MICROBIAL DEGRADATION OF CYPERMETHRIN BY SOIL ISOLATED BACTERIUM *Pseudomonas*sp

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ABSTRACT

The cypermethrin degrading bacteria was isolated from a pesticide contaminated soil(Agriculture field) by enrichment technique with the sole carbon source and energy. Morphological, physiological and biochemical characterization of the bacterium identified *Pseudomonas* species. Biodegradation of waste water containing cypermethrin at different concentrations like 25, 50,75 and 100 mg/l by *Pseudomonas* sp. has maximum reduction of COD at 89.60% and 26 % respectively. The Cypermethrin analysed by HPLC indicated that the isolated bacterium can be used to clean the contaminated pesticide waste water in the environment.

Key words: Biodegradation, Cypermethrin, *Pseudomonas*, COD Reduction, HPLC.

INTRODUCTION

The application of pesticides for pest control in various cropping (rice, sugar cane, paddy, peanuts, wheat, maize, corn etc.,) systems is a general practice in India from 1950's. Insecticides are the dominant group of pesticides used in developing countries like India. Since pesticides are very toxic, they have the potential to adversely affect the health of ecosystem. Insecticides such as Cypermethrin are a group of pyrithroids and they are highly toxic agricultural chemicals widely used in plant protection as well as in urban settings. The applied pesticides persist in the environment (air, soil & water) for variable periods of time. The World Health Organization (WHO, 1990) reported that only 2 to 3 % of applied chemical pesticides are effectively used for preventing, controlling and killing of pests, while rest remain in the soil (EPA2005).

Cypermethrin has beenreported as a highly toxic agent to fish and aquatic invertebrates(Bradbury and Coats, 1989) due to its high lipoaffinityand low solubility. Cypermethrin has beenclassified as a possible human carcinogen by US Environmentalprotection agency (EPA). It adversely affects thecentral nervous system and causes allergic skin reactionsand eye irritation. Problems arising due to toxicity andcarcinogenicity are causing concerns for human health,environment and ecosystem.

Microorganisms play a vital role in degrading pesticides residues (Thomas *et al.*, 1987). The species belonging to genera such as *Achromobacter, Alcaligenes, Arlhobacter, Bordefella, Flavobaclerium, Pseudomonas* and *Xanlhobacler* have been isolated from soil shown to degrade 2,4, dichlorophenoxy acetic acid and 2,4 - dichlorophenol in liquid nutrient media (Ishaq*et al.*, 1994; Guha*et al.*, 1997; Grant *et al.*, 2002. Lee *et al.*, 2004; Shanmugananthan&Mullai, 2009).

In view of this, biological method has been proven to be suitable for treatment of pesticide polluted aquifers that could be impleted either *in situ* or off site in especially designed reactor or waste water treatment plant (KumaranandSivaraman, 1988).Among the different genera of microorganisms for degradation of pesticides, genus *Pseudomonas* has a specialstatus, as the strain of *Pseudomonas* are known to metabolize broad range of organic compounds and utilizes more than100 different substrates as the sole source of carbon, nitrogen, sulfur (Dagley, 1986). Many efforts have beenundertaken to isolate bacteria, capable of biodegradation of synthetic pyrethroid insecticides and a lot of pyrethroiddegradingmicroorganisms have been isolated from polluted soil and water (Sakata *et al*.,1992; Maloeny*et al*., 1993;Halden*et al*.,1999; Nirmali*et al*., 2005; Jilani and Khan, 2006; Tallur*et al*., 2008; Murugesan*et al*., 2010a). Invitro studies have shown that microorganisms have capacity to degrade cypermethrin mainly Pseudomonasaeruginosa more efficacious than other microorganisms (Murugesan*et al*., 2010a and 2010b; Jilani and Khan,2006). These microorganisms were isolated from soil of the pretreated Cypermethrin cotton and Brinjal crop field(Murugesan*et al*., 2010b). In vitro study showed two soil bacteria that are able to degrade cypermethrin insecticide;they are the member of the genera *Pseudomonas* and *Serratia* (Grant, 2001; shanmugananthan and mullai, 2010).In the present study, we report the isolation of a Pseudomonas sp. capable of degradingcypermethrin at higher concentration both in wastewater aswell as in the soil.

MATERIALS AND METHODS

Commercial grade cypermethrin was obtained from local dealer, Laxmi Agro chemicals, Chidambaram, Tamil Nadu. The high purity of cypermethrin standards (99.6 %) was purchased from Sigma Aldrich Ltd., USA. Other chemical reagents and solvents used were of HPLC grade purchased from Ranboxy Ltd. Chennai and all the glassware used was supplied by Borosil, India.

Sample collection for enrichment studies: Soil used for the isolation of cypermethrin degrading bacteria was obtained from agricultural field in Vilagam village near Chidambaram, Tamil Nadu which had an almost 20 year's history of cypermethrin and other pesticides use in pest control activities. The surface soil (0-10 cm) in different places was removed using a spade and placed in plastic bags. The soil was transported to laboratory, sealed in bags and stored at 4°C in refrigerator until use.

Analytical procedure: The cell concentration in batch reactor was determined by weighing the cells retained after sample filtering in Whatman GF/F 0.7 pm filters. Alternatively, the cell growth was quantified by measuring the sample absorbance at 600 nm in a Beckman DU 650 spectrophotometer. COD was determined by potassium dichromate reflux method using Potassium dichromate COD analyzer as described in the standard methods for the examination of water and waste water (APHA *et al* 1998). PH of the sample was measured by pH controller (Model PH22). Cypermethrin was analyzed by High Pressure Liquid Chromotography (HPLC) (Shimadzu, Japan) consisted of a solvent delivery pump LC 20AT, connected with an auto injector model SIL- 6Aand a rheodyne injection valve fitted with a sample loop (20µl). Aguard column filled with pBondapake C18 reverse phase column, effluent was monitored by using UV detector (visible spectro photometer detector SPD-10) the out part of the detector was connected to a chomotoparc (CR6A). Mobile phase consisted for cypermethrin is Methanol and with alcohal. The flow rate was adjusted at 2 ml/min with total elution time of 20 min for each run. The calibration was formed by using pure cypermethrin as a reference for calibrating chart. 20 µl

of the standard and sample was injected in to the liquid chromatogram and peak areas were recorded. The system is suitable if these replicate injections of the standard have a relative standard deviation of 2.0 % or less.

Around 10 g of soil was added to 100 ml of basal medium containing (composition g/l) = 5.8g K,HPO_4,4.5g Kl l,PO_4,2.0 g($(NH_4)2SO_4$,0.16g $MgSO_4$, 0.02 g$CaCl_2$, 0.002 gNa_2MoO_2 0.001 g $FeSO_4$ and 0.001 g $MnCl_2$) with 10 g of cypermethrin and kept on an orbital shaker for 7 days at 150 rpm and ambient conditions. The microorganisms capable of growing in cypermethrin containing basal salt medium were further enriched in enrichment media (pH 6.6-6.8) was prepared (Sutherland *et al* 2000). Again 10 ml ofthe soil suspension in basal medium was transferred in to a fresh flask containing enrichment media with 25 mg/l cypermethrin. The cultures developed in the flask were transferred to fresh enrichment medium containing increased concentrations of cypermethrin till 100 mg/l. The cypermethrin tolerant cultures were selected for further degradation studies.

Experimental set up: Batch experiments were performed in flask containing 100 mg/l cypermethrin was added to enrichment medium inoculated with 1.0 OD (optical density at 600 nm) of culture. These flasks were incubated at ambient condition in static and shaking at 150 rpm for 15 days. A control (with cypermethrin and without bacterial culture) was maintained under similar condition to check the loss of cypermethrin. Samples were collected at 0, 4,7,11 and 15 days and analyzed for growth of microorganisms COD and cypermethrin levels by HPLC.

Optimum growth temperature and pH : The pH range and optimum pH for growth of isolated strain were determined by monitoring the growth (OD 600) of culture in enrichment medium containing maximum of 100 mg/l with different initial pH values (4-9). The temperature range and optimum growth temperature were also determined at 25,30,35,40 and 45°C.

RESULTS AND DISCUSSION

Isolation and identification of Cypermethrin degrading bacteria: Five morphologically different bacterial cultures were isolated from soil sample by enrichment technique in our laboratory. Among the five bacterial isolates, only one strain was growing best and utilized cypermethrin as a sole carbon source and energy was selected for further study. The morphological, physiological and biochemical features showed that it was a Gram negative, aerobic, short rod, mobile oxidase and catalase positive. It grew on Macconkey agar as lactase fermenting microorganism and utilized citrate as the carbon source. The isolate did not produce acid from sugar such as adonital, cellulobio.se, dulcital, insulin, maltose, rhanmose. On the basis of these above result, the bacterium was identified as *Pseudomonas* sp. (Fig.1). The strain could grow in the maximum concentration of cypermethrin (100 mg/l) containing nutrient agar medium in the range of pH (6-9) with optimum at pH 8 and growth was observed within the temperature range of20-45°C, while 35°C was the optimum temperature (data not shown). The several research studies also observed similar results of pH and temperature in high concentrations (Mayo and Noike, 1996; Jilani and Altafkhan, 2004; shanmugananthan and mullai, 2010).

Growth: The OD measurements at 600 nm showed a steady increase in bacterial mass (Fig. 2). Simultaneously HPLC and COD analysis showed a substantial reduction in the level of cypermethrin content (Table 1). A control test without adding pesticides were conducted in

nutrient broth and to evaluate the biodegradation of isolated strain when exposed to different concentrations of cypermethrin (25, 50, 75& 100 mg/l).

Table 1 Degradation of cypermethrin in the presence of *Pseudomonas* stain

S.No	Concentration of Cypermethrin (mg/l)		COD mg/l initial	COD mg/l final	COD degradation %	pH	HPLC %
	Before degradation	After degradation					
1.	25	1.7	5400	560	89.6	8.8	91
2	50	13	9800	2640	73	8.4	74
2.	75	42	13600	6940	48.9	8.1	44
3.	100	76	18400	13290	26	7.9	24

Fig.1 *Pseudomonas* species culture plate isolated from agricultural Held soil

On comparing the growth of isolated strain in the presence of Cypermethrin with that of control, it is observed that bacteria grew faster and OD values were increased upto 15 days of incubation, when 25 mg/1 of cypermethrin was used. Since it has been reported that the aqueous solubility of cypermethrin was very low (Jilani&Altafkhan 2006; Malik *et al.*, 2009), the growth of the strain in a medium containing 50 mg/1 similar up to 4 days incubation and the maximum OD value was 2.3. After 10 days, the growth of microorganism decreased until 15 days, whereas in 100 mg/1, the growth was slowed down, but no inhibition in the growth was observed (Fig. 2; Table 1).

COD and HPLC data: COD and 1IPLC analysis were carried out at fixed intervals as shown in Table 1 (Fig. 3). Different concentrations ranging from 25, 50, 75, and 100 mg/lit were tested. The COD decreased from 5400 to 560 (89.6 % degradation), whereas HPLC reduction was91

%for25mg/lit. When the initial OD was 9800, it was decreased to 2640 (74% degradation). After 15 days incubation, HPLC reduction was 74 % for 50mg/lit. Again the COD was 13600 to 6940 (48.9% degradation),whereas HPLC reduction was 48.9 % for 75mg/lit. The COD strength was high 18400 to 13290 (26 % degradation) and the HPLC data was 24 % observed.

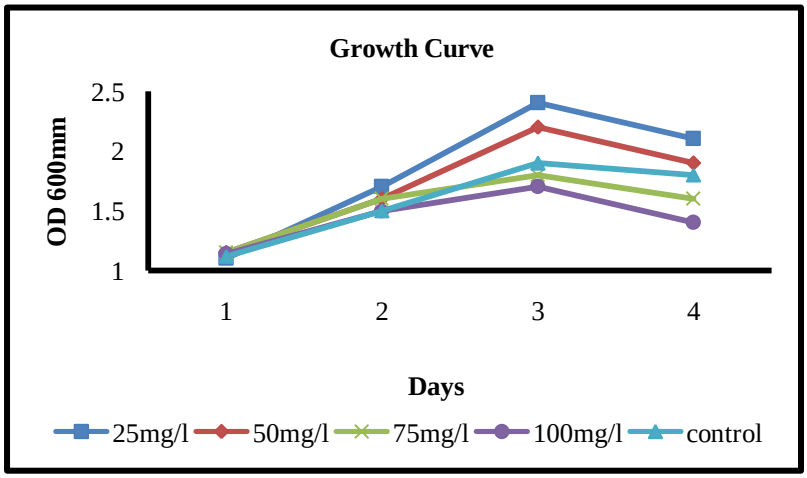

Fig. 2 Growth of *Pseudomonas* in the presence of cypermethrin

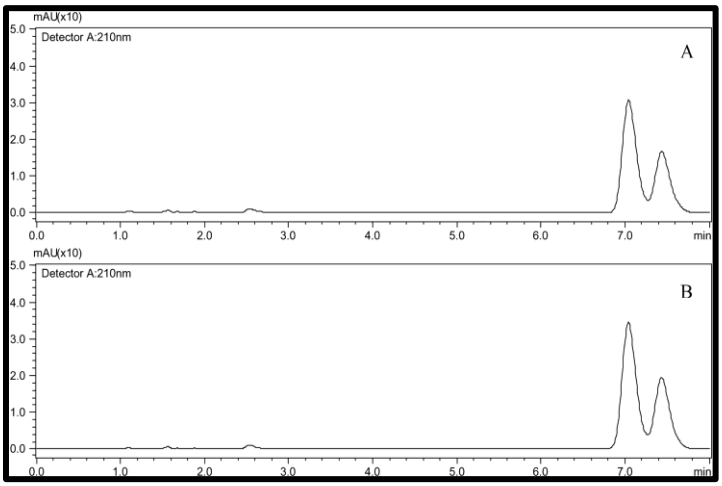

Fig. 3 HPLC chromatograms of cypermethrin standard (A) and cypermethrin pesticide (B)

The bacterial strain *Pseudomonas* sp was isolated from agricultural field and studied for cypermethrin degradation revealed that at high concentration the number of organism decreased or very slightly increased but no inhibition in the growth was observed when compared with control test. Hence, it is concluded that the isolated strain used in the present study has the potential for the treatment of cypermethrin contaminated water and soil.

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