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RESEARCH ARTICLE :

Isolation and characterization of organ phosphorus pesticide degrading bacteria from different crops

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SUMMARY : In the present work total eight bacterial isolates were obtained from insecticide treated maize and ground nut rhizospheric soils by enriching Mineral Salt Medium broth with supplement of Phorate source. These isolates were characterized on the basis of cell morphology, cultural and biochemical properties. Isolates were screened for their phorate degradation capability in liquid cultures. Among the eight phorate degrading bacterial isolates, PDB-1 showed the high population count at different incubation periods (1st day to 5th day) compared to all Phorate degrading bacterial(PDB) isolates. The Phorate degrading bacteria (PDB)-1 isolate utilized the pesticide (Phorate) effectively and showed maximum bacterial count 5.9 cfu $\times 10^6$ ml^{-1}. Phorate degrading efficiency of isolates was determined by measuring the phorate residual concentrations at intervals using Gas chromatographic method. The degradation of Phorate at different concentrations (20, 30 and 40 mg l^{-1}) was examined in the Mineral salt liquidmedium. By this degradation percentage study of Phorate revealed that the Phorate degrading bacterial isolate (PDB-1) degrade the Phorate effectively.

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BACKGROUND AND OBJECTIVES

Modern agriculture is a capital and technology intensive affair that is highly reliant on extensive chemical inputs in order to enhance the production. Plant protection has been an integral part of agricultural crop production. Average field losses in India, caused by insects, diseases, weeds, etc., are estimated to be 10-30%. Consequently, a huge variety of chemical pesticides are popularly used across the globe for pest control purposes. However, increased use of chemicals has lead to environmental pollution. Application of chemicals to soil has resulted in changed soil microflora. Moreover, residual effect of chemicals has also been well-known on plant and animal life. Hence, degradation of pesticides is desirable as soon as thepests are controlled. Microbial processes play animportant role in the biological transformation of pesticides. Saprophytic micro-organisms perform the major degradation or transformation of pesticides

inthe soil.

Phorate {O,O-diethyl S-[(ethylthio) methyl] phosphorodithioate} is a highly toxic organo phosphorus insecticide. Structurally it is thioester of phosphoric acid characterized by a central phosphorus atom. It is extensively used in agriculture, ornamental plants and forests to control sucking and chewing insects (Gallo and Lawryk, 1991). In India, total production of phorate has been estimated to be 4800 MT. This type of large quantity of production indicates larger area of agri land contamination with phorate. The World Health Organization classifies phorate as an extremely hazardous pesticide; also the food and agriculture organization has banned its usage in the developing countries.

An important way to avoid ecological damages and human health problems caused by the presence of pesticides is to reduce their concentrations in the environment, precluding either lixiviation to ground water or possible incorporation to natural food chains **(**Ortiz-Hernandez *et al.*, 2003). Several researchers reported potential bacterial strains like *Pseudomonas* sp., *Arthrobacter* sp., *Bacillus* sp., *Klebsiella* sp., *Serratia marcescens, Enterobacter* sp., *Stenotrophomonas* sp., *Sphingomonas* sp., *Flavobacterium* sp. etc., fungal strains such as *Phanerochaete chrysosporium, Aspergillus terreus, Verticillium* sp , *Pseudomonas* etc. and cyanobacteria like *Anabaena* sp., *Aulosira fertilissima, Phormidium valderianum* for organophosphorous pesticides (chlorpyrifos and phorate) degradation (Dhanya, 2014). Microbes play an imperative role in eliminating toxic substances from environment; furthermore, microbial bioremediation is considered to be a cost-effective tool for the detoxification of xenobiotics (Li *et al*., 2012).

Studies on microbial degradation are useful in the development of strategies for the detoxification of insecticides by microorganisms (Hayatsu *et al*., 2000). The bioremediation of OP-contaminated sites is recognized as a cost-effective and reliable method. Microorganisms play a key role in the degradation of organophosphate compounds and in eventually reducing their toxicity. Many bacteria have been known to degrade different kinds of pollutants including many of the insecticides (Ramanathan and Lalitka Kumari, 1999) and some bacteria capable of degrading OPs have been isolated (Horne *et al*., 2002).

Considering that phorate is one of the most commonly using insecticides for control of pests and insects, the purpose of this experiment was to isolate and characterize phorate degrading-bacteria, to investigate their degradation potential, to assess their adaptation to high concentrations of phorate and to determine their usefulness in biodegradation of contaminated sources.

The purpose of this study was to examine the ability of rhizospheric micro-organisms that could degrade Phorate and to investigate the optimized degradation potential of the organisms.

RESOURCES AND METHODS

Sample collection :

The soil samples were collected from maize and ground nut, fields of chittore district, Andhra Pradesh, India. The samples were collected in sterilized autoclaved glass bottles and brought to lab, coarsely ground, thoroughly mixed and stored at 4^{o}C in polythene bags before use.

Pesticide used :

Commercial-grade insecticide phorate (10% CG purity) was obtained from pesticide market Shamshabad, Rangareddy district Telangana, India. It was used throughout the experimental studies, because it may more closely resemble the active compound that micro-organisms are likely to be exposed to in the soil environment.

Enrichment and isolation of phorate degrading bacterial strains :

Air-dried and sieved (<2 mm) soil samples (10 g) from farm lands were collected and suspended in 250-ml Erlenmeyer flasks containing 50 ml of mineral salts medium (KH_2PO_4 4.8, K_2HPO_4 : 1.2, NH_4NO_3 : 1.0, $MgSO_4.7H_2O$: 0.2, Ca $(NO_3)_2.4H_2O$: 0.04, Fe $(SO_4)_3$: 0.001, pH : 7.0) supplemented with phorate (50 mg l^{-1}). The flasks were incubated on a rotary shaker at 250 rpm for 7 days at 30^{0}C. At periodic intervals, 1ml of culture was taken, made into serial dilutions upto 10^{-8} and enumeration was done by spread plate method. A loop full of bacterial growth from the plates was streaked onto Luria Bertani (LB) agar supplemented with phorate (50 mg l^{-1}) and the plates were incubated at 37^{0}C for 24 h. The individual bacterial colonies that grew on the

medium were sub cultured onto LB agar containing phorate of the same concentration until pure cultures were obtained. Later theses cultures were subjected to morphological, cultural, biochemical and molecular studies.

Characterization of phorate degrading bacteria :

The isolated bacteria were studied for their morphological like gram reaction, pigmentation, cultural characteristics and biochemical characteristics like Indole production, methyl red, voges-praskaure's test, citrate utilization test, oxidase, catalase and sugar fermentation tests (Krieg and Staley, 2010)

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Enrichment and isolation of phorate degrading bacterial strains :

Soil samples were collected from phorate treated rhizospheric soils (Maize and Ground nut) and enriched with 50 $\mu g.ml^{-1}$ of phorate in 10 g of soil mixed with 50 ml MSM broth incubated at 37°C with pH 7 on temperature regulated shaking incubator. Serial dilutions of enriched soil samples ranging from 10^{-1} to 10^{-8} were prepared and spread on LB agar medium plates. The LB agar plates were incubated at 37°C with pH 7 on incubator. Bacterial population count taken at different intervals like 2d, 4d and 7d were taken as 15.7 cfu $\times 10^6$ g^{-1} soil, 16.3 cfu $\times 10^6$ g^{-1} soil, 11.3 cfu $\times 10^6$ g^{-1} soil respectively. Similarly in ground nut crop bacterial count at 2 days, 4 days, and 7 days, were taken population count as 8.7 cfu $\times 10^6$ g^{-1} soil, 10.2 cfu $\times 10^6$ g^{-1} soil and 4.5 cfu $\times 10^6$ g^{-1} soils, respectively. Eight different bacterial colonies were observed on LB agar plates at 10^{-4} dilution. These eight different bacterial isolates were named as PDB-1, PDB-2, PDB-3, PDB-4, PDB-5, PDB-6, PDB-7 and PDB-8 isolates. The eight bacterial colonies were purified by streak plate method on enriched LB agar medium plates.

Similar results were found by Peter *et al.* (2014) isolated the *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Staphylococcus aureus* from rhizospheric soils. These micro-organisms could degrade methyl parathion upto 350 $\mu g\ ml^{-1}$ concentration.

Ali *et al.* (2011) reported similar results and isolated five chlorpyrifos degrading bacterial strains from effluent storage pools of pesticide factories and these isolates exhibited greatest similarity to *Pseudomonas aeruginosa* (AF 137358, AF 531099, AY 264292), *Pseudomonas nitroreducens* (EF107515) and *Pseudomonas putida* (AF 291048).

Morphological and biochemical characterization of phorate degrading bacterial isolates :

The cell morphology, colony morphology, Gram reaction and sporulation, shape was studied for eight phorate degrading bacterial isolates (Table 1). Among eight PDB (phorate degrading bacteria) isolates, four isolates were gram negative, small rods (PDB-3, PDB-

Table 1 : Morphological characters of Phorate degrading bacterial (PDB) isolates

Isolate code	Gram reaction	Cell shape	Sporulation	Cultural characters
PDB-1	Gram +ve	Rod	Sporulation	Light, irregular, non-spreading, glistening, convex, opaque, viscid colony with blue pigmentation
PDB-2	Gram +ve	Rod	Sporulation	Dull white, irregular, spreading, and glistening, convex, opaque, viscid colony with no pigmentation
PDB-3	Gram -ve	Rod	Non-Sporulation	Yellowish green, round, non-spreading, glistening, convex, opaque, viscid colony with green pigmentation
PDB-4	Gram +ve	Rod	Sporulation	Dull white, irregular, spreading, and glistening, convex, opaque, viscid colony with no pigmentation
PDB-5	Gram +ve	Rod	Sporulation	Off white, irregular, non-spreading, smooth, flat, opaque, viscid colony with no pigmentation
PDB-6	Gram -ve	Rod	Non-Sporulation	Dull white, irregular, large, smooth, flat, opaque, viscid colony with no pigmentation
PDB-7	Gram -ve	Rod	Non-Sporulation	Dull white, irregular, non-spreading, smooth, flat, opaque, viscid colony with no pigmentation
PDB-8	Gram -ve	Rod	Non-Sporulation	Dull white, irregular, non-spreading, smooth, flat, opaque, viscid colony with no pigmentation

6, PDB-7, PDB-8),these bacterial isolates shown greenish pigmentation and *Pseudomonas* spp growth characteristics on specified media and biochemical characteristics such as positive for denitrification, H2S and gelatin liquefaction. Remaining four PDB isolates (PDB-1, PDB-2, PDB-4, PDB-5) were gram positive rods. Gram positive rods were showed spore formation ability while gram negative rods were non spore formers. All isolates are single, small rods. All negative isolates are non sporulating and all positive isolates were sporulation ability. The cultural characters of all the isolates were studied on LB agar medium plates. All isolates showed different cultural characters on Luria bertani agar medium plates. PDB-3 colonies had yellowish green pigmentation isolates PDB-5, had white irregular colony, PDB-4, PDB-6, PDB-7, PDB-8, PDB-2, were dull white irregular type colonies, whilePDB-1, madcreamy, irregular colonies on LB agar medium plates. Biochemical characteristics of phorate degrading bacterial isolates had been given in Table 2.

Phorate degrading ability of bacterial isolates :

In this experiment, the growth of eight phorate pesticide degrading isolates was assessed in mineral salt broth containing 2% of pesticides.Among the 8 phorate bacterial isolates, PDB-1showed the high population count at different incubation periods (1st day to 5th day) compared to all PDB inoculants. PDB-1 isolate utilized

Table 2 : Biochemical characteristics of Phorate degrading Bacterial (PDB) isolates

Sr. No.	Isolates	Indole test	MR	VP	Citrate utilization	Catalase test	oxidase test	Starch hydrolysis	Gelatin liquefaction	H_2S test	Carbohydrate utilization						Dinitrification test
											Lac	Suc	Glu	Fru	Mal	Man	
1.	PDB-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	PDB-2	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-
3.	PDB-3	+	+	-	+	+	+	-	+	-	-	+	+	-	-	+	-
4.	PDB-4	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
5	PDB-5	-	+	+	+	+	+	-	+	-	-	-	+		-	-	-
6.	PDB-6	+	-	-	+	+	+	-	+	+	+	+	-	-	-	-	+
7.	PDB-7	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-
8.	PDB-8	-	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+

Table 3 : Population count of PDB in MSM broth containing 2% phorate

Sr. No.	Isolates	Bacterial population (CFU×$10^6 g^{-1}$ soil)				
		1st day	2nd day	3rd day	4th day	5th day
1.	PDB-1	5.9	9.9	9.0	7.6	4.0
2.	PDB-2	3.1	7.2	7.0	5.2	1.2
3.	PDB-3	5.1	7.6	1.9	1.7	1.2
4.	PDB-4	4.0	8.0	2.2	1.4	1.1
5.	PDB-5	1.7	6.1	7.0	1.6	1.4
6.	PDB-6	2.8	3.2	7.1	6.8	4.2
7.	PDB-7	5.8	7.8	2.5	1.4	1.1
8.	PDB-8	4.1	5.2	6.5	4.0	2.2

Table 4 : Degradation percentage of Phorate at different conc. in MSM broth inoculated with PDB-1 at different time intervals

Time of interval	PDB-1					
	Concentration (ppm)	Degradation%	Concentration (ppm)	Degradation%	Concentration (ppm)	Degradation %
Initial	20	0.00	30	0.00	40	0.00
2nd day	15.6	22.0	25.4	15.1	33.4	16.5
4th day	12.09	39.5	20.7	30.8	27.7	30.7
6th day	9.7	51.4	12.7	57.5	26.4	33.9
8th day	7.2	63.9	9.1	69.6	21.7	45.7
10th day	5.1	74.5	3.3	88.8	11.5	71.0
Half life(days)	5.10		2.84		5.90	

the pesticide (phorate) effectively and showed PDB-1 maximum growth bacterial count 5.9 ×10^6cfu ml^{-1} (Table 3).

Degradation of phorate in liquid medium by PDB-1:

In this contents three varied concentrations of phorate MSM brothwere inoculated with PDB-1 isolate and the residual phorate conc. was measured with the help of GC. The degradation of phorate at different concentrations (20, 30 and 40 mg l^{-1}) was examined in the MSL medium on rotary shaker at 150 rpm, 30°C (Table 4).

During first 2 days in incubation the phorate concentration dropped from 20 to 15.6 mg l^{-1}. After 4 and 6 day, phorate was declined to 12.09 and 9.7 mg l^{-1}, respectively and by day 8 and 10 day phorate was declined to 7.2 and 5.1mg l^{-1}. The degradation percentage pattern showed decrease of residues from 2nd day to 10th day and residues were dissipated by 22.0, 39.5, 51.4, 63.9, and 74.5 % at 2, 4 ,6 ,8 and 10 days, respectively. The regression equation was Y = -0.0597 x + 4.3243 R^2= 0.9922 and the Half life was calculated to be 5.10 days (Fig. 1).

At the 30 ppm conc. the Phorate concentration dropped from 30 to 25.4 mg l^{-1} after 2 days. After 4th and 6th day, phorate was declined to 20.7 and 12.7 mg l^{-1}, respectively and by day 8th and 10th day phorate was declined to 9.1 and 3.3 mg l^{-1}. The degradation percentage pattern showed decrease of residues from 2nd day to 10th day and residues were dissipated by 15.1, 30.8, 57.5, 69.6, and 88.8 % at 2, 4 ,6 ,8 and 10 days, respectively. The regression equation was Y = -0.106 x + 4.6986 R^2= 0.9272 and the Half life was calculated to be 2.84 days (Fig. 2).

At the 40 ppm conc the phorate concentration dropped from 40 to 33.4 mg l^{-1} after 2 days. After 4th and 6th day, phorate was declined to 27.7 and 26.4 mg l^{-1}, respectively and by day 8th and 10th day phorate was declined to 21.7 and 11.5 mg l^{-1}. The degradation percentage pattern showed decrease of residues from 2nd day to 10th day and residues were dissipated by 16.5, 30.7, 33.9, 45.7, and 71.0 % at 2, 4 ,6 ,8 and 10 days, respectively. The regression equation was Y = -0.0514 x + 4.6664 R^2= 0.8384and the Half life was calculated to be 5.90 (Fig. 3).

We were noticed initially degradation capacity slow in this study in three different concentrations of phorate. Similar results were shown by Jaya Madhuri and Rangaswamy (2009) they isolated bacterial cultures by selective enrichment technique and identified isolates as

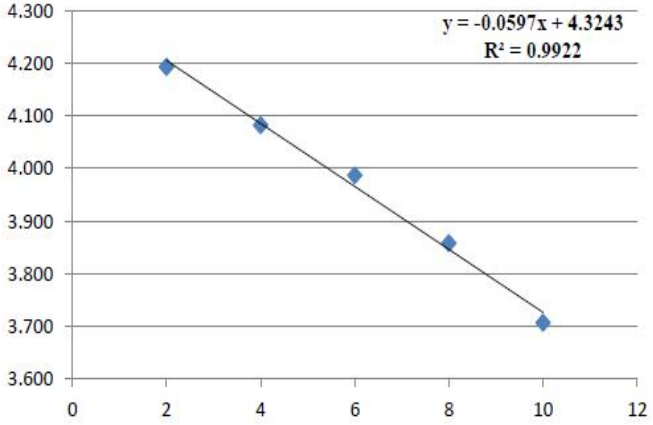

Fig. 1 : Illustration 1 : Phorate degradation at 20ppm

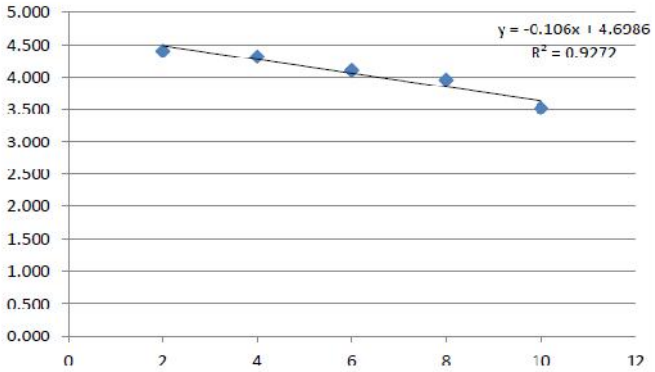

Fig. 2 : Illustration 2 : Phorate degradation at 30ppm

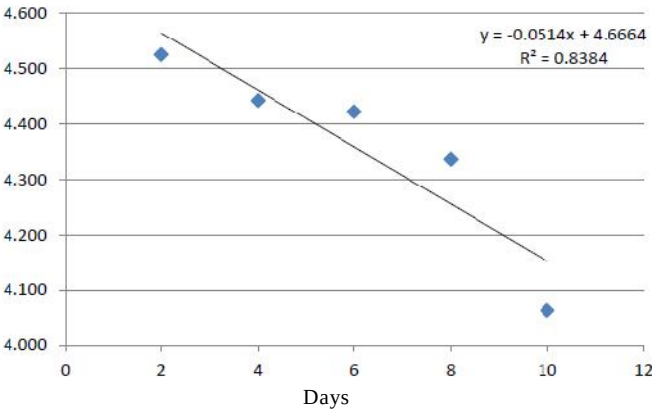

Fig. 3 : Illustration 3 : Phorate degradation at 40ppm

Illustration details : Phorate residual concentrations at different days of intervals

species of *Bacillus* and *Pseudomonas.* These isolates were tested for their ability to degrade the respective insecticides in mineral salts medium. Within 7 days of incubation, nearly 75% of chlorpyrifos and phorate and 50% of dichlorvos, methyl parathion and methomyl were degraded by cultures of soil bacteria. Qualitative analysis of chlorpyrifos and methyl parathion residues by gas chromatography revealed that dichlorvos and phorate were completely degraded by soil isolates at the end of 14 days.

Conclusion :

This study confirmed phorate degrading micro-organisms isolated from chlorpyrifos-applied agricultural crop fields. As indicated by the kinetic constants, the PDB-1 isolate showed different capabilities for the biodegradation of phorate. Moreover, this study would be helpful in the practical application of bioremediation of phorate contaminated waterdue to its low cost and less collateral destruction ofindigenous organisms. Additional work is requiredfor detecting the metabolites, mapping thedegradation pathway and identifying the enzymesinvolved in the biodegradation process.

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REFERENCES

Ali, M.L., Samaneh, K., Morteza, M., Mohsen, M. and Hamid, B. (2011). Isolation and characterization of five chlorpyrifos degrading bacteria. *African. J . Biotech.*, **11** (13): 3140 – 3146.

Dhanya, M.S. (2014). Advances in microbial biodegradation of Chlorpyrifos. *J. En. Res. & Development,* **9**(1).

Gallo, M.A. and Lawryk, N.J. (1991). Organic phosphoru pesticides. In : *Handbook of Pesticide Toxicology Editrion*. Hayes, W.J and Laws, E.R. 3–5. New York: Academic Press.

Hayatsu, M., Hirano, M. and Tokuda, S. (2000). Involvement of two plasmids in fenitrothion degradation by *Burkholderia* sp. strain NF 100. *Appl. Environ. Microbiol.*, **66** (4):1737–40.

Horne, I., Sutherland, T.D. and Harcourt, R.L. (2002). Identification of an opd (organophosphate degradation) gene in an *Agrobacterium* isolate. *Appl. Environ. Microbiol.*, **68** (7) : 33710–43376.

Jaya Madhuri, R. and Rangaswamy, V. (2009). Biodegradation of selected insecticides by *Bacillus* and *Pseudomonas* sps in ground nut fields. *Toxicol. Internat.*, **16** (2) :127-132.

Krieg, N.R. and Staley, J.T. (2010). Bergey's Manual of Systematic Bacteriology, 2nd Ed., Williams and Wilkins Co., Baltimore,

Li, C., Zhang, J., Wu, Z.G., Cao, L., Yan, X. and Li, S.P. (2012). Biodegradation of buprofezin by *Rhodococcus* sp. strain YL - 1 isolated from rice field soil. *J. Agric. & Food Chem.*, **60** : 2531–2537.

Ortiz-Hernandez, M.L., Quintero-Ramirez, R., Nava-Ocampo, A.A. and Bello-Ramirez, A.M. (2003). Study of the mechanism of *Flavobacterium sp.*Forhydrolyzing organophosphate pesticides. *Fun. & Clinical Pharmacol.*, **17**: 717–723

Peter, J.K., Harison, M., Yashab, K., Ajay, K.S. and Shubha, C. (2014). Organophosphate pesticide (methyl parathion) degrading bacteria isolated from rhizospheric soil of selected plants and optimization of growth conditions for degradation. *Internat. J. Res.*, **1**: 1-13.

Ramanathan, M.P. and Lalithakumari, D. (1999) Complete mineralization of methylparathion by *Pseudomonas* sp. A3. *Appl. Biochem. Biotechnol.*, **80**(1):1–12.