RESEARCH ARTICLE

BIOREMEDIATION OF CHLORPYRIFOS USING BACTERIA ISOLATED FROM PESTICIDE CONTAMINATED SOIL

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ABSTRACT

Chlorpyrifos, used as an agricultural pesticide is a cause of great environment pollution and toxicity. Hence exploration of various chlorpyrifos degrading organisms to clean up the pollutant is of immense importance. Present study shows the great efficiency of bacteria in degradation of chlorpyrifos from minimal media where chlorpyrifos is the sole carbon source available for their growth. Four bacterial species isolated from chlorpyrifos contaminated soil showed growth in simulated conditions. These bacterial species were further checked for the extent of degradation. All the four showed degradation above 70%. The most efficient one *Achromobacter spp*. was further studied in detail for degradation along with parallel study of bacteria *Pseudomonas aeruginosa* previously known for degradation capability. Both these species showed degradation above 80%. Selected bacterial species showed promising result in degradation which can be harnessed in clean up technology for chlorpyrifos contaminated environment.

Keywords : Chlorpyrifos, toxicity, bacteria, degradation and clean up technology

INTRODUCTION

Organophosphate pesticides constitute a group of widely used, very heterogeneous compounds that share a phosphoric acid derivative chemical structure (Abo-Amer, Aly E, 2011) wide use of organophosphorus pesticides has created numerous problems, due to environmental pollution. (Metin Diurak and Ferda Ukazanici, 2001).

Chlorpyrifos (CPF) is a type of organophosphorus pesticide and its chemical name is *O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate. It is used worldwide as an agricultural insecticide. The reported half-life of chlorpyrifos in soil varies from 10 to 120 days, with 3, 5, 6-trichloro-2-pyridinol (TCP) as the major degradation product. Attempts to isolate Chlorpyrifos degrading bacteria from chlorpyrifos treated soils have not been very successful. However, chlorpyrifos has been shown to be degraded co metabolically in liquid media by bacteria (Brajesh K. Singh, Allan Walker et al, 2003; Cho CMH, Mulchandani A & Chen W, Getzin LW, 2002).

It has been suggested that the accumulation of TCP, which has antimicrobial properties, prevents the proliferation of chlorpyrifos-degrading micro-organisms in soil (Racke *et al.*, 1990).

In general, microorganisms demonstrate considerable capacity to metabolize many pesticides. They possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds (Bhagobaty, 2007). Bacterial strains, such as *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas* diminuta strain MG, with the capability of hydrolyzing OPs such as diazinon and parathion, were isolated from soils in the Philippines and United States, respectively (McDaniel et al, 1988; Harper et al, 1988). Many microorganisms can specifically hydrolyze the phosphoester bonds of OPs and thus reduce the toxicity of OP pesticides and OP chemical

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warfare agents (e.g. sarin). Studies of Munnecke et al (1974) showed that the rate of enzymatic hydrolysis was two to 450 times faster than that of chemical hydrolysis, when parathion was used as a substrate. Considering that chlorpyrifos is one of the most commonly applied insecticides for control of pests and insects, the purpose of this experiment was to isolate and characterize chlorpyrifos degrading-bacteria, to investigate their degradation potential, to assess their adaptation to high concentrations of chlorpyrifos and to determine their usefulness in biodegradation of contaminated soil.

MATERIAL AND METHODS

Chemicals: Samples of chlorpyrifos (97.0 %, pure analytical grade) were obtained from Gharda Chemical Co., Ltd, India. All other Chemicals were also of analytical grade.

Isolation and culture conditions: The mineral salt medium (MSM, pH 7.2) was prepared by adding 1.5 g K_2HPO_4, 0.5 KH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0.5 g NaCl, 0.2 g $MgSO_4$, 0.02 g $FeSO_4$ into 1L of distilled water. The NA medium was prepared with distilled water containing 1 % Peptone, 0.5 % Meat Extract, 0.5 % NaCl, with pH at 7.

One gram of organophosphorus pesticides contaminated soil was added into the flask containing 100 mL MSM with chlorpyrifos at 0.5 mg/ml and cultivated for 3 days under shaking at 150 rpm at 30°C. Then medium from the flask was inoculated into fresh MSM with chlorpyrifos plates. The colonies on the MSM plate with 0.5 mg/ml chlorpyrifos at 30°C were observed after 24 hrs. Then the colonies were selected and purified and their degrading capability was further tested by inoculation in liquid medium. Chlorpyrifos residue was measured by High-performance liquid chromatography according to the method of CIPAC.

Identification of degrading bacteria: Isolated bacterial strains were identified from laboratory Metropolis Healthcare Ltd.

Inoculum preparation for degradation study: The isolated culture maintained on MSM agar with chlorpyrifos were cultured in the 250 mL flask containing 100mL NB medium supplemented with 0.5 mg/ml chlorpyrifos. Flasks were shaken at 150 rpm and at 30°C for 18 hrs. For all the experiment, 2 ml (OD 0.7nm) of this culture was used.

Bacterial growth and degradation of chlorpyrifos: Degradation experiments were conducted at 30°C and at 150 rpm in MSM supplemented with 0.5 mg/ml chlorpyrifos. The fresh MSM medium without the organism was used as control. The degradation efficiency of the strain isolated were determined and estimated by the removal percentage of chlorpyrifos from the liquid culture.

Extraction of samples for quantification: Samples were recovered from culture flasks at time interval of 0, 1 and 5 days and centrifuged at 10,000 rpm for 15 min to obtain cell free medium. It was further filtered with 0.3 micron filters.

Quantification of chlorpyrifos by HPLC: Chlorpyrifos residue was measured by High-performance liquid chromatography according to the method of CIPAC (CIPAC Handbook 1 C, p. 2028) with following conditions: column C18 (150 x 4.6 mm), programmable variable wavelength UV detector, flow rate: 1 ml/min, mobile phase: Acetonitrile + water + glacial acetic acid (82:17.5:0.5). Injection volume 20 mircolitre chlorpyrifos was detected at 230 wave length.

Selection of efficient strain and its degradation study in formulative market sample along with parallel run strain previously known for degradation : Bacterial strain which showed highest degradation was selected and studied for degradation of chlorpyrifos formulative market sample along with parallel run *Pseudomonas* species which is known to degrade chlorpyrifos. Degradation experiments were conducted at 30°C and at 150 rpm in MSM supplemented with 1 mg/ml formulative chlorpyrifos. The fresh MSM medium without the organism was used as control. The degradation efficiency of the strain isolated were determined and estimated by the removal percentage of chlorpyrifos from the liquid culture.

Extraction of samples for quantification: Left over chlorpyrifos in medium after 1st, 3rd, and 5th day was extracted by liquid extraction using hexane which was further dried and dissolved in acetonitrile and quantified by HPLC

RESULTS AND DISCUSSION:

Table 1: Growth response of four isolated bacteria

NO.	Growth in different concentration(mg/mL) of CPF					
	0.5		0.5		0.5	
B1	+	B1	+	B1	+	B1
B2	+	B2	+	B2	+	B2
B3	+	B3	+	B3	+	B3
B4	+	B4	+	B4	+	B4

(+) Growth (–) No growth

These isolates were identified as gram negative pathogenic organisms (Table 2)

Table 2: Identification of isolated soil bacteria

NO	SAMPLE NO.	NAME
1	B1	*Achromobacter spp.*
2	B2	*Enterobacter cloacae*
3	B3	*Stenotrophomonas maltophilia*
4	B4	*Pseudomonas aeruginosa*

Table 3: Percentage degradation of Chlorpyrifos by test organisms.

No.	Bacteria	24hours		120 hrs	
		Amount remaining in medium	% degradation	Amount remaining in medium	% degradation
1	*Achromobacter spp.*	16.86 %	83.14	0.81 %	99.19
2	*Enterobacter cloacae*	19.18 %	80.82	19.01 %	80.99
3	*Stenotrophomonas maltophilia*	7.32 %	92.68	3.8 %	96.2

Table 4: Percentage degradation of Chlorpyrifos by *Achromobacter spp.* and *Pseudomonas aeruginosa*

No.	Bacteria	% degradation in MS medium		
		24 hours	3rd day	5th day
1	*Achromobacter spp.*	79.46	80.79	87.4
2	*Pseudomonas aeruginosa*	10.40	83.92	84.612

Isolation, adaptation and identification of chlorpyrifos-degrading strain :

During primary screening four strains were isolated that were capable of utilizing chlorpyrifos (0.5 mg/ml) as the sole source of carbon. The isolates, designed B1, B2, B3, B4, were grown in different concentrations of chlorpyrifos (0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml & 3 mg/ml). The four isolated bacteria were adapted to grow in presence of chlorpyrifos and utilize it as sole carbon source for their growth. B1 showed to survive the highest concentration of CPF (Table1).

Biodegradation of chlorpyrifos: The amount of chlorpyrifos remaining in the medium after degradation of CPF was analyzed by HPLC analysis. It was observed that chlorpyrifos gradually decreased over 5 days. The observation (Table 4) showed efficient degradation by all the four bacteria. *Achromobacter* showed maximum degradation to 99.19 % followed by *Stenotrophomonas_maltophilia* 96.2 %, *Enterobacter cloacae* 80.99 % and least by *Pseudomonas aeruginosa* 77.14 %. Good amount of CPF was degraded within 24 hours except by *Pseudomonas aeruginosa*

Selection of efficient strain and its degradation study in formulative market sample along with parallel run strain previously known for degradation.

Achromobacter spp. was selected for further studies as it showed the highest degradation among the four isolated species (Table 3).

Achromobacter spp. was run parallel with *Pseudomonas aeruginosa* which is known to degrade chlorpyrifos to study degradation of formulative market sample of chlorpyrifos (Dursban Chlorpyrifos 20EC). Both the species (Table 4) showed degradation in formulative sample also. They showed degradation above 80%.

CONCLUSION:

The present study reports the isolation and identification of efficient chlorpyrifos degrading bacteria including *Achromobacter spp*. There were four isolates from soil which degraded (77 – 99 %) CPF within 5 days. Utilization of xenobiotic compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. Results from this study suggest that the isolated strains of bacteria are able to grow in medium in the presence of added pesticide and may therefore be used for bioremediation of pesticide contaminated soil. This leads us to believe that the soil with previous exposure to chlorpyrifos contains diverse range of bacteria and fungi having novel organophosphorus hydrolase enzyme system for carrying out enhanced biodegradation of this toxic pesticide.

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