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# Sorption and Metabolism of Metolachlor by a Bacterial Community

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**A stable bacterial community absorbed and transformed the herbicide metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide] from a liquid medium. About 80% of the added ring-[U-$^{14}$C]metolachlor (50 μg/ml) disappeared from the medium and accumulated inside the cells. The ratio of cellular $^{14}$C to $^{14}$C in 1 mg of supernatant reached a value of $1.1 \times 10^4$ in a 10-day-old culture. $^{14}$C remaining in the medium consisted primarily of two dechlorinated products of metolachlor with *m/z* 233 and 263 as determined by mass spectrometry. The $^{14}$C-labeled material absorbed by the cells was strongly bound; only 2% of the $^{14}$C was released into deionized water after shaking for 3 h. Approximately 96% of the $^{14}$C associated with the biomass was extracted with acetone, and high-performance liquid chromatographic analysis of this fraction showed six peaks containing radioactivity. Since no metolachlor was detected by chromatographic analysis, it was concluded that the radioactivity recovered from the cells represented transformed products of metolachlor. Pure cultures isolated from the bacterial mixed culture were less effective in transforming and accumulating metolachlor. These results suggest that it may be advantageous to seed an aquatic environment with a mixture of microorganisms, rather than individual microbial species, as a method for removal or detoxification of metolachlor.**

Although many studies have addressed the significance of bioconcentration and metabolism of a wide variety of pesticides (1, 5, 12), little is known about microbial accumulation of the herbicide metolachlor. In our earlier studies, it was shown that sorption of metolachlor by an actinomycete in a liquid medium was less than 1% (4). Similarly, according to Saxena et al. (10), about 1% of metolachlor was absorbed by growing cells of *Bacillus circulans* or *Bacillus megaterium*, whereas 3 to 5% of the herbicide was taken up by filamentous microorganisms. Using a chemostat, we subsequently obtained a stable mixed bacterial population that is capable of accumulating a substantial amount of metolachlor from a liquid medium. This study was undertaken to determine the nature and extent of sorption and transformation of metolachlor by this bacterial community. A pure culture isolated from this microbial mixture was also included for comparison.

## MATERIALS AND METHODS

**Chemicals.** Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide] (Fig. 1) of technical grade (95.4% purity) and ring-[U-$^{14}$C]metolachlor with a specific activity of 26.1 μCi/mg were gifts of CIBA-GEIGY Corp., Agricultural Division, Greensboro, N.C.

**Organisms and growth conditions.** A stable bacterial community named J4-A was isolated from a 9-week-old metolachlor-enriched chemostat which was inoculated with a primary effluent of municipal sewage (State College, Pa.). The chemostat, equipped with a 500-ml culture vessel with an overflow at 400 ml, was supplied with new medium at a flow rate of 3 ml/h. The medium consisted of 0.5 g of $NH_4Cl$, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 3 g of $Na_2HPO_4$, 2 g of $KH_2PO_4$, 0.1 g of sucrose, 0.01 g of yeast extract, and 5 mg of metolachlor per liter of distilled water. The pH of the medium was 6.9, and the chemostat was incubated at 28°C. About 70% of the metolachlor was transformed in a 9-week-old culture from the chemostat. Scanning electron microscopy and Gram staining indicated that J4-A consisted of three different types of bacteria: (i) short straight rods with rounded ends, 0.4 to 0.6 by 1.5 to 2.3 μm, motile with a single polar flagellum, gram negative; (ii) straight rods, 0.5 to 0.7 by 4 to 5 μm, nonmotile, gram positive; and (iii) curved rods, 0.4 to 0.6 by 3 to 4.5 μm, motile with two polar flagella, gram negative. The dilution plate technique was used to isolate single colonies from J4-A, and 15 isolates thus obtained were tested individually for metolachlor transformation and accumulation capabilities. Isolate B2 (short straight-rod bacterium), which gave the best preliminary results, was used for further study.

The enrichment medium used contained, in 1 liter of medium: 50 mg of metolachlor and 1.2 μCi of ring-[U-$^{14}$C]metolachlor (46 μg), 0.5 g of $NH_4Cl$, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 3 g of $Na_2HPO_4$, 2 g of $KH_2PO_4$, 0.1 g of sucrose, 0.01 g of yeast extract, and 1 g of ethanol. The final pH of the medium was 6.9.

Cultures (J4-A or B2) were grown in 250-ml Erlenmeyer flasks containing 100 ml of the enrichment medium. All flasks were stoppered with a cotton plug and incubated statically at 28°C in the dark. Growth was monitored by measuring optical density of the growth medium at 600 nm with a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.).

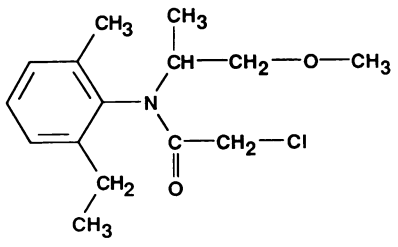


FIG. 1. Chemical structure of metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide].

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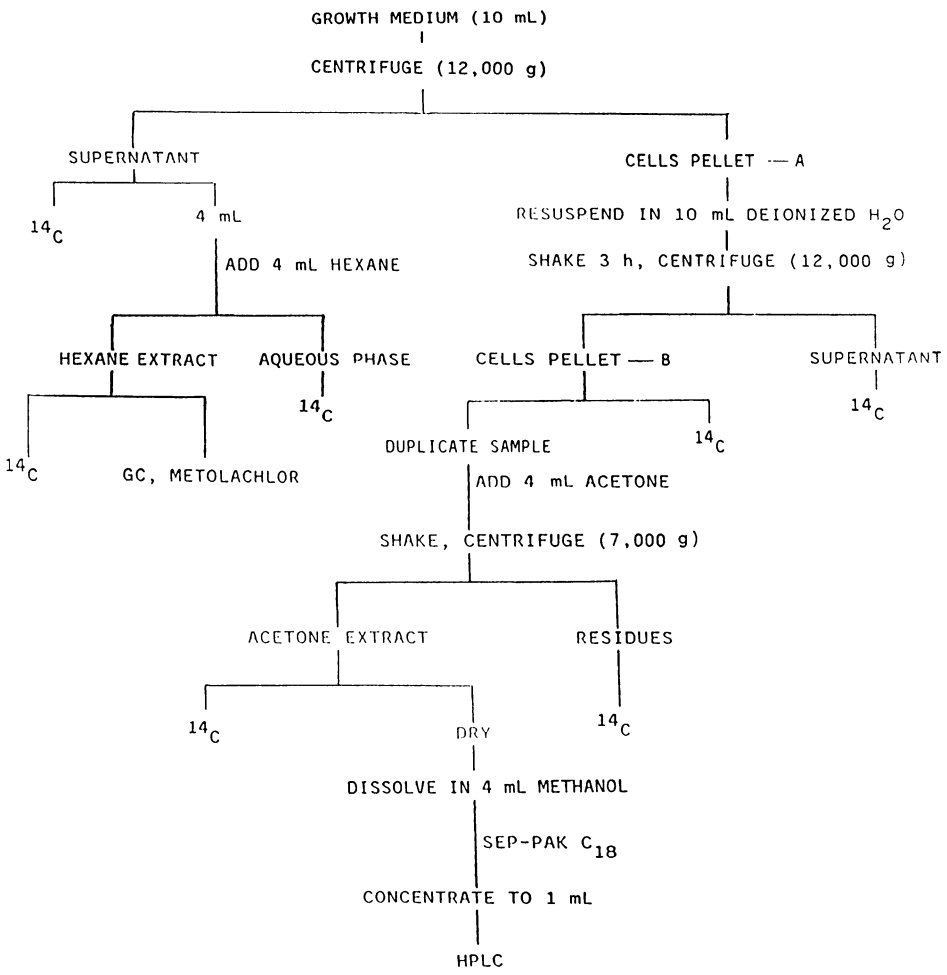



FIG. 2. Scheme of extraction procedure and analysis. GC, Gas chromatography.

**Extraction and determination of metolachlor and radioactivity.** At regular intervals, 10 ml of the growth medium was withdrawn and analyzed according to the procedure shown in Fig. 2. After centrifugation at 12,000 × *g* for 30 min, the supernatant (4 ml) was extracted with an equal volume of hexane, and a 2-μl portion of the organic phase was injected onto a gas chromatograph to assess the disappearance of metolachlor. The cell pellet (pellet A, Fig. 2) was then suspended in 10 ml of deionized $H_2O$ and shaken at 120 rpm for 3 h on a rotary shaker. After recentrifugation, the same

TABLE 1. Accumulation of metolachlor and radioactivity by a bacterial community (J4-A) and by a bacterial isolate (B-2)[a]

| Organisms | Incubation time (days) | Accumulation in: | | | | Accumulation ratio[b] | |
|---|---|---|---|---|---|---|---|
| | | Supernatant | | Cell pellet | | | |
| | | Metolachlor (ng/mg) | $^{14}C$[c] (dpm/mg) | Metolachlor (ng/mg) | $^{14}C$[c] (dpm/mg) | Metolachlor | $^{14}C$ |
| J4-A | 0 | 49.85 | 2.76 | | | | |
| | 3 | 29.74 | 1.71 | 3,571 | 1,407 | 120 | 822 |
| | 4 | 19.05 | 1.13 | 2,750 | 2,362 | 144 | 2,090 |
| | 6 | 8.96 | 0.66 | 732 | 3,251 | 82 | 4,925 |
| | 8 | ND[d] | 0.35 | ND | 3,548 | | 10,137 |
| | 10 | ND | 0.33 | ND | 3,792 | | 11,490 |
| B-2 | 0 | 49.60 | 2.76 | | | | |
| | 8 | 22.42 | 1.44 | 6,540 | 3,322 | 292 | 2,307 |

[a] Data are based on 10 ml of growing culture containing 5.6 to 6.1 mg (dry weight) of cells for J4-A and 4.0 mg of cells for B-2 (average of two experiments with duplicate samples for each treatment).
[b] Ratio of metolachlor or $^{14}C$ in cell pellet (nanograms or disintegrations per minute, respectively, per milligram/metolachlor or $^{14}C$ in supernatant).
[c] From metolachlor or its products.
[d] ND, Not detectable.

process was repeated, and the resulting pellet was designated pellet B. The amounts of $^{14}C$ and metolachlor in the cell-free medium, the deionized water washing solution, the hexane extract, the aqueous phase after hexane extraction, and pellet B were determined.

Pellet B, prepared from a duplicate sample, was extracted with 4 ml of acetone. The levels of radioactivity in the acetone extract and the levels remaining in nonextractable residues after solvent removal were determined. After evaporation to dryness, the acetone extract was dissolved in 4 ml of methanol and passed through a Sep-Pak $C_{18}$ cartridge (Waters Associates, Inc., Milford, Mass.). The eluent was concentrated to 0.5 ml, and 50 μl was analyzed and quantitated by high-performance liquid chromatography (HPLC) for $^{14}C$-labeled metolachlor and $^{14}C$-labeled products.

**Isolation of metabolites.** For isolation of products for chemical characterization, 500 ml of the growth medium was centrifuged at 12,000 × *g* for 30 min, and the supernatant and cell pellet were extracted with methylene chloride and acetone, respectively. Further purification of the products was achieved by thin-layer chromatography (TLC) and HPLC as described below.

**Analytical methods.** Gas-liquid chromatographic analysis was done on a 5890 A gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detector and a Hewlett-Packard 3392 A integrator. A capillary column (RTx-5; 30 m by 0.32 mm, inside diameter) purchased from Restek Corp. (Port Matilda, Pa.) was used. 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$^2$. For isotherm elution, injector, detector, and column temperatures were 250, 275, and 220°C, respectively. For temperature programming used in gas chromatographic-mass spectrometric analysis of metolachlor products, the column temperature was varied from 150 to 250°C at a rate of 4°C/min.

HPLC was performed on a Waters Associates liquid chromatograph. The system consisted of a U6K injector, an M 45 and a 6000 A pump, a model 720 system controller, and a model 440 detector connected to a model 730 data module. A reverse-phase Supelcosil LC-18-DB column (5 cm by 4.6 mm, inside diameter; Supelco, Inc., Bellefonte, Pa.) of 5-μm particle size was used. The mobile phase, eluted at a rate of 1.0 ml/min, was composed of 20% water and 80% methanol for the first 5 min; the methanol concentration was increased to 100% during the next 3 min (gradient curve 8), and this final composition was held for another 10 min before returning to the initial condition for the next injection.

TLC was carried out on 0.25-mm Silica Gel 60F-254 plates (E. Merck AG, Darmstadt, Federal Republic of Germany). The plates were developed in a solvent system of hexane-methylene chloride-ethyl acetate (6:1:3, vol/vol/vol).

Radioactivity was measured in Scintiverse II scintillation cocktail (Fisher Scientific Co., Fair Lawn, N.J.), using a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove Village, Ill.). Samples of cell pellets or silica gel removed from TLC plates were mixed with 15 ml of Scintiverse II in a glass vial to which 3 ml of $H_2O$ was added to make a homogeneous gel before the determination of radioactivity.

Mass spectral data were obtained by electron impact mass spectrometric analysis (70 eV) on a Kratos MS 9/50 mass spectrometer with a direct-insertion probe or on a gas chromatograph-mass spectrometer (Finnigan 3200). Proton nuclear magnetic resonance spectra were recorded on a Bruker WM-360 MHz spectrometer, using deuteriochloroform as the solvent.

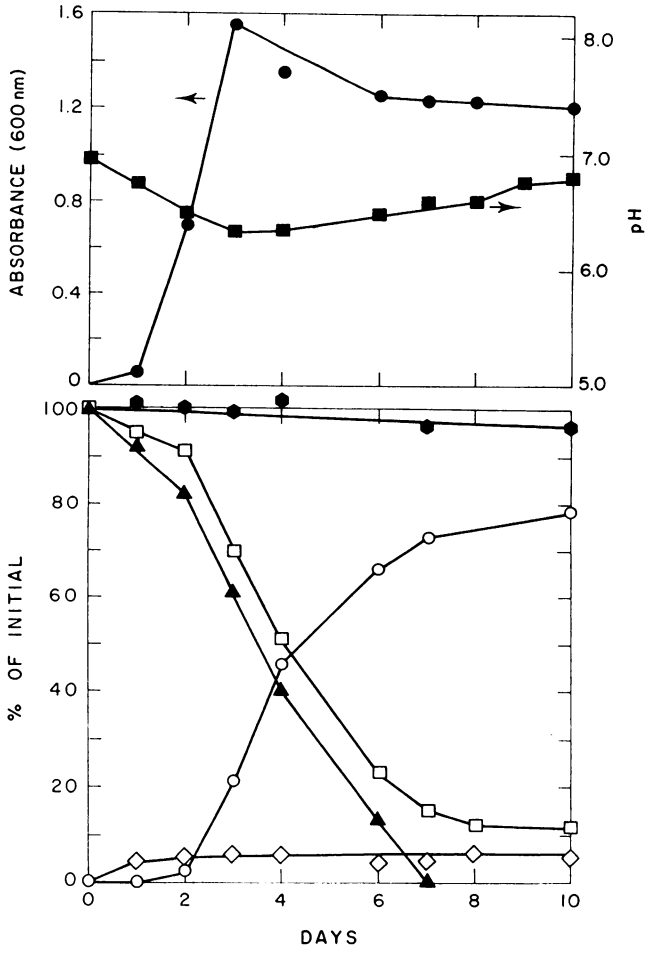



FIG. 3. Transformation of metolachlor by a bacterial community. Symbols: ●, growth; ■, pH; ⬢, total $^{14}C$; □, hexane extract ($^{14}C$); ◇, aqueous phase ($^{14}C$); ▲, hexane extract (metolachlor); ○, pellet ($^{14}C$).

## RESULTS

The rapid disappearance of metolachlor and radioactivity from the growth medium of J4-A corresponded to the increasing accumulation of these substances in the cells. Seven days after inoculation, 15% of the $^{14}C$-labeled material from the supernatant fluid was extractable into hexane and 4% remained in the aqueous phase, but no metolachlor was detected in the hexane fraction. The $^{14}C$ associated with the bacterial cells amounted to 74% of the added chemical in the 7-day-old culture and increased to about 80% after 10 days of incubation (Fig. 3). Low levels of metolachlor were still found in the hexane extract of a 6-day-old culture of J4-A.

TLC separation of the hexane extract from a 7-day-old culture filtrate revealed the presence of two radioactive spots with $R_f$ values of 0.68 and 0.40, which were isolated and designated as products S-1 and S-2, respectively. The yield of product S-1 was three times greater than that of S-2.

Gas chromatographic-mass spectrometric analyses revealed that product S-1 had a mass/charge ratio (*m/z*) of 263 (retention time, 13.25 min) and that S-2 had an *m/z* of 233

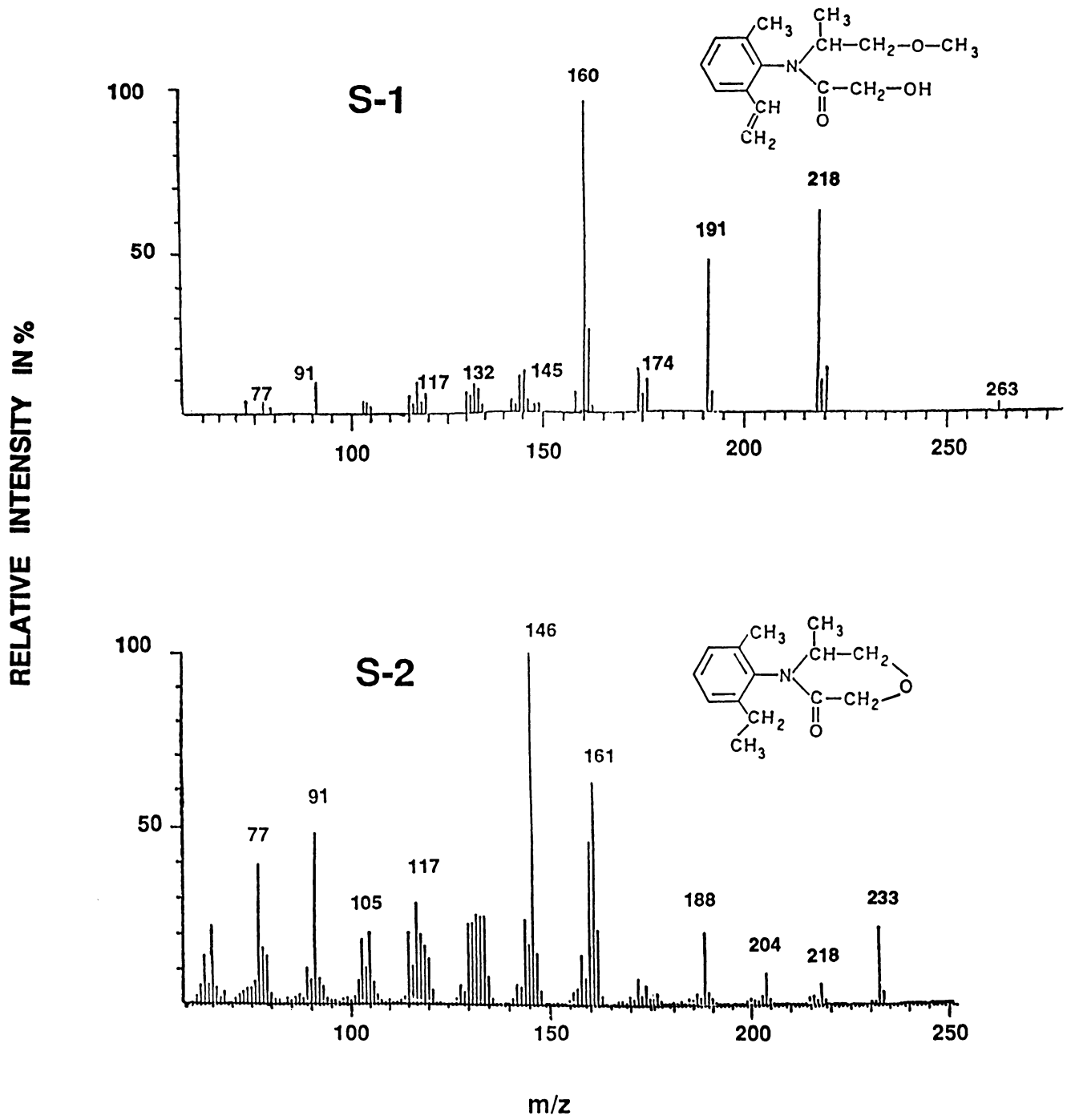



FIG. 4. Mass spectra of two metabolites (S-1 and S-2) of metolachlor isolated from J4-A growth medium.

(retention time, 10.05 min). Mass spectral data of both S-1 and S-2 lacked a chlorine isotopic pattern, which indicated that these products were dechlorinated metabolites of metolachlor (Fig. 4).

S-1 was further purified by HPLC according to the method described by Krause et al. (4). High-resolution mass and nuclear magnetic resonance spectral data of S-1 were identical in all respects with those obtained for a known product previously isolated and characterized after incubation of metolachlor with a *Streptomyces* sp. (S.-Y. Liu and J.-M. Bollag, manuscript in preparation). With an elemental composition of $C_{15}H_{21}O_3N$ (measured, 263.1544; calculated, 263.1522), S-1 is best presented by the structure 2-hydroxy-*N*-(2-methyl-6-vinylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide. Product S-2 had the same retention time and $R_f$ value on both gas-liquid chromatography and TLC, respectively, as did an authentic sample (CGA 40919) provided by CIBA-GEIGY Corp. On the basis of these results and the

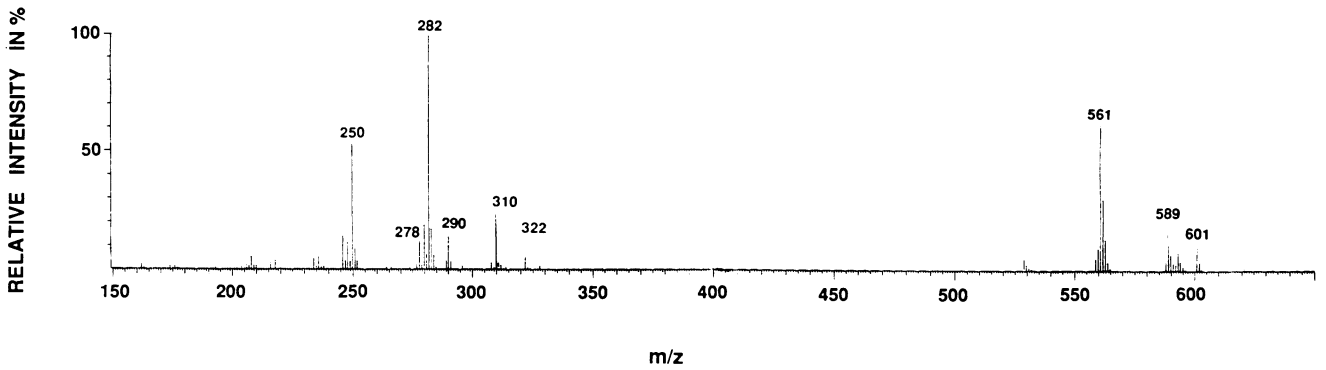



FIG. 5. Chemical ionization mass spectrum of a mixture of transformed products of metolachlor isolated from J4-A cells.

mass spectral data of the compound shown in Fig. 4, we propose S-2 as 4-(2-ethyl-6-methylphenyl)-5-methyl-3-morpholinone.

The amount of metolachlor or $^{14}C$ accumulated by the bacterial cells was indicated as the accumulation ratio (Table 1), which was calculated from the amount of metolachlor or $^{14}C$-labeled material per milligram of cells divided by the amount of the respective substance(s) remaining in 1 mg of the supernatant. The accumulation ratio of $^{14}C$ in the 10-day-old cells of J4-A reached a value as high as $1.14 \times 10^4$. We also tested 15 pure cultures isolated from J4-A for their ability to transform and accumulate metolachlor, but only the optimal results obtained with B-2 are presented. The amount of metolachlor accumulated in the cells of isolate B-2 was greater than the amount found in the mixed culture, but the rate of $^{14}C$-labeled material accumulated by isolate B-2 was only one-fourth that of J4-A after 8 days of incubation (Table 1). Therefore, the pure culture was not as efficient as the mixed culture in taking up and transforming metolachlor.

We also examined the desorption of $^{14}C$-labeled material by resuspending 7-day-old cells of J4-A in deionized water and shaking the cells for 3 h on a rotary shaker. After the cells were pelleted, the process was repeated, and the $^{14}C$ in the pooled supernatant was measured. We found that less than 2% of the $^{14}C$-labeled material was released into the deionized water (data not shown).

About 96% of the $^{14}C$ could be extracted from the 7-day-old cells into acetone, with only approximately 3% of the $^{14}C$ remaining after acetone extraction and rinsing. The acetone extract was concentrated and analyzed by TLC. Essentially all of the applied radioactivity was found in three $R_f$ areas after TLC analysis: 74% at $R_f$ 0.63, 20% at $R_f$ 0.69, and 2% at the origin. A chemical ionization mass spectrum of the sample recovered from the $R_f$ 0.63 spot revealed three protonated molecular ions at $m/z$ 250, 282, and 561 (Fig. 5). Adduct ions ($M^+ + 29$ and $M^+ + 41$) that are common to methane chemical ionization spectra were observed, exhibiting the following molecular ion assignments: $m/z$ 278 ($M^+ + 29$) and $m/z$ 290 ($M^+ + 41$) for $M^+$ 249, $m/z$ 310 ($M^+ + 29$) and $m/z$ 322 ($M^+ + 41$) for $M^+$ 281, and $m/z$ 589 ($M^+ + 29$) and $m/z$ 601 ($M^+ + 41$) for $M^+$ 560. Thus, the presence of three molecular species ($M^+$ 249, $M^+$ 281, and $M^+$ 560) is clearly indicated. Material recovered from a thin-layer plate of $R_f$ 0.69 was also subjected to mass spectrometric analysis, but no conclusive results were obtained.

HPLC analysis of the acetone extract of cells revealed six well-separated radioactive peaks, but no metolachlor was detected (Fig. 6). Each peak was collected, and the distribution of radioactivity was determined. The total $^{14}C$ recovered from these six peaks accounted for 93% of the material injected onto HPLC, which is equivalent to approximately 66% of the initially added [$^{14}C$]metolachlor.

Peaks A through D recovered from the HPLC were subsequently extracted with methylene chloride, and the samples obtained were designated products C-1, C-2, C-3, and C-4. Mass spectral data presented in Fig. 7 showed the lack of a chlorine isotopic pattern for any of the four products; however, the presence of typical characteristic fragmentation ions indicates their relation to metolachlor. Although no clear mechanism has been deduced, product C-1 is possibly formed via a reductive dechlorination of metolachlor. High-resolution mass spectral analysis of product C-1 yielded an elemental composition of $C_{15}H_{23}N_1O_2$ (measured, 249.1730; calculated, 249.1728).

The formation of product C-2 is likely to proceed through the biologically mediated addition of a simple thio compound to a dechlorinated product of metolachlor. High-resolution mass spectral data of product C-2 revealed an elemental composition of $C_{15}H_{23}N_1O_2S$ (measured, 281.1430; calculated, 281.1449). Further evidence for the addition of the sulfur-containing compound was provided by the presence of a sister peak with two mass units higher than that of the parent ion and which was more than 5% of the height of the lower mass peak. According to these results, we tentatively propose metabolite C-2 to be a dechlorinated product of metolachlor with an added thio group (Fig. 8).

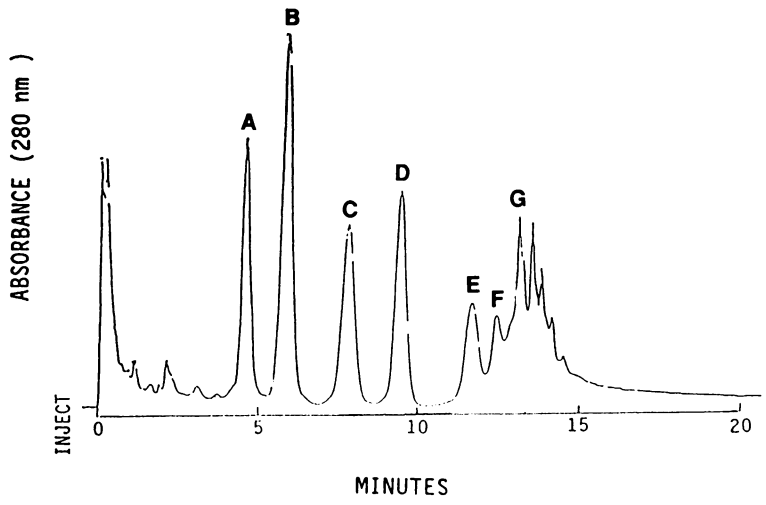
High-resolution mass spectral data indicated that product C-3 had an elemental composition of $C_{33}H_{40}N_2O_4S$ (measured, 560.2677; calculated, 560.2664). It apparently is formed by the conjugation of dechlorinated metolachlor with a more complicated sulfur-containing compound present inside the cells. High-resolution mass spectral data of metabolite C-4 did not indicate the presence of sulfur.

## DISCUSSION

A mixed bacterial culture (J4-A) exhibited a remarkable capacity to take up and accumulate metolachlor from a liquid medium; in addition, it possessed a metabolic potential for transforming the herbicide. The mechanism of binding or accumulation was not determined; it can only be stated that very little removal of metolachlor or its transformation products occurred after repeated washing of the cells with deionized water.

The pure culture, B-2, although able to accumulate metolachlor, was much less effective than the mixed popula-

A




B

| Metolachlor and products | Peak | Retention time (min.) | % of $^{14}C$ injected |
|---|---|---|---|
| Metolachlor | | 1.35[a] | 0 |
| C-1 | A | 4.85 | 12.4 |
| C-2 | B | 6.15 | 31.3 |
| C-3 | C | 7.95 | 29.0 |
| C-4 | D | 9.47 | 12.4 |
| --- | E | 11.55 | 4.1 |
| --- | F | 12.33 | 4.4 |
| Total $^{14}C$ recovered | | | 93.6 |

[a] as determined by coinjection

FIG. 6. HPLC analysis of an extract of 7-day-old J4-A cells grown with metolachlor. (A) Typical chromatogram; (B) summary of data obtained for each peak of the chromatogram.

tion. The synergistic action among members of the microbial community apparently led to better growth and consequently increased accumulation and metabolism of the herbicide. This observation indicates that the mixed culture may constitute a consortium.

Evidence of dechlorination was obtained by mass spectral analyses of products recovered from the medium as well as from the cells. Microbial cells take up xenobiotics, and to protect themselves from the toxic effects, they generally convert the chemicals. Dechlorination is usually the first step in the detoxification of haloaromatic compounds (9). Cells of J4-A are able to tolerate high doses of the dechlorinated products of metolachlor, since rapid growth was observed upon subculturing of the 10-day-old cells, which retained about 80% of the added $^{14}C$-labeled chemicals.

Current studies offer no indication regarding the site of metolachlor accumulation inside the cells. Using electron microscopy, we examined bacterial cells and found no difference in shape between the metolachlor-exposed and nonexposed cells (data not presented). No inclusion bodies were observed in the metolachlor-containing cells. On the basis of electron microscopy, Ludvic et al. (8) suggested that when the yeast *Candida lipolytica* is grown on hydrocarbons, the chemicals penetrate the cell wall and accumulate at the cytoplasmic membrane. Timms and MacRae (11) combined lysozyme treatments of *Klebsiella pneumonia* cells with differential centrifugation to produce various cellular fractions. Their results indicated that approximately 90% of the fensulfothion sulfide formed by the cells remained firmly bound to cell membrane components; very little was associated with the cell wall and the intracellular fraction.

In our experiment, the products recovered from cells of J4-A included dechlorinated metolachlor (product C-1), a thiol compound in which a sulfhydryl group replaced the

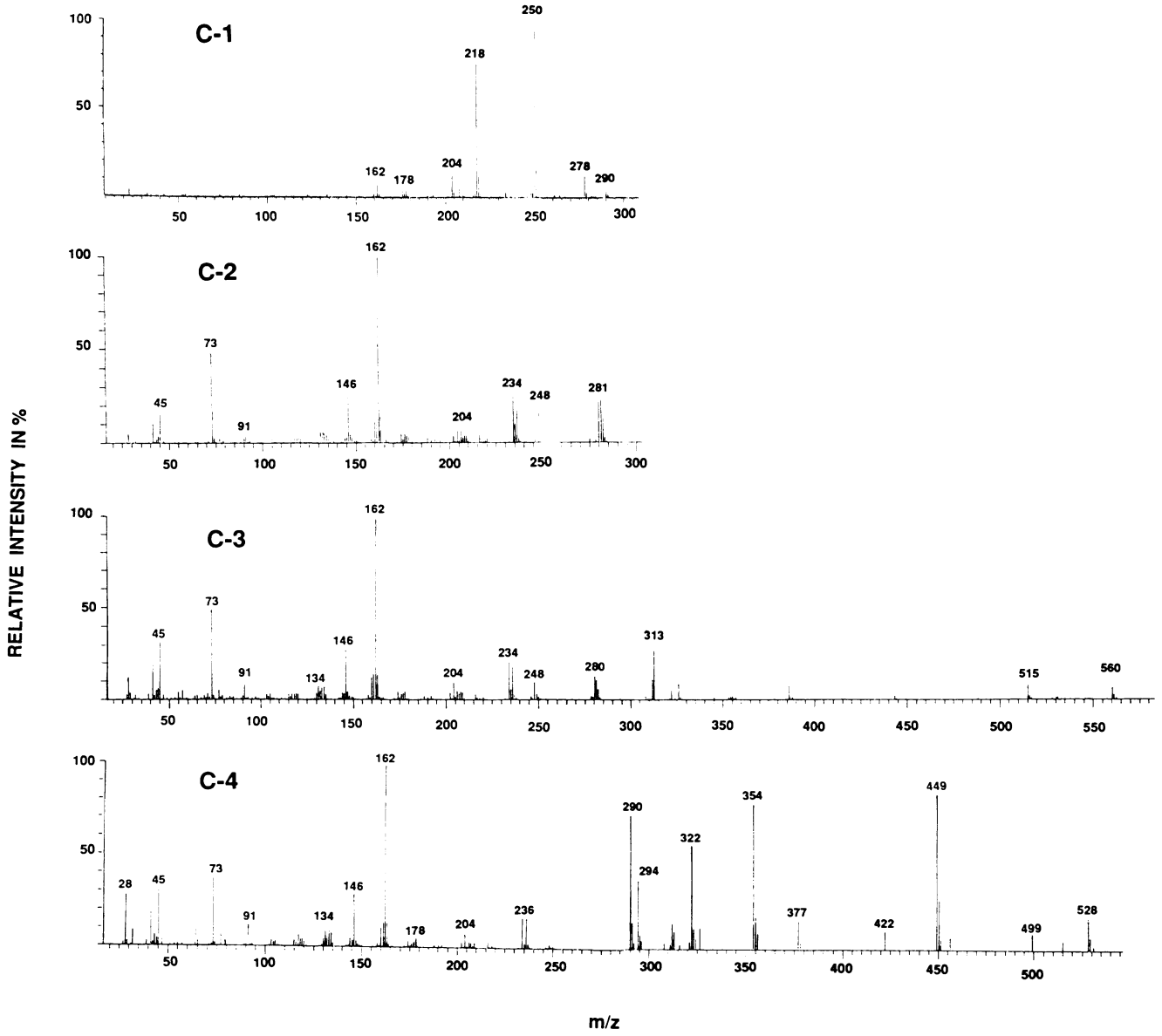



FIG. 7. Mass spectra of four products (C-1, C-2, C-3, and C-4) of metolachlor isolated from J4-A cells (chemical ionization mass spectrum for C-1; electron impact mass spectra for C-2, C-3, and C-4).

chlorine moiety (product C-2), a more complicated sulfur-containing metolachlor conjugate (product C-3), and a non-sulfur-containing metolachlor conjugate (product C-4). These transformed products of metolachlor were also formed to a lesser extent by resting cells of J4-A but not by cells killed by autoclaving or $HgCl_2$, which indicated that the transformation ability demonstrated by J4-A was microbially mediated (unpublished observation).

Volatile sulfur compounds such as alkyl thiols and alkyl sulfides are known to be produced by various microorganisms (3). Glutathione was found to occur in bacteria, yeasts, plants, and animal tissues (2). Leavitt and Penner (6) reported that metolachlor and alachlor conjugate in vitro nonenzymatically with glutathione under anaerobic conditions. They also showed that alachlor reacts anaerobically with cysteine and dithiothreitol. According to Lee et al. (7), the thio group in glutathione replaces the chlorine on the chloroacetyl moiety of propachlor to form a covalent bond between the sulfur and the methylene of the acetyl group. L. L. McGahen (Ph.D. thesis, Michigan State University, East Lansing, 1982) studied the microbial transformation of metolachlor in an anaerobic lake sediment and showed the formation of two products, the dechlorinated metolachlor with and without a thio-methyl moiety.

The modification and bioconcentration of pesticides or their metabolic products in nature are of ecological concern, because these xenobiotics may be resistant to further degradation and thus still pose a threat to higher organisms. However, intentional seeding of bacterial populations, as demonstrated in this study, may prove to be a useful means for the removal of metolachlor and its metabolites from aquatic and terrestrial environments.

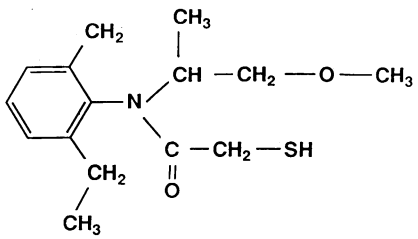


FIG. 8. Chemical structure of metabolite C-2.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Baughman, G. L., and D. F. Paris.** 1981. Microbial bioconcentration of organic pollutants from aquatic systems. Crit. Rev. Microbiol. **8**:205–228.
2. **Boyland, E., and L. F. Chasseaud.** 1969. The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. Adv. Enzymol. **32**:173–219.
3. **Kadota, H., and Y. Ishida.** 1972. Production of volatile sulfur compounds by microorganisms. Annu. Rev. Microbiol. **26**: 127–138.
4. **Krause, A., W. G. Hancock, R. D. Minard, A. J. Freyer, R. C. Honeycutt, H. M. LeBaron, D. L. Paulson, S.-Y. Liu, and J.-M. Bollag.** 1985. Microbial transformation of the herbicide metolachlor by a soil actinomycete. J. Agric. Food Chem. **33**: 584–589.
5. **Lal, R., and D. M. Saxena.** 1982. Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. **46**:95–127.
6. **Leavitt, J. R. C., and D. Penner.** 1979. *In vitro* conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. J. Agric. Food Chem. **27**:533–536.
7. **Lee, J.-K., R. D. Minard, and J.-M. Bollag.** 1982. Microbial metabolism of propachlor (2-chloro-*N*-isopropylacetanilide) in soil suspension. J. Korean Agric. Chem. Soc. **25**:44–54.
8. **Ludvic, J., V. Munk, and M. Dostalek.** 1968. Ultrastructural changes in the yeast *Candida lipolytica* caused by penetration of hydrocarbon into cells. Experientia **24**:1066–1068.
9. **Reineke, W., and H.-J. Knackmuss.** 1988. Microbial degradation of haloaromatics. Annu. Rev. Microbiol. **42**:263–287.
10. **Saxena, A., R. Zhang, and J.-M. Bollag.** 1987. Microorganisms capable of metabolizing the herbicide metolachlor. Appl. Environ. Microbiol. **53**:390–396.
11. **Timms, P., and I. C. MacRae.** 1982. Conversion of fensulfothion by *Klebsiella pneumoniae* to fensulfothion sulfide and its accumulation. Aust. J. Biol. Sci. **35**:661–668.
12. **Timms, P., and I. C. MacRae.** 1983. Reduction of fensulfothion and accumulation of the product, fensulfothion sulfide, by selected microbes. Bull. Environ. Contam. Toxicol. **31**:112–115.