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Isolation and Identification of Microorganisms for the Degradation of Dicamba

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Eight species of soil bacteria from five genera capable of utilizing Dicamba (3,6-dichloro-2-methoxybenzoic acid) as a sole carbon source were isolated and identified. All strains were obtained by enrichment from soil and water with a long history of Dicamba exposure. Strains DI-6, DI-7, and DI-8 removed an average of 97% of the Dicamba from liquid culture in 30 h, with up to 80% of the substrate carbon being converted to CO_2. Degradative activity in liquid culture was observed over a wide pH range. Strains DI-6, DI-7, and DI-8 were able to remove 98% of the Dicamba after 21 days from soil treated with 3.4 μg/g of Dicamba, as compared to 34% from uninoculated soil. The primary soil metabolite of Dicamba, 3,6-dichlorosalicylic acid, was greatly reduced in the inoculated soil with an increase in CO_2 evolution. Thus, these three strains were able to rapidly reduce soil Dicamba concentrations and more completely degrade Dicamba as evidenced by higher rates of mineralization.

The compound 3,6-dichloro-2-methoxybenzoic acid, hereafter referred to as Dicamba, is used as a pre- and postemergent herbicide for the control of annual and perennial broadleaf weeds and several grassy weeds. Dicamba is similar in herbicidal action to phenoxyalkanoic acid herbicides such as (2,4-dichlorophenoxy)acetic acid (2,4-D) but belongs to the class known as the benzoics. Dicamba is chemically stable, and there is considerable evidence suggesting that the degradation of Dicamba, in aerobic soils and water, is biologically mediated (Harger, 1975; Smith, 1973, 1974; Smith and Cullimore, 1975; Scifres et al., 1973). Aerobic soil degradation studies have demonstrated that Dicamba is metabolized to CO_2, with 3,6-dichlorosalicylate being the major metabolite identified (Harger, 1975; Smith, 1973). However, the biological factors involved in the metabolism of Dicamba have not been determined. There are no reports in the literature on the metabolism of Dicamba by a pure culture of microorganisms. The bacterial metabolism of chlorinated benzoates is determined by the number and position of chlorines on the aromatic nucleus (Horvath, 1971). Increased bacterial growth and increased O_2 consumption have been reported when soil organisms capable of growth on *o*-anisate were grown on Dicamba in the presence of *o*-anisate (Ferrer et al., 1985). However, no analytical data on the disappearance of Dicamba were presented.

Organisms capable of degrading Dicamba and/or its 3,5-isomer (3,5-dichloro-2-methoxybenzoic acid) may be useful for facilitating the rapid dissipation of both isomers from the environment (Krueger, 1984). Microbial degradation of Dicamba in the rhizosphere of crops has the potential for protecting susceptible crop species from Dicamba applied for weed control purposes. Dicamba degrading organisms would provide a source of genetic material that might be utilized for the development of Dicamba-resistant higher plants, including crop species (Krueger, 1984).

This study describes the isolation and identification of pure cultures of bacteria capable of utilizing Dicamba as a sole carbon source. Removal of Dicamba from liquid culture over a wide pH range and removal from soil are also demonstrated.

MATERIALS AND METHODS

Chemicals and Microbial Medium. Authentic reference standards used as carbon sources and used for the identification of degradation products had a purity of 98% or greater. [^{14}C]Dicamba (U-phenyl-^{14}C, 11.5 mCi/mmol, radiochemical purity greater than 98%) was synthesized by Pathfinder Labs Inc. To increase solubility, stock solutions of all chemicals used as carbon sources were prepared by titration with NaOH to pH 7.0. All chemical stock solutions were filter sterilized through 0.2-μm Teflon filters before being added to sterile media. All other chemicals were reagent grade or better, and all solvents were glass-distilled quality.

Reduced chlorine medium (pH 7.0) contained the following (in grams/liter): $K_2HPO_4 \cdot 3H_2O$, 1.826; KH_2PO_4, 0.87; $(NH_4)_2HPO_4$, 0.66; $MgSO_4$, 0.097; $MnSO_4 \cdot H_2O$, 0.025; $FeSO_4 \cdot 7H_2O$, 0.005; $CaSO_4$, 0.001. The medium contained less than the detectable level of chlorine (1 ppm) as determined by the method of Bergmann and Sanik (1957). The filter-sterilized carbon source was added after autoclaving. Solid agar Petri plates of this medium were prepared by the addition of 1.5% agar.

Organisms. Soil and water samples were obtained from the storm water retention ponds at the Dicamba manufacturing plant in Beaumont, TX. Microbial strains used for screening are described in Table I.

Microbial Screening Experiments. Microorganisms with known degradative functions (Table I) were inoculated into 50-mL reduced chlorine medium containing 100 μg/mL [^{14}C]Dicamba (58 000 dpm/mL, final volume 50 mL), 3,5-dichloro-2-methoxybenzoic acid, 3,6-dichlorosalicylate, or 3,5-dichlorosalicylate as a sole carbon source. All culture bottles (125-mL amber serum bottles) were

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Table I. Organisms with Known Degradative Capabilities Used for Screening

organism	degradative function[a]	ATCC no. or ref
Pseudomonas fluorescens	aromatic acids$^+$	12633
Pseudomonas sp.	technical chlordane$^+$	25596
Pseudomonas putida	technical chlordane$^+$	25595
Pseudomonas putida	toluene$^+$	33105
Pseudomonas putida	salicylate$^+$	17453
Pseudomonas putida	naphthalene$^+$ salicylate$^+$	17485
Pseudomonas putida	catechol$^+$	17484
Enterobacter aerogenes	produces aryl sulfatase	15038
Cunninghamella elegans	naphthalene$^+$	26269
Pseudomonas putida PP3	produces dehalogenase	Weightman et al., 1979
Pseudomonas putida	3-chlorobenzoic acid$^+$	Chatterjee et al., 1981
Pseudomonas putida	3,5-dichlorobenzoic acid$^+$	Chatterjee et al., 1981
Bacillus brevis	5-chlorosalicylate$^+$	Crawford et al., 1979
Alcaligenes eutrophus	2,4-D$^+$	Pemberton et al., 1979

[a] Plus indicates this organism can metabolize this compound.

sealed with a Teflon septum and incubated at 30 °C on a rotary shaker at 200 rpm.

All cultures were sampled at zero time and 1, 2, 3, and 4 weeks by removing an aliquot through the septum with a sterile syringe. Samples were diluted and plated on nutrient agar to determine total viable cells present. Absorption spectra (scan from 190 to 750 nm) of undiluted, centrifuged samples were used to follow the disappearance of the test chemical. A 0.1-mL sample of medium from each Dicamba culture was radioassayed in duplicate. All liquid scintillation counting was done by an external standard quench correction program. Appropriate background counts were subtracted from all samples.

At 2 weeks all cultures were flushed with CO_2-free air for 30 min at a flow rate of 10 mL/min. Exit air from the cultures containing Dicamba was passed through polyurethane foam to trap organic volatiles and through 0.5 M KOH to trap $^{14}CO_2$. Polyurethane foam was immersed in scintillation cocktail and radioassayed as described. Aliquots of KOH (0.5 mL) were radioassayed as previously described. At 4 weeks, cultures were acidified to pH <1.0 with H_2SO_4 and Dicamba cultures were purged as described.

Isolation of Organisms. A 2.0-mL aliquot of a soil and water mixture from the storm water retention pond of the Dicamba manufacturing plant at Beaumont, TX, was inoculated into sterile medium containing 1000 μg/mL Dicamba as a sole carbon source (final volume 25 mL). Cultures were incubated at 30 °C on a rotary shaker at 150 rpm for 1 week. Aliquots containing 2.0 mL of 1-week-old cultures were inoculated into sterile media containing 1000 μg/mL Dicamba (final volume 25 mL). Cultures were incubated on a shaker for 1 week as described. Aliquots of each of these cultures were plated out on solid media containing 1000 μg/mL Dicamba as a sole carbon source. After several days individual colonies were streaked onto agar containing 1000 μg/mL Dicamba and incubated until colonies appeared. Distinct individual colonies from these streak plate isolates were assigned strain numbers. Colonies were propagated on Dicamba agar and checked for purity by examination at 2000× magnification after fixation and Gram staining. All organisms were identified by biochemical tests using the Flow N/F test system (Flow Laboratories, Inc., McLean, VA) and by the Hewlett-Packard 5898A microbial identification system (Miller and Berger, 1985). Identification tests used included Gram stain, oxidase, motility, gas formation, pyocyanin, fluorescein, glucose fermentation, growth at 42 °C, growth on glucose, xylose, mannitol, lactose, maltose, acetamide, esculin, and urea, hydrolysis of DNA, and production of H_2S and indole.

Microbial Growth Kinetics in Sterile Culture Medium. Dicamba degrading organisms were grown on reduced chlorine medium containing 1000 μg/mL Dicamba. Forty milliliters of each culture was used to inoculate reduced chlorine medium containing 1000 μg/mL [^{14}C]Dicamba (57 000 dpm/mL, final volume 500 mL). Cultures were incubated at 30 °C on a rotary shaker.

All cultures were sampled at 0, 6, 24, and 30 h. At each sampling time, the total viable cell count on nutrient agar was determined as a direct measure of growth. The absorbance at 600 nm was used as a indirect measure of growth. Removal of Dicamba was confirmed by a decrease in absorbance at 274 nm (absorbance maximum of Dicamba in the experimental medium). Aliquots of medium were radioassayed as described. Chlorine was determined by the method of Bergmann and Sanik (1957) so that chlorine released from Dicamba could be calculated.

pH Effects. Four milliliters of each 3-day-old Dicamba degrading culture was used to inoculate liquid chlorine free media containing 1000 μg/mL Dicamba at pH 4.0, 5.0, 6.0, 7.0, and 8.0 (final volume 50 mL). Cultures were incubated at 30 °C on a rotary shaker. All cultures were sampled at zero time and at 24 h. Aliquots of medium were radioassayed in duplicate.

Microbial Growth Kinetics in Soil. Kenyon loam soil was sieved through 2-mm-diameter openings and moistened to field level (18.1% moisture). [^{14}C]Dicamba (in deionized water) was pipetted onto and mixed with the soil to yield a concentration of 3.4 μg/g Dicamba in the moist soil. This is equivalent to an application rate of approximately 2 lb/acre when incorporated into 2 in. of soil. The percentage of sand, silt, and clay, percentage of organic carbon and organic matter, cation-exchange capacity, pH, and moisture capacity of the Kenyon loam soil were determined according to the methods of Weber (1977).

Treated soil (62.2 g of moist soil, equivalent to 50 g of dry soil) was added to 125-mL amber serum bottles. Duplicates of treated soil were inoculated with 1.0 mL of culture from Strains DI-6, DI-7, or DI-8. All three strains had been grown for 2 days in 1 L of media containing 1000 μg/mL Dicamba and then concentrated to 5.0 mL with centrifugation (10 min at 2500*g*). Viable cell counts were conducted on inoculums to determine the total number of cells added to the treated soil. A bottle of treated soil inoculated with 1.0 mL of deionized water served as the control. Al soil treatments resulted in soil moistures that were 75% of the 0.33-bar level. All bottles were sealed with Teflon septums and incubated in darkness at 25 °C for 21 days.

At 1, 3, 7, 14, and 21 days all samples were flushed with CO_2-free air. Exhaust air was bubbled through 1.5 N KOH to trap $^{14}CO_2$. Duplicate 0.5-mL aliquots of KOH were radioassayed. Soil from one replicate of each of the 21-day inoculated soils was analyzed for Dicamba and 3,6-dichlorosalicylic acid according to Sandoz Crop Protection Corp. GC residue Method AM-0766. Recovery (spiked soil samples) and check samples (untreated soil) were also analyzed.

Propagation, Preservation, and Viability. Strains DI-6, DI-7, and DI-8 were grown in a Queue Fermentor (Parkersburg, WV) on 5 L of 1000 μg/mL Dicamba liquid

Table II. Whole-Cell Fatty Acid Profiles of Dicamba Degrading Strains DI-6, DI-7, DI-8 and Two Species of *Pseudomonas*

	peak area, %				
fatty acid	*Pseudomonas* sp. Di-6	*Moraxella* sp. DI-7	*Pseudomonas* sp. Di-8	*Psuedomonas fluorescens*	*Pseudomonas aeruginosa*
C10:0 30H	3.5	3.6	3.8	3.1	3.0
C12:0	3.4	3.3	4.3	4.5	3.5
C12:0 20H	3.6	3.7	3.6	3.1	3.8
C12:0 30H	4.3	4.3	4.9	3.5	3.8
C14:0	0.5	0.5	0.6	0.3	1.2
C16:1 cis9	39.2	35.4	35.0	27.2	13.1
C16:1 trans9	0.0	0.0	0.0	0.0	6.9
C16:0	29.6	30.6	29.0	31.5	23.0
C17:0 cyclo	0.5	4.1	2.9	6.7	0.0
C18:0	0.4	0.3	0.0	0.9	0.4
C18:1 trans9	0.0	0.0	0.0	18.7	41.4
C19:0 cyclo	0.0	0.0	0.0	0.2	0.0
unidentified	15.0	14.1	16.0	0.0	0.0

medium for 3 days. The fermentor was set to maintain the temperature at 30 °C and provide an agitation speed of 250 rpm. A filter sterilized air flow of 1.0 standard L/min maintained a dissolved oxygen concentration of greater than 95% of saturation for all cultures. After 3 days each 5-L culture was concentrated to 50 mL. Aliquots were diluted and plated out on nutrient agar to determine the number of viable cells present. Two milliliter portions of each concentrate were (1) frozen in a dry ice–acetone bath, (2) frozen with an equal volume of glycerol in a dry ice–acetone bath, or (3) frozen in a dry ice–acetone bath and freeze-dried in a Virtis freeze-dryer. All samples were sealed and stored at −70 °C.

Samples of each strain were allowed to thaw at room temperature or reconstituted at zero time and 1, 6, and 12 months after preservation. The number of viable cells present was determined by plate count on nutrient agar. Cultures were inoculated into 25 mL of 1000 μg/mL Dicamba liquid medium to determine the stability of Dicamba degradation activity. Inoculated medium was radioassayed at zero time and at 24 h to determine the extent of Dicamba metabolism.

RESULTS AND DISCUSSION

Isolation and Identification of Dicamba Degrading Organisms. Eight strains that could utilize Dicamba as a sole carbon source were isolated. All strains were Gram-negative and were further identified by biochemical test (as described). Identifications were as follows: strain 1, *Aeromonas sp.*; strain 2, *Pseudomonas sp.*; strain 3, *Xanthomonas sp.*; strain 4, *Alcaligenes sp.*; strain 5, *Moraxella sp.*; strain 6, *Pseudomonas sp.*; strain 7, Moraxella *sp.*; strain 8, *Pseudomonas sp.* Strains 1–5 would only grow slowly on solid (agar) medium containing Dicamba and not in liquid culture. No further investigation was conducted on strains 1–5. Strains 6–8 grew rapidly in liquid media containing Dicamba and were identified further by GC analysis of whole-cell fatty acid profiles. Fatty acid composition of strains 6–8 were similar to each other and to other *Pseudomonas* species (Table II). Differences in the ability of these strains to utilize the various carbohydrates in the biochemical tests indicated that these three strains were distinct organisms.

The organisms isolated that degrade Dicamba represent genera that are commonly found in water, soil, and sewage and are often responsible for the decomposition of man-made chemicals in the environment. Members of the genus *Pseudomonas* are known to posses a wide range of degradative activities (Stanier et al., 1966) and have the ability to develop new degradative activities (Kellogg et al., 1981). Long-term intermittent exposure (approximately 25 years) of microbes to Dicamba in the storm water retention ponds at the Dicamba Manufacturing facility appears to have provided the selective conditions necessary to develop Dicamba degrading capabilities. An excellent example of the development and spread of herbicide degrading activity has been demonstrated for phenoxyacetic acid herbicides. A large number of different genera have been isolated that are capable of degrading these herbicides (Helling et al., 1971).

Screening of Xenobiotic Degrading Organisms. None of the 14 xenobiotic degrading organisms tested (Table I) were able to utilize Dicamba or 3,6-dichlorosalicylate. No increase in cell number was observed, and spectral analysis indicated no loss of the parent compound. No $^{14}CO_2$ was evolved, and no decrease in total radiocarbon was detected in [^{14}C]Dicamba-treated cultures. None of the 14 organisms screened were previously exposed to Dicamba or the dichlorosalicylates, so their inability to utilize these chemicals may not be surprising. The specificity of xenobiotic oxidative enzymes has been reported to be very strict (Chatterjee and Chakrabarty, 1982).

The only growth that was detected was with the *Bacillus brevis* strain that grew on 3,5-dichlorosalicylate. Cell number increased and spectral analysis indicated complete removal of 3,5-dichlorosalicylate in 1 week. This organism was previously shown to degrade 5-chlorosalicylate (Crawford et al., 1979). This organism may be useful for selectively metabolizing the 3,5-isomer from a mixture of 3,6- and 3,5-dichlorosalicylate (Krueger, 1984).

Growth Kinetics in Liquid Culture. When Dicamba degrading organisms (strains DI-6, DI-7, and DI-8) were grown in liquid media containing 1000 μg/mL Dicamba, an average of 97% of the chloride was released after 30 h (Table III). The absorbance of 274 nm (wavelength of maximum absorbance for Dicamba) decreased to zero (Table III). Chloride release and the decrease in absorbance at 274 nm indicate complete removal of Dicamba. Radioassay of media indicates that up to 80% of the substrate carbon is mineralized to CO_2. The substrate carbon remaining in the media was probably incorporated into cell biomass since cell number and turbidity increased (Table III).

Initial reactions in the metabolism of Dicamba by microorganisms and subsequent increases in biomass require an expenditure of energy by the microbe. The evolution of $^{14}CO_2$ from phenyl-labeled [^{14}C]Dicamba suggests that Dicamba is fragmented to compounds that are channeled into oxidative cycles (Krebs cycle) from which the organism can derive useful energy. Mineralization of 2,4-D to CO_2, with a corresponding increase in biomass, has also been demonstrated (Amy et al., 1985).

Effect of pH in Liquid Culture. The effect of pH on Dicamba degrading activity in liquid culture is shown in

Table III. Growth Kinetics of Dicamba Degrading Organisms in Liquid Culture Containing 1000 μg/mL of Dicamba

strain	time, h	viable cells/mL	A_{600}	A_{274}	Dicamba,[a] μg/mL	total Cl, μg/mL	% removal[b]	acidified media, dpm/mL	% removal[c]
DI-6	0	2.1×10^8	0.03	2.36	818	0	0	56 540	0
	6	7.5×10^8	0.03	2.38	828	26	8.1	52i 040	8.0
	24	1.3×10^9	0.14	ND[d]	<10	325	101.2	13 520	76.1
	30	6.8×10^8	0.13	ND[d]	<10	334	104.0	14 360	74.6
DI-7	0	7.8×10^7	0.02	2.38	827	0	0	56 300	0
	6	8.5×10^8	0.02	2.56	887	15	4.7	55 710	1.0
	24	9.8×10^8	0.13	ND[d]	<10	296	92.2	21 630	61.6
	30	1.0×10^9	0.13	ND[d]	<10	315	98.1	18 130	67.8
DI-8	0	1.0×10^8	0.02	2.43	843	0	0	58 490	1.3
	24	1.1×10^9	0.07	ND[d]	<10	151	47.0	40 120	31.1
	30	1.0×10^9	0.13	ND[d]	<10	290	90.3	23 440	59.7

[a] Based on absorbance at 274 nm. [b] Based on chloride release. [c] Bsed on soluble ^{14}C in the media. [d] Limit of detection 10 μg/mL Dicmaba.

Table IV. Activity of Dicamba Degraders at Various pH Values with 1000 μg/mL Dicamba

strain	init pH	% substr C removed after 24 h
DI-6	4.0	1.6
	5.0	21.5
	6.0	38.9
	7.0	61.6
	8.0	61.1
DI-7	4.0	0.0
	5.0	1.0
	6.0	37.0
	7.0	70.0
	8.0	66.0
DI-8	4.0	31.8
	5.0	31.0
	6.0	30.7
	7.0	65.6
	8.0	66.0

Table IV. All three organisms removed approximately the same amount of substrate ^{14}C at pH 7.0 and 8.0. The ability to remove substrate ^{14}C decreases when pH is decreased. However, strain DI-8 was able to remove 31.8% of the substrate ^{14}C even at pH 4.0. The ability of these organisms to degrade Dicamba over a wide range of pH values may make them useful for removing Dicamba from a wide variety of aquatic and soil environments.

Viability of Preserved Cultures. Strains DI-6, DI-7, and DI-8 were able to grow in a bench scale (5-L) fermentor. The cultures decreased from initial pH 7.0 to 6.3 as Dicamba was metabolized. The ability to propagate Dicamba degrading strains as pure cultures in large volumes would be useful for the production of large amounts of inoculums.

Culture viability and Dicamba degrading activity after preservation are demonstrated in Table V. Cultures that were frozen showed the best survival rates. Cultures where less than 1% of the cells survived still showed the ability to degrade Dicamba in liquid culture. Preserving viable cultures of Dicamba degraders would be an important factor in developing inocula for commercial use.

Growth Kinetics in Soil. Initial inoculum size (in cells/gram of field moist soil) for each strain were as follows: strain DI-6, 6.96×10^8; strain DI-7, 2.37×10^8; strain DI-8, 2.69×10^8. Soil characteristics are presented in Table VI.

An average of 63.7% of the Dicamba applied to soil was metabolized to CO_2 in the inoculated soils, compared to only 2.2% in the uninoculated soil after 21 days (Figure 2). The highest degradiation rates occurred between zero and 1 day in all of the inoculated treatments. GC analysis indicates nearly complete removal (98% or greater) of Dicamba and greatly reduced accumulation of 3,6-dichlorosalicylic acid after 21 days (Table VII). In the uninoculated control, 66.2% of the applied Dicamba remained and the concentration of 3,6-dichlorosalicylic acid was equivalent to 19.7% of the applied Dicamba. Dicamba mineralization by microorganisms in soil might also be

Table V. Culture Viability and Dicamba Degrading Activity after Preservation[a]

strain	time, months	% viability after freezing	after freezing with glycerol	after freezing-drying
DI-6	0	51.6	9.5	5.8
	1	24.8	11.6	6.5
	6	4.1	1.3	2.0
	12	0.5	0.7	0.3
DI-7	0	19.7	26.6	2.0
	1	20.0	13.2	0.2
	6	7.1	7.4	0.2
	12	12.8	1.6	0.1
DI-8	0	15.7	0.2	40.7
	1	1.3	0.5	0.3
	6	4.2	0.2	0.2
	12	1.5	0.8	0.7

[a] Tests indicated that at all time periods and after all preservation methods all cultures were able to utilize 1000 μg/mL Dicamba as a sole carbon source in liquid media.

Table VI. Properties of Kenyon Loam Soil

% organic C	2.2
% organic matter (calcd from % organic C)	3.8
cation-exchange capacity (mequiv/100 g)	20.4
pH (deionized water)	6.2
pH (0.01 M $CaCl_2$)	6.0
75% of 0.33-bar level (g H_2O/100 g dry soil)	24.4
bulk density (g/cm^3)	1.6
% sand	34.0
% silt	41.0
% clay	25.0
textural class	loam

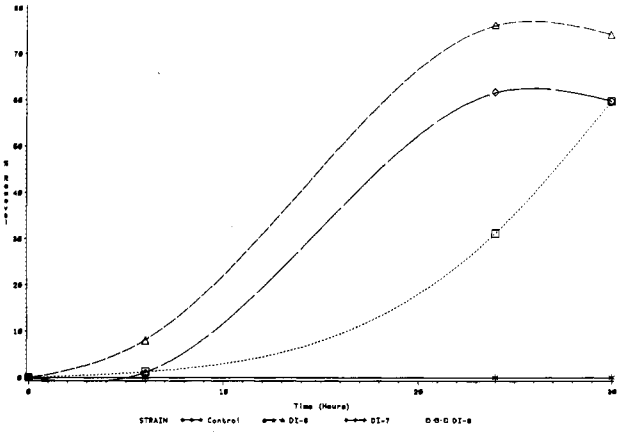

Figure 1. Removal of soluble ^{14}C from liquid media containing 1000 μg/mL [^{14}C]Dicamba by three Dicamba degrading strains and an uninoculated control.

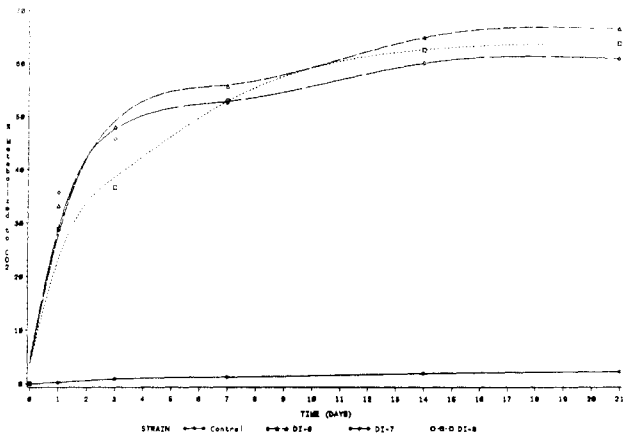

Figure 2. Substrate radiocarbon metabolized to $^{14}CO_2$ from [^{14}C]Dicamba-treated soil (3.4 μg/g) by three Dicamba degrading strains and an uninoculated control.

Table VII. GC Analysis of Dicamba-Treated Soil[a] 21 Days after Inoculation with Dicamba Degrading Organisms

inoculum	Dicamba, μg/g	3,6-dichlorosalicylic acid, μg/g
DI-6	ND[b]	0.014
DI-7	0.019	0.075
DI-8	0.067	0.260
control	2.250	0.670

[a] Initial concentration of Dicamba was 3.4 μg/g. [b] Not detectable, limit of detection for Dicamba and 3,6-dichlorosalicylcic acid 0.01 μg/g each.

influenced by the soil composition, nutrient levels, percent organic matter, and temperature.

CONCLUSIONS

The microbial degradation of herbicides in soil and water can only be useful if degradation products are of less environmental concern than the parent compound. Mineralization of Dicamba by Dicamba degrading organisms to CO_2 and reduced accumulation of 3,6-dichlorosalicylic acid in Dicamba-treated soil indicate that Dicamba is metabolized to compounds that are less of an environmental concern. Complete mineralization of Dicamba would result in reduced environmental exposure to degradation products and reduced potential for leaching of Dicamba or its metabolites to groundwater.

The ability of Dicamba degrading organisms to rapidly remove Dicamba from the soil in the rhizosphere of plants may be useful in developing strategies to protect susceptible crop species from Dicamba. The Dicamba degrading organisms isolated appear well suited for use as a crop inoculum. All three strains (DI-6, DI-7, DI-8) have degradative activity at the 2 lb/acre rate in a typical midwestern soil (Kenyon loam) in the presence of naturally occurring microflora.

Registry No. Dicamba, 1918-00-9; 3,6-dichlorosalicylic acid, 3401-80-7.

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