Biodegradation of Cypermethrin by a Pseudomonas Strain Cyp19 and its use in Bioremediation of contaminated soil

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Citation

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Abstract

Pyrethoids are commonly used insecticide in both agricultural and urban environments. In this paper we describe isolation of a bacterium capable of degrading cypermethrin. The bacterium was isolated from a pesticide contaminated soil by enrichment technique with continuous pressure of cypermethrin as the sole carbon source and energy. Morphological, physiological and biochemical characterization of the bacterium indicated that it was a Pseudomonas species. The degradation ability of the isolate determined by resting cells study revealed that strainCyp19degrades cypermethrin upto 100% within two days. Degradation of cypermethrin in unautoclaved inoculated and autoclaved inoculated soil was 97.5% and 95% respectively after 30-days of incubation studies, whereas it was insignificant in unautoclaved (uninoculated) and autoclaved (uninoculated) soil. The addition of strain Cyp19 (10^6 cells g^{-1}) to soil with a low indigenous population of cypermethrin degrading bacteria treated with 40 ppm of cypermethrin g^{-1} resulted in a higher degradation rate than was observed in noninoculated soils. These results highlight the potential of this bacterium to be used in cleanup of contaminated pesticide waste in the environment.

INTRODUCTION

The contamination of ecosystem is due to pesticide discharges from manufacturing plant, agricultural runoff, leaching, accidental spills and other sources. Pyrithoids are commonly used insecticides in both agriculture and urban settings, their use may further increase as the use of organophosphate insecticides is restricted. The photostable pyrethroids represent over 25% of the world's foliar insecticide market (Leahey, 1985) and have high toxicity to benthic aquatic organisms (Gilliom, 2001 and Hill, 1989). They exhibit high insecticidal activity combined with low mammalian toxicity (Elliott, et al., 1978) consequently, they are replacing some of the more toxic and environmentally persistent organochlorine compounds (Leahey, 1985). The importance of microbial mediated pyrethroid breakdown in soil has been demonstrated by several workers (Chapmen, et al 1981; Williams and Brown., 1978). Many approaches to pesticide treatment have been considered by the researchers (Yu, 2002; Huston and Pignatello, 1999; Arnol, et al., 1995). In view of this, bioremediation i.e. biological method has been proven to be a suitable for treatment of pesticide polluted aquifers that could be implemented either in situ or off-site in especially designed reactor or waste water treatment plants (Tartakouvsjy, et al., 2001; Kumaran and Shivaraman., 1988). However, previous studies (Kaufman et al, 1977; Lord, et al., 1982; Ohkawa et al., 1978) have failed to demonstrate the transformation of pyrethroids by axenic cultures. More recently much effort has been to develop and isolate organisms that can degrade complex compound like biphenyls, polychlorinated biphenyle, DDT, hexachloro cyclo hexane (Lal et al., 1995). Among the different genera of bacteria degrading pesticide, the genus Pseudomonas has special status to metabolize a broad range of organic compounds and therefore an ideal choice for degradative biotechnologies. The purpose of this investigation was to isolate pyrethroid – degrading microorganism.

In the present study, we report the isolation and characterization of a Pseudomonas sp. capable of degrading cypermethrin at higher concentration both in pure culture as well as in the soil.

MATERIAL AND METHODS

SOIL SAMPLE AND CHEMICALS

Soil sample was taken from pesticide and nonpesticide contaminated field of Chaudhary Charan Singh Haryana Agricultural University, Hisar – (Haryana). The pesticide used was purchased from Sigma or Aldrich, USA. Analytical and spectroscopic grade hexane and acetone was purchased from E. Merck, Germany. All other chemicals were of high purity grade commercially available.

ISOLATION OF CYPERMETHRIN DEGRADING BACTERIA

Approximately 10 g of soil sample was suspended in 250 ml minimal medium supplemented with 0.35 mM cypermethrin and incubated at 30 ^{o} C on plateform shaker at 200 rpm. The MM consisted of Na_2HPO_4, 4 g; KH_2PO_4, 2 g; $MgSO_4.7H_2O$, 0.8 g; $(NH_4)_2SO_4$, 0.8 g; trace element solution, 1 ml (per litre); pH 7.0. The composition of trace element solution was (per litre) $Al(OH)_3$, 0.10 g; $SnCl_2.2H_2O$, 0.5 g; KI, 0.05 g; LiCl, 0.05 g; $MnSO_4.H_2O$, 0.8 g; H_3BO_3, 0.50 g; $ZnSO_4.7H_2O$, 0.10 g; $CoCl_2.6H_2O$, 0.10g; $NiSO_4.6H_2O$, 0.10 g; $BaCl_2$, 0.05 g; $(NH_4)_6MO_7O_{24}.4H_2O$, 0.05 g. After 5 days of incubation, 5 ml culture was used to inoculate fresh 0.35 mM cypermethrin containing MM. Subsequently three rounds of enrichment were carried out in MM supplemented with higher concentration of cypermethrin 0.45 mM. Enriched medium was serially diluted and aliquots of 0.1 ml were plated on nutrient agar plates supplemented with 0.45 mM cypermethrin for the isolation of bacterial cultures. Morphologically different types of colonies were streaked for their purification on nutrient agar plates containing 0.45 mM cypermethrin.

IDENTIFICATION AND GROWTH CONDITIONS OF ISOLATE

The identification of bacterium exhibiting the activity of cypermethrin degradation was carried out by its morphological, physiological and biochemical features using the Bergey's Manual of Systematic Bacteriology (Krieg, N. R., 1984).

Minimal medium with 0.4 mM cypermethrin was inoculated with 1% seed culture of isolated strain and incubated at 30ºC under shaking conditions (200 rpm). Growth was observed by measuring absorbance (O.D.) at 600 nm.

RESTING CELL STUDIES

All isolated strains were tested for their ability to degrade cypermethrin by resting cell studies. These isolates were grown in 300 ml nutrient broth supplemented with 0.45 mM cypermethrin in shaking condition at 30 ^{o} C up to mid log phase. Isolates were harvested at 4 ^{o} C and cells were washed twice with MM. These cells were resuspended in 50 ml MM supplemented with 0.4 mM cypermethrin. Aliquots of 10 ml were taken at different time intervals of 0, 12, 24 and 48 hr. Cypermethrin was extracted from cell free supernatant by using a separating funnel with 50 ml hexane (neutral extraction). The pH of the aqueous phase was adjusted to 2.0 with 2N HCl and again extracted with 50 ml hexane: acetone (acidic extraction). The amount of cypermethrin was determined by using gas chromatography (Perkin Elmer Autosystem XL) with a flame ionization detector. The temperature of the injector and detector was 300 ^{o} C and oven temperature was 250 ^{o} C.

BIOREMEDIATION OF CYPERMETHRIN BY STRAIN CYP19

Soil sample used in this study was taken from the non-pesticide contaminated field. The soil was air-dried and ground to powdered form. Samples (120 g) of the autoclaved and unautoclaved soil were spiked with cypermethrin (40 ppm g^{-1}) under aseptic conditions. One set of autoclaved and unautoclaved soil was inoculated with strain Cyp19 (10^{6} cells g^{-1}), and another set of autoclaved and unautoclaved soil without inoculation was kept as control. The inoculum was thoroughly mixed in soils under sterile conditions. Soil samples were incubated at 30 ^{o} C with 45% water-holding capacity in the dark. In this method, 20 g soil sample was taken from both inoculated and uninoculated soils at an interval of 0, 5, 10, 20 and 30 days. Then 0.25 ml of 25% ammonia solution was added to it and mixed well with glass rod. After 30 minutes, when ammonia had evaporated, 0.5 g of activated charcoal and 0.5 g of activated cflorisil was added and mixed thoroughly. The soil was then packed in a 20 mm X 30 cm sintered glass chromatography column containing about 5 cm layer of anhydrous sodium sulphate. The columns were then eluted with 100 ml mixture of acetone: hexane (1: 4). Elutes were dried under nitrogen at 50 ^{o} C, and finally reconstituted in 20 µl hexane for GC analysis. The population of strain Cyp19 was also checked at different time interval by spread plate method using nutrient agar plates containing 0.45 mM cypermethrin.

RESULTS

ISOLATION, IDENTIFICATION AND GROWTH CONDITIONS OF STRAIN CYP19

Morphologically different bacterial cultures were isolated by enrichment in our laboratory. Out of the twenty bacteria isolated, only one strain Cyp19 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 motile, gram negative, aerobic, short rod, oxidase and catalase positive. It grew on MacConkey agar as lactose fermentating microorganism and utilized citrate as the carbon source. The isolate did not produce acid from sugar such as adonitol, cellobiose, dulcitol, insulin,

maltose and rhamnose. On the basis of these results the bacterium was tentatively identified as Pseudomonas sp. The growth of strain Cyp19 in MM supplemented with 0.4 mM cypermethrin is shown in Fig. 1.

DEGRADATION OF CYPERMETHRIN BY STRAIN CYP19 IN RESTING CELL STUDY

To elucidate that strain Cyp19 degrades cypermethrin, resting cell study was performed. After GC analysis, it was found that the initial concentration of cypermethrin 0.4 mM at 0 hr was found to be depleted to 0.1 mM after 24 h. This concentration was further reduced to 0.001 mM after 48 h, indicating almost 100% depletion of cypermethrin as shown in Fig. 2. However, in case of heat killed resting cells no depletion of cypermethrin was observed at any point of time.

DEGRADATION OF CYPERMETHRIN IN SOIL BY STRAIN CYP19

The addition of strain Cyp19 to soil resulted in a more rapid degradation of cypermethrin than by indigenous microflora. Degradation of cypermethrin was insignificant in unautoclaved (uninoculated) and autoclaved (uninoculated) soil after 30-days of incubation studies as shown in Fig. 3. Degradation of cypermethrin was significant in unautoclaved (inoculated) and autoclaved (inoculated) soil, whereas 97.5% and 95% of applied cypermethrin degraded respectively in 30-days of incubation studies. Microbial population of strain Cyp19 reduced from 10^{6} cells g^{-1} of soil to 10^{4} cells g^{-1} of soil in 30 days of incubation study. In the present work the bacterial system successfully degraded cypermethrin in autoclaved and unautoclaved soils indicating that it can survive and compete with the local microflora.

Figure 1

Fig. 1. Growth of strain Cyp19 in minimal medium containing 0.4mM cypermethrin

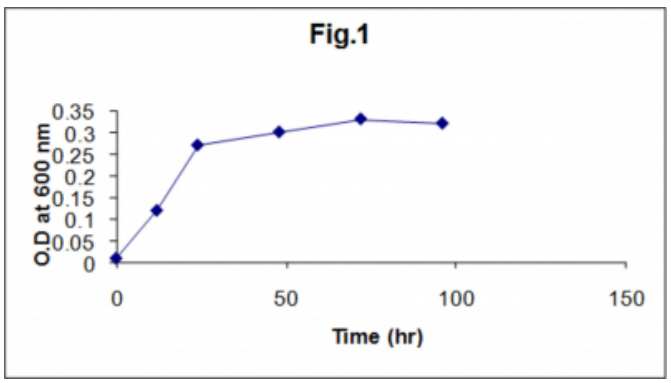
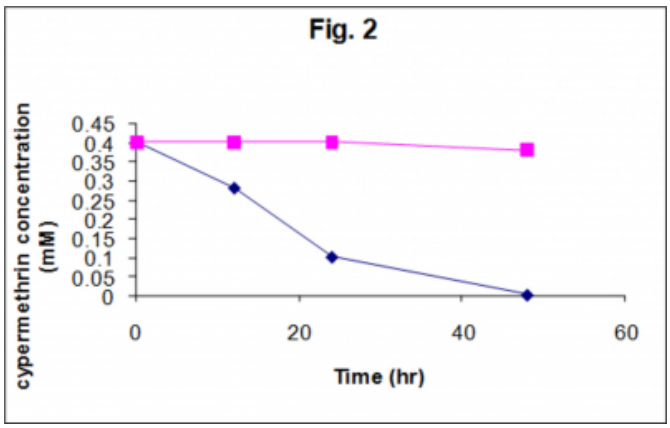


Figure 2

Fig. 2. Degradation of cypermethrin by strain Cyp19 by using gas chromatographic analysis.

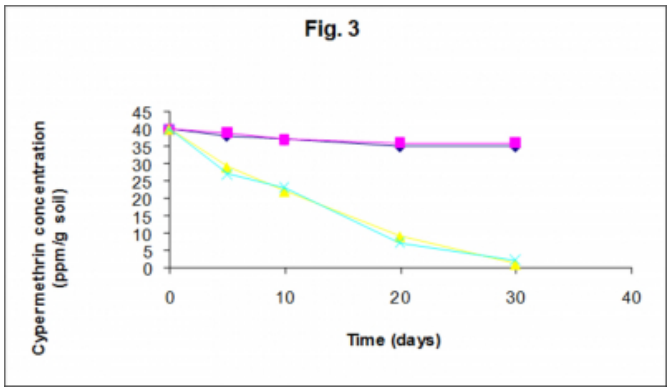


, Degradation of cypermethrin by heat killed strain Cyp19.

, Degradation of cypermethrin by live cells of strain Cyp19.

Figure 3

Fig. 3. Degradation of cypermethrin in soils inoculated with strain Cyp19 at the rate of 10 cells g .



×, autoclaved inoculated soil;

, unautoclaved inoculated soil;

, autoclaved uninoculated soil;

,unautoclaved uninoculated soil.

DISCUSSION

In this study, different bacteria cultures were isolated from pesticide contaminated soil by enrichment technique. Enrichment studies to increase the degradative properties of the mixed cultures were conducted by growing the predominant bacterial isolates. Pesticide concentration in the culture vessel was kept between 0.35 - 0.45 mM to maintain constant pressure on the microorganisms by not allowing substrate limitation. All the isolated cultures were checked

for their ability to degrade cypermethrin by resting cell study. Out of twenty bacteria isolated, only four bacterial cultures were found to degrade cypermethrin. The strain Cyp19 degrade the cypermethrin at much faster rate as compare to other isolates was selected for further study. After morphological, physiological and biochemical analysis, strain Cyp19 was identified as a Pseudomonas sp. The genus Pseudomonades is gram negative, rod shaped, highly oxidative and metabolically versatile, able to degrade aromatic hydrocarbons and pesticide (Ramabathan and Lalithakumari, 1999; Lee, et al., 1998). The strain Cyp19 was grown as shake culture in MM conaintaing cypermethrin to determine that this strain uses the pesticide as sole carbon source. Bacterial degradation of cypermethrin in pure cultures has been reported (Grant, et al., 2002; Lee, et al., 2004 and Malony, et al., 1998). By resting cell study and GC analysis, it was found that strain Cyp19 can degrade 0.4mM cypermethrin concentration within two days completely. Degradation of cypermethrin (40 ppm g^{-1} of soil) was checked by the addition of strain Cyp19. After GC analysis, it was found that in uninoculated soil there was no degradation of cypermethrin. The degradation of cypermethrin was very rapid in Cyp19 inoculated soil. In 30 days of inoculation study, the degradation of cypermethrin was 97.5 % and 95 % in unautoclave inoculated and autoclave inoculated soil respectively.

Successful removal of pesticides by the addition of bacteria have been reported earlier for many compounds, including parathion (Bekhi, et al.,1994), coumaphos (Mulbry, et al., 1996), ethoprop (Karpouzas, et al., 2000), and atrazine (Struthers, et al., 1998). The addition of strain Cyp19 (10^6 cells g^{-1}) to soil with a low indigenous population of cypermethrin degrading bacteria treated with 40 ppm of cypermethrin g^{-1} resulted in a higher degradation rate than was observed in noninoculated soils. In the present work, the isolated cypermethrin- degrading bacterium could be used successfully for the removal of cypermethrin pesticide from contaminated soil and this bacterium can compete and survive with the local microflora.

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