International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 64-71, 2014-2015

Isolation and identification of cypermethrin degrading *Serratia nematodiphila* from cauliflower rhizosphere

Himani Tyagi[1], Pratibha Prashar[1]*

Department of Biotechnology, Faculty of Engineering and Technology, MRIU Faridabad, India

Abstract: Cypermethrin is a popularly used synthetic pyrethroid insecticide in agricultural set up all over the world. Owing to its toxicity for non-target insects as well as other higher organisms including human beings, the presence of cypermethrin in the soil is a matter of concern. This study reports the isolation of a cypermethrin degrading bacterial strain CB2 isolated from the rhizosphere of cauliflower cultivated in soil having a record of long-term cypermethrin usage in district Faridabad, Haryana, India. Based on the morphological features and 16S rRNA gene sequence analysis strain CB2 had been identified as *Serratia nematodiphila*. It utilized cypermethrin as sole carbon source and degraded 97.5% of the insecticide added at an initial concentration of 100 ppm in FTW mineral salt medium in 7 days. On the basis of GC-MS analysis of degradation metabolites released by this strain it was concluded that breakdown of cypermethrin has been carried out through hydrolysis of the carboxyester bond. The optimum temperature and pH for the growth of isolated strain were found as 30^0C and 7.0 respectively. The results thus indicate that the isolated strain may be utilized for bioremediation of cypermethrin contaminated soil after a more detailed on-site study.
Keywords: Cypermethrin, Pyrethroids, *Serratia nematodiphila*, Bioremediation.

Introduction

Modern agriculture is a capital and technology intensive affair that is highly reliant on extensive chemical inputs in order to enhance the production. Consequently, a huge variety of chemical pesticides are popularly used across the globe for pest control purposes. Cypermethrin is a highly active synthetic pyrethroid insecticide categorized as class II pesticide (1) that is commonly used against a wide range of pests in agriculture, public health and animal husbandry. It has been a highly popular chemical pesticide since its introduction in the market in 1977. As far as agriculture is concerned it is primarily used as an effective insecticide against many foliage pests and certain surface soil pests as well. Chemically, it is alpha-cyano-3-phenoxy-benzyl ester of the dichloro analogue of chrysanthemic acid, 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylic acid (2).

Cypermethrin is moderately persistent in the environment as it gets very strongly adsorbed on soil particles thus appreciably limiting its movement and downward leaching through the soil particles. It is highly toxic for freshwater as well as marine fish and invertebrates and is relatively immobile in surface waters. Regarding the non-target insects, cypermethrin presents acute toxicity to many beneficial insects such as honey bees and also to earthworms etc. Further, like all pyrethroids cypermethrin acts as a short-term neurotoxin in humans altering the nerve functions by modifying the normal biochemistry and physiology of nerve membrane sodium channels (3) and by modulating the level of gamma-aminobutyric acid (4). It has been classified as a potential carcinogen by EPA (5) and also implicated with Parkinson's disease (6).

Two major routes of cypermethrin degradation are the photodegradtion and the biodegradation. However, many studies in the past have proved cypermethrin as a reasonably light-stable pyrethroid (7; 8) and cleavage of the ester linkages in cypermethrin molecule through biological processes in soil has been

established as the chief mode for breakdown of this pyrethroid (9). Biodegradation of cypermethrin by a variety of soil and rhizospheric bacteria including some novel strains as well has been reported such as *Catellibacterium* (10), *Azoarcus indigens* (11), *Serratia* (12), *Stenotrophomonas* (13), *Pseudomonas aeruginosa* (14) and many other.

In light of elevated concentrations of toxic pesticide residues in the environment along with the cost intensive and less efficient nature of traditional physico-chemical techniques, effective and eco-friendly bioremediation techniques are critically required for the clean-up of pesticide contaminated sites. This study was hence undertaken to evaluate the cypermethrin degradation capacity of rhizobacteria associated with cauliflower.

Materials and methods

Cypermethrin:

Technical grade cypermethrin for the study was provided by Plant Protection Quarantine and Storage, Department of Agriculture and Co-operation, NH-4, Faridabad, Haryana, India.

Soil samples:

Rhizospheric soil samples were randomly collected from the cauliflower cultivating areas of district Faridabad, Haryana, India. Sampling was carried out in areas having a long history of cypermethrin usage for last many years and the time of sampling was so chosen that the crop was already been sprayed with the pesticide. Plants were uprooted to get the roots and the adhering soil particles. Loosely bound soil was removed with vigorous shaking, the foliage part was discarded and the remaining root and soil samples were transferred to sterile polythene bags, and stored at 4^0C till further use.

Enrichment of soil samples:

Soil adhering to the collected root samples was removed carefully with the help of scalpel and brush and the soil thus obtained was properly mixed. The native microflora in this rhizospheric soil was enriched by the addition of cypermethrin at a concentration of 25 ppm at weekly intervals for six weeks.

Isolation of cypermethrin degrading rhizobacteria:

Enrichment culture technique using FTW mineral salt medium (15) supplemented with cypermethrin as sole carbon and energy source was used for isolation of cypermethrin degrading rhizobacteria. Composition of FTW mineral salt medium (g/l) was K_2HPO_4, 0.255; KH_2PO_4, 0.255; $(NH_4)_2SO_4$, 0.255; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.005; $FeCl_2.4H_2O$, 0.005 and 1.0 ml of trace element solution (16). The Focht trace element solution contained (mg/l): $MgSO_4.H_2O$, 169; $ZnSO_4.7H_2O$, 288; $CuSO_4.5H_2O$, 250; $NiSO_4.6H_2O$, 26; $CoSO_4$,28; and $Na_2MoO_4.2H_2O$, 24.

An aliquot of 10 grams of pretreated soil was added to a 250 ml Erlenmeyer flask containing 100 ml sterilized FTW mineral salt medium supplemented with cypermethrin at a concentration of 25 ppm and incubated at 30^0C under shaking conditions. After a week 10 ml of this culture was transferred to 100 ml of fresh FTW mineral salt medium containing 50 ppm of cypermetrin and incubated under the same conditions. In this way the concentration of cypermetrin was gradually increased from 50ppm to 200 ppm on weekly basis with an increment of 25 ppm. At every incremental step the culture was serially diluted and spread plated on to the corresponding solid FTW mineral salt medium and incubated at 30^0C for 2-4 days. The representative microorganisms growing on the plates were purified following the four-way streaking method. The isolates withstanding highest cypermethrin concentration (200 ppm) were finally selected, re-inoculated into liquid FTW mineral salt medium to confirm cypermethrin utilization and their ability to degrade cypermethrin was determined by gas chromatography (GC).

Identification of cypermethrin degrading isolate:

On the basis of cypermethrin degrading potential isolate CB2 was found to be the most effective strain and it was identified on the bases of its morphological and biochemical characteristics along with 16S rRNA gene sequence analysis. Different morphological features including size, shape, margins, color and consistency

of colony, cell shape, cell arrangement, gram reaction, were examined as described by the standard procedures (17).

Molecular characterization was carried out on the basis of 16s RNA sequencing conducted by Macrogen Inc., Korea. The 16s rRNA region of the isolated strain CB2 was sequenced using universal primers 518F and 800R and compared with the sequences deposited at the National Center for Biotechnology Information (NCBI), using BLAST to establish the percentage nucleotide similarity with these sequences. The bacterial strain was then identified on the basis of calculated percentage nucleotide similarity.

Physiological studies for growth of strain CB2:

In order to determine the optical temperature and pH conditions for the growth of the isolate CB2 three different variants of temperature and pH were tested i.e. an incubation temperature of 25^0C, 30^oC and 35^oC and pH 6.0, 7.0 and 8.0. An aliquot of 1.0 ml of overnight activated culture of the isolate CB2 was inoculated in 50 ml FTW minimal salt medium in triplicate sets and incubated for 48 hours at the above mentioned temperatures i.e. 25^0C, 30^oC and 35^oC after which growth was estimated at 600 nm. A flask containing 50 ml FTW minimal salt medium without bacterial suspension served as the control. Similarly the effect of pH was determined in 50 ml of FTW minimal salt medium maintained at different pH i.e.6.0, 7.0 and 8.0 and incubated for 48 hours at 30^oC.

Biodegradation of cypermethrin by strain CB2:

Degradation of cypermethrin by the selected isolate CB2 was examined in FTW minimal salt medium containing 100 ppm cypermethrin and maintained in triplicates. Overnight activated bacterial culture was inoculated in 250 ml Erlenmeyer flasks containing 100 ml FTW minimal salt medium supplemented with 100 ppm cypermethrin and a flask containing the same medium without bacterial suspension served as the control. Inoculated flasks were incubated at 30^0C under shaking conditions for one week. Samples were withdrawn at regular time intervals to assess the cell growth by recording OD_{600} value using a spectrophotometer and the residual cypermethrin concentration was determined by gas chromatography (GC),Shimadzu QP 2010 system.

Detection of cypermethrin metabolites

Analysis of the cypermethrin metabolites biodegraded by strain CB2 was carried out in cell-free filtrates of FTW minimal salt medium culture broth supplemented with 100 ppm cypermethrin at regular intervals of 24 hours. The regularly withdrawn samples were filtered to remove the biomass and cell-free filtrates thus obtained were acidified with HCl at a concentration of 2 mol/l to reach pH 2.0 and then extracted with ethyl acetate. The organic layer was dehydrated with anhydrous sodium sulphate, and then dissolved in the same volume of methanol (Chromatography grade, Merck, India) (10). These samples were finally filtered using a 0.45 µm membrane and then analyzed by gas chromatography–mass spectrometry (GC–MS) technique using GC-MS-QP-2010 plus, Shimadzu.

The gas chromatograph equipped with a split-splitless injector (split ratios of 50:1) was used for the GC analysis. The oven temperature was initially at 60°C for 2 minutes and then programmed to 300°C at a rate of 20°C $minute^{-1}$ where it was held for 5 minutes. The temperature of injector, transfer line and ionization source was 250°C. The electron impact ionization was tuned at 70 eV and helium was used as carrier gas with an average linear velocity of 1.0 ml min^{-1}. The mass spectra were recorded within 41 to 400 amu to collect the total ion current (TIC) chromatograms.

Statistical analysis

The data obtained for the present investigation were subjected to one-way analysis of variance (ANOVA) for a completely randomized design with $P < 0.05$ being accepted as significant, whenever applicable.

Results

Isolation and identification of cypermethrin degrading bacterial strains

A total of 10 bacterial strains having different morphological features and a capacity to utilize cypermethrin as the sole carbon and energy source for growth, were isolated on FTW minimal salt medium

from the soil samples enriched with cypermethrin. Fifty percent of these bacterial isolates exhibited tolerance up to 150 ppm of cypermethrin in solid as well as liquid FTW minimal salt medium. However, only two isolates i.e. CA4 and CB2 were found to withstand higher concentrations of the pesticide i.e. 200 ppm (Table I).

Table 1. Cypermethrin degrading bacterial strains isolated in cypermethrin enriched FTW minimal salt medium

Serial No.	Isolate	Growth in FTW minimal salt medium containing cypermethrin as the sole carbon and energy source						
		25ppm	50ppm	75ppm	100ppm	150ppm	175ppm	200ppm
1.	CA1	+	+	+	+	+	+	-
2.	CA2	+	-	-	-	-	-	-
3.	CA3	+	+	+	+	+	+	-
4.	CA4	+	+	+	+	+	+	+
5.	CA5	+	-	-	-	-	-	-
6.	CA6	+	-	-	-	-	-	-
7.	CB1	+	+	+	+	+	+	-
8.	CB2	+	+	+	+	+	+	+
9.	CB3	+	-	-	-	-	-	-
10.	CB4	+	-	-	-	-	-	-

+: Growth, –: No growth

On the basis of cypermethrin degrading efficiency strain CB2 was identified as the most effective bacterial isolate. Strain CB2 was a gram-negative, short rod shaped bacterium which gave small, circular and smooth colonies when plated on FTW minimal salt medium. 16S rRNA gene sequence analysis of the 1468 bp segment followed by BLAST indicated 100% nucleotide identity of this isolate with *Serratia nematodiphila* strain P36 (Accession No. FJ662869.1). Thus, strain CB2 was identified as *Serratia nematodiphila*.

Physiological parameters for the growth of strain CB2:

Variation in temperature and pH significantly affected the growth of the isolated strain CB2 ($P < 0.05$). The optimum values of temperature and pH for the growth were established as 30^0C (Figures 1) and 7.0 (Figures 2) respectively, as recorded in terms of absorbance at 600 nm. A shift in the temperature and pH below and above these values resulted in considerable reduction in the growth of strain CB2.

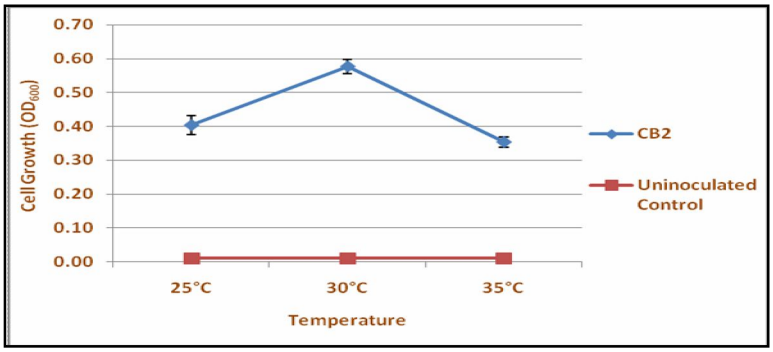

Figure 1. Effect of temperature on growth of strain CB2

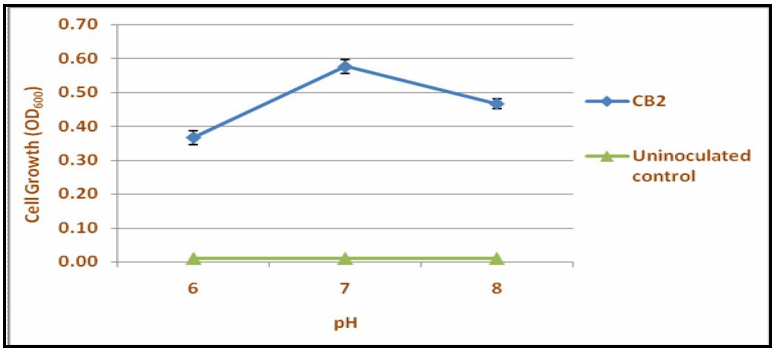

Figure 2. Effect of pH on growth of strain CB2

Biodegradation of cypermethrin by strain CB2:

Strain CB2 was able to grow in FTW mineral salt medium by utilizing cypermethrin as sole carbon source as indicated by the OD_{600} values and it degraded 97.5% cypermethrin added at an initial concentration of 100 ppm in 7 days (Figure 3). As clearly indicated in the graph there was a rapid utilization of cypermethrin in the first 3-4 days and this was accompanied by a corresponding increase in the biomass concentration. After the 5th day a slight fall was recorded in biomass concentration and by this time more than 96% of the cypermethrin had already been utilized. The un-inoculated control did not show any change in biomass concentration. Thus, it may be inferred that cypermethrin was rapidly degraded by the tested strain in 3-4 days at a concentration of 100 ppm by utilizing it as a carbon and energy source.

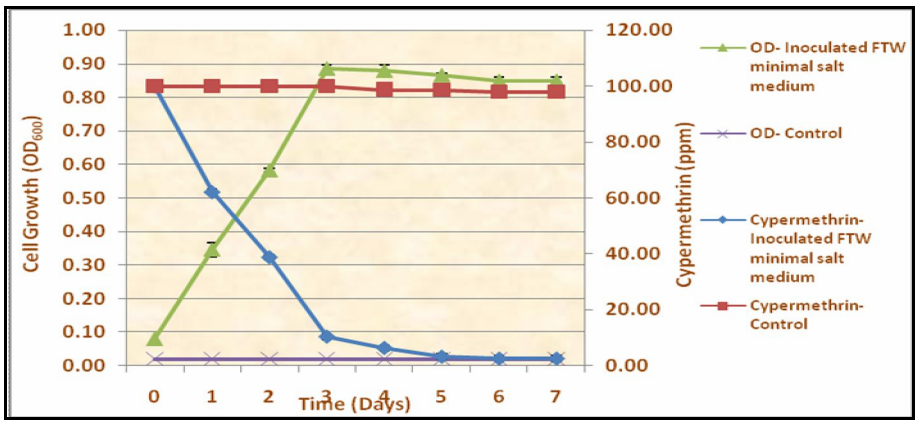

Figure 3. Cell growth and utilization of cypermethrin by strain CB2 in FTW minimal salt medium supplemented with 100 ppm cypermethrin.

Detection of cypermethrin metabolites:

The metabolites produced by strain CB2 as a result of biodegradation of cypermethrin molecule were extracted in methanol and detected by GC-MS. Identification of these compounds was carried out on the basis of their mass spectra matched with the standard compounds from the National Institute of Standards and Technology library database. After 7 days of incubation, the degradation compounds observed were identified as cyclopropanecarboxylic acid at peaks corresponding to a retention time 35.640, 35.782 and 35.866, (+)-*cis*-cypermethrin at retention time 35.921, cyclononasiloxane at retention time 36.622 and 37.545 and 9-eicosyne at retention time 37.483. Thus, it may be inferred from the results that strain CB2 hydrolyzed the carboxyester bond in the cypermethrin molecule to give cyclopropanecarboxylic acid and its derivatives thus degrading cypermethrin to lesser or non- toxic forms.

Discussion

Synthetic pyrethroid insecticides are one of the most commonly used chemical pesticides world over and they have especially gained popularity after EPA banned many of the organophosphate pesticides (18). Pesticide accumulation in the environment and their wide-ranged side effects has always been a matter of concern. Cypermethrin being one of the chiefly used pyrethroid insecticides in agriculture sector has been studied by many research groups across the globe for its biodegradation aspects. Here we report the biodegradation of cypermethrin by *Serratia nematodiphila* isolated from the contaminated soil. Different strains of genus *Serratia* have been reported for biodegradation of cypermethrin including *Serratia plymuthica* and many others (19; 20; 12). Degradation of other synthetic pyrethroid insecticides has also been widely reported by members of this genus for example pyrethroid deltamethrin by *Serratia marcescens* (21); pyrethroid flumethrin (20). Reports on the degradation of organophosphorus insecticides have also been obtained like diazinon by *Serratia liquefaciens* and *Serratia marcescens* (22), methidathion (23), tetrachlorvinphos (24) and malathion by *Serratia marcescens* (25). Some other pesticide groups have also been reported to be degraded by *Serratia* such as chlorinated aliphatic herbicides by *Serratia marcescens* sp SE1 (26). However, this seems to be the first report of cypermethrin degradation by *Serratia nematodiphila*.

Further, most of these strains have shown high efficiency in terms pesticide degradation. For example *Serratia marcescens* strains DeI-1 and DeI-2 degraded 88.3% and 82.8 % deltamethrin respectively, in 10 days (21) while strains of *Serratia liquefaciens* and *Serratia marcescens* were reported to degrade 80-92% diazinon

in mineral salt medium (MSM) supplemented with diazinon (50 mgl^{-1}) as a sole carbon source (22). Similarly *Serratia* spp. strain JC1 and JCN13 isolated from the activated sludge samples of a pesticide manufacturing site degraded 92% beta-cypermethrin within 10 days and 89% within 4 days, respectively under optimal degradation conditions (12). Moreover, certain strains of *Serratia* have been established to simultaneously degrade more than one synthetic pyrethroid insecticide like flumethrin and cypermethrin (20) and organophosphates including chlorpyrifos, coumaphos, parathion, and isazofos when provided as a source of carbon and phosphorus (27). Such strains may prove beneficial in an attempt to remediate the multiple pesticide contaminated sites.

Strain CB2 isolated in this study degraded almost 97.5% cypermethrin when applied at an initial concentration of 100 ppm in a time period of 7 days. Similar degradation rates of cypermethrin have been previously reported by a wide range of bacterial strains isolated from contaminated sites such as 97% degradation by *Catellibacterium* sp. (10), 92% degradation by *Serratia* sp. strain JC1 (12) and 90.4% degradation by *Rhodobacter sphaeroides* (28). Similarly a novel bacterial strain *Ochrobactrum lupine* DG-S-01 was found to degrade over 90% of the initial dose of betacypermethrin (50 mg/l) within 5 days at 30^{o}C and pH 7.0 in mineral salt medium supplemented with glucose, beef extract and yeast extract (29) and another strain *Pseudomonas aeruginosa* CH7 had also been reported for 90 % biodegradation of beta-cypermethrin within 12 days at 25-35^{o}C, pH 6.0-9.0, and a final concentration of beta-cypermethrin 25-900 mg/l (14).

Microbial breakdown and detoxification of cypermethrin and other synthetic pyrethroid has primarily been reported as a result of the hydrolysis of the carboxyl ester linkage of the pesticide molecule by carboxylesterases (30; 12; 31; 10). Another pathway involving reductive dechlorination, oxidation or/and hydrolysis has also been reported to a limited extent (28). In this study we detected the presence of cyclopropanecarboxylic acid and its derivatives during GC-MS analysis of the cypermethrin supplemented broth samples utilized by strain CB2. This clearly indicated that the isolated *Serratia nematodiphila* strain broke down the cypermethrin carboxyl ester linkage and thus utilized it as carbon source thereby leading to its removal from the liquid FTW minimal salt medium. This may also be clearly established from the rapid increase in cellular biomass with a corresponding decrease in cypermethrin concentration during the first 4 days of incubation.

Rhizospheric bacteria have been established as suitable agents for a range of plant growth promoting (32) and pest control activities (33) and at the same time there are certain reports for the remediation of pesticide contaminated soil including cypermethrin by bacteria associated with the rhizosphere of various crops (34; 13). Since rhizospheric bacteria are well adapted to the soil ecosystems and have the capacity to establish themselves effectively in these soils they may prove to be the most suitable option for the bioremediation of the contaminated soils.

Conclusion

The results obtained in this study are quite encouraging and clearly indicate that the isolated strain CB2 that has been identified as *Serratia nematodiphila* may be utilized for the bioremediation of cypermethrin contaminated soil after a more detailed on-site study.

References

1. WHO (2009) The WHO recommended classification of pesticides by hazard and guidelines to classification. International programme on chemical safety.
2. IPCS (2012) Cypermethrin (Environmental Health Criteria 82, 1989). http://www.inchem.org/ documents/ehc/ehc/ehc82.htm.
3. EPA (2008) Reregistration eligibility decision for cypermethrin. http://www.epa.gov/ oppsrrd1/ REDs/cypermethrin_revised_red.pdf.
4. Manna S, Bhattacharyya D, Mandal TK, Dey S (2005) Neuropharmacological effects of alfa-cypermethrin in rats. Indian J Pharmacol 37: 18-20.
5. EPA (2012) Chemicals evaluated for carcinogenic potential. http://npic.orst.edu/chemicals_evaluated.pdf
6. Singh AK, Tiwari MN, Prakash O, Singh MP (2012) A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. Curr Neuropharmacol 10: 64-71.
7. Ruzo LO, Holmstead RL, Casida JE (1977) Pyrethroid photochemistry: decamethrin. J Agric. Food Chem 25: 1385-1389.

8. Day SR, Leahey JP (1980) ^{14} C-cypermethrin: aqueous photodegradation in sunlight. Fernhurst, Imperial Chemical Industries (Unpublished ICI Report No. RJ0154B).
9. Harris CR, Chapman RA, Harris C (1981) Laboratory studies on the persistence and behaviour in soil of 4 pyrethroid insecticides. Can Entomol 113: 685-694.
10. Zhao H, Geng Y, Chen L, Tao K, Hou T (2013) Biodegradation of cypermethrin by a novel *Catellibacterium* sp. strain CC-5 isolated from contaminated soil. Can J Microbiol 59: 311-317.
11. Ma Y, Chen L, Qiu J (2013) Biodegradation of beta-cypermethrin by a novel *Azoarcus indigens* strain HZ5. J Environ Sci Health Part B: Pesticides, Food Contaminants, and Agricultural Wastes 48: 851-859.
12. Zhang C, Jia L, Wang S, Qu J, Li K, Xu L, Shi Y, Yan Y (2010) Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. Bioresour Technol 101: 3423–3429.
13. Dubey KK, Fulekar MH (2013) Investigation of potential rhizospheric isolate for cypermethrin degradation. Biotech 3:33-43. doi 10.1007/s13205-012-0067-3.
14. Zhang C, Wang S, Yan Y (2011) Isomerization and biodegradation of beta-cypermethrin by *Pseudomonas aeruginosa* CH7 with biosurfactant production. Bioresour Technol 102: 7139-7146. doi: 10.1016/j.biortech.2011.03.086
15. Herman DC, Frankenberger WT Jr (1999) Bacterial reduction of perchlorate and nitrate in water. J Environ Qual 28: 1018-1024.
16. Focht DD (1994) Microbiological procedures for biodegradation research. In: Weaver RW et al. (eds) Methods of soil analysis, Part 2, SSSA Book Series 5, pp 407-426.
17. Cappuccino J, Sherman N (2010) Microbiology: A laboratory manual, Ninth edition. Benjamin/Cummings Publishing, CA.
18. Palmquist K, Salatas J, Fairbrother A (2012) Pyrethroid insecticides: use, environmental fate, and ecotoxicology. In: Perveen F (ed) Agricultural and Biological Sciences » "Insecticides - Advances in Integrated Pest Management edited by Insecticides - Advances in Integrated Pest Management. InTech, Europe, pp 251-278.
19. Grant RJ, Daninell TJ, Betts WB (2002) Isolation and identification of synthetic pyrethroid-degrading bacteria. J Appl Microbiol 92:534-540.
20. Grant RJ, Betts WB (2004) Mineral and carbon usage of two synthetic pyrethroid degrading bacterial isolates. J Appl Microbiol 97:656-662. doi:10.1111/j.1365-2672.2004.02358.x
21. Cycon M, Zmijowska A, Piotrowska-Seget Z (2013) Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of *Serratia marcescens*. Int J Environ Sci Technol. doi: 10.1007/s13762-013-0322-0.
22. Cycon M, Wojcik M, Piotrowska-Seget Z (2009) Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. Chemosphere 76:494-501. doi: 10.1016/j.chemosphere.2009.03.023.
23. Li C, Lan Y, Zhang J, Chen Z, Tang D, Xu H (2013) Biodegradation of methidathion by *Serratia* sp. in pure cultures using an orthogonal experiment design, and its application in detoxification of the insecticide on crops. Annals Microbiol 63: 451-459.
24. Ortiz-Hernandez ML, Sanchez-Salinas E (2010) Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in Mexico. Rev Int Contam Ambient 26: 27-38.
25. Kannan V, Vanitha V (2005) Influence of assay medium on degradation of malathion by *Serratia marcescens*. Indian J Biotechnol 4: 277-283.
26. Abel E, Ibrahim N, Huyop F (1990) Identification of *Serratia marcescens* SE1 and determination of its herbicide 2,2-dichloropropionate (2,2-DCP) degradation potential. Malaysian J Microbiol 8: 259-265.
27. Abo-Amer A (2011) Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. J Microbiol Biotechnol 21: 71-80.
28. Yin LB, Zhao LZ, Liu Y, Zhang DY, Zhang SB, Xiao K (2013) Isolation and characterization of cypermethrin degrading bacteria screened from contaminated soil. In: Biodegradation of hazardous and special products. InTech, Europe, pp: 1-16. doi.org/10.5772/56339
29. Chen S, Hu M, Liu J, Zhong G, Yang L, Rizwan-ul-Haq M, Han H (2011a) Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupine* DG-S-01. J Hazard Mater 187: 433-440. doi: 10.1016/j.jhazmat.2011.01.049
30. Tallur PN, Megadi VB, Ninnekar HZ (2008) Biodegradation of cypermethrin by *Micrococcus* sp. strain CPN1. Biodegradation 19:77-82. doi:10.1007/ s10532-007-9116-8.
31. Chen S, Zhang Y, Hu M, Geng P, Li Y, An G (2011) Bioremediation of β-cypermethrin and 3-phenoxybenzoic acid in soils. Proceedings International Symposium on Water Resource and Environmental Protection (ISWREP) 3: 1717-1721. doi:10.1109/ISWREP.2011.5893579

32. Prashar P, Kapoor N, Sachdeva S (2014) Rhizosphere: its structure, bacterial diversity and significance. Rev Environ Sci Biotechnol 13:63-77.
33. Prashar P, Kapoor N, Sachdeva S (2013) Biocontrol of plant pathogens using plant growth promoting rhizobacteria. Sustain Agric Rev 12: 319-360.
34. Ahemad M, Khan MS (2012) Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (*Pisum sativum*)-specific *Rhizobium* sp. strain MRP1. Emirates J Food Agric 24: 334-343.

*****