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Trichloroethylene Metabolism by Microorganisms That Degrade Aromatic Compounds†

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Trichloroethylene (TCE) was metabolized by the natural microflora of three different environmental water samples when stimulated by the addition of either toluene or phenol. Two different strains of *Pseudomonas putida* that degrade toluene by a pathway containing a toluene dioxygenase also metabolized TCE. A mutant of one of these strains lacking an active toluene dioxygenase could not degrade TCE, but spontaneous revertants for toluene degradation also regained TCE-degradative ability. The results implicate toluene dioxygenase in TCE metabolism.

We recently isolated an aerobic gram-negative rod, designated strain G4, that degrades trichloroethylene (TCE) (11). Degradation of TCE by this organism requires the induction of an aromatic degradative pathway (10), which implicates enzymes of the pathway in the cometabolism of TCE. These results suggested that other microorganisms capable of degrading aromatic compounds might degrade TCE. The present study reports on the TCE-degradative ability of other microorganisms, including the natural microflora in environmental water samples as well as pure cultures of several degraders of aromatic compounds. Evidence is presented that a toluene dioxygenase is involved in the TCE-degradative ability of one of these organisms.

All TCE degradation experiments were performed as previously described (10) in sealed 50-ml serum vials containing 10 ml of medium. Water samples from estuarine, river, and groundwater environments were obtained in the Pensacola, Fla., area and were amended with 0.08 M phosphate buffer (pH 7) and 0.02 M ammonium sulfate and were used as both media and sources of inocula for these degradation experiments. In most instances, when samples were amended with toluene or phenol, stimulation of TCE degradation occurred as compared with the analogous tests containing no aromatic compound (Table 1). TCE degradation was greatest when groundwater samples were preincubated with phenol to enrich for phenol degraders prior to the TCE experiments. The results suggest that naturally occurring microorganisms are capable of degrading TCE when induced or stimulated with the appropriate aromatic compounds.

Laboratory strains capable of degrading naphthalene, biphenyl, phenol, and toluene were tested for TCE-degradative ability (Table 2). Cultures of each organism were grown for 20 h in 50 ml of minimal salts broth (11) and all were of approximately equal turbidity. *Pseudomonas* strains except strain mt-2(pWWO) were grown on 0.1% arginine; strain mt-2(pWWO) was grown on 20 mM sodium acetate. *Beijerinckia* sp. was grown on 0.2% sodium succinate plus 0.05% yeast extract. Each culture contained 50 μmol of the appropriate aromatic compound to induce the degradative pathway. Induction of all cultures was confirmed by the oxidation of the appropriate compound as determined polarographically (10). A 1-ml sample of each culture was added to the TCE degradation experiments to which no aromatic compounds were added. Only two of the organisms, strains PpF1 and B5, degraded TCE (Table 2). The degradation involved dechlorination, as indicated by the production of inorganic chloride (10) during TCE degradation by strain PpF1. Both of the active strains degrade toluene by initial dioxygenation of the aromatic ring to form a *cis*-dihydrodiol, which is dehydrogenated to 3-methylcatechol, the substrate for *meta*-ring fission (Fig. 1). All of the organisms tested except *Pseudomonas putida* U convert their respective aromatic growth substrates through the formation by aromatic ring dioxygenases of *cis*-dihydrodiols, which are then dehydrogenated. The one toluene degrader that did not degrade TCE, *P. putida* mt-2(pWWO), oxidizes the methyl group of toluene to form benzoate prior to aromatic ring dioxygenation and dehydrogenation. The phenol degrader, *P. putida* U, apparently converts phenol to catechol by direct hydroxylation (1, 3). Like strain G4 (11), this organism uses *meta*-fission for ring cleavage of catechol. However, unlike strain G4, *P. putida* U does not utilize toluene as a growth substrate.

Two mutants of *P. putida* PpF1 defective in the toluene-degradative pathway were grown overnight on 20 mM lactate medium containing 1 mM toluene and subsequently tested for the ability to metabolize TCE (Table 3). Mutant 106, defective in the first enzyme of the pathway, the toluene dioxygenase (4), did not degrade TCE, whereas mutant 39/D, defective in the second enzyme of the pathway, toluene *cis*-dihydrodiol dehydrogenase (6), degraded TCE. Thus, the ability to degrade TCE appears to be specifically associated with toluene dioxygenase activity and not the result of inactivating early steps in the toluene degradation pathway. In addition, catechol-2,3-dioxygenase does not appear to function in TCE degradation, because mutant 106 contained an active catechol-2,3-dioxygenase under the test conditions, as indicated by the production of 2-hydroxymuconic semialdehyde from catechol (10). Furthermore, the other organisms which were unable to degrade TCE contained catechol-2,3-dioxygenase activities.

Spontaneous revertants of mutant 106, able to utilize toluene, were obtained by plating 6×10^8 cells per plate on agar plates with toluene as the growth substrate (10). The reversion rate was 10^{-8}. Five of the revertants selected at

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TABLE 1. TCE metabolism in environmental samples

Inducer added	TCE remaining (μM)[a] in:								
	Estuarine water			River water			Groundwater		
	Active	Sterile	% Remaining[b]	Active	Sterile	% Remaining	Active	Sterile	% Remaining
None	2.64 ± 0.13	3.05 ± 0.11	87	3.61 ± 0.31	3.93 ± 0.43	92	3.06 ± 0.67	3.58 ± 0.46	85
Toluene (1 mM)	2.30 ± 0.35	3.15 ± 0.28	73	1.86 ± 0.02	4.38 ± 0.24	42	2.06 ± 0.81	4.59 ± 0.81	45
Phenol (1 mM)	2.92 ± 0.46	3.15 ± 0.36	93	3.25 ± 0.66	4.48 ± 0.94	73	1.07 ± 0.58	3.51 ± 0.64	30
Phenol (enrichment)[c]							<0.02[d]	3.51 ± 0.64	<0.6

[a] Determined after 4 days of incubation. All samples initially contained 200 nmol of TCE. Data are the means and the standard deviations of three replicates.
[b] Percentage of the TCE remaining in the active versus the sterile samples.
[c] Samples were preincubated with 2 mM phenol aerobically for 4 days at 25°C prior to the addition of 10 nmol of phenol and 200 nmol of TCE to initiate the TCE degradation experiments.
[d] Minimum detectable level.

random all degraded TCE. The results strongly implicate the toluene dioxygenase in TCE degradation by this organism.

Since mutant 106 did not consume TCE, the toluene dioxygenase must be involved in the initial step of TCE degradation. As previously proposed (10), only one enzyme which can activate molecular oxygen and oxidize TCE may be necessary for its complete dechlorination, since unstable oxoderivatives of TCE can decompose to nonchlorinated compounds (9). However, the present data cannot exclude other enzymes being involved in complete degradation of TCE to innocuous products.

The organisms tested that have ring dioxygenases other than toluene dioxygenase did not degrade TCE. Thus, TCE-degradative activity does not seem to be a general phenomenon of aromatic ring dioxygenases. At present, it is not known whether a toluene dioxygenase is responsible for the TCE-degradative capacity of strain G4.

These results suggest that a number of organisms may possess the potential to degrade TCE. Two *Pseudomonas* isolates, selected independently for the ability to degrade aromatic hydrocarbons, were shown to degrade TCE when properly induced. The natural microflora in several water samples could also be stimulated to degrade TCE when induced with toluene or phenol. A similar condition apparently exists for methanotrophs. TCE was degraded in environmental samples exposed to natural gas (13). Similar results have been recently obtained with pure cultures of methanotrophs (C. D. Little, A. V. Palumbo, S. E. Herbes, and D. M. Genung, Abstr. Annu. Meet., Am. Soc. Microbiol. 1987, Q105, p. 299). Evidently, the recalcitrance of TCE in the environment is due to a lack of the appropriate inducing conditions for the required enzymes and not to the lack of naturally occurring bacteria with the potential for degradation. Thus, it seems likely that TCE is aerobically biodegraded in situ at TCE-polluted sites that are also contaminated with the appropriate growth substrates for aromatic compound- or methane-degrading microorganisms.

TABLE 2. TCE metabolism by microorganisms that degrade aromatic compounds[a]

Organism	Reference	Aromatic inducer	TCE remaining (μM)[b]
Beijerinkia sp.	8	Biphenyl	2.54 ± 0.1
P. putida U	1, 3	Phenol	2.04 ± 0.2
P. putida NCIB 9816	2	Naphthalene	2.34 ± 0.1
P. putida mt-2(pWWO)	5, 12	Toluene	2.13 ± 0.1
P. putida B5	–[c]	Toluene	<0.02[d]
P. putida PpF1	4, 7	Toluene	<0.02
None		None	2.73 ± 0.02

[a] Cultures were induced with the indicated aromatic compounds as described in the text. A 1-ml sample of each induced culture and 200 nmol of TCE were added to each TCE degradation on experiment. Data are the means and standard deviations of three replicates.
[b] After 24 h of incubation.
[c] D. A. Kunz and P. J. Chapman, personal communication.
[d] Minimum detectable level.

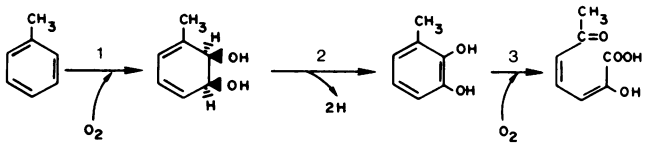

FIG. 1. Pathway for catabolism of toluene by *P. putida* PpF1 and B5. Enzymes indicated are: 1, toluene dioxygenase; 2, *cis*-dihydrodiol dehydrogenase; 3, catechol-2,3-dioxygenase.

TABLE 3. Metabolism of TCE by mutants of *P. putida* PpF1 unable to degrade toluene[a]

P. putida strain	Reference	Defective enzyme	TCE remaining (μM)
Parent	7	None	<0.02[b]
106	4	Toluene dioxygenase	2.98 ± 0.09
39/D	6	Dihydrodiol dehydrogenase	<0.02
None			3.84 ± 0.13

[a] Toluene at 1 mM replaced phenol in these TCE metabolism experiments.
[b] Minimum detectable level.

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LITERATURE CITED

1. **Bayly, R. C., S. Dagley, and D. T. Gibson.** 1966. The metabolism of cresols by species of *Pseudomonas*. Biochem. J. **101:** 293–301.
2. **Catterall, F. A., K. Murray, and P. A. Williams.** 1971. The configuration of the 1,2-dihydroxy-1,2-dihydronaphthalene formed in the bacterial metabolism of naphthalene. Biochim. Biophys. Acta **237:**361–364.
3. **Dagley, S., and D. T. Gibson.** 1965. The bacterial degradation of

catechol. Biochem. J. **95**:466–474.
4. **Finette, B. A., V. Subramanian, and D. T. Gibson.** 1984. Isolation and characterization of *Pseudomonas putida* PpF1 mutants defective in the toluene dioxygenase enzyme system. J. Bacteriol. **160**:1003–1009.
5. **Franklin, F. C. H., M. Bagdasarian, M. M. Bagdasarian, and K. N. Timmis.** 1981. Molecular and functional analysis of the TOL plasmid pWWO from *Pseudomonas putida* and cloning of genes for the entire regulated aromatic ring *meta* cleavage pathway. Proc. Natl. Acad. Sci. USA **78**:7458–7462.
6. **Gibson, D. T., M. Hensley, H. Yoshioka, and T. J. Mabry.** 1970. Formation of (+)-*cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene by *Pseudomonas putida*. Biochemistry **9**:1626–1630.
7. **Gibson, D. T., J. R. Koch, and R. E. Kallio.** 1968. Oxidative degradation of aromatic hydrocarbons by microorganisms. I. Enzymatic formation of catechol from benzene. Biochemistry **7**:2653–2662.
8. **Gibson, D. T., R. L. Roberts, M. C. Wells, and V. M. Kobal.** 1973. Oxidation of biphenyl by a *Beijerinckia* species. Biochem. Biophys. Res. Commun. **50**:211–219.
9. **Miller, R. E., and R. P. Guengerich.** 1982. Oxidation of trichloroethylene by liver microsomal cytochrome P-450: evidence for chlorine migration in a transition state not involving trichloroethylene oxide. Biochemistry **21**:1090–1097.
10. **Nelson, M. J. K., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard.** 1987. Biodegradation of trichloroethylene and the involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. **53**:949–954.
11. **Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard.** 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. **52**:383–384.
12. **Williams, P. A., and K. Murray.** 1974. Metabolism of benzoate and the methylbenzoates by *Pseudomonas putida* (*arvilla*) mt-2: evidence of the existence of a TOL plasmid. J. Bacteriol. **120**:416–423.
13. **Wilson, J. T., and B. H. Wilson.** 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. **49**:242–243.