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Received for review June 5, 1989. Accepted December 15, 1989.

Registry No. PPO, 9002-10-2; ethanol, 64-17-5; 1-propanol, 71-23-8; 2-propanol, 67-63-0; 1-butanol, 71-36-3; 2-methylpropanol, 78-83-1; 2-butanol, 78-92-2; 2-methyl-2-propanol, 75-65-0; 1-pentanol, 71-41-0; 2-methylbutanol, 137-32-6; 2-pentanol, 6032-29-7.

Involvement of Microorganisms in Accelerated Degradation of EPTC in Soil

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Accelerated EPTC (*S*-ethyl dipropylcarbamothioate) degradation was confirmed in a mixed culture of microorganisms derived from a soil with enhanced degradation (history soil) by using ¹⁴C-labeled EPTC. The antibacterial agent chloramphenicol (*D*-(-)-*threo*-2,2-dichloro-*N*-[β -hydroxy- α -(hydroxymethyl)-*p*-nitrophenethyl]acetamide) markedly suppressed ¹⁴CO₂ evolution while the antifungal agent cycloheximide (4-[(2*R*)-2-((1*S*,3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide) did not, suggesting that soil bacteria play a significant role in enhanced EPTC degradation. A fast EPTC bacterial degrader (FD1) strain and a slower one (SD1), which were isolated by a soil enrichment technique from a history soil, were capable of utilizing EPTC as a sole carbon source. Vernolate (*S*-propyl dipropylcarbamothioate), butylate (*S*-ethyl bis(2-methylpropyl)carbamothioate), or cycloate (*S*-ethyl cyclohexylethylcarbamothioate) were also degraded by these bacteria in a pattern similar to that in a soil with enhanced degradation. Inoculation of nonhistory soil with FD1 strain induced accelerated degradation of the herbicide in the soil at rates similar to those in field soils exhibiting EPTC accelerated degradation.

Accelerated biodegradation of pesticides in soil following repeated application and consequent loss of their efficacy against the target pest has been demonstrated with

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carbamothioate herbicides, as well as with many other pesticides (Kaufman et al., 1985; Roeth, 1986; Katan and Aharonson, 1989). This phenomenon has been shown to be linked with previous application of the same pesticide (history soils) or a structurally similar one and accompanied by an adaptation and/or enrichment of specific pesticide-degrading soil microorganisms. Although it is well accepted that soil microorganisms play a significant role in the degradation of soil-incorporated carbamothio-

ates (Kaufman, 1967; Wilkinson, 1988), few studies has been conducted to isolate and identify the microorganisms involved or to elucidate modes of degradation. Lee (1984) isolated EPTC (*S*-ethyl dipropylcarbamothioate) degrading microorganisms from history soil by enrichment culture techniques and found that the bacterial isolates lost their ability to degrade EPTC after prolonged storage. He hypothesized that this loss was due to the disappearance of some indigenous plasmid and concluded that the role of fungi in EPTC accelerated degradation is more important than that of bacteria. Only recently, Tam et al. (1987) and Mueller et al. (1988) have described the isolation from carbamothioate-adapted soils and characterization of bacterial strains that effectively degraded the herbicides and could grow on carbamothioates as a sole carbon source. Furthermore, they demonstrated that the degradative capability is associated with the indigenous plasmids harbored by these bacteria. Moorman (1988) found no significant difference in number of EPTC-degrading microorganisms in soils adapted or non-adapted to EPTC degradation. He suggested that the observed increases in the rate of EPTC metabolism were caused by physiological or enzymatic alterations, rather than an increase in degrader population. With use of soil perfusion and other enrichment methods, a variety of microbial degraders were isolated from soils with accelerated degradation of 2,4-D [(2,4-dichlorophenoxy)acetic acid] (Torstensson et al., 1975), carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) (Karns et al., 1986), aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxy] (Read, 1987), and isophenofos (isopropyl *O*-[ethoxy(isopropylamino)phosphonothioyl]salicylate) (Racke and Coats, 1987).

Recently, we reported (Tal et al., 1989a) the accelerated degradation of carbamothioate herbicides in Israeli soils following repeated use of vernolate (*S*-propyl dipropylcarbamothioate). We also investigated (Tal et al., 1989b) the fate of [¹⁴C]EPTC in these soils and examined the possibility of controlling the process by disinfection means and chemicals. The objectives of the current study were (a) to isolate EPTC-degrading microorganisms from soil that acquired accelerated degradation of vernolate, (b) to assess their ability to degrade other carbamothioate herbicides, and (c) to examine their capacity to degrade EPTC in nonhistory soil.

MATERIALS AND METHODS

Chemicals. Analytical (98–100%) EPTC and carbonyl-labeled [¹⁴C]EPTC (specific activity 10 mCi/mmol), were obtained from ICI Americas (Stauffer Chemical Co.). Cycloheximide (4-[(2*R*)-2-[(1*S*,3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexyl]-2-hydroxyethyl] glutarimide) and chloramphenicol (*D*-(-)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide) (Sigma) were also of analytical grade. The radioactive herbicide was purified by thin-layer chromatography (TLC) on precoated 0.25-mm Silica Gel F plates (Merck, Darmstadt, West Germany). The plates were developed to height of 15 cm with hexane-acetone (3:1, v/v) solvent system (*R_f* 0.80). The obtained radiopurity was greater than 98%.

Soils. Soil of Netzer-Sireni (Xerorthent; 71.8% sand, 8.9% silt, 19.3% clay, 1.4% organic matter; 20% field capacity, pH 7.6) with a 6-year history of vernolate treatments was collected from the depth of 5–10 cm. This soil has the capacity of enhanced degradation of EPTC (Tal et al., 1989a). Control (nonhistory) soil was taken from an adjacent field with no previous exposure to carbamothioates. The soils were screened through a 2-mm sieve, placed in polyethylene bags, and stored at 5 °C in the dark until used.

Isolation, Selection, and Enumeration of Microorganisms. The total numbers of bacteria + actinomycetes, fungi, and actinomycetes present in soils or in mixed cultures derived

from soils were determined by a standard plate dilutions technique. Aliquots (0.1 mL) of the dilutions were plated on the following selective media. Nutrient (8 g/mL; Bacto Nutrient Difco) agar was employed for culturing soil bacteria, rose bengal agar (Martin, 1950) was used for culturing soil fungi, and an alkalized water medium described by Ho and Ko (1979) was used for culturing actinomycetes. Counts were performed after incubation in the dark at 25 °C for 4, 7, and 10 days for bacteria, fungi, and actinomycetes, respectively. Degradation capacity was examined for microorganisms isolated directly either from soil or from mixed cultures. In the first case, aliquots of each soil dilutions were spread on solid basal salts medium (BSM) (Spain et al., 1980), containing 700 mg of K₂HPO₄, 500 mg of NH₄Cl, 112 mg of MgSO₄·2H₂O, 14 mg of CaCl₂, 5 mg of ZnSO₄, 2 mg of Na₂MoO₄·2H₂O, and 50 mg of EPTC/L of distilled water (pH 7.0). After 5 days of incubation, 50 individual bacterial colonies from each soil were chosen randomly and transferred to nutrient medium supplemented with 50 μg/mL EPTC. In the second case, in order to isolate possible EPTC degraders from mixed cultures, aliquots were spread on solid BSM supplemented with EPTC at 50 μg/mL. Sixty bacterial colonies and 25 fungal colonies from each mixed culture were chosen randomly and subcultured on the appropriate selective media supplemented with EPTC at 50 μg/mL. The obtained individual cultures in both cases were further examined for their ability to degrade EPTC, as described below.

Enrichment Cultures and [¹⁴C]EPTC Degradation in Mixed Culture. An aqueous suspension of soil microorganisms was prepared by shaking a 50-g sample of soil with 200 mL of BSM supplemented with EPTC at 50 μg/mL. After 12 h of shaking at 25 °C, the soil was separated from supernatant by centrifugation (1000g × 5 min). A 50-mL aliquot of the supernatant was placed in a biometer flask (Bartha and Pramer, 1965) containing 10 μg/mL analytical EPTC (in 0.5 mL of ethanol) and [¹⁴C]EPTC (0.5 μCi) dissolved in 0.5 mL of acetone. Both ethanol and acetone were evaporated from the flask prior to the addition of the culture suspension. Some of the flasks were also treated with aqueous solutions of either cycloheximide or chloramphenicol at a final concentration of 100 μg/mL. A 10-mL portion of CO₂ trapping solution (0.5 N KOH) was inserted in the side arm of the flask. Polyurethane plugs (20-mm diameter, 10-mm thickness, density 0.022 g/cm³) were placed in the two flask outlets to trap volatile substances as described by Kearney and Kontson (1976), and the flasks were incubated in the dark at 25 °C under continuous shaking. The trapping solution was changed periodically, and aliquots (0.5 mL) were transferred to vials containing scintillation fluid (4.5 mL; Insta Gel II, Packard). Radioactivity was measured by liquid scintillation counting (LSC), with use of a liquid scintillation spectrometer (Kontron Betamatic). LSC data were corrected for quenching and background. Simultaneously, the polyurethane plugs in each flask were changed, cut to small pieces, and shaken in 20 mL of hexane. After 30 min, 0.5 mL of extract was taken for ¹⁴C quantification by LSC. In a preliminary experiment using TLC, we confirmed that the radioactivity extracted from the polyurethane plugs was [¹⁴C]EPTC, and only traces of [¹⁴C]-EPTC were detected in the KOH solution. The amount of radioactivity remaining in culture was determined periodically in aliquots (0.5 mL) by LSC.

Carbamothioate Herbicide Degradation in Culture and Bacterial Growth Assays. Bacterial isolates were each inoculated into culture tubes (25-cm height and 15-mm diameter) containing 20 mL of BSM supplemented with 50 μg/mL carbamothioate herbicide as the sole carbon source. The tubes were stoppered with aluminum foil wrapped corks and incubated on a rotary shaker at 80 rpm in the dark at 25 °C. Periodically, samples (5 mL) of the culture were withdrawn, transferred to a vial, and vigorously shaken with 10 mL of hexane. After partitioning, aliquots (5 μL) of the upper layer (hexane) were analyzed by gas-liquid chromatography (GLC) and the percentage of remaining herbicide (as percent of applied) was determined. In this system in which closed tubes were used, herbicide volatilization was negligible. In parallel to EPTC degradation analyses, bacterial growth studies were also performed. Aliquots (0.5 mL) of the culture used for GLC analyses were serially diluted, plated on nutrient agar medium, and

counted for viable cells as previously detailed.

To determine whether fungal isolates have the ability to degrade EPTC, each was grown on solid BSM (20 mL) containing 50 $\mu\text{g}/\text{mL}$ EPTC. Periodically, five agar disks, 10-mm diameter, were removed and extracted in 10 mL of hexane. Aliquots (5 μL) of the hexane layer were analyzed by GLC.

Characterization of EPTC Degraders and Their Maintenance. Bacterial colonies utilizing EPTC as a sole carbon source were characterized with standard microbial characterization techniques (Buchanan and Gibbons, 1974). The pure cultures were grown on nutrient agar supplemented with 50 $\mu\text{g}/\text{mL}$ EPTC and stored at 4 $^{\circ}\text{C}$ until use.

Conditioning Accelerated EPTC Degradation in Non-history Soil by Inoculation with Bacteria. Soil (1000 g) from Netzer-Sireni that had never been exposed to carbamothioates (nonhistory) was treated with 10 $\mu\text{g}/\text{g}$ EPTC and enriched with bacterial cell suspension (10^7 cells/g of soil; OD 0.2 at 420 nm) of the indicated strain. The soil was thoroughly mixed, moistened to 75% of field capacity, and incubated in the dark at 25 $^{\circ}\text{C}$. Periodically, the whole volume of soil was thoroughly mixed and subsamples of soil (20 g) were removed and extracted with a 60-mL mixture of acetone-hexane (1:2, v/v) for 1 h on a rotary shaker. Distilled water (80 mL) was added and the shaking continued for an additional 30 min. After soil particles had settled, 25 mL of the upper layer (hexane) were sampled, filtered through a filter paper (Whatman No. 1), and dried over anhydrous sodium sulfate. When required, the filtrates were concentrated on a vacuum rotary evaporator at room temperature. To determine the EPTC residues, aliquots (5 μL) of hexane extract were analyzed by GLC.

Gas-Liquid Chromatography (GLC). The carbamothioate herbicides were determined by using a Varian Model 6000 chromatograph equipped with a thermionic specific detector and a glass column (180 \times 0.2 cm (i.d.)) packed with 3% OV-17 on 80-100-mesh Gas Chrom Q. Temperatures of the injection port, column, and detector were 220, 145, and 230 $^{\circ}\text{C}$, respectively. Gas flow rates were 4.5, 175, and 40 mL/min for hydrogen, air, and nitrogen, respectively. The peak heights of samples were measured and compared with those obtained from similar injections of solutions containing known concentrations of herbicide. In cultures spiked in the range 0.5-50 $\mu\text{g}/\text{mL}$, the recoveries of EPTC, vernolate, butylate (*S*-ethyl bis(2-methylpropyl)-carbamothioate), and cycloate (*S*-ethyl cyclohexylethylcarbamothioate) were $92 \pm 5\%$, $89 \pm 6\%$, $90 \pm 4\%$, and $87 \pm 5\%$, respectively, and the limit of detection was approximately 0.01 $\mu\text{g}/\text{mL}$.

Experimental Design and Data Analyses. Experiments were in a completely randomized design with three replications. Data were analyzed by the *F*-test and Duncan's multiple-range test. All experiments were conducted three times with similar results. Results shown represent data from one of three experiments.

RESULTS AND DISCUSSION

Quantification of the Microbial Populations in Soils. In a previous study we have shown that soil microorganisms are involved in the accelerated degradation of EPTC and other carbamothioate herbicides (Tal et al., 1989b). The total numbers of bacteria + actinomycetes, fungi, and antinomycetes was similar in both history and nonhistory soils not previously treated with herbicide (Figure 1). The overall numbers of these microorganisms were in the range commonly found in cultivated soils (Alexander, 1977). In order to assess directly the numbers of microbial degraders in the soil, individual colonies of bacteria, growing on dilution plates from both soils, were tested for their capacity to degrade EPTC. A total of 50 isolates of bacteria from each soil were tested, but none was found to degrade the herbicide. This might be due to the low proportion of the degraders in the total population of microorganisms. Moorman (1988), using the most probable number method, did not find an increase in numbers of EPTC degraders in history soils. He suggested that accelerated degradation is a result of selec-

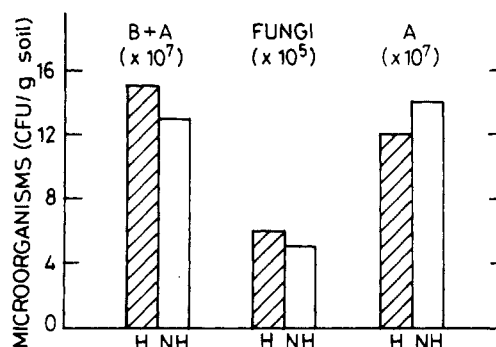


Figure 1. Counts of microorganisms in Netzer-Sireni soil with (H) or without (NH) accelerated degradation of EPTC: B = bacteria; A = actinomycetes.

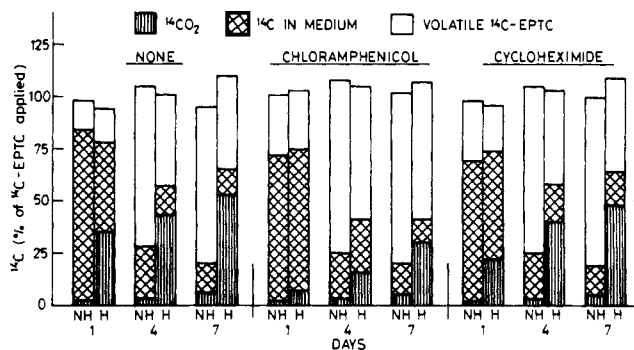


Figure 2. Effect of chloramphenicol and cycloheximide on the degradation of [¹⁴C]EPTC in mixed culture of microorganisms derived from Netzer-Sireni soil with (H) or without (NH) accelerated degradation of EPTC.

tive stimulation of specific microbial groups present among the general population and/or of physiological and enzymatic alteration in the adapted microflora. Therefore, in the following studies, soil enrichment methods were employed to magnify the population of the EPTC degraders.

Degradation of [¹⁴C]EPTC in a Mixed Culture of Soil Microorganisms. [¹⁴C]EPTC was applied to mixed cultures of microorganisms derived from history soil. During 7 days of incubation, over 50% of the [¹⁴C]EPTC was evolved as ¹⁴CO₂ (Figure 2), whereas in cultures derived from nonhistory soil only 5% of the applied radioactivity was released as ¹⁴CO₂ after a similar incubation period. Simultaneously, the radioactivity content of the mixed-culture medium declined with time of incubation. After 1 day of incubation, the amount of soluble ¹⁴C in the medium was 39% lower in the history soil as compared to nonhistory soil, apparently due to rapid conversion of EPTC to CO₂. The amount of volatile [¹⁴C]EPTC captured in the polyurethane traps was lower in the culture derived from history soil (Figure 2). Addition of the antibacterial antibiotic chloramphenicol to the culture inhibited ¹⁴CO₂ evolution from cultures derived from history soil. The antifungal antibiotic cycloheximide slightly affected ¹⁴CO₂ evolution during the first day of incubation, while no inhibitory effect was observed after 4 and 7 days of incubation. A similar effect regarding chloramphenicol and cycloheximide was also observed in a previous study of EPTC degradation in a soil with enhanced degradation (Tal et al., 1989b). Similar results were reported by Racke and Coats (1987) with the insecticide isophenofos.

Quantification of Soil Microorganisms and EPTC Degraders in Mixed Culture. Enumeration of the microbial flora present in the EPTC enriched medium following 7 days of incubation indicates that the total

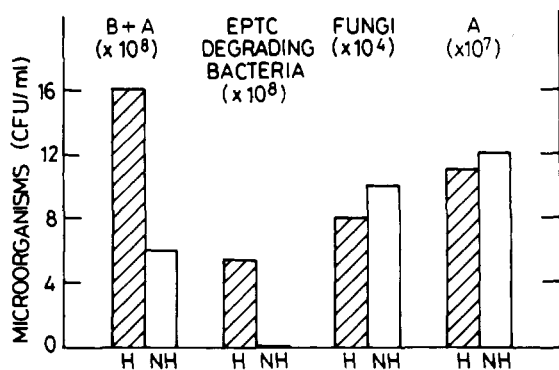


Figure 3. Counts of microorganisms in mixed culture supplemented with 50 µg/mL EPTC from Netzer-Sireni soil with (H) or without (NH) accelerated degradation of EPTC: B = bacteria; A = actinomycetes.

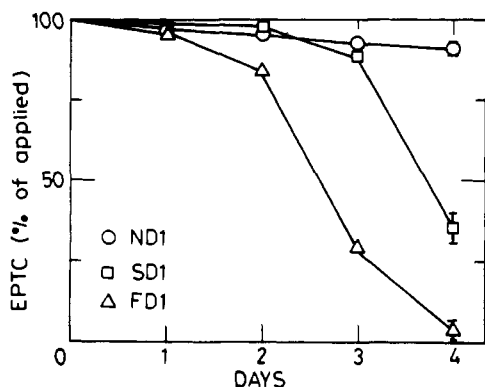


Figure 4. EPTC degradation by three soil bacterial strains, in a basal salts medium culture where the herbicide was the sole carbon source. Results are presented as percentage of noninoculated control (in which 4% of the applied EPTC dissipated during 4 days). Key: ND1 = nondegrader strain; SD1 = slow degrader strain; FD1 = fast degrader strain. Vertical bars represent 1 SE.

number of bacteria + actinomycetes present in the medium derived from history soil was significantly ($P = 0.05$) higher than that found in culture derived from nonhistory soil (Figure 3). Sixty colonies of bacteria were randomly sampled from the mixed culture derived from either history or nonhistory soil, and each was examined for their capacity to degrade EPTC. Eighteen colonies (30%) derived from the mixed culture of history soil were found to be EPTC degraders, while no such degraders were detected among a similar number of colonies sampled from nonhistory soil (nondegrader = ND). Accordingly, the number of EPTC degraders in the culture was calculated from the total population as shown in Figure 3. These numbers do not represent the actual size of the population of the degraders in soil since the assessment was done in an enrichment culture. However, they indicate that the relative abundance of degraders in a history soil is much higher than that in a nonhistory soil, in which the population of the degraders was below the threshold of detection. Of the 18 isolates that were found to be EPTC degraders, 12 of them degraded EPTC relatively faster (fast degrader = FD) than the other six isolates (slow degrader = SD). The rate of degradation by bacterial FD and SD isolates is demonstrated in Figure 4; 50% of the applied EPTC was degraded within 2.5 and 3.8 days, respectively, whereas the degradation by ND isolate was negligible. All the FD isolates were Gram-positive, rod-shaped bacteria. All the SD isolates were Gram-negative. It is yet unknown whether isolates of the bacterial degraders represent a single organism or a variety of organisms. In this study, sealed tubes were used for

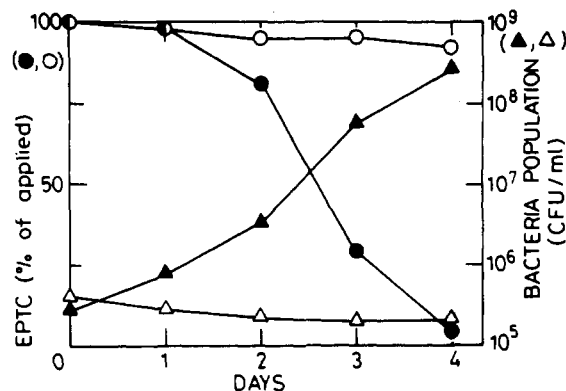


Figure 5. Degradation of EPTC and growth of bacteria in culture where the herbicide was the sole carbon source. The results for EPTC degradation are presented as a percentage of noninoculated control (in which 4% of the applied EPTC dissipated during 4 days). Key: Open symbols = nondegrader isolate; closed symbols = fast degrading isolate.

bacterial growth, and therefore loss due to volatilization was negligible.

There were no significant ($P = 0.05$) differences between mixed cultures derived from either history or nonhistory soil regarding the numbers of fungi or actinomycetes. The population of bacteria in history soil was 10-fold higher than those of actinomycetes. Most of the fungi belonged to *Penicillium sp.* and *Fusarium sp.* None of the 25 isolates of fungi from each soil that were examined were found to be able to degrade EPTC.

It should be noted that, in spite of the important role of bacterial degraders in the enhanced degradation process, they occupy only a small fraction of the total microflora in the soil since none of the 50 bacterial isolates that were directly isolated from a history soil could degrade EPTC. Therefore, the use of enrichment culture was necessary for the detection of the bacterial degraders. Numerous enrichment methods, in both soil and culture, were used to isolate microorganisms associated with enhanced degradation of EPTC (Lee, 1984; Tam et al., 1987; Moorman, 1988), 2,4-D (Torstenson et al., 1975), carbofuran (Karns et al., 1986), isophenofos (Racke and Coats, 1987), and aldicarb (Read, 1987). Lee (1984) isolated from EPTC history soil fungi and bacteria belonging to various species and concluded that fungi are more dominant than bacteria in EPTC degradation. In the present work we could not detect fungal EPTC degraders. Recently, Tam et al. (1987) reported on the isolation and genetic characterization of a bacterial strain that was able to degrade EPTC effectively. Initially, this strain was determined as an *Arthrobacter sp.*, but later it was identified as a *Rhodococcus sp.* (Tam et al., 1988). Mueller et al. (1988) have isolated a *Flavobacterium sp.* strain capable of utilizing carbamothioate herbicides as a primary carbon source. Both works linked the bacterial carbamothioate-utilizing abilities with plasmid involvement. The possible association of degradation capacity of the bacteria found in our study with plasmids deserves to be investigated.

Degradation of Carbamothioate Herbicides and Growth of Degraders in Culture with Herbicide as the Sole Carbon Source. The fast-degrading bacterial strain FD1 degraded EPTC very quickly, and after a short lag period of 1 day, the degradation increased until all the EPTC was completely exhausted after 4 days of incubation (Figure 5). Similar rates of degradation of EPTC were recorded for the other FD strains (data not shown). Counts of the viable cells showed that the bacterial population increased 1000-fold during 4 days of incubation.

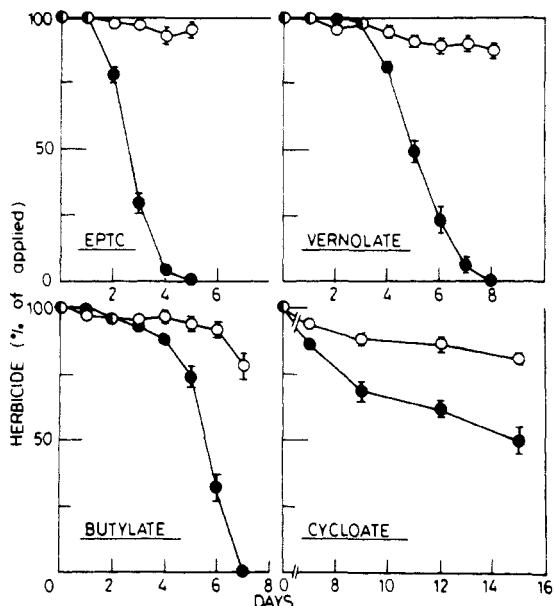


Figure 6. Degradation of carbamothioate herbicides in basal salts medium by a fast-degrading isolate (●) and by a nondegrading isolate (○). Results for each herbicide are presented as a percentage of noninoculated control. Dissipation from noninoculated control for EPTC during 5 days, vernolate during 8 days, butylate during 7 days, and of cycloate during 15 days were 4, 6, 6, and 8%, respectively. Vertical bars represent 1 SE.

In contrast, only a small decrease in the amount of the applied EPTC (ca. 5%) was observed in the culture of the nondegrading bacterium (ND1), with no significant increase in their number.

When FD1 and ND1 isolates were grown on either EPTC, vernolate, butylate, or cycloate as the sole carbon source, EPTC dissipation was the fastest with a 1-day lag period and half-life ($t_{1/2}$) of 2.5 days (Figure 6). Vernolate and butylate were degraded at a lower rate with 3–4 days of a lag period and a $t_{1/2}$ of 5 and 5.5 days, respectively. Cycloate dissipation, however, was much slower with a $t_{1/2}$ of 15 days, indicating structural specificity of the microbial enzymatic system responsible for the degradation process. Very small dissipation of carbamothioate herbicides was detected in the case of nondegrading isolate. These results are in accordance with our previous observation on the dissipation rate of different carbamothioate herbicides in vernolate history soil (Tal et al., 1989a). This cross-capacity to degradation may also indicate the possible utilization of alternative substrates as energy sources for the degraders when the challenge compound is absent.

Induction of EPTC-Enhanced Degradation in Nonhistory Soil by Inoculation with EPTC Degraders. Inoculation of nonhistory soil with the EPTC degrader (FD1) accelerated degradation of EPTC and resulted in its complete disappearance within 3 days (Figure 7). On the other hand, noninoculated soil or soil inoculated with nondegrading bacteria (ND1) resulted in a slow and normal dissipation rate of the herbicide so that after 12 days of incubation 30% of the EPTC was still present. Thus, inoculation of a nonhistory soil by an isolate of bacteria capable of rapid degradation of carbamothioate herbicides in culture also induces accelerated dissipation of EPTC at a rate similar to that found in history soil.

Several findings in this study support the conclusion that bacteria are important in the accelerated degradation of EPTC in soil. The involvement of bacteria in the enhanced degradation of EPTC was shown by test-

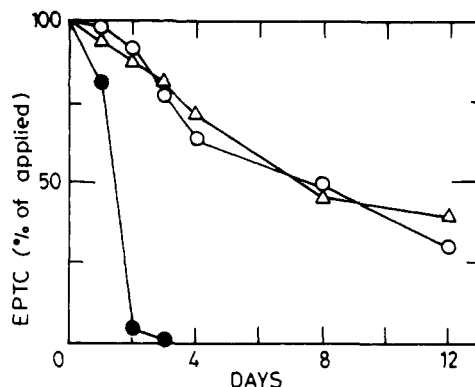


Figure 7. Dissipation of EPTC from nonhistory soil from Netzer-Sireni inoculated with a fast-degrading bacterium at 10^7 CFU/g (●), noninoculated soil (△), and soil inoculated with a nondegrading isolate (○).

ing the degradation capacity of randomly chosen isolates of bacteria (Figure 3). Under these experimental conditions, fungi were not found to degrade EPTC. However, the possible involvement of actinomycetes in this process should not be excluded. Enhanced degradation in mixed cultures was inhibited by an antibacterial agent but not by an antifungal one both in culture (Figure 2) and in soil (Tal et al., 1989b). The pattern of degradation of various carbamothioates in culture of an isolate of a bacterial degrader (Figure 6) was similar to that observed in soil with enhanced degradation (Tal et al., 1989a). Degradation of [14 C]EPTC was associated with evolution of 14 CO₂ both in a field soil with enhanced degradation (Tal et al., 1989a) and in a mixed culture originated from history soil (Figure 2). The evolution of high quantities of 14 CO₂ is indicative of microbial involvement in the enhanced EPTC degradation process in the history soil. Finally, a nonhistory soil acquires enhanced degradation upon inoculation with a bacterial degrader (Figure 7). Our bacterial cultures still maintained their degradation capacity after 9 months of storage and several subculturings.

Our results may also suggest that by exploiting such microorganisms with high degradation capability (or enzymes derived from them) it could be possible to decontaminate undesirable pesticide residues from agricultural fields or from industrial spills, by inoculating with these microorganisms. However, the feasibility of such treatment under field conditions remains to be investigated.

ACKNOWLEDGMENT

We thank B. Miaullis of ICI Americas (Stauffer Chemical Co.) for supplying the [14 C]EPTC and to Palimport Ltd., Israel, for providing the formulated chemicals used in these studies. We thank G. Kritzman for his help in the characterization of the bacterial isolates.

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Received for review March 28, 1989. Revised manuscript received November 7, 1989. Accepted January 8, 1990. This study was supported in part by a grant from the National Council for Research and Development, Israel, and the Gesellschaft für Strahlen und Umweltforschung MbH, München, Germany.