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Research Article

Optimization of Cypermethrin Degradation By Bacterial Cultures Isolated From Soil

Oly Roy and Usha Malavali Subbaiah*

Department of Microbiology, Centre for P.G. Studies, Jain University, 3rd block, jayanagar, Bangalore-560011

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ABSTRACT

Present study was carried out to identify potential bacteria which is capable of degrading cypermethrin. cypermethrin is a pyrethroid pesticide which is harm to environment. This study is aimed to bioremediate agricultural soil using bacterial strain isolated from soil.Agricultural soils were enriched with pesticides and collected for isolation of bacteria capable of degrading cypermethrin. One gram of soil sample is inoculated into 100 ml of mineral salt medium supplemented with 1% cypermethrin as sole source of carbon for isolation of cypermethrin degrading bacteria. After seven days percent degradation of pesticide was estimated by measuring cypermethrin concentration at 595 nm. Out of 64 bacteria isolated from 32 soil samples, strain JUO17b and JUO20a showed more efficiency in degrading cypermethrin compared to other strains. Strain JUO17b showed 89% degradation and JUO20a showed 78% degradation of cypermethrin by the end of 7 days. Optimization of pesticide degradation by the same cultures was carried out using various parameters such as pH, temperature, shaking speed, inoculum concentration, pesticide concentration, carbon and nitrogen sources. Both the strains showed optimum degradation at a temperature of 35°C, pH 7, shaking condition of 150 rpm, inoculum concentration of 2 ml and pesticide concentration of 2% in presence of 100 mg/l each of glucose and yeast extract over a period of 24 hrs. Under optimized condition strain JUO17b could show 100% degradation of cypermethrin, whereas strain JUO20a showed 92% degradation of cypermethrin. Further, strain JUO17b was identified as *Serratia nematodiphila* based on 16s rRNA sequencing and phylogenetic tree analysis.

INTRODUCTION:

Pyrethroid insecticides were first developed over 40 years ago at a time when concerns were growing about the persistent nature of organochlorine pesticides. Pyrethroid pesticides are synthetic analogues of naturally occurring pyrethrins; a product of flowers from pyrethrum plant. Pyrethroids work by targeting the sodium channels in neuronal membranes of insects. Synthetic Pyrethroid insecticides have been used for more than 20 years to control insect pests in a variety of crops, but they have become increasingly popular following outright bans or limitations on the use of cholinesterase-inhibiting insecticides (1, 2). Cypermethrin is broad spectrum synthetic Pyrethroid insecticides which use on variety of agricultural and greenhouse food/feed crops (Fig. 1). Cypermethrin, one of a handful of light-stable synthetic Pyrethroid, is registered to control cockroaches, fleas and other indoor pests and food processing plants and also in agriculture to control pests on cotton, fruits and vegetables. About 90% of the cypermethrin manufactured worldwide is used to combat pests feeding on cotton crops. Depending on the specific product formulation, EPA classifies pesticides containing cypermethrin as toxicity class II or III (I = most toxic, IV = least toxic).Considerable amount of work has been done on cypermethrin degradation by bacteria (3, 4) and fungi (5) isolated from agricultural soil and other sources. With the aim of isolating efficient strain of bacteria capable of degrading cypermethrin and to optimize various parameters to increase the degradation capability of screened bacteria the present investigation has been taken up.

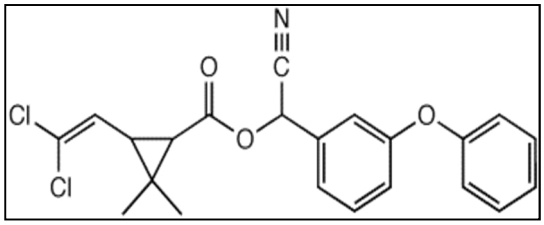

Fig. 1: Structure of Cypermethrin

Materials and Methods

Sample collection: Thirty-two soil samples were collected in sterile polythene bags from different agricultural sites in and around Bangalore having a history of repeated application of pesticides. Samples were refrigerated at 4˚C until use.

Isolation and enrichment: Bacteria capable of degrading cypermethrin were isolated by adding 1 g of soil into 100 ml of mineral salts medium (6) adjusted to pH 7 containing 1% cypermethrin as a sole source of carbon. Flasks were incubated at 37°C under static condition for 30 days with intermittent addition of pesticide. At the end of incubation pure cultures were obtained by streaking a small amount of the growth media on mineral salts agar medium with 1% cypermethrin. Pure cultures were maintained on the same agar medium.

Screening: Loopfuls of cultures were inoculated separately into mineral salts medium with pH 7 and 1% cypermethrin and the flasks were incubated at static condition for 10 days. The isolates were screened based on the growth of bacteria in mineral salt media supplemented with 1% cypermethrin and based on the amount of cypermethrin degraded by the isolates after completion of incubation.

At the end of incubation, percentage degradation of cypermethrin by each bacterium was estimated according to Janghel et al. (2007) using LCV (leucocrystal violet) as an indicator (7). Leucocrystal violet was prepared by dissolving 250 mg of LCV in 200 ml of distilled water and 3 ml of orthophosphoric acid and made up the volume upto 1000 ml. Cypermethrin were extracted from the cultures by acidifying the media (pH 5) and then by mixing with equal volume of ethyl acetate. To 1 ml of extract, 1 ml of 20% NaOH was added and incubated at room temperature for 10 min. Then 1 ml of 0.1 % KI and 1 ml of LCV reagent were added to it and incubated at room temperature for 15 min. Absorbance was measured at 595 nm against a reagent blank which contained all the reagents except sample which was replaced by ethyl acetate. Percent degradation of the pesticide by the cultures was calculated using the formula:Percent degradation = [(Initial concentration-final concentration)/ initial concentration] X 100 Bacteria showing highest degradation were selected for further studies.

Optimization of cypermethrin degradation: Optimization of cypermethrin degradation by the two isolates JUO17b and JUO20a was carried out by one-factor-at-a-time experiments.

optimization of degradation of cypermethrin by these two isolates was carried out with respect to various parameters like pH (5-9), temperature (25˚C-45˚C), inoculum size (1-10 ml), pesticide concentration (1-10%) shaking speed (50-200 rpm), additional carbon sources at 100 mg/l concentration

(glucose, starch, lactose, sucrose, maltose) and nitrogen sources at 100 mg/l concentration (potassium nitrate, yeast extract, beef extract, sodium nitrate, peptone). Every 24 hrs, degradation efficiencies of both the isolates were monitored upto 7 days.

Identification of bacteria: The isolates JUO17b and JUO20a which were screened out based on percent degradation of cypermethrin were identified up to genus level according to Bergey's manual of systematic bacteriology (8). The isolate JUO17b was identified up to species level based on nucleotide sequencing and phylogenetic tree analysis which was outsourced from Bhatt Bio-tech India (P) Ltd., Bangalore. Then the gene sequence was deposited in NCBI gene bank for accession number.

Results

Isolation and screening of cypermethrin degrading bacteria: Out of thirty two soil samples collected from various agricultural fields in and around Bangalore, 64 isolates capable of degrading cypermethrin were obtained (Table 1). Based on the growth on mineral salt medium 32 isolates were screened out for cypermethrin degradation. On gram staining majority of the isolates were found to be gram negative in nature though few were gram positive. Out of 32 isolates, strain JUO17b and JUO20a showed more efficiency in degrading cypermethrin pesticide compared to other strains. Strain JUO17b showed 89% degradation and strain JUO20a showed 78% degradation of cypermethrin by the end of 7 days.

Table 1: Percent degradation of cypermethrin by different bacterial isolates

SL. No.	Sample codes	Gram staining	Percent degradation (%)	SL. No.	Sample codes	Gram staining	Percent degradation (%)
1	JUO1a	Gram negative rods in chain	71	24	JUO13a	Gram negative rods in single	67.5
2	JUO1b	Gram positive rods in single	31	25	JUO13b	Gram positive rods in chain	41
3	JUO2a	Gram negative rods in single	76	26	JUO14a	Gram negative rods in single	72
4	JUO2b	Gram positive rods in chain	35	27	JUO14b	Gram positive rods in single	35
5	JUO3a	Gram negative rods in single	72.5	28	JUO15a	Gram negative rods in single	71
6	JUO3b	Gram positive rods in single	33	29	JUO15b	Gram positive rods in chain	38
7	JUO4a	Gram negative rods in single	69	30	JUO16a	Gram negative rods in single	69
8	JUO4b	Gram positive rods in single	28	31	JUO16b	Gram negative cocci in cluster	62
9	JUO5a	Gram negative rods in single	77	32	JUO16c	Gram positive rods in single	41
10	JUO5b	Gram positive rods in single	39	33	JUO17a	Gram positive rods in chain	33
11	JUO6a	Gram negative rods in single	73	34	JUO17b	Gram negative rods in single	78
12	JUO6b	Gram positive rods in chain	41	35	JUO18a	Gram negative rods in single	70
13	JUO7a	Gram negative rods in single	69.5	36	JUO18b	Gram positive rods in single	37
14	JUO7b	Gram positive rods in single	37.5	37	JUO19a	Gram negative rods in single	77.5
15	JUO8a	Gram negative rods in single	75	38	JUO19b	Gram negative rods in single	61
16	JUO8b	Gram positive rods in single	32	39	JUO19c	Gram positive rods in single	30
17	JUO9a	Gram negative rods in single	74	40	JUO20a	Gram negative rods in single	89

18	JUO9b	Gram negative cocci in cluster	68	41	JUO20b	Gram positive rods in single	38
19	JUO9c	Gram positive rods in chain	38	42	JUO21a	Gram negative rods in single	70
20	JUO10a	Gram negative rods in single	64	43	JUO21b	Gram positive rods in single	29
21	JUO11a	Gram negative rods in single	73	44	JUO22a	Gram negative rods in single	77.5
22	JUO12a	Gram negative rods in single	68	45	JUO22b	Gram positive rods in single	75
23	JUO12b	Gram positive rods in single	31	46	JUO23a	Gram negative rods in single	71

Table 1: Percent degradation of cypermethrin by different bacterial isolates cont..

SL. No.	Sample codes	Gram staining	Percent degradation (%)	SL. No.	Sample codes	Gram staining	Percent degradation (%)
47	JUO23b	Gram positive rods in chain	41	56	JUO28a	Gram negative rods in single	66
48	JUO24a	Gram negative rods in single	76	57	JUO28b	Gram positive rods in single	31
49	JUO24b	Gram positive rods in single	36	58	JUO29	Gram negative rods in single	71
50	JUO25a	Gram negative rods in single	75	59	JUO30a	Gram negative rods in single	68
51	JUO25b	Gram positive rods in chain	31	60	JUO30b	Gram positive rods in chain	28
52	JUO26a	Gram negative rods in single	61	61	JUO31a	Gram negative rods in single	65
53	JUO26b	Gram positive rods in single	39	62	JUO31b	Gram positive rods in chain	30
54	JUO27a	Gram negative rods in single	77	63	JUO32a	Gram negative rods in single	66
55	JUO27b	Gram positive rods in single	32	64	JUO32b	Gram positive rods in single	36.5

Optimization of degradation: Results of optimization of cypermethrin degradation by one-factor-at-a-time experiment is given below.

JUO17b and JUO20a showed almost same response towwards effect of pH, temperature, inoculum size, pesticide concentration, shaking speed, effect of additional carbon and nitrogen sources.

Effect of various pH (pH5 to pH9) on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 2. Both the organisms showed highest degradation at pH 7 (JUO20a-78%, JUO17b-89%) and lowest degradation at pH 5 (JUO20a-55%, JUO17b-57%).

Effect of various temperature (25˚C to 45˚C) on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 3. Both the organisms showed highest degradation at 35˚C (JUO20a-77%, JUO17b-87%) and lowest degradation at 25˚C (JUO20a-60%, JUO17b-65%).

Effect of various inoculum size (1%v/v to10%v/v) on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 4. Both the organisms showed highest degradation at 5%v/v (JUO20a-81%, JUO17b-92%) and lowest degradation at 1%v/v (JUO20a-77%, JUO17b-87%).

Effect of various cypermethrin concentration (1% to 10%) on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 5. Both the organisms showed highest degradation at 1% cypermethrin concentration (JUO20a-79%, JUO17b-90%) and lowst degradation at 10% cypermethrin concentration (JUO20a-50%, JUO17b-54%).

Effect of various shaking speed (50 rpm to 200 rpm) on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 6. Both the organisms showed highest degradation at 100 rpm (JUO20a-85%, JUO17b-

98%) and lowest degradation at 200 rpm (JUO20a-43%, JUO17b-57%).

Effect of various carbon sources (glucose, sucrose, lactose, maltose, starch) at a concentration of 100mg/l on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 7. Both the organisms showed highest degradation at 100 mg/l glucose concentration (JUO20a-92%, JUO17b-97%) and lowest degradation at 100mg/l lactose and sucrose concentration (JUO20a-80%, JUO17b-90%).

Effect of various nitrogen sources (beef extract, yeast extract, peptone, KNO_3 and $NaNO_3$) at a concentration of 100mg/l on the growth of bacteria and degradation of cypermethrin by JUO17b and JUO20a are presented in figure 8. Both the organisms showed highest degradation at 100mg/l yeast extract concentration (JUO20a-89%, JUO17b-94%) and lowest degradation at 100mg/l of sodium nitrate concentration (JUO20a-80%, JUO17b-80%).

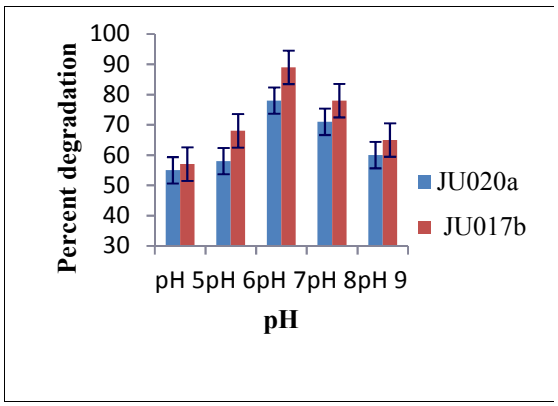

Fig. 2: Effect of pH on degradation of cypermethrin by JUO17b and JUO20a

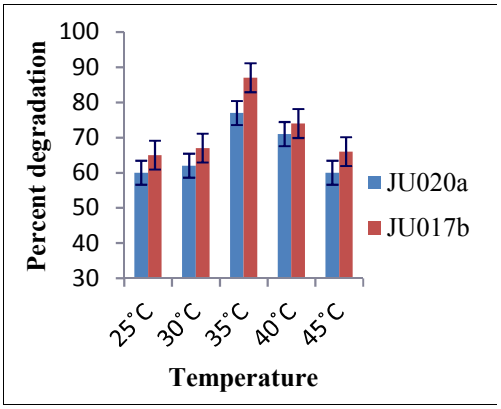

Fig. 3: Effect of temperature on degradation of cypermethrin by JUO17b and JUO20a

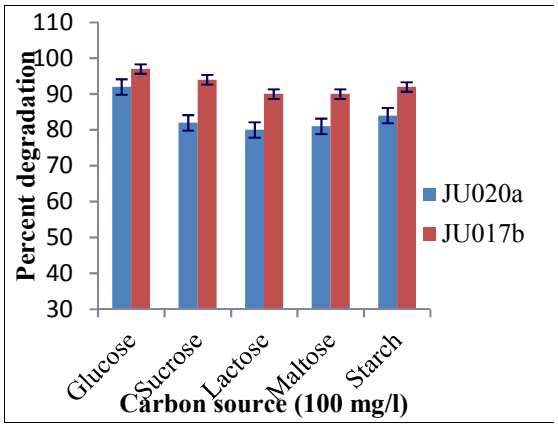

Fig. 6: Effect of different carbon sources on degradation of cypermethrin by JUO17b and JUO20a

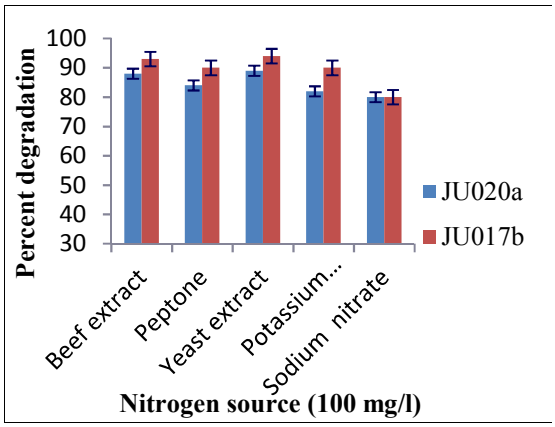

Fig. 7: Effect of different nitrogen source son degradation of cypermethrin by JUO17b and JUO20a

Taxonomic identification of bacteria: After one-factor-at-a-time optimization process JUO17b showed 100% egradation of cypermethrin where JUO20a showed 92% degradation. Based on biochemical tests (Table 2) JUO17b was identified as *Serratia* sp. and JUO20a was identified as *Enterobacter* sp. Based on the nucliotide sequence and phylogenetic tree analysis JUO17b was identified as *Serratia nematodiphila* and the sequence is deposited in gene bank with the accession number BankIt1832681 seq1 KT153595.

Table 2: Biochemical characterization of JUO17b and JUO20a

S.No.	Biochemical test	JUO17b	JUO20a
1	Gram stain	Gram's negative rods	Gram's negative rods
2	Motility	Positive	Positive
3	Methyl red	Negative	Negative
4	Voges Proskauer test	Positive	Positive
5	Indole	Negative	Negative
6	Citrate utilization test	Positive	Positive
7	H_2S production	Negative	Negative
8	gelatine hydrolysis	Positive	Negative
9	Lipase production	Positive	Negative
10	dNAse production	Positive	Negative
11	Catalase	Positive	Positive
12	Oxydase	Negative	Positive
13	Lysine utilization	Positive	Negative
	Tentative identification	*Serratia* spp.	*Enterobacter* spp.

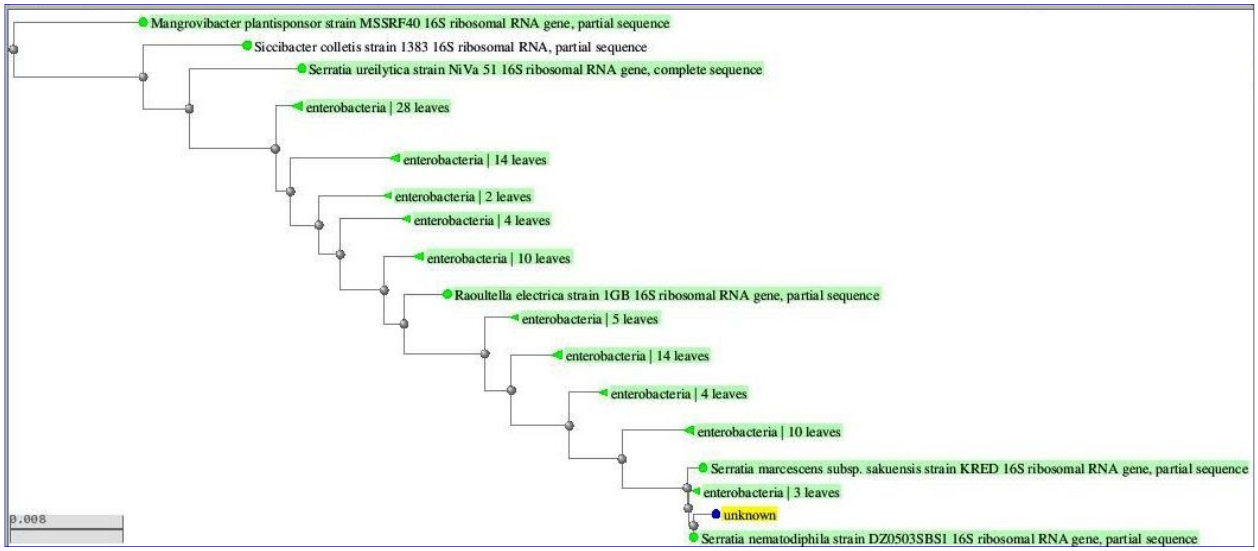

Fig. 8: Phylogenetic tree of the isolate JUO17b

Discussion

In the present study 64 isolates were obtained from 32 soil samples which are capable of degrading cypermethrin. Screening was carried out based on the degradation capability of bacteria which is similar to the method followed by Janghel et al. ((7). JUO17b and JUO20a showed higher degradation capability than other isolates. Under optimized condition JUO17b showed highest degradation which was identified as *Serratia nematodiphila.* A similar result was obtained by Yin et al.(9). Lin et al. (10) isolated *streptomysis* spp from waste water which was capable of degrading up to 90 % of cypermethrin in one day at temperature of 26˚C - 28˚C. This streptomysis sp. can't tolerate temperature higher than 34˚C. It can degrade 250 mg/l concentration of cypermethrin whereas *Serratia nematodiphila* is capable of degrading almost 300 mg/l concentration of cypermethrin. A fungal strain isolated from soil is capable of degrading cypermethrin (50 mg/l) up to 54.83% in 7 days. In this case high temperature and alkaline pH favours the degradation process (5). In 2014 Akbar *et al.* isolated and identified three bacterial strain (*Acinetobacter, Brevibacillus* and *Sphingomonas*) which are highly efficient in degradation of cypermethrin (11). These bacterial strains were able to degrade 85% of cypermethrin (100 mg/l) within

10 days. Whereas under optimized condition *Serratia nematodiphila* can degrade up to 100% cypermethrin within 3 to 4 days.

Conclusion

From the present study it can be conclude that *Serratia nematodiphila* isolated from soil can degrade cypermethrin efficiently. So this organism can be use for bioremediation of cypermethrin contaminated soil.

Conflict of Interest: Authors has no conflict of interest.

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