EPTC Degradation by Isolated Soil Microorganisms

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Microorganisms capable of degrading the herbicide EPTC (*S*-ethyl *N*,*N*-dipropylcarbamothioate) were isolated from three soils with and without histories of carbamothioate use. All EPTC-degrading isolates belonged to the genus *Rhodococcus*. All three isolates in pure culture systems degraded 50 μg mL^{-1} of technical EPTC in as little as 14 h at low cell densities and used the molecule as a sole source of carbon and energy. Growth of the isolates in rich media led to the frequent loss of EPTC-degrading ability, although plasmids encoding for EPTC degradation could not be identified. [^{14}C]-EPTC experiments suggested that the degradation of EPTC proceeds by initial attack at the carbonyl linkage, followed by degradation of the dipropylamine side chain.

Keywords: *Herbicide; metabolism; hydrolysis; biodegradation; plasmids; carbamothioate*

INTRODUCTION

The phenomenon known as enhanced pesticide degradation refers to the accelerated decomposition of a compound in soil that has a previous history of application of that compound versus the degradation rate in a soil having never been exposed to that pesticide or similarly structured ones. The rapid degradation of a pesticide often leads to loss of efficacy because the level of pesticide falls below that required to kill target pests. To date, there are more than 25 pesticides including insecticides, herbicides, and fungicides known to exhibit accelerated degradation in "problem" soils. One of the most widely studied of these compounds is EPTC (*S*-ethyl *N*,*N*-dipropylcarbamothioate), a herbicide used extensively for control of grassy weeds in corn. Rahman et al. (1979) was the first to report the occurrence of enhanced degradation in New Zealand soils with repeated use of the carbamothioate herbicides. Other investigators in various parts of the United States and in other countries have since observed similar phenomena with carbamothioates (Obrigawitch et al., 1982; Skipper et al., 1986; Harvey, 1987; Rudyanski et al., 1987; Bean et al., 1988; Tal et al., 1989a).

The mechanisms or causes of enhanced degradation are not well understood. Several theories have been proposed for the occurrence of enhanced degradation (Audus, 1949). One theory suggests that microorganisms with the capability of degrading the compound exist, and the rapid degradation which follows is a result of the build-up of populations of such microorganisms. Another suggests an enzymatic inducement with the lag period observed before rapid degradation corresponding to the time required to fully develop an enzyme potential. Moorman (1988), using a most probable number technique, reported that larger EPTC-degrading populations did not exist in soils exhibiting accelerated degradation of EPTC, and that increased rates of metabolism were responsible for accelerated degradation rather than increased numbers of microorganisms. Mueller et al. (1989) reported greater numbers of actinomycetes capable of degrading butylate (*S*-ethyl *N*,*N*-diisobutylcarbamothioate) in soils with previous histories of butylate or vernolate (*S*-propyl *N*,*N*-dipropylcarbamothioate) application, and greater numbers of vernolate-degrading bacteria in soils with a history of vernolate use. However, there were no significant differences in the number of EPTC-degrading microorganisms in EPTC history soils. Recently, however, several investigators (Tam et al., 1987; Mueller et al., 1988) have reported that the carbamothioate degradative ability of bacterial isolates was plasmid-encoded, suggesting the possibility of transfer of the genetic material encoding for EPTC degradation in the environment as a possible explanation for enhanced degradation of EPTC.

The objectives of this study were to isolate microorganisms capable of degrading EPTC, to investigate EPTC metabolism, and to investigate whether the EPTC-degrading ability of these microorganisms is plasmid-encoded.

MATERIALS AND METHODS

Isolation of EPTC Degraders. Three Ohio soils were used for batch culture enrichments for the isolation of EPTC-degrading microorganisms. Brookston soil was obtained from a corn field with a 4-year history of EPTC application. It was a clay loam (Typic Agriaquoll) and contained 4.5% organic carbon and had a pH of 7.5. Dekalb soil was from a corn field with an unknown history and was a channery loam (Typic Dystrochrept) and contained 1.8% organic carbon and had a pH of 6.5. Jimtown soil was from a forest site with no known carbamothioate herbicide applications. It was a loam (Aeric Ochraqualf), had 2.1% organic carbon and a pH of 7.0. One gram of field-moist soil, which had been stored at 4 °C, was added to 50 mL of a minimal salt medium containing the following (g L^{-1}): $(NH_4)_2SO_4$, 5.0; K_2HPO_4, 0.8; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $CaSO_4$, 0.1; and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.001 (Kaufman and Kearney, 1965). Technical grade (98%) EPTC (Stauffer Chemical Co.) was added as the sole carbon source at a rate of 100 or 200 μg mL^{-1}. At 7–10 day intervals when the medium became turbid, 1 mL was inoculated into flasks containing fresh medium. After three transfers, cultures were plated onto nutrient agar (NA) plates. Colonies were streaked

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on NA plates to ensure purity, and then grid-plated onto minimal salts plates with EPTC as the sole carbon source. Colonies appearing on the minimal salt + EPTC plates were again transferred to minimal salts + EPTC plates to avoid carryover of nutrients.

Identification and Characterization of Bacteria. Phase-contrast microscopy was used to examine the morphology of the bacterial isolates. Gram staining was done by standard techniques. The ability of the isolates to use various carbon sources was tested in the basal salts medium given below. The fatty acid profiles of the isolates were performed on a Hewlett-Packard gas chromatograph with a HP5898A Microbial I.D. system using Aerobic Library, version 3.0, produced by Microbial I.D. (Newark, DE).

EPTC Degradation in Broth Culture. Colonies appearing on minimal salts + EPTC plates were initially screened for growth on EPTC by transferring them to minimal salts broth containing EPTC (200 μg mL^{-1}) as the sole carbon source. Those cultures that became turbid were tested for EPTC degradation by gas chromatographic analysis by inoculating them into 25 mL of fresh minimal salts medium + EPTC in screw-cap, sidearm 250-mL flasks. Cultures were allowed to grow at 28 °C until they reached an optical density (600 nm) of 0.05 as measured by a Bausch & Lomb Spectronic 20. At this time, technical EPTC was added to achieve a final concentration of 50 μg mL^{-1}. At various intervals, optical density measurements were taken, and simultaneously, 1-mL aliquots were aseptically withdrawn and extracted by shaking for 2 min with 1 mL of HPLC grade toluene. The upper toluene layer was removed and dried over anhydrous sodium sulfate for gas chromatographic analysis.

Resting Cell Suspension Studies. For resting cell suspension studies a basal salt medium (BSM) described by Tomasek and Karns (1988) was used which consisted of 50 mM potassium phosphate, pH 7.0, 15 mM $(NH_4)_2SO_4$, 0.8 mM $MgSO4{\cdot}7H_2O$, 0.18 mM $CaCl_2{\cdot}2H_2O$, 5 μM $MnCl_2{\cdot}6H_2O$, 2 μM $FeSO_4{\cdot}7H_2O$, 8 μM $NaMoO_4{\cdot}2H_2O$, and 1 μM each $CuCl_2$, $ZnCl_2$, $CoSO_4{\cdot}7H_2O$, $NiSO_4{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $Al_2(SO_4)_3nH_2O$, and H_3BO_3. Wild-type cultures were grown in BSM + analytical (99.8%) EPTC (BSME) at a concentration of 150 μg mL^{-1} as the sole C source for five to seven days until turbid, whereas EPTC$^-$ strains were grown in either BSM + dipropylamine (BSMDPA) or BSM + glycerol to log phase (36–40 h). All cultures and resting cell suspensions were incubated at 28 °C with shaking. Cells were harvested by centrifugation (6000*g*), washed twice in 50 mM potassium phosphate buffer (pH 7.0) + 1 mM magnesium sulfate, and resuspended in the same buffer to an OD of 170 (EPTC-grown) or 400 (DPA- or glycerol-grown) Klett units (no. 66 filter). Five milliliters of the cell suspensions was added to 50 mL flasks containing 5 mL of a 200 μg mL^{-1} EPTC solution in the same buffer achieving a final concentration of 100 μg of EPTC mL^{-1}. At various intervals, 0.5 mL aliquots were removed and extracted by shaking for 1 min with 0.5 mL of hexane. The hexane layer was pipetted into a separate vial for analysis by gas chromatography. All degradation experiments were completely randomized block designs with two replications.

Gas Chromatographic Analysis. The toluene extracts were analyzed for EPTC with a Varian 3700 gas chromatograph equipped with a Varian thermionic nitrogen specific detector. Injector and detector temperatures were 200 and 300 °C, respectively. The column, maintained at a temperature of 175 °C, was 3% OV-17 on 100/120 Supelcoport 3 m in length. The carrier gas was helium, at a flow rate of 28 mL min^{-1}. The hydrogen and air flow rates to the detector were 4.5 and 180 mL min^{-1}, respectively. The minimum detectable level of EPTC was 0.05 μg mL^{-1}. Gas chromatographic analyses were later simplified by using hexane extraction and a Chrompack (Bridgewater, NJ) wall coated, fused silica capillary column, 26 m in length, with a CP Sil 19 CB liquid phase and a temperature program from 80 to 140 °C at the rate of 20 °C min^{-1}. EPTC was quantitated by comparing peak areas to those generated with standards of known concentrations.

Maintenance of Bacterial Isolates. Bacterial isolates were maintained initially on the minimal salts agar slants supplemented with 200 μg mL^{-1} EPTC as the sole C source. Later, they were maintained on agar slants of the basal salts medium (BSM) + EPTC at the same rate. Due to the frequent loss of ability to degrade EPTC when maintained on nutrient agar or BSM supplemented with other C sources and due to the slow rate of growth, the isolates were kept in BSME (150 μg mL^{-1}) broth culture and transferred every five to thirty days depending on the frequency of use. Freshly transferred cultures (4–5 days old) were used in all studies. EPTC$^-$ strains were maintained in BSMDPA broth (200 μg mL^{-1}) or on agar slants of the same media.

Plasmid Isolation Techniques. Bacterial isolates (grown in BSME) were used as inocula for cultures grown to mid-log phase in BSMDPA (20 mM) and then harvested by centrifugation (6000*g*). Bacterial pellets were frozen (−30 °C) until use. To facilitate lysis, pellets were pretreated with acetone according to the DNA isolation procedure of Heath et al. (1986). The large-scale plasmid DNA isolation procedure of Hansen and Olsen (1978) was used with the following modifications. A 1.5–2.5 g cell pellet was suspended in 6.0 mL of 0.25 M sucrose in 0.05 M Tris (pH 8.0). Lysozyme (20 mg mL^{-1}) was added in 1.0 mL of 0.25 M Tris (pH 8.0) and incubated at 37 °C for at least 30 min. At room temperature, 2.5 mL of both EDTA (0.25 M, pH 8.0) and SDS (20% [w/v] in TE [10 mM Tris, pH 8.0, 1 mM EDTA]) were added, followed by 0.75 mL of NaOH (3 M). Four rounds of heat pulses of 5 min at 60 °C followed by 5 min on ice were used. Neutralization and renaturation were accomplished with 6.0 mL of Tris (2 M, pH 7), 3.0 mL of SDS (20% (w/v) in TE), and 6.0 mL of NaCl (5 M). After centrifugation, one-third volume of PEG (42% (w/v) in 0.01 M sodium phosphate, pH 7.0) was added. Pellets were redissolved in 8 mL of TE buffer and 8.1 g of CsCl, and 1 mL of ethidium bromide (5 mg mL^{-1}) was added for ultracentrifugation (105000*g* for 40 h). EtBr was removed from bands obtained from CsCl gradients by butanol extraction, the DNA was reprecipitated, and then dissolved in 100 μL of sterile water. Twenty microliter samples were added to tracking dye and loaded onto a horizontal 0.6% agarose Tris borate (TBE) minigel (Maniatis, 1982). Electrophoresis was carried out at 100 V for 90 min. The gel was stained with EtBr and photographed.

DNA/DNA Hybridization. The plasmid DNA from all four *Rhodococcus* isolates from the agarose minigels was transferred to reinforced nitrocellulose (Optibind, Schleicher & Schuell, Keene, NH) by Southern blotting (Schatz, 1989). Probe DNA was generated by digesting plasmid DNA from *Rhodococcus* strain TE1 (Tam, Behki, and Kahn, 1987) with the restriction endonuclease *Eco*RI following the manufacturer's instructions (Bethesda Research Laboratories, Gaithersburg, MD). A 5.8 kb *Eco*RI fragment from the small plasmid (approximately 6 kb) from TE1 was recovered from a 0.6% TBE gel using a NA45 DEAE membrane according to the manufacturer's instructions (Schleicher & Schuell). The probe DNA was ^{32}P-labeled (100 μCi mmol^{-1}) using an Oligolabelling Kit (Pharmacia, Uppsala, Sweden). Unincorporated nucleotides were separated from the labeled DNA probe with a Stratagene (La Jolla, CA) push column. The filter was hybridized and washed at 65 °C (high stringency corresponding to >80% homology) using method IV of the GeneScreen instruction manual (New England Nuclear, Wilmington, DE). The filter was put on X-ray film for exposure times of 1 h to overnight. The nitrocellulose filter was boiled to remove the TE1 probe according to the manufacturer's instructions (Schleicher & Schuell) and was rehybridized with a ^{32}P-labeled 5.8 kb *Eco*RI fragment from the 6 kb plasmid from isolate DE1 by the methods previously described.

Isolation of Mutants Deficient in EPTC Degradation. Mutants unable to utilize EPTC as a sole carbon source (EPTC$^-$) were isolated by several techniques. Cells grown on EPTC were inoculated into tubes of nutrient broth (NB) containing a series of 2-fold dilutions of the mutagen, mitomycin C (100, 50, 25, 12.5, ..., 0.098 μg mL^{-1}) and allowed to grow for 3 days. The tube with the highest concentration of mitomycin C exhibiting growth was diluted and plated on nutrient agar to give isolated colonies. Cells grown on EPTC were also grown in NB treated with acridine orange, or in NB incubated at an elevated temperature (32 °C) as described by

Tam et al. (1987), and then plated on NA as above. Spontaneous EPTC$^-$ mutants were isolated by inoculating 2 mL of a culture grown on EPTC into 50 mL of NB and allowing them to grow overnight at 28 °C before plating onto NA. In all cases the colonies from the nutrient agar plates were patched onto a 10 × 10 grid on plates of NA (to recover colonies), BSME agar (to detect isolates unable to utilize EPTC), and BSMDPA agar (to detect mutants unable to grow on EPTC due to defects in metabolism). Selected isolates that grew poorly on BSME agar were transferred to BSME broth to confirm the EPTC$^-$ phenotype.

[^{14}C]EPTC Degradation Experiments. Turbid cultures of wild-type and EPTC$^-$ strains grown in BSME or BSMDPA, respectively, were used as an inocula for mid-log phase cultures in BSM with either glycerol or DPA as the C source. Cells were harvested by centrifugation, washed twice in phosphate buffer given above, and resuspended to an optical density of 400 Klett units (no. 66 filter). Five milliliters of these suspensions was added to biometer flasks (Bartha and Pramer, 1965) containing 5 mL of phosphate buffer (given above) with 200 μg mL^{-1} of either 1-propyl or carbonyl ^{14}C-labeled EPTC with a specific activity of 1 μCi mg^{-1}. Controls consisted of the same system without cells. The labeled compounds were gifts from Stauffer Chemical Co. and were greater than 97% pure as determined by autoradiography following thin layer chromatography using a hexane–ethyl acetate (3:2) developing system (R_f = 0.6). A polyurethane plug was inserted into the sidearm of each biometer flask to trap volatilized EPTC (Kearney and Kontson, 1976). The flasks were incubated at 28 °C with gentle shaking. $^{14}CO_2$ was collected in the reservoir containing 10 mL of 0.5 M NaOH which was changed at various times over the course of the experiments. Three milliliters of the NaOH was added to vials containing 7 mL of Beckman Ready-Solv cocktail and were counted in a Beckman scintillation counter after dark-adapting the samples overnight. Experiments were done in duplicate, each experiment being a completely randomized design with two replicates.

RESULTS AND DISCUSSION

Isolation and Characterization of EPTC-Degrading Organisms. Numerous colonies appeared on the nutrient agar plates inoculated with biomass from the last batch enrichment cultures from each of the three soils. The majority of these isolates were unable to grow on BSME plates after two transfers. However, several colonies of similar morphology from each soil exhibited good growth on EPTC plates and were transferred to BSME broth culture. The isolate from each soil that produced turbidity most quickly in BSME was selected for further study. These same isolates were unable to grow on BSM in the absence of added carbon sources. The microorganisms were designated BE1, DE1, and JE1 from the Brookston, Dekalb, and Jimtown soils, respectively.

All three isolates were buff-colored actinomycetes of somewhat similar morphology. On nutrient agar BE1 was a small, flat colony which did not exceed 1 mm in diameter; DE1 was a larger (2–3 mm), flat, drier-looking colony; and JE1 formed colonies which were intermediate in size but quite elevated. They were all gram positive short rods which formed branches that appeared to break up into coccoidal elements. The isolates all utilized glucose, sorbitol, glycerol, citrate, succinate, lactate, acetate, propanol, and propionic acid as sole carbon and energy sources, but grew poorly or not at all on xylose and formic acid. The fatty acid profiles obtained from these organisms identified them as members of the genus *Rhodococcus* but no species classification was possible.

Growth of two of the isolates at the expense of EPTC is shown in Figure 1. The starting concentration of 50 μg mL^{-1} was degraded by isolates JE1 and DE1 over an 8–12 h period with a simultaneous increase in the number of bacterial cells as evidenced by the increase in turbidity of the cultures. Control flasks demonstrated that loss of EPTC due to volatilization was minimal (<5%) over the course of the experiment. BE1 degraded EPTC at a similar rate (data not shown). These results are in agreement with those of Tam et al. (1987, 1988), who reported degradation of 30 μg mL^{-1} EPTC over an 8-h period with similar increases in cell density for their isolate, designated TE1, which was also a *Rhodococcus* sp. (originally identified as *Arthrobacter* sp.). Resting cell suspensions prepared from EPTC-grown cells of isolates BE1, DE1, and JE1, as well as those from the Canadian isolate TE1, degraded EPTC without a lag period at comparable rates (Figure 2). Again, loss of EPTC due to volatilization or chemical hydrolysis was minimal (<12%) over the course of this experiment.

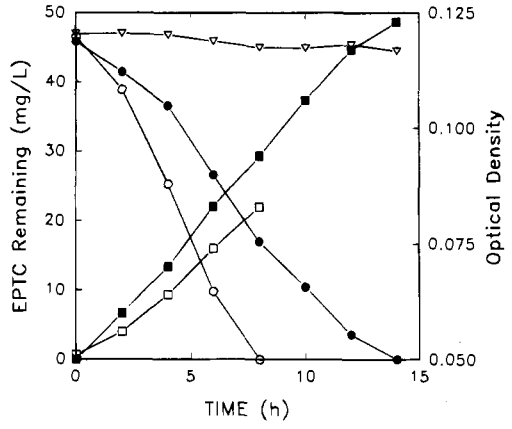

Figure 1. EPTC degradation and utilization for growth by wild-type isolates JE1 and DE1: (▽) EPTC remaining in solution in uninoculated control; (circles) EPTC remaining in solution; (squares) optical density (600 nm); (solid symbols) DE1; (open symbols) JE1.

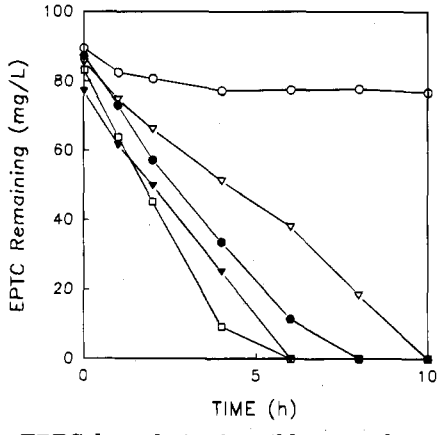

Figure 2. EPTC degradation by wild-type isolates in resting cell suspensions: (○) uninoculated control; (▽) DE1; (●) BE1; (▼) JE1; (□) TE1.

Genetic Analysis of EPTC Degradation. There was a high spontaneous loss of EPTC-degradation capability in the three newly isolated *Rhodococcus* strains when they were grown in nutrient broth for a short period (Table 1). Between 77 and 91% of the colonies isolated from nutrient broth exhibited poor or no growth on EPTC plates. Treatment with the plasmid curing agent, acridine orange, or an elevated temperature (32 °C) did not affect the frequency of loss of EPTC-degrading ability (Table 1). The EPTC$^-$ phenotype of

Table 1. EPTC$^+$ Colonies Obtained after Curing Treatments[a]

treatment	BE1	DE1	JE1
nutrient broth	9	14	23
acridine orange	7	14	15
32 °C temperature	7	16	22

[a] Of 100 colonies grid-plated from nutrient agar to BSME.

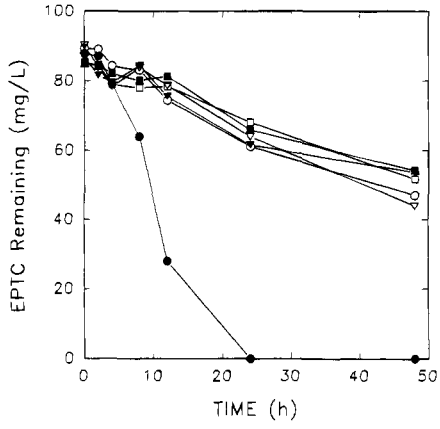

Figure 3. EPTC degradation by wild-type and cured strains of BE1: (○) uninoculated control; (●) wild-type BE1; (▽) mitomycin C; (▼) nutrient broth; (□) acridine orange; (■) 32 °C.

several of these isolates was confirmed by resting cell studies such as the one shown in Figure 3 for BE1. After a 2-h lag, the wild-type cells degraded EPTC in the medium at an accelerated rate while the EPTC$^-$ derivatives did not remove EPTC faster than the no-cell control. Both wild-type EPTC degraders and the EPTC$^-$ derivatives were grown in BSM with glycerol as the sole carbon source for these experiments. The fact that there was a lag period observed when glycerol-grown cells were used for resting cell suspensions, but there was no lag when EPTC-grown cells were used (Figure 2), suggests that the gene(s) encoding the EPTC degradation enzyme(s) in these isolates are induced by EPTC or some metabolite of EPTC.

Tam et al. (1987) demonstrated that the EPTC degradation phenotype was associated with a plasmid of 50.5 MDa (80 kilobases (kb)) with their isolate, TE1. In addition, Mueller et al. (1988) demonstrated that the genes encoding for the degradation of the carbamothioate herbicide, butylate, were encoded on a >100 kb plasmid in a strain of *Flavobacterium*. The presence of one or more of the genes required for EPTC degradation on plasmids in BE1, DE1, and JE1 might explain the high frequency of loss of the EPTC degradation phenotype in these strains. However, we have been unable to implicate a particular plasmid in the degradation of EPTC by the three *Rhodococcus* species we describe here. Nor were we able to isolate the 80 kb plasmid from isolate TE1 although the organism was capable of degrading EPTC. Several different plasmid isolation techniques were tried, and several plasmids were identified in these strains, however, we saw no differences in the plasmid profiles of EPTC$^+$ and EPTC$^-$ strains. Using a modification of the plasmid isolation method of Hansen and Olsen (1978), we consistently identified plasmids of >100 kb in size and one of 6 kb in both EPTC$^+$ cells and their EPTC$^-$ derivatives (data not shown). Agarose gel electrophoresis of plasmid DNA from each strain that had been digested with several restriction endonucleases, *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, *Bgl*II, *Dra*I, *Sal*I, *Sma*I, or *Ssp*I also failed to demonstrate any clear differences between EPTC$^+$ strains and their EPTC$^-$ derivatives (data not shown).

The plasmid profiles obtained from the three isolates, BE1, DE1, and JE1, were very similar to each other and to that which we obtained from the Canadian isolate, TE1 (Figure 4A). To determine whether these organisms were genetically similar, fragments of the small, easily isolated, 6 kb plasmid from DE1 and the Canadian isolate TE1 were used to probe Southern blots of plasmid DNA from each of the EPTC-degrading *Rhodococcus* strains in DNA/DNA hybridization experiments. As shown in Figure 4B, the fragment from TE1 (the organism from a Canadian soil with a 4-year history of EPTC use) hybridized to the 6 kb plasmid from BE1 (the organism from a Ohio soil with a 4-year history of EPTC use) but not the 6 kb plasmid from JE1 or DE1 (soils with no known history of EPTC use). The DNA fragment from DE1 hybridized to the 6 kb plasmid from JE1, but not to BE1 or TE1 (Figure 4C). It is interesting to note that the small plasmids from two strains isolated from soils with histories of EPTC use showed homology with one another but not with organisms from soils with no known EPTC history, however, the significance of this pattern of hybridization is unclear. This experiment demonstrates the potential folly in comparing the plasmid profiles of microorganisms from different sources. In this case, the small plasmids from all four organisms were identical in size (6 kb), and even showed an identical *Eco*RI restriction digest pattern. Without DNA/DNA hybridization studies or comparison of the restriction fragment pattern obtained with several restriction enzymes, these plasmids may have been mistaken as identical.

[^{14}C]EPTC Degradation Studies. Resting cell suspensions of isolates grown in BSM + glycerol were used for studies on the degradation of ^{14}C-carbonyl- and 1-^{14}C-propyl-labeled EPTC by isolates BE1 and the EPTC-deficient mitomycin C mutant, BE1MC. The data in Figure 5 show that there was little spontaneous breakdown of EPTC as less than 0.2% of the total label added was evolved as $^{14}CO_2$ over the course of the experiment in the no-cell controls. Levels of $^{14}CO_2$ similar to the no-cell controls were evolved from the EPTC$^-$ strain, BE1MC (data not shown). With the wild-type isolate, BE1, there was a lag period of approximately 4 h before $^{14}CO_2$ was evolved from the carbonyl-labeled EPTC, which was followed by a rapid release of $^{14}CO_2$ (Figure 5). Approximately 45% of the total activity was released as $^{14}CO_2$ by 50 h. However, when 1-propyl-labeled EPTC was used, there was a longer lag period of approximately 8 h before release of $^{14}CO_2$ was evident (Figure 5). This lag period was, again, followed by a period of rapid degradation and evolution of $^{14}CO_2$, with approximately 32% being released by 50 h. Because of the volatile nature of EPTC significant amounts of label were trapped in the foam plug used to capture undegraded EPTC vapors (data not shown). These data suggest that the EPTC molecule is first hydrolyzed at the ester linkage which would result in the formation of CO_2, ethyl mercaptan, and dipropylamine. The longer lag associated with the CO_2 release from the propyl-labeled compound indicates that induction of the biochemical mechanisms required for dipropylamine utilization is distinct from induction of the enzymes required for cleavage of the EPTC molecule at the carbonyl moiety.

When the cells were grown in BSM + dipropylamine rather than with glycerol as the carbon source a

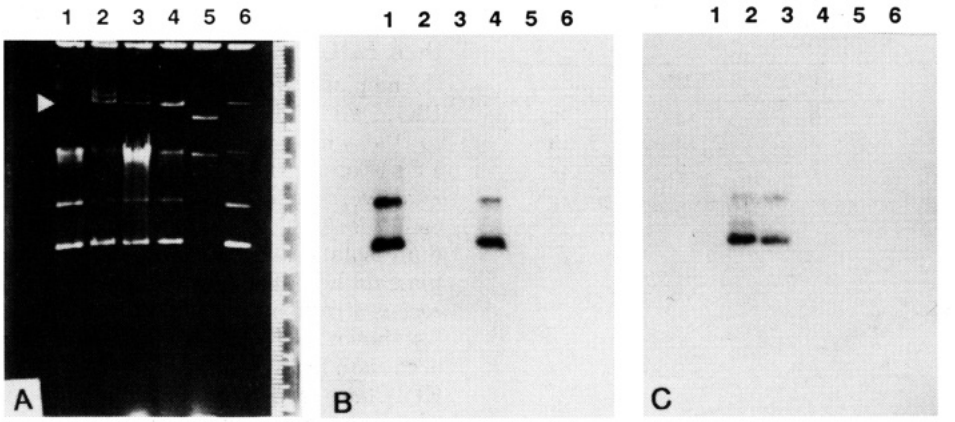

Figure 4. Genetic analysis of wild-type isolates: (A) plasmid profiles; (B) DNA hybridization with TE1; (C) DNA hybridization with DE1. (Lanes: 1, BE1; 2, DE1; 3, JE1; 4, TE1; 5, RP4; 6, R144. The uppermost bands in lanes 5 and 6 correspond to plasmids of 57 and 98 kb, respectively.

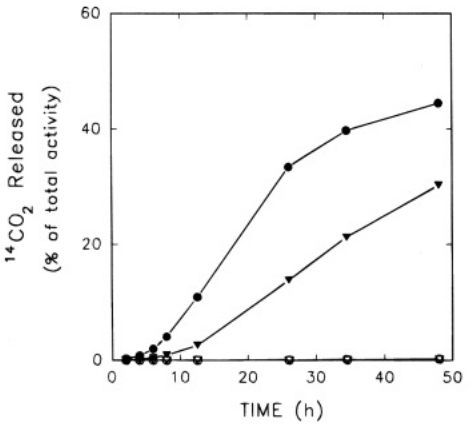

Figure 5. Degradation of [^{14}C]EPTC by wild-type strain BE1 and no-cell controls grown in BSM + glycerol: (○) control carbonyl label; (▽) control propyl label; (●) BE1 carbonyl label; (▼) BE1 propyl label.

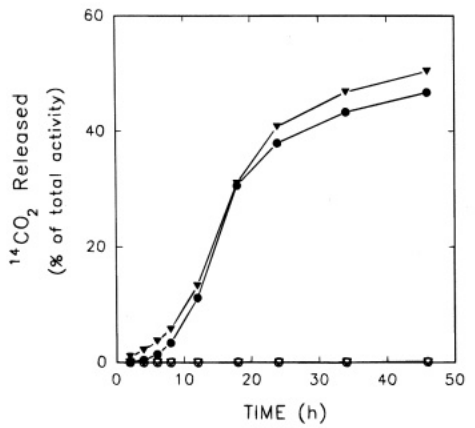

Figure 6. Degradation of [^{14}C]EPTC by wild-type strain BE1 and no-cell controls grown in BSM + dipropylamine: (○) control carbonyl label; (▽) control propyl label; (●) BE1 carbonyl label; (▼) BE1 propyl label.

different pattern of $^{14}CO_2$ evolution by isolate BE1 was observed (Figure 6). $^{14}CO_2$ was released at approximately the same rate from the two labeled compounds. At 46 h, 47 and 50% of the total activity added was evolved as $^{14}CO_2$ for the carbonyl- and propyl-labeled compounds, respectively. The same 4-h lag period was observed with the carbonyl-labeled EPTC, however, the lag observed for $^{14}CO_2$ release from the 1-propyl-labeled EPTC was also only 4 h. It appeared as though the cells which were grown on dipropylamine were preinduced for rapid degradation of the propyl side chain but that this degradation would only take place after the induction of enzymes needed to hydrolyze EPTC.

Little is known regarding the pathways of EPTC metabolism by microorganisms although decomposition in soils is thought to be microbially mediated (Kaufman, 1967; Fang, 1969; Lee, 1984). Fang (1969) proposed that carbamothioate metabolism by microorganisms could occur through hydrolysis at the ester linkage resulting in the formation of CO_2, a mercaptan, and an alkylamine. Sulfoxidation and hydroxylation reactions prior to carbamate linkage cleavage have been proposed for microorganisms (Wilson and Rodebush, 1987; Dick et al., 1990). Recently, Tal et al. (1989b) reported that a small fraction (1−3%) of the ^{14}C found in the organic extraction phase of soils receiving ^{14}C-carbonyl EPTC occurred as oxidized metabolites, the sulfoxide and the sulfone. The data presented in Figures 5 and 6 do not demonstrate whether oxidation to the unstable sulfoxide or sulfone occurs prior to cleavage of the carbamate linkage; however, they do suggest that cleavage occurs before degradation of the dipropylamine side chain. Dick et al. (1990) observed the presence of *N*-depropyl-EPTC in culture medium and suggested that more than one microbial degradative pathway may exist. Wilson and Rodebush (1987), working with the EPTC degradation inhibitor, dietholate, also have suggested that different pathways may be operable in history and nonhistory soils.

Conclusions. The isolation of several microorganisms capable of degrading EPTC from the same genus of actinomycetes from soils both with and without a history of EPTC application may suggest that EPTC-degrading actinomycetes are widespread in the environment. These isolates were capable of rapid degradation of EPTC and were able to use the herbicide as a sole source of carbon and energy. The instability of the EPTC-degrading trait suggests that the enzyme(s) may be plasmid-encoded, although plasmids responsible for EPTC degradation were not identified. [^{14}C]EPTC experiments suggested that EPTC degradation by isolate BE1 proceeds initially by cleavage at the carbonyl group followed by degradation of the dipropylamine side chain.

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