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KINETIC STUDIES OF DEGRADATION OF TRIPHENYLTIN PESTICIDES BY *PSEUDOMONAS PUTIDA* No. C

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ABSTRACT

A newly isolated bacteria, Pseudomonas putida *no. C, was able to degrade triphenyltin hydroxide (TPTOH), chloride (TPTCl) and acetate (TPTOAc) pesticides in vitro. Glucose was found to be necessary for the process. The rates of the disappearance of the pesticides parallel the rates of increase in the bacterial cell numbers. There was also a lag period of about 4 hours during the early stage of the degradation. Only a small amount of the pesticides were to be found attached to the cell surface and organelles.*

INTRODUCTION

Triorganotin compounds are widely used as pesticides and their fate in the environment is of important concern. Certain triorganotin compounds have been found to be degraded to less toxic di- and mono- tin derivatives in the soil.[1] A bacterial strain, *Pseudomonas putida* no.C, has been isolated from soil samples around Bangkok and it has been found that this bacteria is capable of degrading triphenyltin compounds *in vitro.*[2] This paper investigates the kinetics of the degradation of triphenyltin hydroxide, chloride and acetate by the bacteria, as well as the affect of various carbon sources towards the rates.

MATERIALS AND METHODS

Triphenyltin hydroxide (TPTOH) was obtained from Ventron Corp. Triphenyltin chloride (TPTCl) and triphenyltin acetate (TPTOAc) were obtained from Aldrich Chemical Co.

Minimal medium for the bacterial growth has the following composition (grams per liter): Na_2HPO_4 15 g, KH_2PO_4 3.0 g, NaCl 0.5 g, NH_4Cl 1.0 g, trace element 1.0 ml, 1.0 M $MgSO_4 \cdot 7H_2O$ 2.0 ml, 1.0 M $CaCl_2$ 0.1 ml. The pH was adjusted to 7.4.

10 ppm stock solutions of the triphenyltin pesticides (TPTOH, TPTCl, TPTOAc) were prepared by dissolving 10 mg of each compound in sufficient amount of 1.0% DMSO (dimethylsulphoxide) and the solution then added to 1.0 liter of the minimal

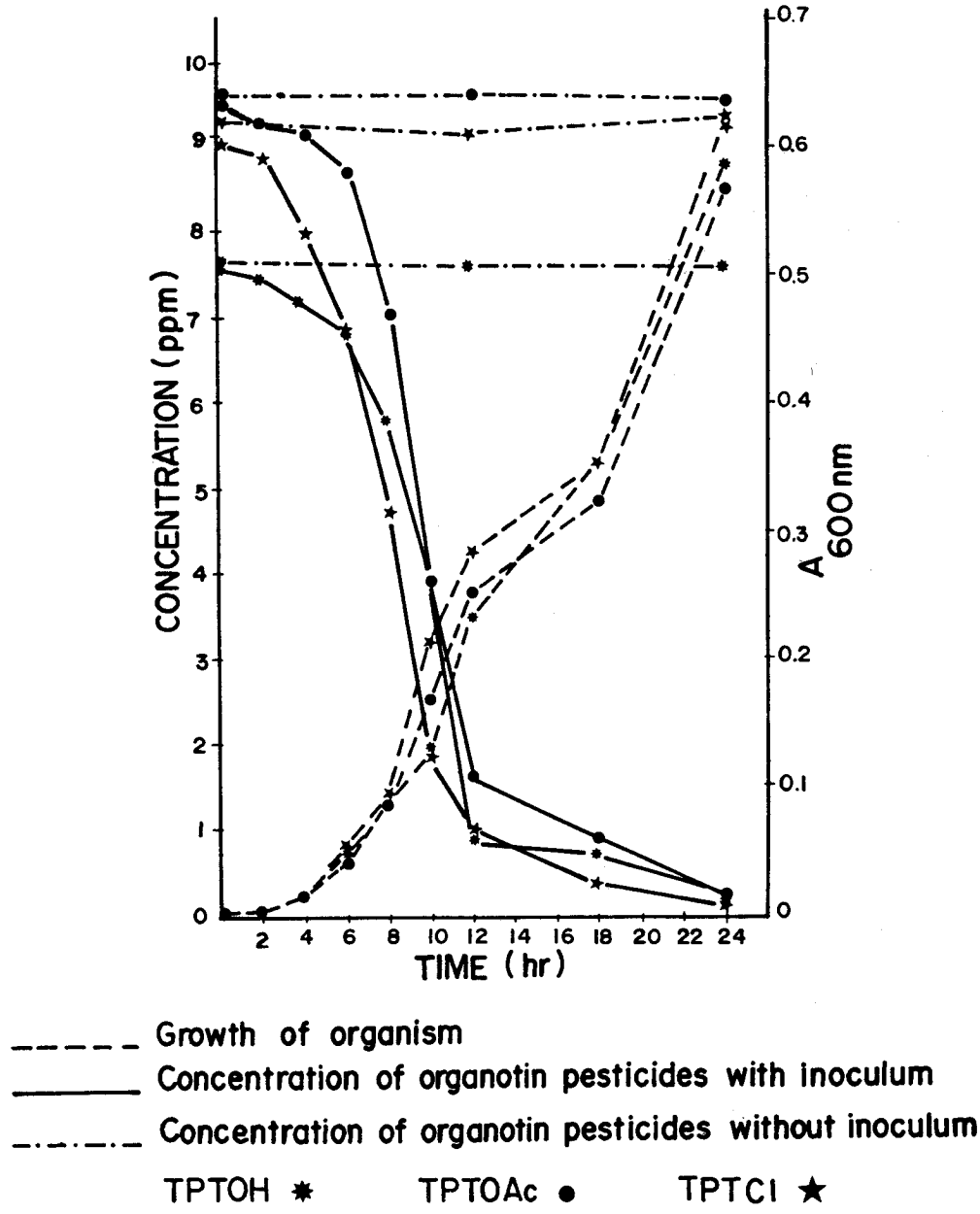

Fig. 1 Growth (--------) of *P. putida* no. C and the concentration of TPTOH TPTOAc, and TPTCl at various time intervals in incubation flask with inoculum (-----) and without inoculum (.....). TPTOH TPTOAc, TPTCl

medium. The medium was sterilized by filtering through sterile 0.45 μm Millipore membrane filter.

A. Kinetics of degradation of organotin pesticides by the isolated *P. putida* no. C.

The isolated bacteria was inoculated into 500 ml Erlenmyer flasks containing 150.0 ml minimal medium, 1% glucose, and a triphenyltin pesticide. These cultures were incubated at 30°C on a rotary shaker set at 200 rpm. Samples were withdrawn from each flask at 0, 2, 4, 6, 8, 10, 12, 18 and 24 hr after inoculation. Each sample was centrifuged at 10,000 rpm for 10 minutes. The supernatant was analysed for the amount of remaining pesticides by the spectrofluorometric method.[3] During the experiment, growth of the culture was also monitored by measuring the absorbance at 600 nm using Spectronic 20 Spectrophotometer (Milton Roy Co.).

The control flask received similar treatment, but was not inoculated with the bacteria.

B. Determination of the amount of triphenyltin pesticides absorbed by the isolated bacteria.

Biodegradation tests were performed as in section A. After 24 hr the sample was collected and centrifuged. The pellet was washed 4 times with the minimal medium. The washed media were separately collected and analysed for the absorbed amount of the triorganotin pesticide on the cell surfaces. The cells were then disrupted by sonication for 7.5 minutes using 30 second periods at 15 second intervals by using Soniprep (Soniprep 150 Ultrasonic Disintegrator, MSE Sonicator Instruments Manor Royal, England), and centrifuged afterward. The supernatent was analysed for the amount of the pesticide inside the cells. The debris was extracted with 3.0 ml toluene to remove triorganotin which might be bound to the cell's components. The extracted toluene was analysed by the spectrofluorometric method for the concentration of the triphenyltin pesticide.

C. Biodegradation of organotin pesticides in the presence of various carbon sources.

One percent of the following carbon sources namely, sucrose, xylose or starch were used instead of 1% glucose. Ten ml of samples were withdrawn from each flask at the beginning and the end of the incubation period (24 hr). They were then centrifuged, and the supernatants were analysed for the concentration of the organotin by the spectrofluorometric method.

RESULTS

A. Kinetics of the degradation of TPTOH, TPTOAc, and TPTCl by the isolated *P. putida* no. C.

The growth pattern of *P. putida* no. C and its ability to remove TPTOH, TPTOAc, and TPTCl from the medium are shown in Fig.1, which are the average values from three separate experiments. The concentrations of the three triphenyltin pesticides decreased with time, and this parallels the concurrent increase in the number of bacteria in the cultures. There is no significant difference between the decay plots for the three pesticides, and all show an initial lag period during the first 4-6 hours. All the pesticides (initial concentration 7.35, 9.25 and 8.90 ppm) were essentially degraded within 24 hours.

B. The absorption of the pesticides, TPTOH, TPTOAc and TPTCl, in various cell compartments of *P. putida* no.C

Experiments were carried out to determine the presence of the pesticides in the various cell compartments of the bacteria. After 24 hrs incubation with the pesticides, the cultures were divided into 4 fractions, namely, supernatant, the washing solutions (first, second and third), broken cells, and cell debris. The results are shown in Table 1. The amount of pesticide absorbed to the various cell compartments is highest for TPTOH (14%) and much less for TPTOAc and TPTCl (4.1% and 2.5%, respectively). In the supernatent there was only 2-3% of pesticides.

DISCUSSION

The kinetics of the degradation of the three triphenyltin pesticides by *P. putida* no. C show similar pattern. The concentration of the pesticides decreased with time whilst the bacteria grew in number. There was a lag period of about 4 hrs in the early incubation period in which the rate of growth of the bacteria and the rate of removal of the organotin compounds were low. This may be due to the need for the bacteria to adapt themselves to the new environment and consequently the reproduction rate is slow in this phase. Between 6-12 hrs there was a rapid increase in bacterial growth as well as a marked decrease in the pesticide concentration. Almost 80% of the organotin compounds have now been removed from the incubation medium. After 24 hrs essentially all the compounds have been degraded.

The result of this study agrees with that of Barug,[1] who reported the degradation of bis (tributyltin) oxides by *P. aeruginosa.*

The very similar kinetic patterns of the degradation of the three organotin pesticides would seem to indicate that the important determinant factor is the organic group. The anionic part of the compound (X group in RSnX) affects the solubility and volatilty of the compounds.[4] TPTOH has a much lower solubility in water than TPTOAc and TPTCl (< 1 ppm for TPTOH, 2.9 ppm for TPTOAc and 5.2 ppm for TPTCL.[3]) This may account for the higher amount of TPTOH found attached to the bacterial cells (see Table 1).

This experiment also showed that the triorganotin compounds that were removed from the incubation solution were not absorbed to any great extent to various cellular compartments of the bacteria. It is expected that the pesticides were degraded into di- or mono- tin compounds or other metabolites, which are not sensitive to the fluorescence technique used to monitor the triorganotin compounds. This result corresponds with that of Barnes *et al.,*[5] who studied the degradation of fungicide by fentin.

The capability of *P. putida* no. C to degrade the triphenyltin compounds requires the presence of a carbon source and it was found that glucose and sucrose are far superior than starch or xylose (see Table 2). Data in the literature concerning the effect of organic compounds on microbial degradation of xenobiotic are conflicting. Some authors report an inhibiting effect, whereas others show that they have a stimulating influence

TABLE 1 The amount of triphenyltin pesticides in supernatent and various cell fractions of *Pseudomonas putida* no. C.

Cell fractions	Amount of organotin					
	TPTOH		TPTOAc		TPTCl	
	ug	%	ug	%	ug	%
Supernatant	0.25	3.37	0.20	2.56	0.10	1.26
First washing	0.20	2.70	0.10	1.28	0.10	1.26
Second washing	0.15	2.02	0.05	0.64	0.04	0.50
Third washing	0.10	1.35	0.04	0.51	0.03	0.38
Broken cell	0.20	2.70	0.03	0.38	0.02	0.25
Cell debris	0.35	4.73	0.10	1.28	0.01	0.13
Control	7.40	1.00	7.80	1.00	7.95	1.00

TABLE 2 Removal of organotin compounds by *Pseudomonas putida* no.C using various carbon sources.

Carbon sources	Concentration of organotin								
	TPTOH			TPTOAc			TPTCl		
	ppm 0 hr	ppm 24 hr	%	ppm 0 hr	ppm 24 hr	%	ppm 0 hr	ppm 24 hr	%
glucose	7.4	0.1	98.6	7.8	0.1	98.7	7.95	0.05	99.4
sucrose	7.4	0.9	87.8	7.8	1.4	82.1	7.95	1.5	81.1
starch	7.4	4.45	66.9	7.8	2.8	64.1	7.95	3.45	56.6
xylose	7.4	6.45	12.8	7.8	8.8	12.8	7.95	7.0	11.9

on the biodegradation process.[6] This stimulating action has been named "cometabolism". For example, the herbicide ordram was degraded by cultures of *Bacillus sp.* in the presence of ethanol. Golovleva[6] mentioned that organic compounds of low molecular weight generally stimulate the biodegradation of xenobiotics. The results of this work supports this view, for glucose is a better carbon source than sucrose and both are more efficient than starch. However xylose, a smaller molecule than glucose, has been found to be much less effective than glucose. This may be explained by the fact that the bacteria may use a different metabolic pathway for xylose.

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