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Metabolism of DDT Analogues by a *Pseudomonas* sp.

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A *Pseudomonas* sp. rapidly metabolized several nonchlorinated analogues of DDT, with the exception of 2,2-diphenylethanol, as the sole carbon source. Several of the mono-*p*-chloro-substituted diphenyl analogues were also metabolized as the sole carbon source by the bacterium. The resulting chlorinated aromatic acid metabolites were not further metabolized. The isolate was unable to metabolize *p,p'*-dichlorodiphenyl analogues as the sole carbon source.

The increasing public concern about the environmental pollution caused by DDT because of its long persistence in nature has resulted in legal restrictions on the use of this pesticide. A major problem with DDT is the ultimate disposal of surplus supplies that have accumulated in storage over the years without causing any adverse effects on the environment. An attractive approach is to isolate microorganisms capable of metabolizing the pesticide and to develop a fermentation process to convert these compounds to innocuous products. To develop such a biological disposal system for DDT, we used several methods to attempt to isolate from various samples microorganisms capable of growth on DDT or DDT analogues as the sole carbon source.

In various ecosystems microorganisms cause only modest changes in the DDT molecule (1). The structurally related metabolites resulting from DDT degradation also are known to persist in nature. Pfaender and Alexander (4) reported that pure cultures of bacteria decompose DDT extensively only through a cometabolic process. Focht and Alexander (2) reported that bis-(*p*-chlorophenyl)methane, a product of the microbial metabolism of DDT, could be degraded by a strain of *Hydrogenomonas*. Based on these findings, we tested several chlorinated and nonchlorinated analogues of DDT to determine their extent of biodegradation by a *Pseudomonas* sp. isolated in our laboratories and to gain a further understanding of microbial decomposition of DDT.

MATERIALS AND METHODS

Isolation and growth. A bacterium capable of utilizing 1,1-diphenylethane, a nonchlorinated analogue of DDT, as its sole carbon source was obtained from sewage by the enrichment culture technique. The isolated culture was grown in mineral salts medium containing, in grams per liter: diphenylethane, 0.36; $(NH_4)_2SO_4$, 1.32; Na_2HPO_4, 1.42; KH_2PO_4, 0.54; $MgSO_4 \cdot 7H_2O$, 0.50; and $Ca(NO_3)_2$ and $FeSO_4$, 0.5×10^{-3}, in distilled water; the final pH was 7.0. In a test of whether the diphenylethane-grown isolate was capable of metabolizing various analogues of DDT as the sole carbon sources for growth, 1 ml of a 48-h, diphenylethane-grown culture was added to 50 ml of mineral salts medium in 250-ml Erlenmeyer flasks containing 0.36 g of the test compound per liter. The cultures were incubated on a shaker at 28°C. Periodically, the culture flasks were checked for turbidity and change in color. If growth was observed, the medium was analyzed for the disappearance of the substrate and the appearance of any detectable metabolites.

Chemicals. DDT was obtained from Montrose Chemical Corp. of California, Torrance, Calif. It was purified to >99% by recrystallization (methanol). Aldrich Chemical Co., Milwaukee, Wis., supplied diphenylmethane, bis(*p*-chlorophenyl)acetic acid (98% purity), 2.2-bis(*p*-chlorophenyl)-1,1-dichloroethane (>99% purity), and 1,1-diphenyl-2,2,2-trichloroethane (>99% pure). 4,4'-Dichlorobenzophenone (99% pure) was obtained from Dow Chemical Co., and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane was purchased from Polyscience Corp., Evanston, Ill. The following chemicals were synthesized by the Pharmaceutical Chemistry department at Stanford Research Institute (R. Bramhall, manuscript in preparation): 1,1-diphenylethane, 2,2-diphenylethanol, 1-(*p*-chlorophenyl)-1-phenylethane, 1-(*p*-chlorophenyl)-1-phenylethene, bis(*p*-chlorophenyl)methane, 1,1-bis(*p*-chlorophenyl)ethane, 1,1-bis(*p*-chlorophenyl)ethene, 1-(*p*-chlorophenyl)-1-phenylethanol, 1,1-bis(*p*-chlorophenyl)ethanol, 1,1-bis(*p*-chlorophenyl)-2-chloroethane, and 1,1-bis(*p*-chlorophenyl)-2-chloroethane. The purity of these compounds was >99%, as evidenced by a single peak when gas chromatography was performed.

Analytical procedures. Quantitative analysis was performed by gas chromatography using the internal standard method of quantitation. A 50-ml culture sample was acidified with 4 N HCl and extracted with 2 × 50-ml portions of diethyl ether. The

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ether extracts were combined, dried over anhydrous $MgSO_4$ and concentrated by rotary evaporation to approximately 3 ml. An appropriate internal standard (*trans*-stilbene, lindane, or DDT) was added, and the solution was silylated with *N,O-bis*(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co.). Both silylated and unsilylated samples were analyzed by gas chromatography on a Varian 204 gas chromatograph equipped with flame ionization detectors. Analyses were performed under the following conditions; glass column (6 ft [ca. 182.9 cm] by 2 mm) packed with 4% SE-30 plus 6% OV-210 on 80/100-mesh GasChrom Q; 100–230° at 4°/min; injector, 150°; detector, 300°; flow rate, 50 ml of N_2 per ml. An Autolab 6300 digital integrator was used for peak area determinations.

Gas chromatographic/mass spectral (gc/ms) data were obtained on an LKB 9000 gc/ms equipped with a PDP-12 computer. Absorption spectra were obtained with a Cary 15 spectrophotometer.

RESULTS

An organism capable of growth on diphenylethane as the sole carbon source was identified as a *Pseudomonas* sp. on the basis of its morphological and biochemical characteristics (5). Diphenylethane was rapidly metabolized to 2-phenylpropionic acid (identified by gc/ms comparison with an authentic sample obtained from Aldrich Chemical Co.), which was further degraded by the bacterium (Fig. 1). The rates of degradation of diphenylethane and 2-phenylpropionic acid were determined to be 1.05 and 0.34 μmol/h, respectively, by determining the slopes from the curves in Fig. 1 (curves A and C). Curve B represents the sum of the rates of formation and degradation of 2-phenylpropionic acid (0.67 μmol/h), from which the rate of formation of 2-phenylpropionic acid was calculated to be 1.01 μmol/h (0.67 μmol − [−0.34 μmol/h]). Thus, 2-phenylpropionic acid is formed nearly as rapidly as diphenylethane is metabolized.

The bacterium also metabolized diphenylmethane rapidly to produce phenylacetic acid, which was further metabolized.

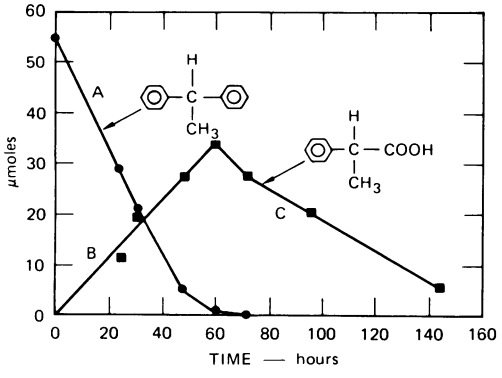


FIG. 1. *Degradation of 1,1-diphenylethane.*

1-(*p*-Chlorophenyl)-1-phenylethane also was found to undergo degradation as the sole carbon source, yielding 2-(*p*-chlorophenyl)propionic acid as the only metabolite (Fig. 2). This metabolite was identified as the trimethylsilyl ester by comparative gc/ms techniques with an authentic sample (Aldrich Chemical Co.). During the first 2 days of growth, the bacterium degraded the substrate at a rate of 20 μmol/day. 2-(*p*-Chlorophenyl)propionic acid was produced at an almost equal rate (19 μmol/day). After 6 days, the substrate had been quantitatively converted to 2-(*p*-chlorophenyl)propionic acid. The metabolite accumulated in the medium and did not undergo further degradation.

The compound 1-(*p*-chlorophenyl)-1-phenylethene, an analogue of DDE [1,1-bis(*p*-chlorophenyl)-2,2-dichloroethene] was utilized by the bacterium as the sole carbon source to yield 2-(*p*-chlorophenyl)-2-propenoic acid as the only metabolite detected. The metabolite was identified as the trimethylsilyl ester by gc/ms techniques.

The bacterium also metabolized 1-(*p*-chlorophenyl)-1-phenylethanol as the sole carbon source. Degradation of the molecule produced 2-(*p*-chlorophenyl)-2-hydroxypropionic acid as a metabolite, which was identified as the ditrimethylsilyl derivative by gc/ms (Fig. 3). The formation of 2-(*p*-chlorophenyl)-2-hydroxypropionic acid reached its maximum on day 4, and thereafter it was found to be slowly degraded during 8 days of incubation. Another metabolite, atrolatic acid (2-hydroxy-2-phenylpropionic acid), was identified by gc/ms and results from the cleavage of the chlorine-containing aromatic ring. However, only minor amounts of this metabolite were produced, indicating a preferential attack on the nonchlorinated ring.

The compound 2,2-diphenylethanol, which is analogous to 1-(*p*-chlorophenyl)-1-phenylethanol, except for the position of the hydroxyl

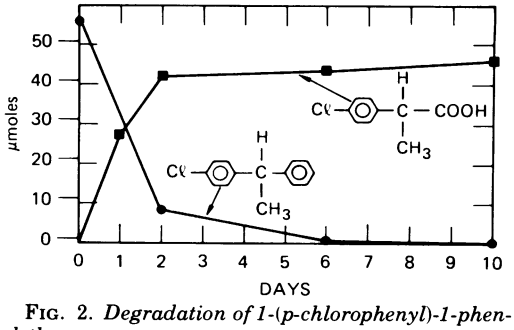


FIG. 2. *Degradation of 1-(p-chlorophenyl)-1-phenylethane.*

group and halogen substitution, was not degraded, and the starting material was quantitatively recovered.

Several analogues of DDT and the intermediates suggested to be involved in DDT metabolism (6, 7) were tested for possible metabolism by this *Pseudomonas* sp. The organism did not metabolize several of the chlorinated analogues of DDT; Table 1 summarizes the results. Attempts to isolate cultures capable of metaboliz-

TABLE 1. *Ability of Pseudomonas sp. to metabolize DDT and DDT analogues*

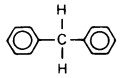
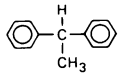
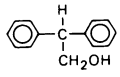
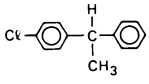
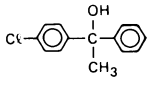
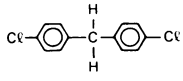
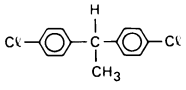
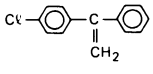
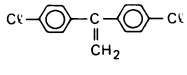
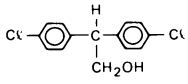
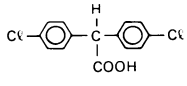
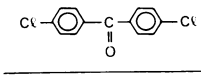
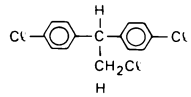
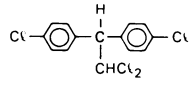
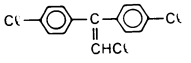
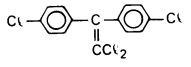
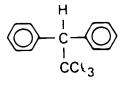
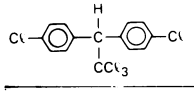
Compound	Growth as Sole Carbon Source	Metabolite Detected
	+	Phenylacetic acid
	+	2-Phenylpropionic acid
	-	None
	+	2-(p-Chlorophenyl)-propionic acid
	+	2-(p-Chlorophenyl)-2-Hydroxypropionic acid
	-	None
	-	None
	+	2-(p-Chlorophenyl)-2-propionic acid
	-	None
	-	None
	-	None
	-	None

TABLE 1—*Continued*

Compound	Growth as Sole Carbon Source	Metabolite Detected
	-	None
	-	None
	-	None
	-	None
	-	None
	-	None

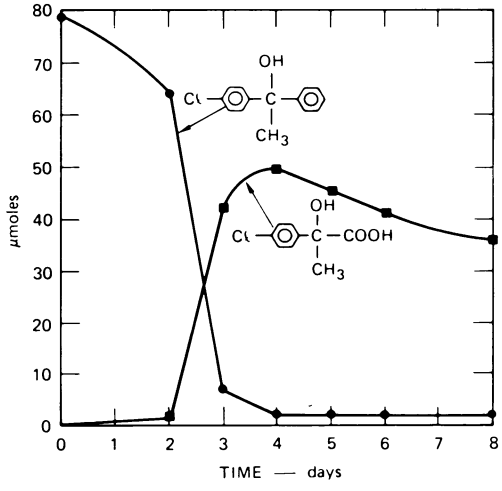


FIG. 3. *Degradation of 1-(p-chlorophenyl)-1-phenylethanol.*

ing several of the chlorinated analogues of DDT, by the enrichment procedure, did not yield any successful organisms.

DISCUSSION

The biodegradation of chlorinated and nonchlorinated analogues of DDT with our *Pseudomonas* sp. isolate usually involved fission of an aromatic nucleus. There was no evidence that either of the alkyl carbons in the diphenylmethane or diphenylethane derivatives was

attacked first. With the exception of 2,2-diphenylethanol, the nonchlorinated compounds 1,1-diphenylethane and diphenylmethane were rapidly metabolized, with the formation of 2-phenylpropionic acid and phenylacetic acid, respectively. These metabolites were further degraded by the bacterium.

The *p*-chlorophenyl compounds 1-(*p*-chlorophenyl)-1-phenylethane, 1-(*p*-chlorophenyl)-1-phenylethanol, and 1-(*p*-chlorophenyl)-1-phenylethene were metabolized rapidly as sole carbon sources. The unsubstituted aromatic rings were cleaved first, resulting in the corresponding *p*-chlorophenylcarboxylic acids. Further metabolism of the chlorinated aromatic acids was not normally observed, except for 2-(*p*-chlorophenyl)-2-hydroxypropionic acid, which was found to undergo only moderate decomposition. However, these compounds should undergo rapid metabolism by other microorganisms in the environment. Pfaender and Alexander (4) reported that an *Arthrobacter* sp. and sewage cultures metabolized *p*-chlorophenylacetic acid rapidly.

None of the *p,p'*-dichloro-substituted ring compounds were metabolized as sole carbon sources. Although 1,1-diphenyl-2,2,2-trichloroethane has no chlorine substituents in the aromatic rings, it was not metabolized by this organism.

These results indicate that both aryl-chlorine substitution and α-carbon substitution are important in determining biodegradability by this bacterium. Focht and Alexander (3) postulated that both aromatic and α-carbon substituents render DDT inert to aerobic attack by a *Hydrogenomonas* sp. through electron deactivation of the aromatic ring. This postulate was found to be true with our *Pseudomonas* sp., which prefers aromatic ring attack on the nonchlorinated ring when a choice is available [such as in 1-(*p*-chlorophenyl)-1-phenylethane)]. However, ring deactivation by α-carbon substituents such as —COOH, —CCl_3, —$CHCl_2$, and —CH_2Cl, although slight, must be reevaluated in light of the finding that moving a hydroxyl group from the α- to β-position in diphenylethane leads to nonbiodegradability by this bacterium. This suggests that other factors besides electron deactivation may play an important role in the biodegradation of DDT and DDT analogues.

ACKNOWLEDGMENT

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