Microbial Metabolism of 2,4,5-Trichlorophenoxyacetic Acid in Soil, Soil Suspensions, and Axenic Culture

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In the decomposition of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in soil and soil suspension, 2,4,5-trichlorophenol appeared and then disappeared. No 2,4,5-T decomposition was evident in soil or soil suspension sterilized by γ irradiation. A soil isolate of *Pseudomonas fluorescens* destroyed about 70% of the 2,4,5-T added to a glucose–inorganic salts medium in 80 h, and nearly 60% of the herbicide that was metabolized was recovered as 2,4,5-trichlorophenol. The bacterium did not use the trichlorophenol as a carbon source, and it did not release $^{14}CO_2$ from uniformly ring-labeled 2,4,5-trichloro[^{14}C]phenol or uniformly ring-labeled 2,4,5-[^{14}C]T. Soil suspensions converted 8% of labeled 2,4,5-T and 40% of labeled 2,4,5-trichlorophenol to $^{14}CO_2$ in 25 days. 2,4,5-Trichlorophenol was converted by microorganisms in the soil suspensions to products that were identified as 3,5-dichlorocatechol, 4-chlorocatechol, and succinate by gas chromatography and mass spectrometry and to products that were tentatively identified as *cis,cis*-2,4-dichloromuconate, 2-chloro-4-(carboxymethylene)but-2-enolide, and chlorosuccinate by gas and thin-layer chromatography. On the basis of these results, a pathway of 2,4,5-T decomposition is proposed.

The herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been widely used for the selective control of weeds and as defoliants. Because of the large quantities of these herbicides that are applied to soil and the potential toxicity of the breakdown products to nontarget organisms, the fate of 2,4-D and the products of its metabolism have attracted considerable attention. The initial attack on 2,4-D appears to involve the microbial cleavage of the ether linkage to yield 2,4-dichlorophenol, a reaction that has been observed to occur in samples of soil and water with sediment (Alexander, 1974) as well as in axenic microbial cultures (Loos et al., 1967). Studies of a strain of *Arthrobacter* have disclosed that the 2,4-dichlorophenol that is thus formed is oxidized to 3,5-dichlorocatechol, which in turn is converted to a dichlorinated muconic acid, the corresponding monochlorobutenolide, and chloromaleylacetic acid. The subsequent steps probably involve the sequential formation of 2-chloro-4-ketoadipate, chlorosuccinate, and succinate (Bollag et al., 1968a,b; Duxbury et al., 1970; Sharpee et al., 1973; Tiedje et al., 1969). Essentially the same pathway appears to occur in *Pseudomonas* sp., except that 6-hydroxy-2,4-dichlorophenoxyacetate and 2- and 4-chlorocatechols are also generated (Evans et al., 1971).

In contrast, little is known of the products of microbial metabolism of 2,4,5-T. Horvath (1970) reported that *Brevibacterium* sp. cometabolized 2,4,5-T to yield an organic product tenatively identified as 3,5-dichlorocatechol, and 2,4,5-trichlorophenol (2,4,5-TCP) is produced during the breakdown of the pesticide in soil (Alexander, 1974). Although the phenol is degraded further (Alexander, 1974), the pathway remains totally unknown.

The present study was thus undertaken to determine the products of microbial attack in order to establish the pathway of metabolism of 2,4,5-T.

MATERIALS AND METHODS

Materials. 2,4,5-T was obtained from Dow Chemical Co., Midland, MI, and 2,4,5-, 2,3,4-, 2,3,6-, 2,3,5-, 3,4,5-, and 2,4,6-trichlorophenols and catechol were from Eastman Organic Chemicals, Rochester, NY. Uniformly ring-labeled 2,4,5-[^{14}C]T (sp act., 1.61 mCi/mmol) and 2,4,5-TCP (sp act., 4.0 mCi/mmol) were purchased from California Bionuclear Corp., Sun Valley, CA. The purities of the ^{14}C-labeled compounds were 98.0 and 98.5%, respectively, as determined by thin-layer chromatography. Unlabeled 2,4,5-T was recrystallized twice in benzene before use, and the purity of the compound was greater than 99% as determined by thin-layer chromatography. 3,5-Dichlorocatechol, *cis,cis*-2,4-dichloromuconate, 2-chloro-4-(carboxymethylene)but-2-enolide, chlorosuccinate, succinate, 6-hydroxy-2,4-dichlorophenoxyacetic acid, and 4-chlorocatechol were obtained from Dr. J. M. Duxbury, Cornell University. Thin-layer and gas–liquid chromatography indicated that these chemicals were 85–95% pure. All compounds were dissolved in 95% ethanol prior to addition to media. Standards were prepared with pesticide-grade ethyl acetate (Fisher Scientific Co., Rochester, NY). All other chemicals were of the highest purity available commercially.

Glassware. Glassware was cleaned by rinsing in distilled water, followed by a 24-h immersion in 20% (v/v) HNO_3. The nitric acid was removed by thorough washing in tap water, followed by distilled water.

Incubation Conditions. The inorganic salts solution and the bacteria will be separately described (Rosenberg and Alexander, 1980). The basal medium consisted of the inorganic salts solution supplemented with 5 mM glucose. Because the greatest rate and extent of transformation of phenoxy herbicides in the four soils studied occurred in a Philippine soil (Maahas clay, pH 6.8, 3.0% organic matter, 0.27% total nitrogen), it was used to investigate the metabolite formed in the transformation of 2,4,5-T and 2,4,5-TCP. The soil was amended with 10 μg of 2,4,5-T/g of soil and incubated for 4 months at 29 °C in 250-mL Erlenmeyer flasks, at which time 2,4,5-T and 2,4,5-TCP could not be detected by gas chromatography. The soil samples (100 g) were then flooded with 150 mL of basal medium, the flasks were mixed for 2 h at 29 °C and 150 rpm, the contents of the flasks were allowed to settle, and then 20-mL portions were transferred to either 125-mL baffled Erlenmeyer flasks or 250-mL biometer flasks (Bellco Glass, Vineland, NJ). These suspensions were amended to final concentrations of 10 μg of unlabeled

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2,4,5-T or 2,4,5-TCP/mL in the baffled Erlenmeyer flasks or 31 nCi (5.0 μg) of uniformly ring-^{14}C-labeled 2,4,5-T/mL or 0.22 μCi (12 μg) of uniformly ring-^{14}C-labeled 2,4,5-TCP/mL in the biometer flasks.

The flasks were incubated on a rotary shaker (150 rpm) at 29 °C. The $^{14}CO_2$ evolved from the biometer flasks was trapped in 0.1 N KOH contained in the sidearm, the trapping solution being replaced at appropriate time intervals. One-milliliter portions of the trapping solution were added to 15 mL of Aqueous Counting Scintillant (ACS, Amersham/Searle Corp., Arlington Heights, IL) in scintillation vials (Kimble, Toledo, OH), and the radioactivity was counted. At the same time, 50-μL portions of the inoculated suspensions were placed in scintillation vials with 15 mL of ACS, and the radioactivity was counted.

The transformation of unlabeled 2,4,5-T was determined in Maahas clay. For this purpose, 100-g portions of soil were air-dried and passed through a 2-mm sieve. The herbicide (2.5 μg) in ethanol was added to the reaction flasks, the solution was evaporated to about 0.5 mL, and 1.0 mL of distilled water was added. The soil was then introduced into the flask and mixed with the herbicide, and distilled water was finally added to bring the soil to 70% of field capacity. The soil was aerated at a rate of 100 mL/min with water-saturated air that was first freed of CO_2 by passage through Ascarite. The temperature was 23–25 °C. In an attempt to enhance 2,4,5-T degradation, one set of flasks was amended with 1.0 mg each of sodium benzoate and glucose/g of soil. For all experiments, possible nonbiological transformation of 2,4,5-T and 2,4,5-TCP was assessed using soil inoculum, cell suspensions, or soil treated with 6 Mrad of γ irradiation. Controls without added herbicide or 2,4,5-TCP were also prepared, incubated, and analyzed as described.

To determine the products of 2,4,5-T degradation, a bacterium obtained from Maahas clay was grown in the basal medium amended with 20 μg of unlabeled 2,4,5-T or 2,4,5-TCP/mL or with either 31 nCi of uniformly ring-^{14}C-labeled 2,4,5-T/mL or 0.22 μCi of 2,4,5-TCP/mL. The organism was grown in either 125-mL baffled Erlenmeyer flasks or 250-mL biometer flasks at 29 °C on a rotary shaker operating at 150 rpm.

Analysis of Unlabeled Metabolites. The compounds were extracted from the mixtures inoculated with soil or the axenic bacterial culture with equal volumes of pesticide-grade ethyl acetate after acidifying to pH 2 with 1 N HCl. The extraction was repeated three times, and the solvent phases were dried with anhydrous Na_2SO_4. On the basis of tests with known concentrations of 2,4,5-T and 2,4,5-TCP, the extraction procedure removed more than 90% of the chemicals from the sample. The solvent was concentrated to about 0.5 mL with dry N_2 and treated with diazomethane (Daughton et al., 1976).

2,4,5-T and possible metabolites were extracted periodically from the soil by adding 100 mL of a solvent system containing ethyl acetate, diethyl ether, and acetone (5:5:1) to 25 g of soil. The soil was shaken for 60 min, the particles were allowed to settle, and the solvent phase was removed. The solvent was dried with anhydrous Na_2SO_4, concentrated under a stream of dry N_2 to about 5 mL, and treated with diazomethane. The efficiency of recovery of 2,4,5-T from the soil was 85% when tested with known concentrations of the herbicides. For gas chromatography and combined gas chromatography–mass spectrometry, 2,4,5-TCP and possible metabolites formed from 2,4,5-T were prepared in ethyl acetate and derivatized with diazomethane. Analysis was by gas–liquid chromatography or gas chromatography–mass spectrometry.

Analysis of Labeled Metabolites. The samples were extracted, dried, and concentrated (to 2 mL) as described above, and 50-μL portions were withdrawn for the determination of radioactivity. For chromatographic analysis, 50 μL of extract was spotted on thin-layer silica gel (GF) Redi/plates (Fisher Scientific Co., Pittsburgh, PA). Each spot was overlaid with 50 μL containing 150 μg of a possible microbial product generated from the parent chemical. The spots were allowed to dry, and the plates were developed in a solvent system of isopropyl alcohol, ethyl acetate, and ammonium hydroxide (7:9:4). The chromatograms were examined under UV light, the spots containing the metabolites were scraped off the plate, and the resulting material was placed in scintillation vials containing 15 mL of ACS. The radioactivity was then counted.

A second set of thin-layer chromatography plates was developed, dried in air, and placed against Kodak no-screen SB-5 X-ray films (Eastman Kodak Co., Rochester, NY) for 3–4 weeks to detect radioactive areas. The films were developed as described by Wang and Willis (1965) with Kodak X-ray developer and rapid fixer.

Analytical Methods. The disappearance of 2,4,5-T and 2,4,5-TCP was determined by measurement of the UV absorbance of the solution at 292 and 310 nm, respectively, using 1.0-cm quartz cuvettes and a Beckman DU-2 spectrophotometer. All readings were corrected for material absorbing at the same wavelengths. Phenol was determined by the method of Chrastil (1975) using a Bausch and Lomb Spectronic-20 spectrophotometer. Chloride was estimated by the technique of Bergmann and Sanik (1957), and the values obtained were corrected for the chloride in the basal medium. Radioactivity was determined by counting in a Beckman liquid scintillation counter, Model LS-100C. All counts were corrected for quenching and background.

Gas chromatographic analysis was perfomed with a Perkin-Elmer gas–liquid chromatograph, model 3920B, equipped with a flame ionization detector. The packing was 3% OV-17 on 100/120 mesh Gas-Chrom W (HP) in a 1.83-m by 2-mm (i.d.) glass column (Supelco, Inc., Bellefonte, PA). The operating temperatures were 215 °C for the injector and 250 °C for the interface (detector). The column was maintained for 2 min at 60 °C and programmed at 8 °C/min to 250 °C, or it was operated isothermally at 135 °C. The flow rate of the helium carrier gas was 30 mL/min. The quantities of 2,4,5-T and its possible metabolites were determined by comparison with standard curves prepared from the authentic chemicals.

Mass spectra were obtained with a Finnigan 3300 mass spectrometer, electron impact 70 eV, coupled with a Finnigan 3300 gas chromatograph via a heated single-stage jet separator and using a glass column identical with the one described previously except that it was U-shaped and 1.53 m long. The spectra were compared with those of authentic compounds.

RESULTS

The metabolism of 2,4,5-T and 2,4,5-TCP by the mixed populations in a soil suspension was determined by loss of UV absorbance at 292 and 310 nm, respectively, and by gas chromatographic analysis of 2,4,5-T. This mixture of organisms from soil rather than soil itself was used to facilitate the demonstration of microbial transformations in the preliminary studies. The data suggested that 2,4,5-T disappeared slowly, and gas chromatographic analysis showed that only about 10% was lost after 25 days (Figure 1). The solution gave a reaction in the Chrastil (1975) test,

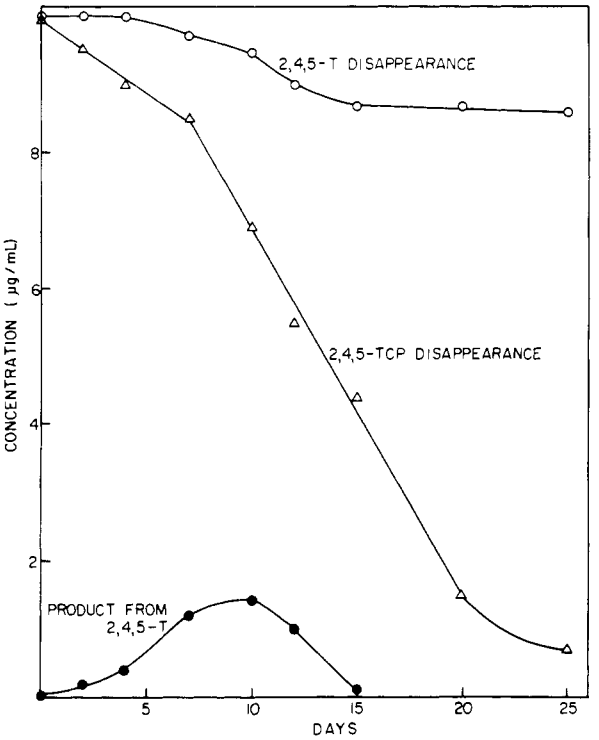

Figure 1. Changes in the concentrations of 2,4,5-T and 2,4,5-TCP in a soil suspension containing 10 μg of 2,4,5-T or 2,4,5-TCP/mL. The product formed from 2,4,5-T absorbed maximally at 310 nm.

suggesting a phenol, and it also contained a product absorbing light at 310 nm, which is the wavelength at which 2,4,5-TCP absorbs maximally. The absorption maxima for 2,4,5-, 2,3,4-, 2,3,6-, 2,3,5-, 3,4,5-, and 2,4,6-trichlorophenols were 310, 283, 289, 280, 305, and 317 nm, respectively. Authentic 2,4,5-TCP added to the soil suspension disappeared rapidly, and more than 80% of the compound was lost at 25 days as judged by decline in UV absorbancy. About 75% of the chlorine in the herbicide was liberated as free chloride. After 25 days, the soil suspension receiving 2,4,5-T was extracted with ethyl acetate and saved for gas chromatography–mass spectrometry. In soil suspensions sterilized with γ irradiation, no 2,4,5-T decomposition was evident.

The breakdown of uniformly ring-^{14}C-labeled 2,4,5-T and uniformly ring-^{14}C-labeled 2,4,5-TCP was also determined by measuring the evolution of $^{14}CO_2$ and the decrease of ^{14}C in the soil suspension. The amount of ^{14}C in the soil suspension containing labeled 2,4,5-T did not decrease until day 16, and after 25 days about 12% of the ^{14}C had disappeared from the liquid (Figure 2). Concurrent with the decrease in radioactivity in the suspension was the evolution of $^{14}CO_2$, and about 8% of the initial radioactivity had been evolved as $^{14}CO_2$ by day 25. In contrast, when the soil suspension was incubated with labeled 2,4,5-TCP, the radioactivity in the liquid started to decrease at 4 days, and nearly 50% of the ^{14}C had disappeared from the suspension by day 25. As the ^{14}C derived from 2,4,5-TCP was lost from the suspension, $^{14}CO_2$ was evolved until more than 40% of the initial radioactivity was recovered as $^{14}CO_2$ by day 25. The suspension was saved for thin-layer chromatography and autoradiography.

A bacterium was isolated from Maahas clay by inoculating soil into a medium containing 100 mg of 2,4,5-T, 0.30 g of glucose, 0.30 g of glycerol, 0.30 g of sodium succinate, 0.50 g of $(NH_4)_2SO_4$, 0.20 g of KCl, 0.20 g of $MgSO_4 \cdot 7H_2O$, 0.10 g of NaCl, 50 mg of $CaCl_2 \cdot 2H_2O$, and 20 mg of $FeCl_3 \cdot 6H_2O$ per liter and 12 mM potassium phosphate buffer.

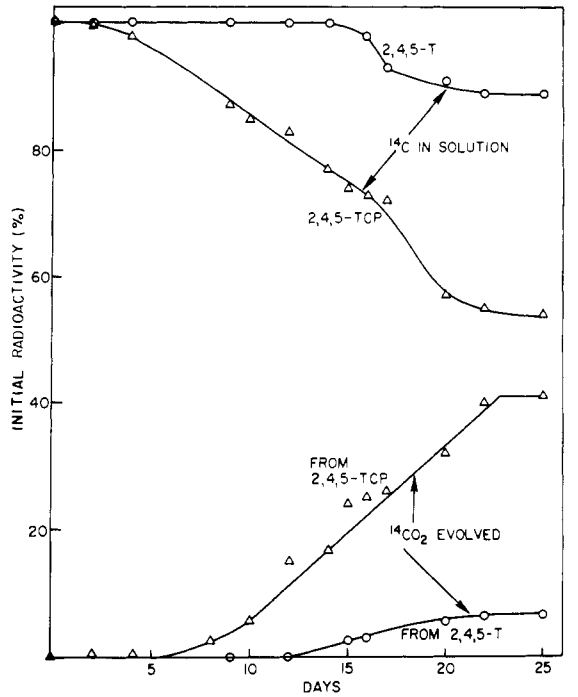

Figure 2. Metabolism of labeled 2,4,5-T or 2,4,5-TCP and evolution of $^{14}CO_2$ by soil suspensions.

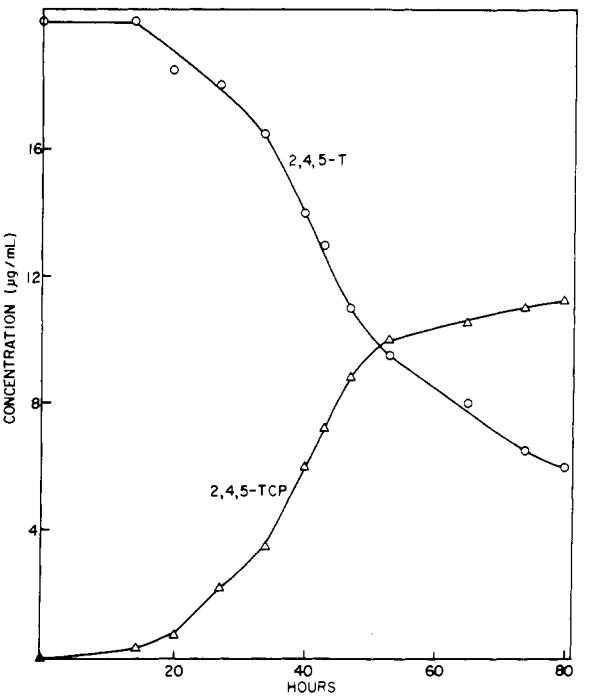

Figure 3. Changes in the concentrations of 2,4,5-T and 2,4,5-TCP effected by a bacterium grown in basal medium amended with 20 μg of 2,4,5-T.

When measurements of the UV absorption indicated that the 2,4,5-T was destroyed and a change in visible turbidity was evident, the enrichment culture was transferred to fresh medium. After two successive transfers, the enrichment was streaked on a solid medium of the same composition. The isolate was identified as *Pseudomonas fluorescens* based on its physiological and morphological characteristics. The bacterium was grown in the basal medium amended with 20 μg of unlabeled 2,4,5-T and 0.5 μCi of uniformly ring-^{14}C-labeled 2,4,5-T/mL. When compared to sterile controls, a decrease in 2,4,5-T concentration was evident at 20 h, and about 70% of the

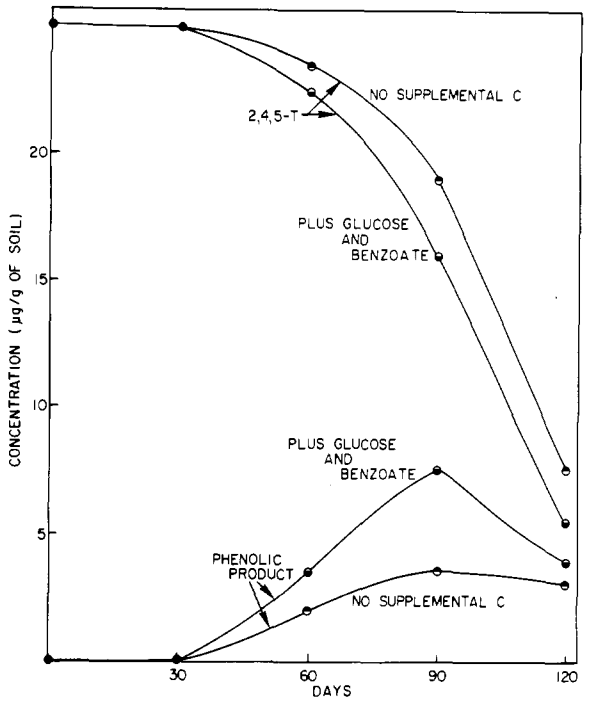

Figure 4. Changes in the concentrations of 2,4,5-T in Maahas clay amended with 25 μg of 2,4,5-T/g of soil and formation of a phenolic product. The soil was either treated with 1.0 mg of glucose and sodium benzoate/g or not so amended.

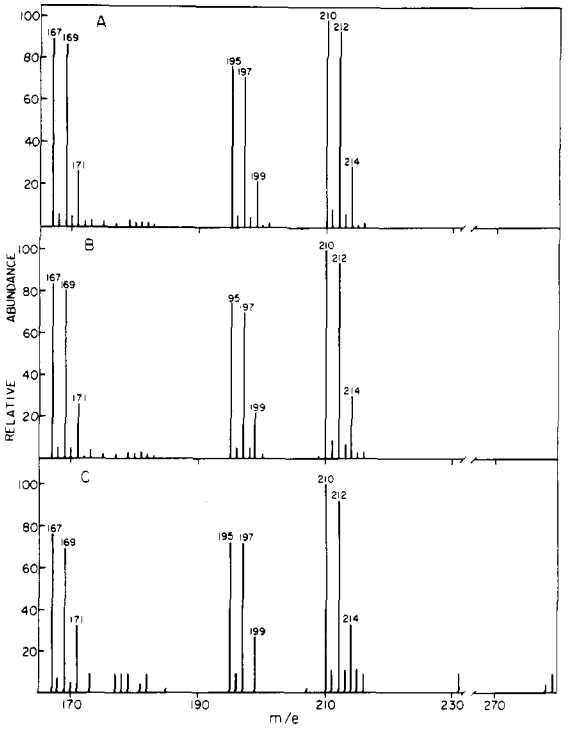

Figure 5. Mass spectra of the methyl derivatives of authentic 2,4,5-TCP (A) and of a product extracted from a bacterial culture (B) and Maahas clay soil (C) incubated with 2,4,5-T.

chemical had disappeared at 80 h (Figure 3). The analysis of 2,4,5-T was by measuring absorbancy at 292 nm and by gas chromatography of samples of the solution that had been extracted with ethyl acetate and derivatized. At about 20 h, the solution contained a substance absorbing maximally at 310 nm, giving a positive phenol reaction, and having the same retention time as 2,4,5-TCP by gas chromatographic analysis. On the assumption that the unknown was 2,4,5-TCP and by using 2,4,5-TCP as a standard for quantification, the yield of the presumed 2,4,5-TCP reached a value of nearly 60% of the maximum that could be produced from 2,4,5-T. No free chloride was released into the medium.

The isolate of *P. fluorescens* could not use 2,4,5-TCP as a carbon source for growth. Moreover, it did not metabolize 2,4,5-TCP in the basal medium supplemented with 20 μg of unlabeled 2,4,5-TCP/mL or with 2.0 μCi (7.7 μg) of labeled 2,4,5-TCP/mL. The bacterium did not liberate $^{14}CO_2$ from labeled 2,4,5-T and 2,4,5-TCP when growing in the basal medium. Thus, the isolate metabolized 2,4,5-T and converted it to a product that might be 2,4,5-TCP, but it could not degrade the phenol further. The cultures grown in the basal media amended with unlabeled 2,4,5-T were extracted with ethyl acetate, derivatized, and saved for further analysis.

The degradation of 2,4,5-T was determined in Maahas clay amended with 25 μg of the herbicide/g of soil. Some of the soil samples were supplemented with 1.0 mg each of glucose and sodium benzoate/g of soil. Portions (25 g) of the soil samples were removed at 30, 60, 90, and 120 days and extracted, and the extracts were analyzed by gas chromatography. The disappearance of 2,4,5-T was not evident until day 60 regardless of whether the soil had received the glucose–benzoate mixture (Figure 4). This supplement thus did not enhance 2,4,5-T metabolism in the first few days, when the two available carbon sources were likely being utilized. The rate of 2,4,5-T loss appeared to be greater in the carbon-supplemented soil than in the soil receiving 2,4,5-T alone, but the difference was slight and because only two soil replicates were examined, it is not known whether the difference was statistically significant. No 2,4,5-T decomposition was evident in soil sterilized by γ irradiation.

A product was found in the extracts of soil that had been incubated with 2,4,5-T for 60 days. This compound gave a positive test for phenol, and it had the same retention time (270 s) by gas chromatography as authentic 2,4,5-TCP. The concentration of the compound rose with time and then decreased somewhat after 90 days. No other products were found by gas chromatography of the extracts of the 2,4,5-T-amended soil as compared with soil not receiving the herbicide. The extract was saved for further analysis.

Derivatized extracts of the soil suspension, *P. fluorescens* culture, and soil that had been incubated with 2,4,5-T were analyzed by gas chromatography. The methyl derivative of a product in each of these extracts had the same retention time as the methylated derivative of authentic 2,4,5-TCP. The retention times for 2,4,5-, 2,3,4-, 2,3,6-, 2,4,6-, 3,4,5-, and 2,3,5-trichlorophenols were 270, 180, 350, 240, 265, and 105 s, respectively, on the OV-17 column operated isothermally at 135 °C. Although 2,4,5- and 3,4,5-trichlorophenols had similar retention times, their mass spectra differed significantly. Figure 5 shows the spectra for authentic 2,4,5-TCP (A), the metabolite from the *P. fluorescens* culture (B), and the product obtained from the soil (C). The product from the soil suspension incubated with 2,4,5-T had an identical spectrum with that of the other two metabolites. The compounds had molecular ions with m/e of 210 and fragmentation patterns identical with that of authentic 2,4,5-TCP. Thus, microorganisms in axenic culture, soil suspension, and Maahas clay converted 2,4,5-T to 2,4,5-TCP.

Because the soil suspension liberated $^{14}CO_2$ from labeled 2,4,5-TCP, the phenol was further metabolized; hence, the extracts from these suspensions were analyzed for possible products formed from 2,4,5-TCP. The derivatized extract from the inoculum amended with unlabeled 2,4,5-TCP was

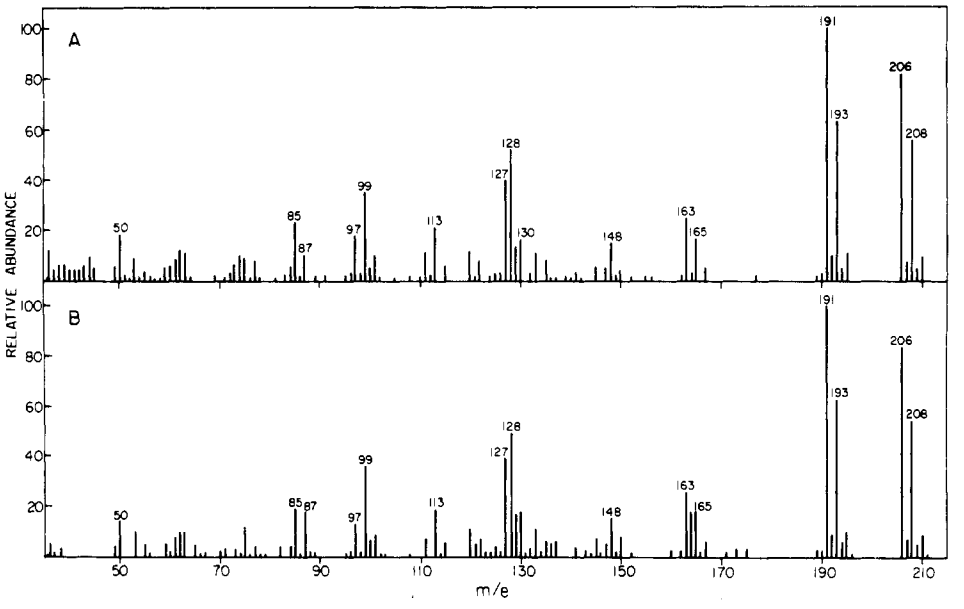

Figure 6. Mass spectra of the methyl derivatives of authentic 3,5-dichlorocatechol (A) and of a product formed in soil suspension incubated with 2,4,5-TCP (B).

analyzed by gas chromatography, and the retention times were compared with retention times of authentic 3,5-dichlorocatechol, *cis,cis*-2,4-dichloromuconate, 2-chloro-4-(carboxymethylene)but-2-enolide, chlorosuccinate, succinate, and 4-chlorocatechol. These compounds have been previously identified as products generated in the metabolism of 2,4-dichlorophenoxyacetate (Bollag et al., 1968a,b; Duxbury et al., 1970; Sharpee et al., 1973; Tiedje et al., 1969). On the basis of comparisons with retention times for these derivatized standards, all of the products were found in the extract of the soil suspension incubated with 2,4,5-TCP. The retention times were 16.3, 13.1, 16.7, 10.0, 8.6, and 13.4 min, respectively, on the temperature-programmed OV-17 column.

Mass spectra were obtained for three of these products. One was identical with the mass spectrum of authentic 3,5-dichlorocatechol (Figure 6). The compound showed a molecular ion with m/e of 206, although the major peak occurred at 191, which probably represents the loss of a methyl group and the formation of $-OCH_2O-$ from the oxygens of the catechol.

The spectrum of the second product was identical with that of 4-chlorocatechol (Figure 7), and the product had a molecular ion with m/e of 172 and a fragmentation pattern identical with that of authentic 4-chlorocatechol. The third product did not show the expected molecular ion at m/e of 146, but it did have a base peak at m/e 115 and a fragmentation pattern identical with that of authentic dimethyl succinate (Figure 8).

The other three metabolites that were tentatively identified by gas chromatography were further analyzed by thin-layer chromatography and autoradiography. Extracts from the soil inoculum amended with labeled 2,4,5-TCP were spotted on silica gel plates, and these spots were overlain with 50 μL of a solution containing each of the authentic chemicals. A second set of plates was used for autoradiography. The authentic chemicals were located on the chromatograms by their UV absorption or, in the case of chlorosuccinate, by the use a spray for halogenated compounds. The plates were first sprayed with a solution of 1.0 g of $AgNO_3$ in 100 mL of 0.5 N NH_4OH solution, they were dried briefly, and then the plates were sprayed with 0.1% ethanolic fluorescein solution (Fisher Scientific Co.). These spots were then scraped off and transferred

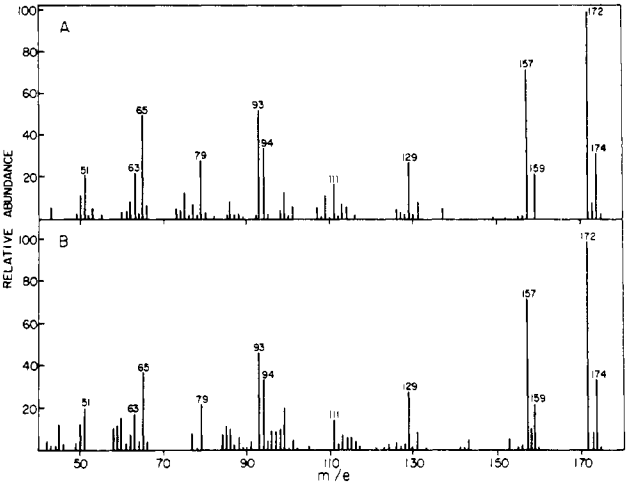

Figure 7. Mass spectra of the methyl derivative of authentic 4-chlorocatechol (A) and of a product formed in soil suspension incubated with 2,4,5-TCP (B).

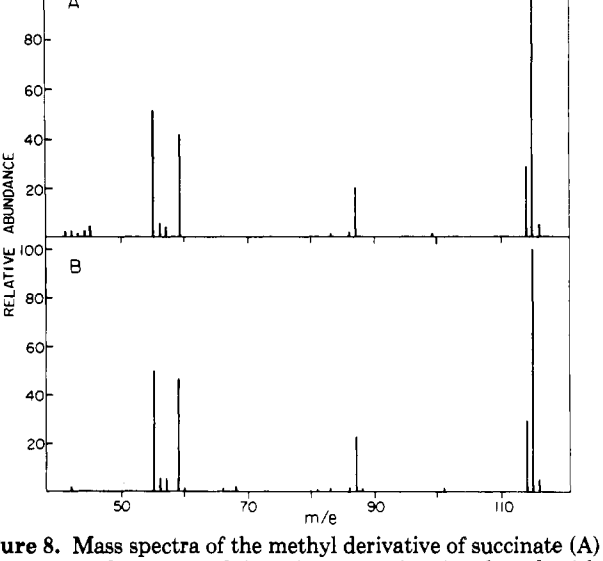

Figure 8. Mass spectra of the methyl derivative of succinate (A) and of a product formed in soil suspension incubated with 2,4,5-TCP (B).

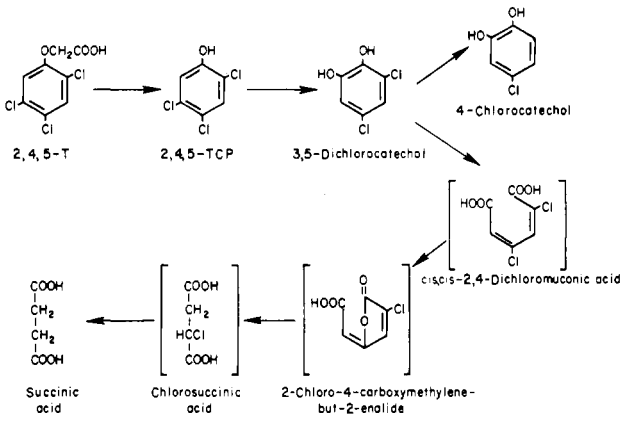

Figure 9. Proposed pathway for the microbial metabolism of 2,4,5-T.

to scintillation vials containing 15 mL of ACS solution. The radioactivity would have been generated by 2,4,5-TCP metabolism. On the basis of radioactivity on the X-ray film and spot formation, the products appeared to be *cis,cis*-2,4-dichloromuconate, the chlorobutenolide, and chlorosuccinate, the R_f values for which were 0.37, 0.25, and 0.55, respectively. No such products were found in the sterile soil suspensions incubated with 2,4,5-TCP; hence, the products are derived from microbial metabolism.

DISCUSSION

The breakdown of 2,4,5-T in soil has been observed by a number of investigators. For example, Yoshida and Castro (1975) detected the microbial destruction of 2,4,5-T in two Philippine soils 12 weeks after addition of the herbicide. Koch (1975) reported the evolution of $^{14}CO_2$ from ring-labeled 2,4,5-T applied to Johnson Island coral, and Sharpee (1973) showed microbial activity on 2,4,5-T in a temperate soil as well in model aquatic ecosystems. The most extensive microbial destruction of a trichlorophenoxy herbicide was reported by Ou and Sikka (1977), who showed that 2-(2,4,5-trichlorophenoxy)propionate was converted to CO_2. In the present study, 2,4,5-TCP, 3,5-dichlorocatechol, 4-chlorocatechol, and succinic acid were identified as products by mass spectrometry. In prior work, it has been found that 2,4,5-TCP is produced from 2,4,5-T in soil and water (Sharpee, 1973) and during the utilization of 2-(2,4,5-trichlorophenoxy)propionate by a mixed microbial culture (Ou and Sikka, 1977). On the basis of a positive Arnow-catechol test and thin-layer chromatography, Horvath (1970) proposed that *Brevibacterium* sp. generated 3,5-dichlorocatechol from 2,4,5-T. However, the present inquiry provides definite evidence for the microbial formation of 3,5-dichlorocatechol, and several other intermediates formed in the metabolism of 2,4,5-TCP also have been tentatively identified. On the basis of these findings, 2,4,5-T appears to be acted on by an initial cleavage of the ether linkage to yield 2,4,5-TCP, which is then converted to 3,5-dichlorocatechol. The benzene ring is then apparently opened to yield products tentatively identified as *cis,cis*-2,4-dichloromuconate, 2-chloro-4(carboxymethylene)but-2-enolide, and chlorosuccinate, and succinate is the final product of dehalogenation (Figure 9). The pathway of metabolism beyond 3,5-dichlorocatechol thus is analogous to the sequence described for 2,4-D degradation (Loos, 1975). The precursor of 4-chlorocatechol is likely the dichlorocatechol, but is not presently clear how the dichlorocatechol is generated from 2,4,5-TCP because both a dehalogenation and a hydroxylation are required.

The data presented here suggest that 2,4,5-T was acted on by cometabolism by the isolate. An isolate able to use the herbicide as a carbon source for growth has yet to be obtained. However, a soil suspension contained organisms able to transform 2,4,5-TCP to succinic acid, indicating that the 2,4,5-T is ultimately converted to compounds that undoubtedly serve as carbon and energy sources for microorganisms. The usually long persistence of 2,4,5-T in soil is thus likely a result of the inability of the small cometabolizing population to replicate by using the pesticide as a carbon source, but a product of the cometabolism that can be used as a carbon source would not be found in nature in appreciable concentrations because that product would serve as a substrate for microbial growth.

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