APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1982, p. 514–516
0099-2240/82/080514-03$02.00/0

Vol. 44, No. 2

Biodegradation of 2,4,5-Trichlorophenoxyacetic Acid in Soil by a Pure Culture of *Pseudomonas cepacia*

D. K. CHATTERJEE, J. J. KILBANE, AND A. M. CHAKRABARTY*

Department of Microbiology, University of Illinois Medical Center, Chicago, Illinois 60612

Received 25 February 1982/Accepted 27 April 1982

A pure culture of *Pseudomonas cepacia* AC1100 was able to degrade and grow in presence of 2,4,5-trichlorophenoxyacetic acid in soil. At optimum temperature (30°C) and moisture content (15 to 50% [wt/vol]) strain AC1100 could degrade as much as 95% of 2,4,5-trichlorophenoxyacetic acid at high concentration (1 mg/g of soil) within 1 week.

The extensive use of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as a component of Agent Orange in Vietnam and as a herbicide in the United States and other countries has created toxicological problems (3) leading to a restricted usage of this compound in the United States and other countries (2). The persistence of 2,4,5-T has contributed significantly to this pollution problem since, until recently, this compound was known to be biodegraded very slowly and only by cooxidative metabolism (4, 6, 8). We recently reported the isolation of a strain of *Pseudomonas cepacia*, AC1100, capable of utilizing 2,4,5-T (in synthetic mineral media) as a sole source of carbon and energy (5). The effectiveness of genetically manipulated strains to degrade environmental pollutants from the open environment has been questioned because of the presence of various easily assimilable carbon sources in soil, as well as due to competition by indigenous microorganisms (7). Here we report the results of our investigation to determine whether strain AC1100 can efficiently degrade 2,4,5-T in soil and, if so, to determine the optimum conditions for degradation.

To test the ability of *P. cepacia* AC1100 to degrade 2,4,5-T in soil, topsoil from a flowerbed of the University of Illinois Medical Center campus was dried at 37°C, freed of stones and debris, and divided into two portions. Half of the soil was contaminated with 10 to 1,000 μg of 2,4,5-T (sodium salt) per g of soil, whereas the other half of the soil remained untreated. These soil samples were then used in experiments to determine the effect of 2,4,5-T concentration, bacterial concentration, moisture, and temperature on the ability of strain AC1100 to degrade 2,4,5-T in soil. Detailed methods for 2,4,5-T determination, chloride release, and viable cell counts have been described previously (5). Substantial degradation of 2,4,5-T in soil occurred in the presence, but not in the absence, of strain AC1100. Moreover, strain AC1100 was able to degrade 100 μg of 2,4,5-T per g of soil or 1,000 μg of 2,4,5-T per g of soil with almost equal efficiency, allowing 75% or greater degradation (as measured by spectrophotometric or gas chromatographic method or both) within 4 days (data not shown). Table 1 shows the effect on 2,4,5-T of varying the bacterial concentration in soil. The presence of strain AC1100 in the soil was required to obtain 2,4,5-T degradation, and increasing the strain AC1100 concentration increased 2,4,5-T degradation. The optimum temperature for the degradation of 2,4,5-T in soil by *P. cepacia* AC1100 was 30°C (Table 2), which is also the optimum temperature for the growth of strain AC1100 in a liquid or solid medium (5). The moisture content of soil was found to affect the efficiency of strain AC1100-mediated 2,4,5-T degradation, the optimal moisture content was found to be approximately 25%, and decreased

TABLE 1. Effect of various concentrations of *P. cepacia* AC1100 on the degradation of 2,4,5-T in soil[a]

Bacterium cells per g of soil	Incubation period (days)	2,4,5-T degradation (%)	
		Cl^- release	Physical loss[b]
	5	ND[c]	ND
2.2×10^5	5	54	50
2.2×10^6	5	63	66
2.2×10^7	5	78	81

[a] Sodium salt of 2,4,5-T was present at 1,000 μg/g of soil; the soil was hydrated to 20% of its dry weight and incubated at 30°C.

[b] The physical loss of 2,4,5-T reported is an average of spectrophotometric (absorbance at 288 nm) and gas chromatographic determinations as described previously (5), using soil extract as a control for both determinations. The lower limit of detection of 2,4,5-T by the spectrophotometric (Gilford model 2600) method was approximately 20 μg/ml in water or soil extract.

[c] ND, Not detectable.

TABLE 2. Soil degradation of 2,4,5-T at various temperatures and moisture contents by *P. cepacia* AC1100

Bacterium[a] cells per g of soil	Incubation temp (°C)	Soil water content (%)	2,4,5-T degradation (%)	
			Cl^- release	Physical loss[b]
2×10^{7}[c]	20	20	52	52
2×10^{7}[c]	30	20	82	73
2×10^{7}[c]	37	20	61	56
2×10^{7}[c]	42	20	30	32
2×10^{7}[d]	30	15	87	78
2×10^{7}[d]	30	25	100	95
2×10^{7}[d]	30	50	92	89
2×10^{7}[d]	30	75	60	58
2×10^{7}[d]	30	90	61	57

[a] Control experiments without strain AC1100 in the soil demonstrated no detectable loss of 2,4,5-T under identical conditions. The concentration of 2,4,5-T used was 1,000 μg/g of soil.

[b] The physical loss of 2,4,5-T reported was an average of spectrophotometric (absorbance at 288 nm) and gas chromatographic determinations as described previously (5).

[c] Bacteria were incubated for 5 days at different temperatures.

[d] Bacteria were incubated for 7 days at various moisture contents of the soil.

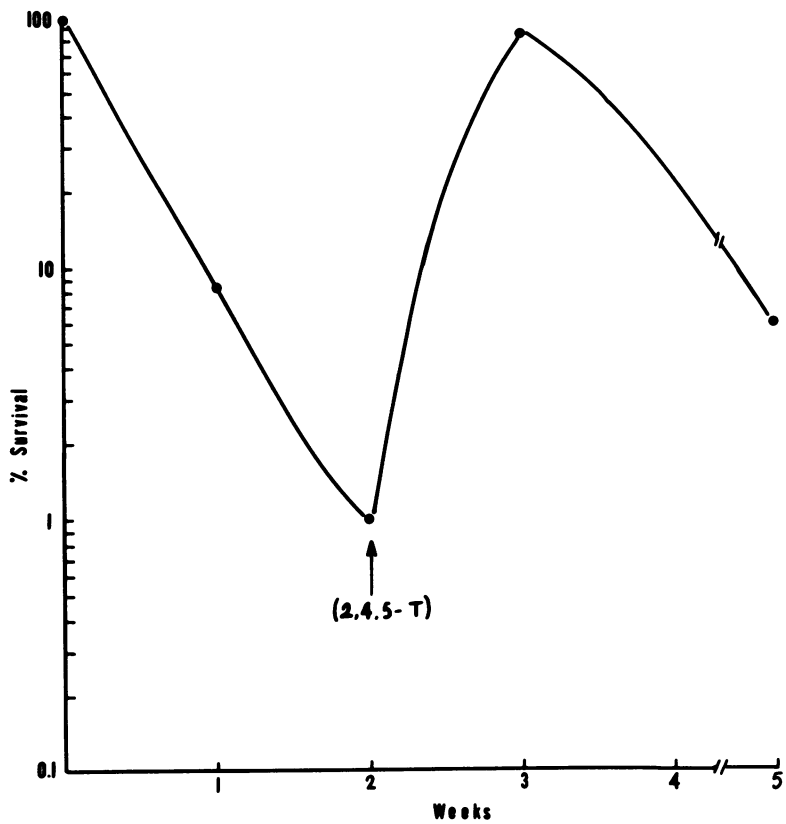

FIG. 1. Survival of strain AC1100 in soil in the absence and presence of 2,4,5-T. Soil containing no 2,4,5-T was inoculated with 6.7×10^6 AC1100 cells per g of soil and incubated for 2 weeks at 30°C with 20% soil moisture content, at which time 2,4,5-T was added at a concentration of 1 mg/g of soil and then incubated for an additional 3 weeks. At the start of the experiment and at the end of each 1-week interval a portion of the soil was removed, and the number of colony-forming units on a synthetic medium, with 2,4,5-T as the only source of carbon (5), was determined.

2,4,5-T degradation rates were found in soils containing either lesser or greater moisture contents (Table 2).

The survival of strain AC1100 in soil in the presence and absence of 2,4,5-T was monitored, and as Fig. 1 shows, the AC1100 population was highly dependent upon the concentration of 2,4,5-T in the soil.

In the absence of 2,4,5-T in the soil, the titer of strain AC1100 fell precipitously. However, the addition of 2,4,5-T at the end of the second week caused a rapid rise in the titer of strain AC1100, with the concomitant loss of 2,4,5-T during the next week (Fig. 1). The fact that the number of strain AC1100 cells increased dramatically upon the addition of 2,4,5-T illustrates that strain AC1100 was not merely surviving for a time in soil, but was capable of healthy growth in soil supported by the degradation of 2,4,5-T. Once the 2,4,5-T was exhausted (95% in about a week), a rapid decrease in the survival rate of the bacterium was observed.

In conclusion, our data demonstrate that a laboratory-bred bacterium capable of utilizing a known recalcitrant compound such as 2,4,5-T can effectively degrade and remove more than 95% of the compound from the soil within a week. The presence of indigenous microflora (determined at about 2×10^8 cells per g of soil) or various other carbon sources did not appear to significantly affect the ability of the strain to completely decompose 2,4,5-T from the soil. Previous studies on the decomposition of parathion by acclimated bacteria under field conditions have demonstrated the usefulness of such cultures in removing this compound from the contaminated areas (1). Our studies reconfirm the effectiveness of specially designed bacterial strains for the removal of toxic chemicals from the environment.

This research was supported by a grant from the National Science Foundation (PCM-13558) and by a Reproductive Hazards in the Workplace Research grant no. 15-2 from the March of Dimes Birth Defects Foundation.

LITERATURE CITED

1. **Barles, R. W., C. G. Daughton, and D. P. H. Hsieh.** 1979. Accelerated parathion degradation in soil inoculated with acclimated bacteria under field conditions. Arch. Environ. Contam. Toxicol. **8**:647–660.
2. **Firestone, D.** 1978. The 2,3,7,8-tetrachlorodibenzo-paradioxin problem: a review, p. 39–52. *In* C. Ramel (ed.), Chlorinated phenoxy acids and their dioxins, vol. 27. Ecol. Bull., Stockholm.
3. **Grant, W. F.** 1979. The genotoxic effects of 2,4,5-T. Mutat. Res. **65**:83–119.
4. **Horvath, R. S.** 1979. Microbial cometabolism of 2,4,5-trichlorophenoxyacetic acid. Bull. Environ. Contam. Toxicol. **5**:537–541.
5. **Kilbane, J. J., D. K. Chatterjee, J. S. Karns, S. T. Kellogg, and A. M. Chakrabarty.** 1982. Biodegradation of 2,4,5-trichlorophenoxyacetic acid by a pure culture of *Pseudomonas cepacia*. Appl. Environ. Microbiol. **44**:72–78.
6. **McCall, P. J., S. A. Vrona, and S. S. Kelly.** 1981. Fate of uniformly carbon-14 ring labeled 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid. J. Agric. Food Chem. **29**:100–107.
7. **Pierce, G. E.** 1981. Potential role of genetic engineering in microbial degradation, p. 204–210. *In* M. Keenberg (ed.), Proceedings of 1981 Battelle Conference on Genetic Engineering, volume IV. Battelle Seminar and Studies Program, Seattle, Wash.
8. **Rosenberg, A., and M. Alexander.** 1981. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) decomposition in tropical soil and its cometabolism by bacteria *in vivo*. J. Agric. Food Chem. **28**:705–709.