Degradation of Methyl Parathion by a Mixed Bacterial Culture and a *Bacillus sp.* Isolated from Different Soils

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A mixed bacterial culture and a *Bacillus sp.* capable of degrading methyl parathion (MP) were isolated from different soils. Although both cultures extensively degraded MP to its final oxidation products, CO_2 and H_2O, the mixed culture degraded MP only in the presence of a second carbon source such as glucose or yeast extract. None of the isolates could individually degrade the chemical. A *Pseudomonas sp.* isolated from the mixed culture was found to have capacity to degrade *p*-nitrophenol, which is the hydrolysis product of MP. The *Bacillus sp.* utilized MP as a sole source of carbon for growth. Possible implications of our findings with respect to the microbial degradation of MP in soil are discussed.

Methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate, MP) has been increasingly used as an insecticide since the 1970s in place of an analogous chemical, parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate). However, information on microbial degradation and fate of MP in the environment is not as extensively available as for parathion. It was reported as early as 1973 that three soil bacteria, a *Bacillus sp.* and a *Pseudomonas sp.* (Siddaramappa et al., 1973), and a *Flavobacterium sp.* (Sethunathan and Yoshida, 1973), hydrolyzed parathion to *p*-nitrophenol (PNP). A number of bacteria capable of hydrolyzing parathion, including a mixed bacterial culture (Munnecke and Hsieh, 1974), an *Arthrobacter sp.* and a *Bacillus sp.* (Nelson, 1982), and *Pseudomonas diminuta* (Serdar et al., 1982) were subsequently reported. In contrast, brief mention of a strain of *Bacillus subtilis* capable of degrading MP was reported in a 1977 review article by Laveglia and Dahm (1977). An aquatic bacterium, a *Flavobacterium sp.*, when grown in nutrient broth, also degraded MP (Lewis et al., 1985). However, it was not clear whether the degradation was simply a hydrolysis process or proceeded beyond the hydrolysis step. A *Pseudomonas sp.*, which possessed an organophosphate-degradinggene similar to those reported for *P.diminuta* and a *Flavobacterium sp.* (Serdar et al., 1982), was reported by Chaudhry et al. (1988). The organism hydrolyzed both MP and parathion to PNP. Microorganisms that utilize MP as a sole source of carbon and energy for growth have not been reported to date.

In this study, we have isolated from soil a mixed bacterial culture and a *Bacillus sp.* capable of mineralizing MP to carbon dioxide. The *Bacillus sp.* utilized MP as a sole source of carbon for growth. We have also investigated the degradation pathways of MP by both the axenic culture and the mixed culture. In addition, we have investigated a bacterium, isolated from the mixed culture, that degrades PNP.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled [^{14}C]methyl parathion (MP) was purchased from California Bionuclear Corp. (Sun Valley, CA), with a specific activity of 65 MBq/mmol. The labeled chemical was purified by preparative thin-layer chromatography (TLC) to better than 98% radiopurity. Uniformly ring-labeled [^{14}C]-*p*-nitrophenol (PNP) was prepared by alkaline hydrolysis of purified [^{14}C]MP as described previously by Ou (1985). Analytical-grade MP (99.9%), analytical-grade parathion (99.1%), analytical-grade EPN (*O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate, 98.5%), and technical-grade fenitrothion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, 95.1%) were obtained from the Environmental Protection Agency (Research Triangle Park, NC). Technical-grade MP (80%) was provided by Monsanto Chemical Co. (St. Louis, MO). Our sample of technical-grade MP also contained 17% xylene and 3% unknowns. Before use, xylene in the technical-grade MP was allowed to evaporate. Thus, the technical-grade MP, when used, was better than 95% purity. Analytical-grade PNP (99%) was purchased from Eastman Kodak Co. (Rochester, NY).

Soils and the Enrichment of MP-Degrading Microorganisms. Arredondo fine sand (Grossarenic Paleudult) and Cecil sandy loam (Typic Hapludult) were used for this study. The Arredondo soil consisted of 1.7% of organic C at pH 5.5; and the Cecil soil had 0.9% organic C with pH 5.6. Key properties of the two soils have been reported previously (Ou et al., 1978, 1985).

For enrichment of MP-degrading microorganisms in Arredondo and Cecil soils, technical-grade MP was initially applied to the two soils (100 g) at a concentration of 10 μg/g. The soil–water contents for Arredondo soil and Cecil soil were maintained at 0.08 and 0.10 mL/g, respectively. MP was then continuously applied at an interval of 2–3 weeks, and the amount of chemical added was gradually increased. At the final application, MP was applied at a concentration of 50 μg/g. After 4 months of incubation at room temperature (20–24 °C), 10 g of each sample were transferred to 100 mL of sterile basal mineral medium in 250-mL Erlenmeyer flasks, with or without supplement by glucose (500 μg/mL). The basal mineral medium consisted of (per liter of deionized water) 4.8 g of K_2HPO_4, 1.2 g of KH_2PO_4, 1 g of NH_4NO_3, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.04 g of $CaCl_2$, and 0.001 g of $Fe_2(SO_4)_3$. The flasks were incubated in an environmental rotary shaker at 200 rpm and at 25 °C. MP concentration in the basal mineral medium was 50 μg/mL. Once every 3 weeks, 10 mL from each sample was transferred to fresh medium containing MP (50 μg/mL). After three consecutive transfers, turbidity developed in the Cecil soil seeded MP–basal mineral medium without supplementing with glucose. No turbidity, however, developed in the Arredondo soil seeded MP–basal mineral medium without supplementing with glucose. For confirming the presence of MP-degrading microorganisms, a small amount of [^{14}C]MP (32 Bq/mL) was added to the flasks containing soil-seeded MP-basal mineral medium. The flasks were incubated at 25 °C for 24 h, and evolved $^{14}CO_2$ was trapped in KOH (8 mol/L). The KOH was placed in stainless-steel vials hung under rubber stoppers. Each flask was tightly closed with a stopper. ^{14}C activity in the KOH traps was determined by

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scintillation counting. $^{14}CO_2$ was evolved from the Cecil soil seeded samples without supplement of glucose and from the Arredondo soil seeded samples supplemented with glucose.

Isolation and Maintenance of MP-Degrading Microorganisms. MP (50 μg/mL)–basal mineral agar plates were used for isolation and purification of MP-degrading microorganisms from enriched samples, which had been shown to have capacity to degrade [^{14}C]MP to $^{14}CO_2$. Bacterial colonies developed on the plates, which had been inoculated with a small amount of enriched Cecil soil suspensions. A small amount of bacterial mass from one colony was transferred to a new plate. After two such transfers, a small amount of cell mass from one colony was inoculated to a sterile 125-mL Erlenmeyer flask containing 25 mL of filtered (0.22-μm) analytical-grade MP (50 μg/mL)–basal mineral medium. Turbidity developed after 24 h of incubation. Even after 10 consecutive transfers, turbidity always developed in the liquid medium. It was then concluded that the bacterium utilized MP as a sole source of carbon for growth. The bacterium was tentatively identified as a *Bacillus sp.* It was a Gram-positive, spore-forming motile rod, both oxidase-positive and catalase-positive. The organism was maintained in filtered analytical-grade MP (50 μg/mL)–basal mineral medium and on MP–basal mineral agar slants. Parathion, fenitrothion, and EPN, which are chemically analogous to MP, were also used for observing the capacity of the organism to utilize and to hydrolyze these chemicals.

Microbial colonies were not visible on the MP (50 μg/mL)–basal mineral agar plates inoculated with mixed culture, if derived from Arredondo soil seeded MP–basal mineral medium supplemented with glucose. The mixed culture was maintained in 100 mL of basal mineral medium containing 50 mg of technical-grade MP and 50 mg of glucose or 50 mg of yeast extract. The mixed culture consisted of at least 13 rod-shaped bacteria. On the basis of their morphological, physiological, and biochemical characteristics, these bacteria were tentatively identified as four *Pseudomonas sp.*, four *Acinetobacter sp.*, one *Alcaligenes sp.*, one *Arthrobacter sp.*, one *Corynebacterium sp.*, and two unknowns (a Gram-positive and a Gram-negative). The mixed culture was found to grow on PNP without supplement with a second carbon such as glucose. A bacterium capable of utilizing PNP as a sole source of carbon was isolated from the mixed culture on PNP–basal mineral agar plates. The bacterium was identified as a *Pseudomonas sp.*

Metabolism of [^{14}C]MP by the *Bacillus sp.* and the Mixed Bacterial Culture. Two-day-old *Bacillus sp.* grown in filtered MP (50 μg/mL)–basal mineral medium was inoculated to 100 mL of filtered basal mineral medium containing analytical-grade MP (1–10 μg/mL) and [^{14}C]MP (92 Bq/mL) in 250-mL Erlenmeyer flasks. Each flask was tightly closed with a rubber stopper, a stainless-steel vial containing KOH being hung under the stopper. This was used to trap evolved $^{14}CO_2$. Hexane was used to extract MP from culture fluids, and an equal volume of chloroform and ethyl ether was used to extract MP and metabolites. ^{14}C activity in the water and organic solvent extracts was quantified by liquid scintillation counting. MP and metabolites were determined by TLC autoradiographic assay, followed by liquid scintillation counting as described previously by Ou (1985) and Ou et al. (1983). ^{14}C activity in the KOH was determined by liquid scintillation counting. At the same time, aliquots of the KOH were acidified with concentrated HCl to pH <1. ^{14}C activity in the acidified samples was near background levels. This suggests that ^{14}C activity trapped in the KOH was principally associated with $^{14}CO_2$.

Since the mixed bacterial culture required a second carbon source in order to degrade MP, glucose (500 μg/mL) or yeast extract (500 μg/mL) was added to filtered MP (10 μg/mL)–basal mineral medium. The same procedures as used for studying metabolism of MP by the *Bacillus sp.* were employed.

A similar procedure used for determination of $^{14}CO_2$ evolution from [^{14}C]MP was used for determination of $^{14}CO_2$ evolution from [^{14}C]PNP by PNP-degrading organisms. ^{14}C activity in culture fluids was extracted with an equal volume of chloroform and ethyl ether. Parent chemical and metabolites were determined by TLC autoradiography and scintillation counting. PNP in the culture fluids was also monitored spectrophotometrically at 420 nm.

Table I. Degradation of MP and Growth of the *Bacillus sp.* in Basal Mineral Medium Containing 50 μg/mL of [^{14}C]MP

time, h	optical density[a]		% distribution		% applied ^{14}C		
	420 nm	550 nm	MP	PNP	hexane	water	total
0	0	0.002	100	0	100	0	100
0.5	0.50	0.001	45	55	31	70	101
1	0.82	0.001	22	78	NT[b]	NT	NT
2	0.98	0.002	1	99	2	97	99
4	1.14	0.002	0	100	1	92	93
8	0.95	0.005	0	100	1	72	73
24	0.03	0.013	ND[c]	ND	0	28	28

[a] No addition of [^{14}C]MP. [b] Not tested. [c] Not detected.

RESULTS AND DISCUSSION

Degradation by the *Bacillus sp.* When a small amount of 36-h-old *Bacillus sp.* grown in MP–basal mineral medium was inoculated into the MP–basal mineral medium to give an initial cellular density of 6×10^5 cells/mL, faint yellow color developed almost immediately. The yellow color could not be extracted with hexane but could be extracted with an equal volume of chloroform and ethyl ether. The chemical associated with the yellow color was subsequently identified by TLC to be PNP. The organism appeared to produce active MP-hydrolase, as MP at 1 μg/mL was instantly hydrolyzed to PNP. As soon as the *Bacillus sp.* was inoculated, all ^{14}C activity became nonextractable by hexane. Without inoculation of the *Bacillus sp.*, all applied ^{14}C activity remained in the form of [^{14}C]MP; i.e., it could be extracted by hexane. Even at 10 μg/mL, 97% of the applied [^{14}C]MP was hydrolyzed by the organism to PNP in 30 min and, after 2 h, only PNP was detected in the culture fluids. PNP was completely degraded in 4–6 h. After 24 h of incubation, 64% of the applied ^{14}C activity was evolved as $^{14}CO_2$ and 35% of the added ^{14}C actvity remained in the culture fluid. The latter was found to be associated with microbial mass.

Table I shows the turbidity of *Bacillus sp.* (550 nm) grown in MP (50 μg/mL)–basal mineral medium, optical density at 420 nm, percent of applied ^{14}C in the hexane and water phases, and the inferred distribution of MP and PNP. Maximal optical absorbance for PNP is 420 nm. MP at this concentration provided good growth for the *Bacillus sp.* as indicated by increase in culture turbidity and rapid disappearance of MP, as well as rapid formation and subsequent rapid disappearance of its hydrolysis product PNP. PNP was the only chemical detected in the chloroform–ethyl ether extracts. the only chemical detected in the hexane extracts was MP. In 2 h, 98% of the [^{14}C]MP was degraded. After 4 h, [^{14}C]MP could not be detected by TLC autoradiographic assay, even though 1% of the applied ^{14}C was detected in the hexane extract. PNP disappeared completely between 8 and 24 h. In a separate experiment, it was found that 67, 70, and 72% of the applied [^{14}C]MP (50 μg/mL) were mineralized to $^{14}CO_2$ in 24, 48 and 72 h, respectively. After 72 h, the remaining ^{14}C (29%) in the culture fluid was principally associated with bacterial mass.

It was found that MP-grown cells of the *Bacillus sp.* also hydrolyzed parathion and fenitrothion to PNP and 3-methyl-*p*-nitrophenol, respectively. Similar to PNP, 3-methyl-*p*-nitrophenol in water at neutral and alkaline pH forms a yellow color. Thus, 3-methyl-*p*-nitrophenol appeared to be the end degradation product of fenitrothion by the *Bacillus sp.* Neither fenitrothion nor its hydrolysis product 3-methyl-*p*-nitrophenol could serve as a sole source of carbon for the organism, however. The

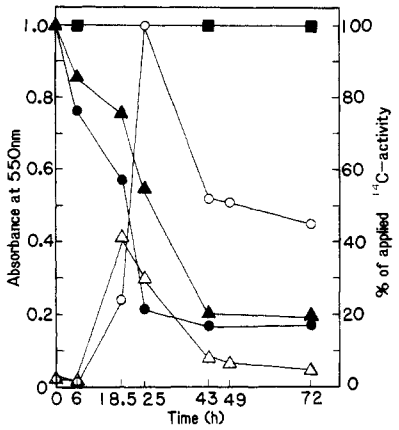

Figure 1. Growth of the mixed culture and disappearance of ^{14}C activity from a basal mineral medium containing [^{14}C]MP and either glucose or YE. Growth: ○, grown in glucose; △, grown in YE. Disappearance of ^{14}C activity: ●, from glucose; ▲, from YE. ■, control, basal mineral medium plus [^{14}C]MP only.

Bacillus sp. also did not utilize parathion as a sole source of carbon for growth. After initial hydrolysis and utilization of the liberated PNP by the *Bacillus sp.*, degradation of the chemical did not proceed further. Furthermore, the MP-grown *Bacillus sp.* did not hydrolyze EPN to PNP. EPN is chemically analogous to parathion, except that one of the two ethyl groups is substituted by a benzene ring. This suggests that MP-hydrolase produced by the MP-grown *Bacillus sp.* is only partially specific and may hydrolyze some chemicals analogous to MP. However, these chemicals appear not to induce appropriate hydrolases for completing the hydrolysis process.

Unlike a *Pseudomonas sp.* (Chaudhry et al., 1988) and a *Pseudomonas sp.* (Munnecke and Hsieh, 1974) that both eventually lost their capacity to utilize MP and parathion, respectively, for growth, but retained capacity to hydrolyze MP and parathion to PNP, the *Bacillus sp.* did not lose its capacity to utilize MP, even after more than 1 year of maintenance in MP–basal mineral medium.

MP Degradation by the Bacterial Mixed Culture. [^{14}C]MP at 10 μg/mL was rapidly degraded by the mixed culture, as indicated by the disappearance of ^{14}C activity from the culture fluids and the formation of gaseous $^{14}CO_2$. Figure 1 shows the disappearance of ^{14}C activity in conjunction with growth of the mixed culture in [^{14}C]MP–basal mineral medium supplemented with glucose or yeast extract. The mixed culture grew better in glucose than in yeast extract, and [^{14}C]MP subsequently disappeared more rapidly from the basal mineral medium supplemented with glucose. After 43 h of incubation, ^{14}C in the culture fluids remained unchanged or little changed. After the culture fluids had been fractionated into hexane and water phases (Table II), the patterns of ^{14}C distribution from the culture fluids supplemented with glucose and yeast extract were distinctly different, despite the fact that ^{14}C activity in both the glucose and yeast exract rapidly declined (see Figure 1). At 6 h and earlier, ^{14}C activity in both the glucose and yeast extract was completely or nearly completely extracted by hexane. At 18.5 h, about half of the ^{14}C activity in the presence of glucose could be extracted by hexane, whereas more than 70% of the ^{14}C activity in yeast extract went to the hexane phase. After 25 h, no ^{14}C activity in the glucose-amended medium was found to be associated with the hexane phase, whereas 40–67% of the ^{14}C activity in yeast extract went to the hexane phase. TLC autoradiographic analysis revealed that the only chemical detected in the hexane fractions from both the glucose and the yeast extract was the parent chemical MP. ^{14}C activity in the water phase was principally associated with bacterial mass (80% to near 100%).

Table II. Distribution of ^{14}C Activity in Hexane and Water Phases following Extraction of the Basal Mineral Medium Containing 10 μg/mL of [^{14}C]MP and either Glucose or YE

time, h	^{14}C activity, %					
	glucose		YE		control[a]	
	water	hexane	water	hexane	water	hexane
0	0	100	0	100	0	100
6	0	100	4	96	0	100
18.5	46	54	28	72	ND[b]	ND
25	100	0	57	43	0	100
43	100	0	33	67	3	97
49	100	0	45	55	ND	ND
72	100	0	60	40	7	93

[a] [^{14}C]MP in the basal mineral medium. [b] Not determined.

Table III. $^{14}CO_2$ Production from [^{14}C]MP (10 μg/mL) by the Mixed Culture in Basal Mineral Medium Supplemented by either Glucose or YE

time, days	$^{14}CO_2$ production, % applied ^{14}C	
	%/day	cum %
1	31.8[a] (12.3)[b]	31.8[a] (12.3)[b]
2	10.5 (35.5)	42.3 (47.8)
3	5.8 (15.9)	48.1 (63.7)
5	3.6 (3.9)	55.3 (71.5)
9	1.2 (1.1)	60.1 (75.7)

[a] Grown in glucose. [b] Grown in YE.

^{14}C activity in supernatants of the water phase, after extraction with chloroform–ethyl ether, was found to be associated with TLC polar product(s) (R_f 0), with the exception of the mixed culture grown for 18.5 h in glucose. In this sample, a small amount of PNP was detected. It was likely that since glucose and yeast extract constitute somewhat different sources of carbon, the relative growth of bacteria in the mixed culture would not be identical for the two media. The mixed culture grown in yeast extract, despite rapid initial degradation of MP, did not completely degrade MP. A small amount of MP still remained in this culture medium even after 72 h. The level of ^{14}C activity in the sterile basal mineral medium without the mixed culture did not change over the 72-h period, though 7% of the [^{14}C]MP was chemically hydrolyzed during this period, presumably to PNP. Since no TLC polar product was detected from the degradation of MP by the *Bacillus sp.* it is likely that TLC polar product(s) may be the lysis products of ^{14}C cellular components of the bacteria from the mixed culture, such as amino acids, peptides, nucleotides, nucleic acids, etc.

Similar to the disappearance of ^{14}C activity, [^{14}C]MP in the glucose was initially mineralized more rapidly to $^{14}CO_2$ by the mixed culture than in the yeast extract (Table III). However, after 9 days, more total $^{14}CO_2$ had been produced in the yeast extract (75.7% vs. 60.1%). At the end of the incubation period (9 days), 30.8 and 25.8% of the applied ^{14}C remained in the glucose and in the yeast extract, respectively.

The mixed culture completely solubilized MP at application rates of 25 000 and 50 000 μg/100 mL in 3 and 4 days, respectively, presumably to CO_2 and bacterial mass. Prior to the inoculation, only 20 and 10% of the MP at 25 000 and 50 000 μg/100 mL were initially solubilized.

Failure of individual isolates of the mixed culture to degrade MP suggests that the degradation of MP was due to a concerted effort by the organisms. Once these bacteria in the mixed culture were separated, they lost

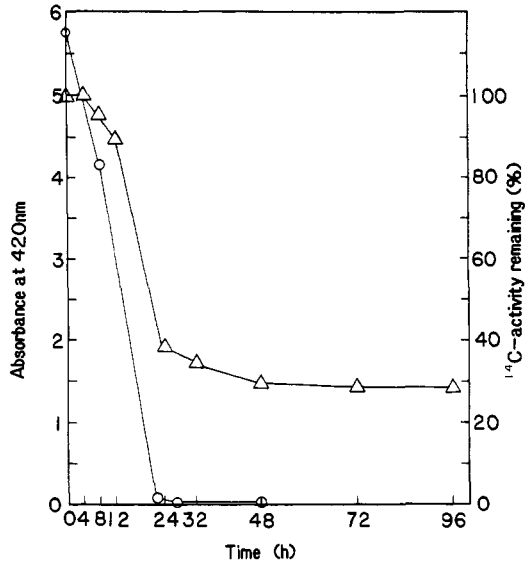

Figure 2. Degradation of [^{14}C]PNP by a *Pseudomonas sp.*: ○, absorbance at 420 nm; △, % of applied ^{14}C activity remaining in the culture fluid.

Table IV. $^{14}CO_2$ Production from [^{14}C]PNP by a *Pseudomonas sp.* in the Basal Mineral Medium Containing 100 μg/mL of PNP

time, days	rate $^{14}CO_2$ prodn, %/days
1	51.5
2	11.2
3	2.9
4	1.9
total	67.5

the capacity to degrade MP. Since one of the bacteria in the mixed culture (a *Pseudomonas sp.*) utilized PNP as a sole source of carbon for growth, it is likely that some bacteria in the mixed culture may be involved in the hydrolysis of MP to PNP, which then was rapidly degraded by the *Pseudomonas sp.* This is not the first time that individual isolates from a mixed culture have failed to degrade a target chemical, despite the fact that a parent mixed culture did. A bacterial mixed culture consisting of two bacteria cometabolically degraded the herbicide silvex to CO_2 and H_2O (Ou and Sikka, 1977). However, individual isolates as well as a mixture of the two isolates transferred to the same medium failed to degrade the herbicide. A thermophilic bacterial mixed culture consisting of two bacteria grew on hexadecane–basal mineral medium, but individual colonies transferred to the same medium failed to grow (Sukatsch and Johnson, 1972).

PNP Degradation by the *Pseudomonas sp.* Isolated from the Mixed Culture. [^{14}C]PNP, at 100 μg/mL, was rapidly degraded by the *Pseudomonas sp.* as indicated by a rapid decrease in the absorbance at 420 nm and rapid disappearance of ^{14}C activity from the culture fluids, as well as by $^{14}CO_2$ evolution (Figure 2; Table IV). Absorbance at 420 nm by the culture fluids had completely disappeared after 24 h, and at the same time, ^{14}C activity had rapidly declined and more than half of the applied ^{14}C activity had been evolved as $^{14}CO_2$. After 96 h of incubation, 67.5% of the applied ^{14}C activity was mineralized to $^{14}CO_2$, and the remaining ^{14}C activity (26.4%) in the culture fluid was essentially associated with bacterial mass. Total ^{14}C recovery was 93.9%.

The only chemical detected in the chloroform–ethyl ether extracts was PNP, and after 24 h, less than 1% of the applied ^{14}C activity could be thus extracted. This suggests that PNP was rapidly utilized by the *Pseudomonas sp.*, converting to CO_2 and microbial mass.

The fact that a mixed bacterial culture capable of degrading MP through cometabolism was isolated from one soil (Arredondo fine sand), while an axenic bacterial culture, a *Bacillus sp.* capable of utilizing MP as a sole source of carbon for growth was isolated from another soil (Cecil sandy loam), suggests that MP in soil can be degraded by cometabolism involving a group of bacteria, can serve as a sole source of carbon for an organism, or both. In any case, MP in soil can be completely degraded to the final oxidation products CO_2 and H_2O (Ou, 1985; Ou et al., 1983). Furthermore, microorganisms capable of hydrolyzing structurally similar chemicals such as parathion or fenitrothion may concurrently hydrolyze MP, resulting in the formation of PNP. Since PNP degraders are abundant in the environment (Spain et al., 1980), PNP would be rapidly degraded.

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LITERATURE CITED

Chaudhry, G. R.; Ali, A. N.; Wheeler, W. B. Isolation of a Methyl Parathion-Degrading *Pseudomonas* sp. That Possesses DNA Homologous to the opd Gene from a *Flavobacterium* sp. *Appl. Environ. Microbiol.* **1988**, *54*, 288–293.

Laveglia, J.; Dahm, P. A. Degradation of Organophsophorus and Carbamate Insecticides in the Soil and by Soil Microorganisms. *Annu. Rev. Entomol.* **1977**, *22*, 483–513.

Lewis, D. L.; Hodson, R. E.; Freeman, L. F. III. Multiphasic Kinetics for Transformation of Methyl Parathion by *Flavobacterium* Species. *Appl. Environ. Microbiol.* **1985**, *50*, 553–557.

Munnecke, D. M.; Hsieh, P. H. Microbial Decontamination of Parathion and *p*-Nitrophenol in Aqueous Media. *Appl. Microbiol.* **1974**, *28*, 212–217.

Nelson, L. M. Biological-Induced Hydrolysis of Parathion in Soil: Isolation of Hydrolyzing Bacteria. *Soil Biol. Biochem.* **1982**, *14*, 219–222.

Ou, L.-T. Methyl Parathion Degradation and Metabolism in Soil: Influence of High Soil-Water Contents. *Soil Biol. Biochem.* **1985**, *17*, 241–243.

Ou, L.-T.; Sikka, H. C. Extensive Degradation of Silvex by Synergistic Action of Aquatic Microorganisms. *J. Agric. Food Chem.* **1977**, *25*, 1336–1339.

Ou, L.-T.; Rothwell, D. F.; Wheeler, W. B.; Davidson, J. M. The Effect of High 2,4-D Concentrations on Degradation and Carbon Dioxide Evolution in Soils. *J. Environ. Qual.* **1978**, *7*, 241–246.

Ou, L.-T.; Rao, P. S. C.; Davidson, J. M. Methyl Parathion Degradation in Soil: Influence of Soil-Water Tension. *Soil Biol. Biochem.* **1983**, *15*, 211–215.

Ou, L. T.; Rothwell, D. F.; Mesa, M. V. Soil Sterilization by 2450 MHz Microwave Radiation. *Proc.—Soil Crop Sci. Soc. Fla.* **1985**, *44*, 77–80.

Serdar, C. M.; Gibson, D. T.; Munnecke, D. M.; Lancaster, J. H. Plasmid Involvement in Parathion Hydrolysis by *Pseudomonas diminuta*. *Appl. Environ. Microbiol.* **1982**, *44*, 246–249.

Sethunathan, N.; Yoshida, T. A *Flavobacterium* sp. That Degrades Diazinon and Parathion. *Can. J. Microbiol.* **1973**, *19*, 873–875.

Siddaramappa, R.; Rajaram, K. P.; Sethunathan, N. Degradation of Parathion by Bacteria Isolated from Flooded Soil. *Appl. Microbiol.* **1973**, *26*, 846–849.

Spain, J. C.; Pritchard, P. H.; Bourquin, A. W. Effects of Adaptation on Biodegradation Rates in Sediment/Water Cores from Esturaine and Freshwater Environments. *Appl. Environ. Microbiol.* **1980**, *40*, 726–734.

Sukatsch, D. A.; Johnson, M. T. Bacterial Cell Production from Hexadecane at High Temperatures. *Appl. Microbiol.* **1972**, *23*, 543–546.

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Photodecomposition of Metalaxyl in an Aqueous Solution

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UV irradiation of metalaxyl in aqueous solution resulted in 70% substrate transformation in 5 days, with rates of transformation affected by irradiation time, pH, and substrate concentration. Addition of 1% acetone accelerated photodecomposition, while riboflavin and methylene blue had no effect. After 5 days of irradiation of metalaxyl at pH 6.8, two products (A and B) were formed: A contained 3% and B 6% of the initial radioactivity. The two compounds were isolated by TLC and their structures identified by mass and NMR spectroscopy. Irradiation of A resulted in the formation of B. In each case photolysis caused a rearrangement of the *N*-acyl group to the 4-position on the aromatic ring.

Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester] is the active ingredient of the fungicide Ridomil. Since its introduction in 1977, it has been widely used for the control of plant diseases caused by oomycetous fungi of the order Peronosporales. The primary mechanism of action of metalaxyl is an inhibition of RNA-polymerase activity of various oomycetous species. Research on mobility and metabolism of metalaxyl in soil and microbial degradation of the fungicide have been reviewed by Cohen and Coffey (1986).

In our previous studies we investigated the microbial transformation of metalaxyl by the fungus *Syncephalastrum racemosum* and found that the major transformation mechanism involves benzylic or aromatic ring hydroxylation of the fungicide (Zheng et al., 1989). There is little information available regarding UV degradation of metalaxyl. A project report of CIBA-GEIGY Corp. has documented that, after irradiation of an aqueous solution of metalaxyl for 7 days using artificial sunlight, 5% of the added chemical was converted to metalaxyl acid [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine] and 17% to unidentified polar compounds (Burkhard, 1979).

The purpose of our research was to examine the effect of UV irradiation on the transformation of metalaxyl, to isolate and identify the transformation products, and to elucidate the mechanisms of photolytic transformation reactions.

MATERIALS AND METHODS

Chemicals. Metalaxyl (technical grade) of 96.4% purity and [*ring*-U-^{14}C]metalaxyl with a specific activity of 39 μCi/mg was supplied by Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC. Riboflavin (RF), methylene blue (MB), and benzophenone (BP) were purchased from Sigma Chemical Co. (St. Louis, MO). These chemicals were added as freshly prepared solutions in deionized water or dissolved directly into the metalaxyl solutions (200 mg/L for RF, 100 mg/L for MB and BP or 1% acetone) prior to irradiation.

UV Irradiation of Metalaxyl and Recovery of Samples. Ultraviolet irradiation was performed with a 30-W germicidal lamp (Angstrom 2537, General Electric). Open dishes (250 mL), each containing 200 mL of the reaction solution, were placed 30 cm beneath the UV light. The reaction solutions were irradiated for 1–5 days at 30 °C. The samples containing photosensitizer were irradiated for 4–8 h under the same conditions. Controls were placed in a dark chamber and incubated for the same period of time; no chemical changes were observed.

Because of volume changes due to evaporation, it was necessary to adjust each reaction solution to the original volume before extraction. Ethyl acetate was used as extractant. The reaction solution was shaken for 1 min with an equal volume of ethyl acetate in a separatory funnel. The efficiency of extraction exceeded 94% as indicated by radioactivity measurements of the aqueous and solvent phase. The distribution of radioactivity in the reaction mixture, in the ethyl acetate extract, and in the aqueous phase was determined by liquid scintillation spectrometry.

Experiments with Various Metalaxyl Concentrations and pH Values of the Reaction Solutions. Photodecomposition studies with various concentrations of metalaxyl (5, 10, 20, 30, 40, 50 mg/L) were carried out in a citrate–phosphate buffer (0.2 M Na_2HPO_4/0.1 M citric acid) at pH 6.8. To investigate the effect of pH on the photodecomposition of metalaxyl, 50 mg/L of the substrate was prepared in citrate–phosphate buffers of pH 2.8, 4.8, and 6.8 and in a Tris buffer of pH 8.8 (0.1 M Tris/0.1 M HCl). Following the first ethyl acetate extraction of the reaction solution (pH 4.8, 6.8, 8.8), the remaining aqueous phase was adjusted to pH 2.8 with 2 M HCl and reextracted with an equal volume of ethyl acetate. The two ethyl acetate extracts were pooled and evaporated to dryness on a

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