterile medium containing 0.1% (wt/vol) ascorbic acid was 0.6 nmol h^{-1} ml^{-1} at 50°C. The metabolism of TeCH by *R. chlorophenolicus* slowed down to zero when the temperature was further increased from 50 to 55°C.

Induction of *para*-hydroxylating enzymes. We showed earlier that the enzymes for the degradation of chlorophenols

FIG. 4. Degradation of polychlorinated phenols and TeCH at different partial pressures of O_2 by *R. chlorophenolicus*. Symbols: (○) PCP, (●) 2346-TeCP, (□) 235-TCP, (■) TeCH.
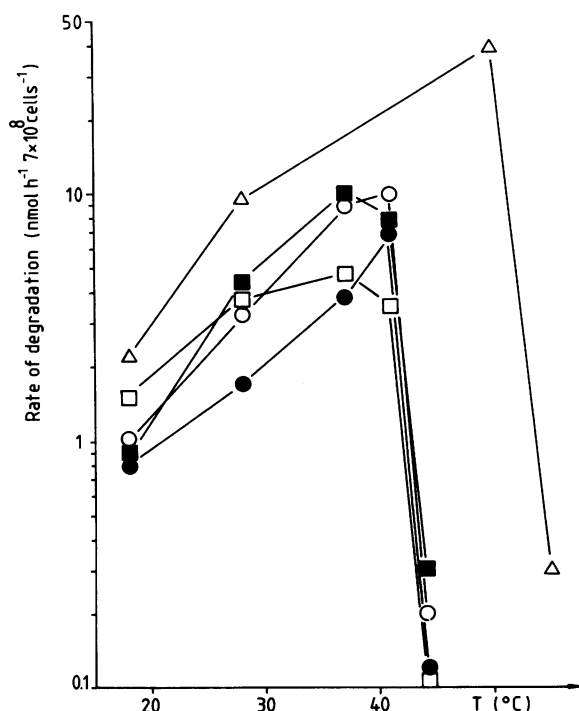
FIG. 5. Dependence of initial rates of degradation of polychlorinated phenols and TeCH on temperature by R. chlorophenolicus. Symbols: (O) PCP, (●) 2346-TeCP, (□) 235-TCP, (■) 2356-TeCP, (△) TeCH.

FIG. 6. Formation of TeCH and TCH through dechlorination and para-hydroxylation of different polychlorinated phenols.

no chloramphenicol added, all of the compounds were readily degraded by the noninduced bacteria in 20 h (Table 3).

DISCUSSION

R. chlorophenolicus PCP-I is a polychlorophenol degrader (2a). In this paper we show that it hydroxylated PCP, 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP. The novel hydroxyl group was set in position para to the preexisting hydroxyl, whether or not there was a chlorine substituent in position 4 of the substrate. 2356-TeCP,
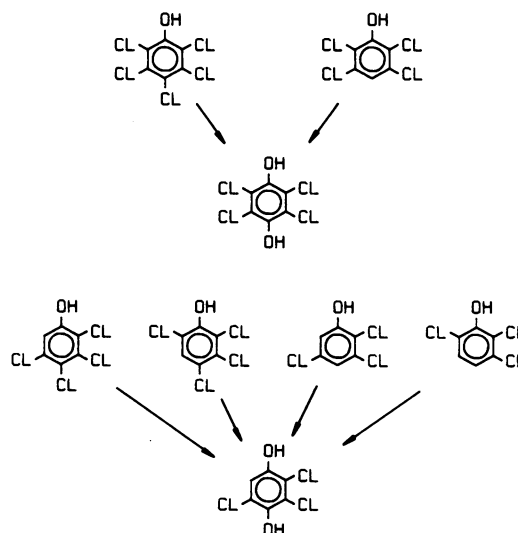
235-TCP, and 236-TCP were hydroxylated by R. chlorophenolicus, whereas 4-chlorinated phenols (PCP, 2345-TeCP, and 2346-TCP) were both dechlorinated and hydroxylated. Figure 6 illustrates the central role of TCH and TeCH in the metabolism of chlorophenols. All of the chlorophenols that were converted into TeCH or TCH were good substrates, whereas the other chlorophenols, which would para-hydroxylate into di- or monochlorohydroquinones, were poor substrates for R. chlorophenolicus (2a). Reiner et al. found a mutant of their PCP-degrading bacterium KC-3 to accumulate TeCH from PCP and suggested that the wild-type parent degraded PCP via TeCH (21). Juhl et al. (10) recently showed that the enzymes in human and rat liver homogenates also converted PCP into TeCH. The production of hydroquinones from tetra- and -trichlorophenols was not, as far as we know, demonstrated earlier.

The para-hydroxylation of chlorophenols by R. chlorophenolicus is a hydrolytic reaction. This was shown with a 4-chloro-substituted phenol (2345-TeCP) and with a chlorophenol with position 4 free (235-TCP). In both cases the

TABLE 3. Inducers in the metabolism of polychlorinated phenols and hydroquinones by R. chlorophenolicus

Inducer	Metabolism of compound in the presence of chloramphenicol[a]							
	PCP	2345-TeCP	2346-TeCP	2356-TeCP	235-TCP	236-TCP	TCH	TeCH
None (control)[b]	+	+	+	+	+	+	+	+
None	−	−	−	−	−	−	−	−
PCP	+	+	+	+	+	+	+	+
2345-TeCP	+	+	+	+	+	+	+	+
2346-TeCP	+	+	+	+	+	+	+	+
2356-TeCP	+	+	+	+	+	+	+	+
235-TCP	+	+	+	+	+	+	+	+
236-TCP	+	+	+	+	+	+	+	+
TCH	−	−	−	−	−	−	+	+
TeCH	−	−	−	−	−	−	+	+

[a] The concentration of 2345-TeCP was 5 μM, and that of all other compounds was 10 μM. +, More than 98% of the compound was removed in 20 h; −, less than 20% of the compound was removed in 20 h.
[b] Without chloramphenicol.

hydroxyl set in position 4 was derived from a water molecule ($H_2^{18}O$). 4-Chlorobenzoate has been shown to be dehalogenated and hydroxylated by *Pseudomonas* sp. and *Arthrobacter* sp. with H_2O as the oxygen donor, as was shown by incorporation of water-derived ^{18}O (17, 18). A strain of *Aspergillus niger*, in contrast, was found to *para*-hydroxylate benzoate by using O_2 (19). Some strains of *Pseudomonas* and *Arthrobacter* were found to metabolize mono- and dichlorinated phenols, benzoates, and benzenes via chlorinated catechols (3, 9, 15, 20). Both the chlorocatechol-producing and the chlorocatechol-degrading enzymes were O_2-incorporating oxygenases with relaxed specificity, and dehalogenation took place after the ring cleavage.

The conditions required by *R. chlorophenolicus* for dehalogenation and *para*-hydroxylation of different polychlorinated phenols were identical. The degradation of different chlorophenols similarly required the presence of molecular oxygen, although this oxygen was not incorporated into the phenol. Perhaps *para*-hydroxylation requires energy, which is generated by *R. chlorophenolicus* only in the presence of molecular oxygen, or alternatively O_2 as an electron acceptor may be essential in restoring the activity of the enzymes or cofactors used in *para*-hydroxylation. The rate of degradation of different chlorophenols similarly decreased to zero when the temperature was raised from 41 to 44°C; in contrast, TeCH was readily degraded up to 50°C. This suggests that the temperature-sensitive step in the degradation of chlorophenols at 44°C is the *para*-hydroxylation reaction; the downstream reactions proceeded readily at higher temperatures, since no sign of accumulated intermediates was found during the degradation of TeCH at 50°C. Perhaps the *para*-hydroxylating enzyme(s) or some essential cofactor is inactivated at 44°C.

Synthesis of the enzyme(s) for the *para*-hydroxylation of PCP, 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP was under joint control. Any one of these induced the degradation of all of the others and also of TeCH and TCH. The hydroquinones, on the other hand, did not induce the synthesis of the *para*-hydroxylating enzyme(s). This suggests that the true inducer of the hydroxylating enzyme is the polychlorinated substrate (chlorophenol) itself rather than some dechlorinated, later intermediate. In 2,4,5-trichlorophenoxyacetic acid-degrading *Pseudomonas cepacia* AC1100 (14) the conversion of 2,4,5-trichlorophenoxyacetic acid into 245-TCP was catalyzed by constitutively expressed enzyme(s), whereas the enzymes for the metabolism of 245-TCP were induced by 245-TCP (7, 11, 12). *P. cepacia* was also shown to dehalogenate PCP and many other chlorophenols, but the cells had to be preincubated with 245-TCP or with 2,4,5-trichlorophenoxyacetic acid (11–13). A PCP-degrading bacterium, KC-3, isolated by Chu and Kirsch (4), was found to degrade 2346-TeCP, 2356-TeCP, 235-TCP, 236-TCP, and 246-TCP without lag when the cells were preincubated in the presence of PCP (5). However, when the cells were preincubated with 246-TCP, a lag phase preceded the degradation of all the chlorophenols but 246-TCP; moreover, 235-TCP was not attacked at all (5). It seems that in *P. cepacia* and in KC-3 the regulation of the metabolism is very specific; only the compound used in enrichment can serve as an efficient inducer. Such narrow specificity was not seen in *R. chlorophenolicus*, although it was isolated from a mixed culture enriched for many years with PCP; not only PCP but also 2345-TeCP, 2346-TeCP, 2356-TeCP, 235-TCP, and 236-TCP induced readily the chlorophenol-metabolizing enzymes in *R. chlorophenolicus*.

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