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Biodegradation of Cypermethrin by using Indigenous Bacteria Isolated from Surface Soil

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Abstract. Cypermethrin is a synthetic pyrethroid pesticide, widely used to control pests in cotton and vegetable crops. A current environmental concern is the contamination of aquatic ecosystem due to pesticide discharges from manufacturing plant, agricultural runoff, leaching, accidental spills and other sources. An effective pesticide waste treatment technology is needed to prevent water pollution and to comply with increasing regulatory pressures. The microorganisms play a significant role in detoxifying pesticides in the environment. There are few reports on the degradation of pyrethroid insecticides in soils. This study may provide basis for prevention and control of pesticides pollution. The ability of five bacterial isolates (*Pseudomonas aeruginosa*, *P.fluorescens*, *Bacillus licheniformis*, *Alcaligenes* sp. and *Corynebacterium* sp.) isolated from cultivated field to degrade cypermethrin was studied using enrichment technique, with varying concentrations of cypermethrin in the medium. The *P.aeruginosa* was enumerated with and without adding cypermethrin. The concentration of cypermethrin and its intermediates during the biodegradation experiment analyzed on GC-MS.In these five different bacterial colonies, degradation was found to occur to a great extent only in the presence of *P.aeruginosae* culture. *P.aeruginosae*, *Pseudomonas fluorescens* and *B.licheniformis* were found active in utilizing cypermethrin (3%) whereas *Corynebacterium* sp. and Alcaligenes sp. were moderately active in utilizing cypermethrin (0.5 and 1 %).These findings suggest that the utilization of cypermethrin by *Pseudomonas* sp. and Bacillus licheniformis may be feasible and this treatment option for the removal of pesticide from the soil and degradation observed only in the presence of microorganisms.

Keywords: Cypermethrin, Biodegradation, Surface soil, Indigenous bacteria

1. Introduction

Cypermethrin has moderate persistence in soils. Under laboratory conditions, cypermethrin degrades more rapidly in soils and in aerobic conditions [10].The half-life of cypermethrin is 4 days to 8 weeks [11].Cypermethrin is co metabolized by bacteria in soil. Isolation of indigenous bacteria capable of metabolizing pyrethroid insecticides has received considerable attention because these bacteria provide an environmentally friendly method of in situ [6, 8]. In some contaminated environments, autochthonous microbial populations have evolved over time to adapt to these contaminants. These sites are therefore the most appropriate ecological niches to find and isolate strains capable of degrading organophosphate insecticides [7, 9]. Cypermethrin was selected as it belongs to a group of bioresistant compounds which are not normally removed by conventional treatment plants. The research aim was to identify the potential microbial strain able to utilize cypermethrin from the soil.

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2. Materials and Methods

The soil samples were collected from cultivated field in north of Iran. These fields had been already treated with cypermethrin for about three past years. Soil samples were collected at different sites of the field and then, these soil samples were transferred to sterile polythene bag and used for analysis. The bacterial culture capable of degrading cypermethrin was isolated from agricultural soil using enrichment technique, with varying concentrations of cypermethrin in the medium. The soil sample(5 - 10 g) from an agricultural site was inoculated into 500 ml of nutrient broth operating at 250 rpm for seven days at room temperature (ranged from 25 - 28°C). At daily intervals one loop full of enrichment culture from the flasks was streaked on nutrient agar plates supplemented with cypermethrin (0.5 - 3%) and incubated at 35°C for 24 - 48 h. Individual colonies were sub cultured into nutrient agar plates containing same concentration of cypermethrin until pure culture was isolated. The isolates were maintained at 4°C and sub cultured after every three months. The identification and characterization of the isolates was performed using morphological, cultural and biochemical characteristics up to the stage of genus[1]. Then, genomic DNA compared with the 16S rRNA gene database at the National Centre for Biotechnology Information (NCBI). The enumeration of viable cell count was performed with and without adding pesticide. Aliquot (2.5 mL) of 24 h culture grown in nutrient broth was inoculated into 25 mL nutrient broth flask containing different concentrations of cypermethrin and tested their ability to degrade supplemental substrate (the pesticide). Control flasks of equal volume of nutrient broth medium containing culture but no pesticide were run in parallel to confirm that significant die off was not occurring over the period of each test.Growth of the isolate was determined by viable cell enumeration immediately after inoculation and at 2, 4, 6 and 24 h later. Miles and Misra technique was used for bacterial growth study. Sample of bacterial culture (1 mL) was drawn at regular intervals and serial dilutions (10-5-10-8) of bacterial culture with and without addition of pesticide (control) was performed using 9 mL sterile saline blank (0.85 % NaCl; pH = 7).Appropriate dilutions of bacterial samples were plated in triplicate on nutrient agar medium. Each plate divided into four segments and used for several dilutions. Three drops of culture were placed in each section of nutrient agar plate and were allowed to dry followed by incubation at 35 °C for 24 h.After incubation the viable colonies were counted using the method described by Collins and Lynes (1985) and results were reported accordingly.

3. Results

The aim of the study was to determine the total heterotrophic and Cypermethrin resistant bacterial population. The physic-chemical characteristics of soil were carried out. The data in Table 1 were indicated presence of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium and magnesium in soil. The total heterotrophic bacterial populations isolated from the soil contaminated with cypermethrin was represented. The data indicates the presence of bacteria, fungi and actinomycetes in soil in Table 2.Nearly 18 bacterial colonies were isolated and identified. Among them 35.5% are gram positive bacteria and the rest 64.5% are gram negative bacteria. The findings showed that five bacterial isolates were active in dye removal based on the screening results. The most effective isolate, which showed the highest biodegradation potential for tested pesticide in aerobic condition, were selected for further studies. These different colonies were identified on nutrient agar medium enriched with Cypermethrin according to, Bergey's Manual of Determinative Bacteriology (1994). In table 3, One of the largest, most rapidly growing colonies of bacterial isolates was *P.aeruginosae* that selected for a series of growth curve experiments .Growth curve experiment was performed with 0.5, 1.5 and 3.0 % dose of cypermethrin to determine the viable count of *P. aeruginosa* the optimum concentration of Cypermethrin that supports growth of isolates was also evaluated in Figure 1. A control test without adding pesticide in nutrient broth was conducted in order to evaluate the mineralization potential of isolated strains when exposed to different concentrations of Cypermethrin. In table 4, The bacterial isolates were identified as a member of the genus *Pseudomonas aeruginosa*, *P.fluorescens*, *Bacillus licheniformis*, *Alcaligenes* sp. and *Corynebacterium* sp. *P.aeruginosa* was predominant (27.4%) followed by *P.fluorescens* (23.8%) and *Bacillus licheniformis* (13.2%), *Alcaligenes* sp. (5.5%) and *Corynebacterium* sp. (4.5%).The isolated native bacteria exhibited remarkable resistance to the cypermethrin .The generic composition of total viable bacterial strains at different concentrations of cypermethrin has been shown in Figure 2.This study revealed that except

Corynebacterium sp. and *Alcaligenes sp*. .All other species including *P.aeruginosa*, *P.fluorescens* and *Bacillus licheniformis* utilized cypermethrin as a carbon source at 1 to 3% concentrations. On comparing the growth of *P. aeruginosa* in presence of Cypermethrin with that of control, it becomes clear that the *P.aeruginosa* grows faster and a higher number of cells were observed in 0.1, 0.5 and 3% concentration of Cypermethrin that the phase of acclimation of *P.aeruginosa* continued up to almost 2 h., after the initial inoculation. The count at 0 h. was 7x105 CFU/mL and then started increasing slowly. At 8h., the total viable count was 78x108 CFU/mL with generation time of 57 min.At 24h., the total viable count at control was 189 ± 10.53 x 107 CFU/ml. The findings showed that the total viable counts significantly increased, indicating that the culture after remaining in the lag phase for 2 h. entered into the phase of positive acceleration. The growth of *P.aeruginosa* in the presence of Cypermethrin continued and proved that even high concentration of Cypermethrin is not toxic to *P.aeruginosa*, however, its mineralization potential decreased, which prolonged the lag phase. The concentration of cypermethrin and its intermediates during the biodegradation experiment carried out and analyzed quantitatively and qualitatively on GC-MS. The data showed that cypermethrin was hydrolyzed to 3-phenoxy benzaldehyde and 3-phenoxy-benzyl alcohol.

Table 1: Physico-Chemical characteristics of cultured soil

Parameters	**soil**
PH	7.7 ±0.1
Moisture	85%±0.2
Alkalinity/100gms	0.7 meq
Dissolved Oxygen	13 mg/kg
BOD	2±0.5 mg/kg
COD	1215±0.2mg/kg
Temperature	23^{0C} ± 2
Cation Exchange Capacity/100gms	300meq
Magnesium	976 mg/kg
Potassium	231 mg/kg
Chloride	149 0mg/kg
Calcium	2385 mg/kg
Sulphate	1.0 mg/kg
Kjeldahl Nitrogen	1290mg/kg
Phosphorus	0.74 mg/kg
Organic Carbon	5.12 %

Table 3: Incidence of total heterotrophic bacterial population and cypermethrin resistance at (0.5 and 3%) Concentrations in cultivated field

S/NO	Dilutions	Total viable counts(CFU/g)	Cypermethrin resisting bacterial counts (CFU/g)	
			Cypermethrin concentration	
			3%	0.5%
1	10^4	7.55 x 10^6	3.25 x 10^6	2.22 x10^6
2	10^5	6.25 x 10^7	3.22 x 10^7	1.45 x10^7
3	10^6	5.12 x 10^8	2.78 x 10^8	1.25 x10^8
4	10^7	3.20 x 10^8	2.21 x 10^8	1.1 x10^8
5	10^8	2.1 x 10^8	1. 5 x 10^8	0.94 x10^8

Table 2: Microbial characteristics of soil

Parameters	Soil
Total viable count/g	1116
Total coliform count/g	790
Total Yeast & Mould count/g	92
Actinomycetes count/g	77
Pseudomonas count/g	<75
E.coli count/g	Nill
Anaerobic bacterial count	Nill
Thermophilic bacterial count	<14
Anaerobic spore count	<18
Thermophilic spore count	<20
Anaerobic thermophilic spore	Nill
Nocardia	<30
Salmonella spp.	Present
Enterobacter spp.	Present
Pseudomonas spp.	Present
Corynebacterium spp.	Present
Flavobacterium spp.	Present
Alcaligenes spp.	Present
Serratia spp.	Present
Bacillus licheniformis	Present
Bacillus spp.	Present
Alcaligenes spp.	Present
Providencia spp.	Present
Agrobacterium spp.	Present
Mucor spp.	Present
Aspergillus spp.	Present
Penicillium spp.	Present

Table 4. Cypermethrin resistance pattern of native bacterial isolates

Bacterial Isolates	Cypermethrin resisting bacterial counts (CFU/g)					
	Cypermethrin concentration					
	0.5%	1%	1.5 %	2.0 %	2.5 %	3%
P. aeruginosa	+	+	+	+	+	+
P.fluorescence	+	+	+	+	+	-
B.licheniformis	+	+	+	-	-	-
Corynebacterium sp.	+	-	-	-	-	-
Alcaligenes sp.	+	-	-	-	-	-

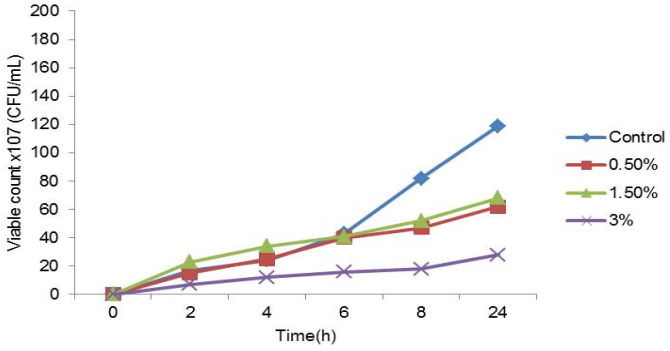

Fig. 1: Growth of *P.aeruginosa* in the presence of Cypermethrin

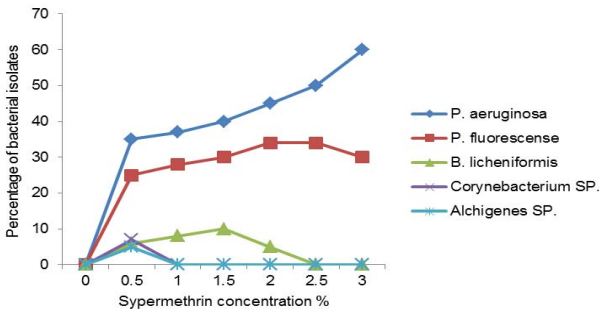

Fig. 2: Generic composition of total viable bacterial strains isolated from cultivated field at 0.01 to 1

4. Discussion

Based on the results from this study and that by and other researchers it may be concluded that *P.aeruginosa* strain is able to grow in the presence of added pesticide and therefore it could be effectively used for the treatment of pesticide contaminated soil or water [3]. However, further research is necessary to understand the fundamental mechanism of enhancement and inhibition in microbial degradation at high concentration of pesticides. The present research findings described that this may be the first instance in which high concentration of cypermethrin degradation has been achieved in short retention time of 24 h.Although, transformation of pyrethrins (50 mg/L) by pure culture of *pseudomonas fluorescence* in the presence of tween 80 under aerobic conditions with a half-life of less than 5 days[2,5] . It is reported that technical grade of cypermethrin can be reduced from 60 mg/L to 6 mg/L by *pseudomonas sp.* in 20 days [4]. The overall findings suggest that using of native isolates for the degradation of cypermethrin is reasonable treatment option for the removal of pesticide wastes from contaminated soil or wastewater as biodegradation observed only in the presence of acclimated microorganism under aerobic conditions. In result, surface soil treatment technique used for bioremediation of pesticides using soil microflora would be an effective treatment technology for other group of pesticides and its effluents.

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