Microbial Dechlorination of the Herbicide Metolachlor

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We studied the dechlorination of metolachlor by four microorganisms, *Streptomyces* sp., *Phanerochaete chrysosporium*, *Rhizoctonia praticola*, and *Syncephalastrum racemosum*, in a growth medium containing 0.35 mM metolachlor. A significant amount of the herbicide was dechlorinated by all cultures, resulting in the formation of seven dechlorinated products as determined by high-performance liquid chromatography and mass spectrometric analyses. Transformation mechanisms included dehalogenation with subsequent hydroxylation of the chloroacetyl group; further reactions led to ring formation between the acetyl group and the benzylic ethyl side chain. Dehalogenation in conjunction with demethylation at the *N*-alkyl substituent and hydroxylation at the aralkyl side chain were also observed. *Streptomyces* was the most active culture in dechlorinating metolachlor; 41% of the added herbicide was recovered as dechlorinated products after a 16-day incubation period. Dechlorinated products from *P. chrysosporium*, *R. praticola*, and *S. racemosum* amounted to 28.4, 26.8, and 13.5%, respectively, of the originally added metolachlor. Upon incubation of the dechlorination product G or I with *Streptomyces*, both compounds were further transformed, indicating metabolism of the primary products.

INTRODUCTION

Studies from our laboratory have shown that the herbicide metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] can be extensively transformed by an *Actinomyces* sp., *Mucor racemosus*, and a *Fusarium* sp. and, to a lesser extent, by *Bacillus circulans* and *B. megaterium* (Krause et al., 1985; Saxena et al., 1987). Incubation of metolachlor with the soil actinomycete resulted in the formation of a number of products that were hydroxylated at the aralkyl side chains as well as at the *N*-alkyl substituent, but no evidence of dechlorination of metolachlor was obtained. Furthermore, there was no noticeable loss of [^{14}C]metolachlor from the axenic cultures and no proliferation of microbial populations when the herbicide was applied as the sole source of carbon or nitrogen. Therefore, we concluded that the transformation of metolachlor by the organisms was the result of cometabolic reactions and that no mineralization of the aromatic moiety of the herbicide had occurred.

Subsequently, however, we demonstrated dehalogenation and mineralization of metolachlor by indigenous microbial populations in a soil perfusion system (Liu et al., 1987) and dechlorination of metolachlor by a stable bacterial community in batch enrichment cultures (Liu et al., 1989). Single cultures of *Streptomyces* sp. and *Syncephalastrum racemosum* that were isolated from metolachlor-contaminated soil were also able to dechlorinate metolachlor. McGahen and Tiedje (1978) also reported dechlorination of metolachlor by resting cells of *Chaetomium globosum*. Their results were based solely on GC–MS data; no products were isolated for chemical characterization, and the extent of dechlorination was not determined. Because dechlorination of xenobiotics is often a significant initial step in the detoxification of hazardous compounds (Reineke and Knackmuss, 1988), it is important to know more about microbial dechlorination of metolachlor.

The objective of the present study was to examine four microbial strains for their ability to dechlorinate metolachlor, to isolate and identify the formed products, and to determine the mechanism of transformation.

MATERIALS AND METHODS

Microbial Strains and Culture Conditions. A strain of *Streptomyces* sp. and *S. racemosum* were isolated from metolachlor-perfused soils (Liu et al., 1987). *Rhizoctonia praticola* was from our culture collections, and *Phanerochaete chrysosporium* was obtained from Dr. M. Tien of the Biochemistry Department, The Pennsylvania State University.

The growth medium for the *Streptomyces* sp. was composed of (per liter of Milli-Q water), 100 mg (0.35 mM) of metolachlor and 1.59×10^2 kBq of [^{14}C]metolachlor (ring uniformly labeled, with a specific activity of 2.73×10^5 kBq/mmol), 2.0 g of KH_2PO_4, 3.0 g of Na_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $(NH_4)_2SO_4$, 0.05 g of $Ca(NO_3)_2$, 0.4 g of yeast extract, and 20 g of sucrose. The medium had a pH of 6.8. The three fungal cultures were grown in a medium that had essentially the same composition as the above except that it contained 0.5 g of Na_2HPO_4 and 2.0 g of KH_2PO_4, with a pH of 6.2. All cultures were grown in 250-mL Erlenmeyer flasks each containing 70 mL of the medium and incubated at 28 °C on a rotary shaker operating at 120 rpm.

Analytical Procedures. The disappearance of metolachlor was monitored at regular intervals by GC analysis. Three milliliters of culture filtrate was extracted with an equal volume of hexane, and 2 μL of the organic phase was analyzed by GC. To determine the distribution of radioactivity, 10 mL of the culture filtrate was extracted with the same volume of ethyl acetate, and aliquots (0.5 mL) of both the organic and the aqueous phases were measured for radioactivity. To determine the radioactivity in the biomass, 16-day-old cells from each flask were collected on Whatman No. 4 filter paper by using Büchner funnels. Subsequently, the cell biomass was suspended in 100 mL of 0.1 M phosphate buffer (pH 6.8) and, after shaking thoroughly, it was again collected by filtering. The process was repeated twice, and after the final wash, the cell biomass was suspended in 25 mL of Milli-Q water and homogenized; 0.5-mL portions of the homogenate were removed for radioactivity determination.

For quantitative analysis of products, 100 mL of culture filtrate was extracted with the same volume of ethyl acetate. The organic phase was evaporated to dryness on a rotary evaporator, and residues were dissolved in 2 mL of methanol. The sample in methanol was filtered through a 0.45-μm nylon filter membrane (Fisher Scientific), and 25 μL was analyzed by high-performance liquid chromatography (HPLC). Samples eluted from HPLC were collected every 15 s with a Foxy fraction collector (Isco, Inc., Lincoln, Nebraska). Each 0.5-mL fraction was subsequently measured for radioactivity, and the sum of radioactivity for each

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peak was determined. HPLC was also employed to isolate large amounts of products for structure characterization.

Chloride ion was measured by the method of Iwasaki et al. (1956). The culture filtrates were first passed through a Sep-Pak C_{18} cartridge to remove colored compounds, which interfered with the measurement of chloride ions by the spectrophotometric method. To 5 mL of culture filtrate were added 1 mL of an "iron" solution (12.05 g of ferric ammonium sulfate in 100 mL of 9 M HNO_3) and 1.5 mL of mercuric thiocyanate solution (100 mg of mercuric thiocyanate in 100 mL of dioxane–ethyl alcohol solution, 2:1 v/v). Absorption was measured at 460 nm with a Model 2000 spectrophotometer (Bausch and Lomb, Inc., Rochester, NY). A calibration curve that was linear in the range 0.05–10 ppm was prepared by using NaCl as a standard.

HPLC was performed on a Waters Associates instrument. The system consisted of a U6K injector, an M 45 and a 6000 A pump, a Model 680 automated gradient controller, and a Model 480 detector. Compound separation was achieved with a Supelcosil LC-8 DB column (15 cm × 4.6 mm) of 5-μm particle size from Supelco, Inc. (Bellefonte, PA). Initial sample elution was performed by using solvent A, which consisted of methanol and water (44:56 v/v), at 2 mL/min for the first 17 min. During the next 3 min, solvent A was reduced from 100 to 65%, while solvent B (acetonitrile) was increased from 0 to 35% by using a curve 5 gradient elution. From 20 to 24 min, solvent B was brought to 100% (by use of gradient curve 2) and maintained at that level for another 1 min before the mobile phase was returned to the initial conditions. The column was ready for the next injection after a 5-min equilibration.

Gas–liquid chromatographic analysis was done on a 5890 A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector and a Hewlett-Packard 3392 A integrator. A capillary column (RTx-5, 30 m × 0.32 mm) purchased from Restek Corp. (Port Matilda, PA) was used. For analysis of metolachlor, samples were injected in the split mode with an 80:7 split ratio. Helium was used as the carrier gas at a fixed pressure of 48 lb/in^2. The injector, detector, and column temperatures were 230, 255, and 200 °C, respectively. For product analyses, the column temperatures were programmed from 150 to 250 °C, at 4 °C/min.

Thin-layer chromatography (TLC) was carried out on 0.25-mm silica gel 60 F_{254} plates (EM Science, Darmstadt, W. Germany) in a solvent mixture of hexane–methylene chloride–ethyl acetate (6:1:3 v/v).

Mass spectral data were obtained by electron impact mass spectrometric analysis (70 eV), using a direct insertion probe on a Kratos MS-9/50 mass spectrometer, or by a directly coupled gas chromatograph–mass spectrometer (Finnigan 3200). In some cases, molecular weights were confirmed by chemical ionization mass spectrometry using isobutane. Proton nuclear magnetic resonance spectra were recorded on a Bruker 360-MHz spectrometer, using deuteriochloroform as solvent.

Radioactivity was measured in Scintiverse II scintillation cocktail with a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove, IL). For routine analysis, samples were counted in 5 mL of cocktail. For radioactive materials scraped from TLC plates or prepared from cell homogenates, samples were counted in 10 mL of the scintillation cocktail containing 2.5 mL of distilled water to make a homogeneous gel before counting.

RESULTS

GC analyses of the growth medium indicated that after a 4-day incubation, transformation of metolachlor was accelerated with all four microorganisms, while little metolachlor disappeared from the control medium. The metolachlor remaining in the culture medium of *S. racemosum*, *Streptomyces* sp., *R. praticola*, and *P. chrysosporium* was 11.3, 15.6, 20.8, and 28.3%, respectively, after 16 days of incubation (Figure 1). Thereafter, the amount of metolachlor either remained constant (*S. racemosum* and *R. praticola*) or continued to decline at a slower rate (*P. chrysosporium* and *Streptomyces* sp.).

Mass balance studies showed that there was almost no loss of radioactivity during the course of the experiment. However, the increase of radioactivity in the aqueous phase was greatly affected by microbial activity. Substantial amounts of ^{14}C remained in the aqueous fraction after extraction of the growth medium with ethyl acetate following the 16-day incubation, indicating that polar and water-soluble metabolites were formed as a result of biological activity (Table I). To determine the dechlorination of metolachlor by the four microorganisms, we measured the amount of chloride released into the culture medium. The results showed that as much as 43.2% of the total Cl of the initially added metolachlor could be found in the medium of *Streptomyces* sp. (Table I).

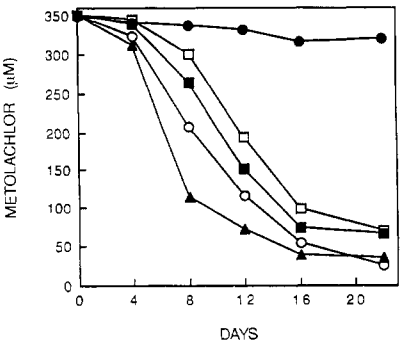

Figure 1. Disappearance of metolachlor from the growth medium of four microorganisms. (●) Control; (□) *P. chrysosporium*; (■) *R. praticola*; (○) *Streptomyces* sp.; (▲) *S. racemosum*.

Table I. Recovery of Chloride and Radioactivity from Growth Medium (16-Day-Old), Cell Biomass, and the Distribution of Radioactivity after Solvent Fractionation of the Culture Fluid

	% of initial ^{14}C				
microorganism	growth medium	cell biomass	ethyl acetate phase	aq phase	chloride release in %
control (without microorganisms)	100	0	99.2	0	0
Streptomyces sp.	98.3	2.1	80.6	15.4	43.2
P. chrysosporium	97.6	2.5	72.3	23.3	29.7
R. praticola	95.8	3.2	70.4	25.7	29.8
S. racemosum	94.3	4.7	66.5	28.2	15.6

Separation of ethyl acetate extracts by HPLC and determination of radioactivity in all fractions collected from HPLC revealed the presence of a number of metabolites. On the basis of the similarity of the HPLC separation profiles of these metabolites from various cultures, peaks of radioactivity having the same retention time were labeled metabolites A–J according to the order of elution (Figure 2). Essentially the same metabolites were formed by the different organisms, but the yield of a particular product varied among the cultures. Dechlorination of metolachlor was determined on the basis of the absence of the typical monochlorine isotopic cluster in the mass spectra of samples isolated from *Streptomyces* sp., as well as from the other cultures.

As shown in Table II, seven dechlorinated products, namely, metabolites B and D–I, were observed. In addition, there were four chlorine-containing metabolites (A, C, J, and K). The radioactivity recovered from each peak was converted to percent of the initially added radioactive chemical. *Streptomyces* sp. was most active in dechlorinating metolachlor. The yield of dehalogenated products from the *Streptomyces* sp. accounted for 41.2% of the initially added radioactivity. The dechlorinated products generated by *P. chrysosporium* and *R. praticola* were found to be 27.8 and 26.4%, respectively, of the original substrate. Only about 13.5% of the initial substrate was dechlorinated by *S. racemosum*. The predominant transformation product of *S. racemosum* was

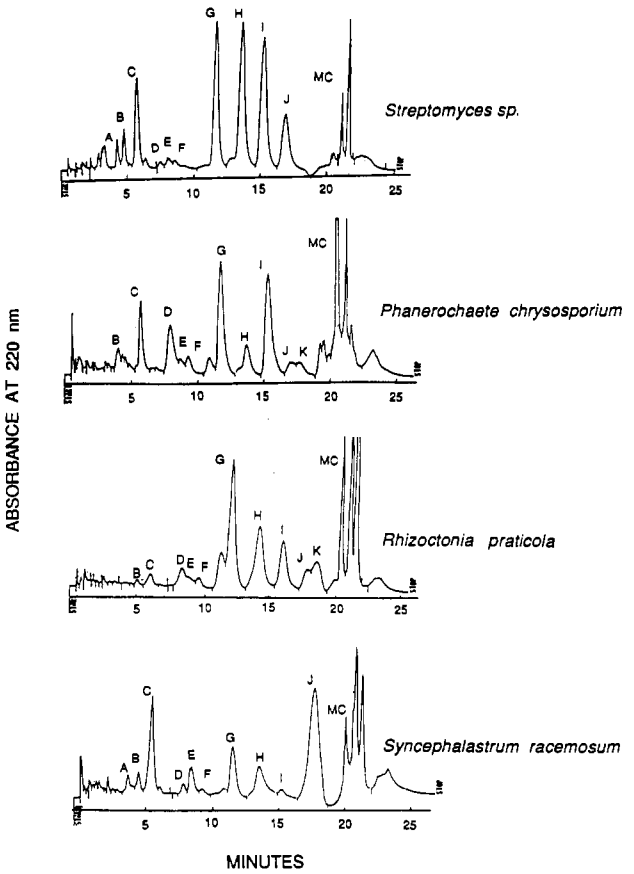

Figure 2. HPLC chromatograms of ethyl acetate extractable metabolites formed from the metabolism of metolachlor by four microorganisms. Samples were obtained from 16-day-old cultures.

metabolite J, which accounted for 25.6% of the added chemical. The other three cultures also yielded metabolite J, which accounted for about 2.5–9% of the initial chemical.

On the basis of HPLC coelution and comparison of mass spectra with those of authentic samples previously isolated from an actinomycete (Krause et al., 1985), metabolite J was identified as 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methylethyl)acetamide (a demethylated product), metabolite A as 2-chloro-*N*-[2-(hydroxyethyl)-6-(hydroxymethyl)phenyl]-*N*-(2-methoxy-1-methylethyl)acetamide, and metabolite K as 2-chloro-*N*-[2-ethyl-6-(hydroxymethyl)phenyl]-*N*-(2-methoxy-1-methylethyl)acetamide.

The molecular structure assignment for metabolite C was based solely on low-resolution mass spectrometric data, which had characteristic fragments of m/z 211 (M^+), 162, 147, and 132. The presence of the $M^+ + 2$ peak and the typical isotopic cluster indicates it is a chlorine-containing compound.

Molecular structures for the three minor dechlorinated products B, E, and F were determined by GC–mass spectrometric analyses. Metabolite B possessed mass spectral fragments of m/z 205 (M^+), 160, 145, and 132; metabolite E of m/z 233 (M^+), 218, 204, 188, 161, 160, and 146; and metabolite F of m/z 233 (M^+), 218, 191, and 160.

The three major dechlorinated products, namely, metabolites G–I from *Streptomyces* sp., and metabolite D from *P. chrysosporium* were purified and subjected to high-resolution mass spectrometric analyses (Table III). Additionally, proton NMR spectrometry was employed for structure elucidation of metabolites D, G, and I (Figure 3). The numbering system presented in Figure 3 is based on retaining each carbon's identity as found in metolachlor. The proper chemical name of each metabolite reflects the use of the formal numbering system found in the literature.

Metabolite D had an M^+ at m/z 233, a molecular formula of $C_{14}H_{19}NO_2$. The compound showed three aromatic hydrogens as well as phenyl, ethyl, and methyl groups as present in the metolachlor spectrum. The presence of H-1′ (3.79) sharing a 6.4-Hz coupling with methyl-3′ at 1.14 ppm as well as couplings to both H-2′ protons at 4.06 and 3.79 ppm indicated this portion of the metabolite D resembled metolachlor as well. The obvious lack of a methoxy group and the downfield shift of the H-2″ protons to 4.41 and 4.36 ppm suggested an ether ring closure eliminating methyl chloride. The nuclear Overhauser effect (NOE) data (Table IV) supported this proposed structure. Of particular interest is the 2% NOE observed between H-2′ (4.06 ppm) and H-2″ (4.36 ppm), which is consistent with a diaxial interaction in the amidic ring. According to this information, metabolite D could be identified as *N*-(2-ethyl-6-methylphenyl)-5-methyl-3-morpholinone. The compound could also exist as atropomers as a result of hindered rotation about the amidic–phenyl bond.

Metabolite G possessed a 1.2% parent ion at m/z 263 corresponding to the molecular formula $C_{15}H_{21}NO_3$. A chemical ionization mass spectrum of the compound revealed the protonated molecular ion at m/z 264 and an $M^+ + 29$ peak, which is common to methane chemical ionization spectrum. The proton NMR spectrum of metabolite G showed the substituted phenyl ring to be intact except for the loss of one of the H-7 benzylic protons. The residual H-7 hydrogen was shifted downfield to 5.12 ppm, and the adjacent methyl group at C-8 appeared as a doublet (1.62 ppm) with a 6.4-Hz coupling. This 2-methoxy-1-methylethyl information suggested oxidation had occurred at the benzylic site. As in metolachlor, the 2-methoxy-1-methylethyl side chain was intact. Metabolite G thus must possess an ether lactam ring as shown in Figure 3.

High-resolution mass spectra data of metabolite H (Table III) together with NMR spectrometric analysis (data not shown) indicate that the compound is probably an isomer of metabolite G.

High-resolution mass spectral data of metabolite I had an M^+ of m/z 265; this ion is less than 1% of the base peak representing $C_{15}H_{23}NO_3$. This small parent intensity combined with the mass difference of 18 amu between metabolite I and metolachlor suggested that hydroxylation occurred at the former Cl site. The proton NMR spectrum fully supports this concept. As with metabolite D, atropomers can exist. Thus, metabolite I could be identified as *N*-(2-ethyl-6-methylphenyl)-2-hydroxy-*N*-(2-methoxy-1-methylethyl)acetamide.

The time course of formation of the three dechlorinated products (G–I) in the growth medium of a *Streptomyces* sp. is shown in Figure 4. The greatest accumulation of three dechlorinated products (G–I) was observed after 16 days of incubation; thereafter, these compounds gradually declined, suggesting further transformation of the formed intermediates.

To determine whether the decrease in the levels of metabolites G and I after 16 days of incubation indeed represented further metabolism of the intermediates, the ability of *Streptomyces* sp. to transform metabolites G and I was examined. Upon incubation of metabolite G or I with a *Streptomyces* sp., both metabolites were observed to be transformed. The rate of metabolite I transformation was found to be more rapid than that of metabolite G, with mass balance data from 12-day-old samples indicating that about 80% of metabolite I was transformed, as

Table II. Qualitative and Quantitative Analyses of Metolachlor and Its Metabolites after HPLC Separation[a]

Rt, min	product	m/z	presence of chloride[b]	% of initial ^{14}C			
				Streptomyces sp.	*P. chrysosporium*	*R. praticola*	*S. racemosum*
4.40	A	315	+	2.0			1.7
5.82	B	205	-	2.4	1.7	0.7	1.1
6.80	C	211	+	8.6	5.4	2.0	8.4
8.08	D	233	-	1.0	5.3	2.4	1.3
8.99	E	233	-	1.4	0.9	0.5	2.1
9.90	F	233	-	1.6	1.2	0.6	0.5
12.14	G	263	-	12.8	9.3	11.5	4.1
14.22	H	263	-	11.5	2.6	6.7	3.0
15.92	I	265	-	10.3	7.4	4.4	1.4
17.68	J	269	+	9.0	2.5	5.8	25.6
19.20	K	299	+		3.0	8.5	
21.21	metolachlor	283	+	17.5	27.6	21.3	11.9
% ^{14}C recovered from all products				78.1	66.9	64.4	61.1
% ^{14}C recovered as dechlorinated products				41.0	28.4	26.8	13.5

[a] Samples were prepared from ethyl acetate extractable fractions of a 16-day-old culture medium. [b] On the basis of mass spectrometric analysis showing a cluster of peaks typical for the isotopic pattern of chlorine atom.

Table III. High-Resolution Mass Spectral Data for Metabolites D, G, and I

metabolite	measured mass	rel intensity, %	composition of M^+ and fragment ions
D	233.1419	23.62	$C_{14}H_{19}NO_2$
	218.1185	7.00	$C_{13}H_{16}NO_2$
	188.1082	21.24	$C_{12}H_{14}NO$
	161.0883	100.00	$C_{11}H_{15}N$
	146.0822	62.51	$C_{10}H_{12}N$
G, H	263.1544	1.25	$C_{15}H_{21}NO_3$
	218.1164	33.77	$C_{13}H_{16}NO_2$
	191.0934	27.77	$C_{11}H_{13}NO_2$
	174.0917	6.32	$C_{11}H_{12}NO$
	160.1114	100.00	$C_{11}H_{14}N$
	145.0877	5.84	$C_{10}H_{11}N$
	132.0819	4.94	$C_9H_{10}N$
I	265.1674	0.21	$C_{15}H_{23}NO_3$
	220.1331	53.18	$C_{13}H_{18}NO_2$
	193.1165	12.64	$C_{11}H_{15}NO_2$
	162.1183	100.0	$C_{11}H_{16}N$
	146.0832	12.28	$C_{10}H_{12}N$
	134.0967	7.76	$C_9H_{12}N$

compared to only 40% of metabolite G (Figure 5). Greater than 99.5% of the radioactivity was recovered from the ethyl acetate fractions of the respective control samples, which contained only the starting material (data not shown). Thin-layer chromatograms of the ethyl acetate fraction from 12-day-old culture fluids revealed, in addition to the unconverted substrate (metabolite G R_f 0.67; metabolite I R_f 0.69), the presence of three new radioactive spots. The products derived from metabolite G were designated G-A (R_f 0.34), G-B (R_f 0.21), and G-C (R_f 0) and from metabolite I I-A (R_f 0.31), I-B (R_f 0.21), and I-C (R_f 0). G-A contained 10.9% of the initial ^{14}C, possessed a molecular ion of m/z 233, and coeluted with metabolite E on HPLC. Less than 2% of the ^{14}C was recovered in products G-B and G-C. No mass spectral data were obtained for these two compounds. The radioactivity found in metabolites I-A, I-B, and I-C constituted 14.2, 11.0, and 2.3%, respectively, of the initially added ^{14}C. The mass spectral data for I-A showed m/z 267 (M^+), 256, 220, and 162. The molecular weight of I-A thus indicated that, in addition to demethoxylation at the *N*-alkyl group of metolachlor, hydroxylation had occurred at a methyl side chain of metabolite I. Metabolite I-B had a mass of m/z 233 and coeluted with metabolite D on HPLC.

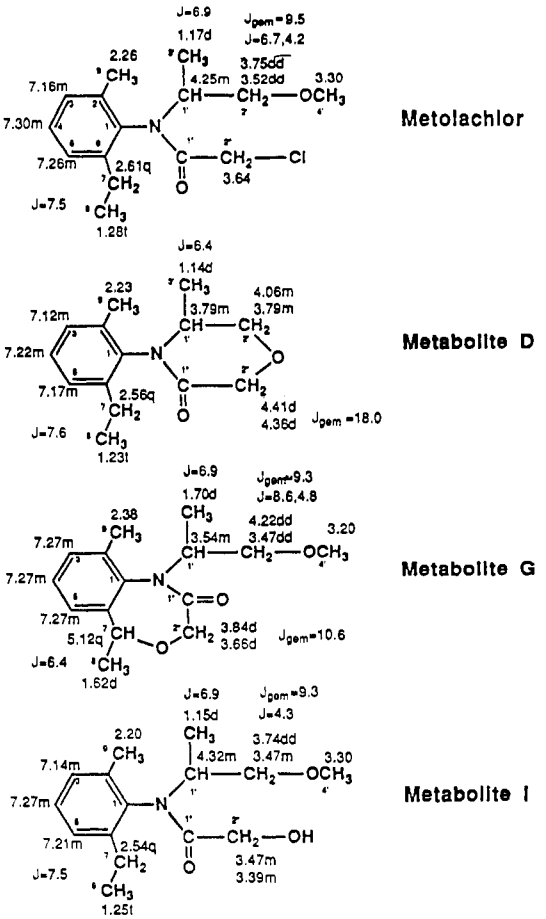

Figure 3. Proton NMR spectra of metolachlor and three dechlorinated products.

DISCUSSION

In the present study, we have demonstrated the dechlorination of metolachlor by four microorganisms. This finding, together with our previous results in which no dechlorination of metolachlor was observed from a number of axenic cultures, seems to indicate a strain-specific phenomenon in microbial dechlorination of metolachlor. The extent of dechlorination and the yield of a particular dechlorination product, as well as the formation of metabolites generated from the transformation mechanisms such as demethylation and/or hydroxylation at the aromatic side chains, differed from one organism to another. The present study shows that dechlorination

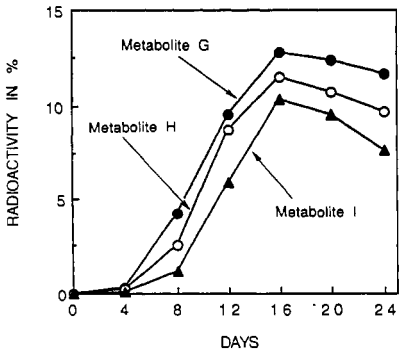

Figure 4. Formation of three dechlorinated products of metolachlor by a *Streptomyces* sp.

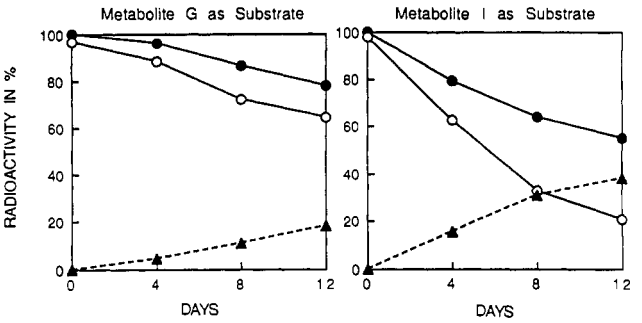

Figure 5. Transformation of two dechlorinated products of metolachlor by a *Streptomyces* sp. The starting material for metabolite G was 12.5 μM and for metabolite I 12 μM. (○) Substrate remaining (determined by TLC); (●) ^{14}C in ethyl acetate extract; (▲) ^{14}C in aqueous phase.

Table IV. Nuclear Overhauser Effect (NOE) for Metabolites D, G, and I

metabolite D	metabolite G	metabolite I
H-5 to H-7 (2%)	H-5 to H-7 (14%)	H-5 to H-7 (4%)
H-7 to H-5 (4%)	H-3 to H-9 (4%)	H-5 to H-8 (4%)
H-8 to H-5 (6%)	H-8 to H-4′ (5%)	H-3 to H-9 (6%)
H-3 to H-9 (6%)	H-8 to H-$2'_{4.22}$ (6%)	H-1′ to H-9 (2%)
H-9 to H-3 (11%)	H-$2'_{4.22}$ to H-8 (10%)	H-$2'_{3.74}$ to H-9′ (2%)
H-3′ to H-9 (4%)	H-$2'_{4.22}$ to H-4′ (7%)	H-$2'_{3.74}$ to H-4′ (4%)
H-9 to H-3′ (3%)	H-1′ to H-7 (14%)	H-$2'_{3.47}$ to H-7 (6%)
H-9 to H-1′ (4%)	H-$2'_{3.47}$ to H-3′ (3%)	H-$2'_{3.47}$ to H-9 (7%)
H-1′ to H-9 (3%)	H-4′ to H-8 (6%)	H-4′ to H-$2'_{3.74}$ (2%)
H-1 to H-7 (6%)	H-4′ to H-$2'_{4.22}$ (7%)	H-7 to H-$2'_{3.47}$ (2%)
H-7 to H-1′ (2%)	H-7 to H-3′ (9%)	H-7 to H-1′ (1%)
H-3′ to H-2′ (8%)	H-7 to H-5 (32%)	H-7 to H-5 (3%)
H-$2'_{4.06}$ to H-$2''_{4.36}$ (2%)	H-7 to H-1′ (21%)	H-9 to H-3′ (2%)
	H-3′ to H-7 (11%)	H-9 to H-$2'_{3.47}$ (2%)
	H-3′ to H-$2'_{3.47}$ (7%)	H-9 to H-1′ (3%)
	H-9 to H-3 (52%)	H-9 to H-3 (10%)
		H-8 to H-$2''_{3.39}$ (1%)
		H-8 to H-5 (9%)
		H-3′ to H-9 (3%)
		H-$2'_{3.47}$ to H-3′ (3%)

was the major transformation mechanism of metolachlor with *Streptomyces* sp., *P. chrysosporium*, and *R. praticola*. The ability of a microorganism to dechlorinate metolachlor did not seem to require its previous acclimation to the herbicide. Of the three microorganisms, *Streptomyces* sp. had been previously exposed to metolachlor, and it was the most active culture in dechlorinating the herbicide. However, *S. racemosum*, which was also isolated from metolachlor-perfused soils, showed the least activity in dechlorinating metolachlor. The predominant metabolic pathway used by *S. racemosum* was demethylation with subsequent hydroxylation of the *N*-alkyl group to form a product (J) that contained one-fourth of the applied chemical. Demethylation of metolachlor was less prevalent in the other three cultures. Hydroxylation of the aromatic side chains or hydroxylation in conjunction

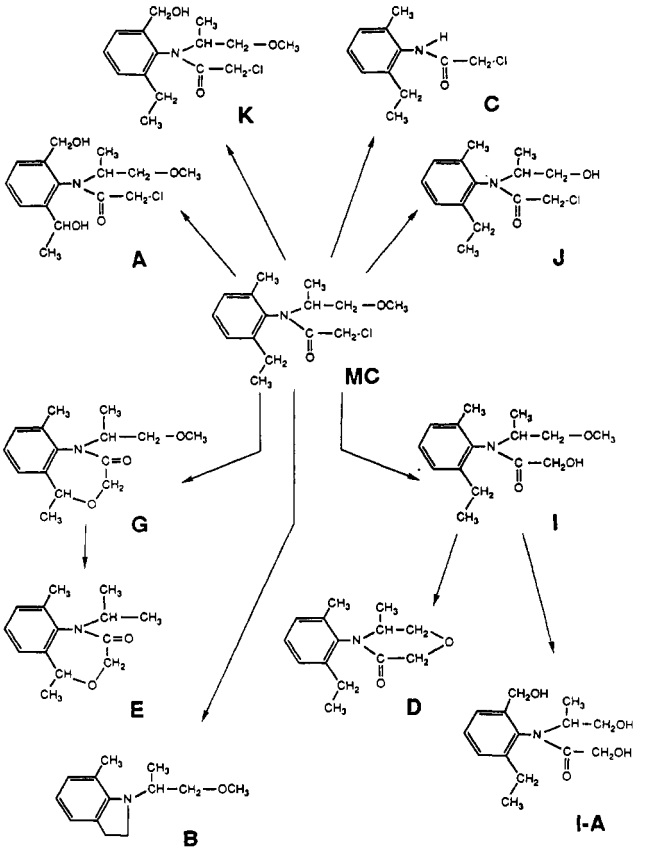

Figure 6. Suggested scheme of microbial degradation of metolachlor.

with demethylation was found to be only a minor pathway in the transformation of metolachlor by *Streptomyces* sp., *P. chrysosporium*, and *S. racemosum*. However, a fair amount of hydroxylated product (K), amounting to 8.5% of the added substrate, was produced by *R. praticola*.

Dechlorination with subsequent hydroxylation of the chloroacetyl group, as demonstrated in the present study, can be referred to as a hydrolytic reaction since the halogen was exchanged for a hydroxyl group generated from water. We did not observe reductive dehalogenation of metolachlor in the present study. Reductive dehalogenation of metolachlor was previously reported in investigations with a bacterial community in a batch culture and with indigenous microorganisms in a soil perfusion experiment by Liu et al. (1989) and also by McGahen (1982) in her studies on degradation of metolachlor by soil sediments.

From the present study, it is clear that the decreasing extractability of the ^{14}C-labeled metolachlor was the result of biological activity. Since a large portion of polar and water-soluble products remained in the aqueous fraction, it would be of interest to elucidate the transformation mechanisms that lead to the formation of compounds with increasing polarity and to determine the toxicity of these products. Research in this area is especially important since water-soluble products are more likely to leach through soil and pose a potential threat to groundwater (Cohen et al., 1986). However, it should also be noted that polar products are usually more susceptible to further transformation, and additional investigations are necessary to determine the ultimate fate of these water-soluble compounds.

Evidence of metolachlor dechlorination by the four microorganisms was obtained not only by the measurement of Cl ion released into the medium but also by the determination of the formed dechlorinated products by

HPLC. In general, the percentage of chloride released into the medium was determined to be 1–2% greater than the percentage of dechlorinated metabolites measured by HPLC. This discrepancy is not surprising since some dechlorinated products remaining in the aqueous phase may not have been accounted for by the present method of HPLC analysis.

On the basis of the metabolites characterized, we have proposed the scheme of metolachlor transformation in Figure 6. Our results, however did not agree with some of the intermediates suggested in the pathway studies of McGahen and Tiedje (1978). In their study the two initial steps of metolachlor dechlorination appeared to be (a) dechlorination in conjunction with dehydrogenation of the ethyl side chain and (b) dechlorination leading to the formation of a tetrahydroquinoline. Neither dechlorination mechanism was found in the present study, which explained that different pathways were generated by different organisms.

Dechlorination of metolachlor followed by cyclization of the acetyl group with the ethyl side chain resulted in the formation of the metabolites G and H. This transformation pathway of metolachlor was previously not reported, but a similar reaction with alachlor was described (Chesters et al., 1989). We could observe a further transformation of the formed intermediates using the dechlorinated products I and G as substrates.

Dechlorination is an important reaction since it usually results in the detoxification of a pesticide as in the case of metolachlor (LeBaron et al., 1988). Our results suggest that metolachlor can be dechlorinated by microorganisms, but the accumulation of dechlorinated products differed with the various microorganisms.

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Registry No. Metabolite A, 121073-76-5; metabolite B, 65513-68-0; metabolite C, 32428-71-0; metabolite D, 120375-14-6; metabolite E, 131068-70-7; metabolite G, 131068-71-8; metabolite I, 131068-72-9; metabolite J, 65513-61-3; metabolite K, 96394-97-7; metachlor, 51218-45-2.