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ISOLATION AND CHARACTERIZATION OF DICHLOROVOS DEGRADING BACTERIAL STRAIN *PSEUDOMONAS STUTZERI* SMK

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ABSTRACT: A gram negative bacterium, capable of degrading dichlorovos (2, 2 dichlorovinyl dimethyl phosphate) was isolated from pesticide contaminated agriculture soil by enrichment technique. The morphological, biochemical and 16S r RNA gene sequence analysis confirmed that the isolate is *Pseudomonas stutzeri* smk.

KEYWORDS: Biodegradation, *Pseudomonas stutzeri,* FTIR, HPLC.

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1. INTRODUCTION

The pest causes significant agronomic damage .The damage caused by such pest are controlled by use of integrated pest management .which involves the use of different pesticides of which insecticides form a major part .(Theodoridis et al.2005) But unplanned and extensive use of pesticides leads to an accumulation of huge amount of residues in the environment .Due to uptake and accumulation of these toxic compounds in food chain and drinking water causes serious environmental and health hazards.(Mohammed ,2009) The organophosphate pesticides are the group of highly toxic, heterogeneous compounds widely used for pest control. There are currently 140 OP compounds being used as pesticides and as plant growth regulators around the world. (Kang et al, 2006). In agriculture, these synthetic OP compounds are widely used as insecticides .These

compounds acts as acetylcholine substitutes and inhibits the acetylcholine esterase enzyme. (Bakry et al, 2006). In humans, OP poisoning causes various clinical effects like neck muscles weakness and diarrhea. (Serdar and Gibson, 1985, Grimselyet.al, 1998). Exposure to these OP compounds causes cancer, endocrine disruption, birth defects and neurological damage. (Miller and Sharp, 1998, IARC, 2001AISDR, 2005). Among OP insecticide Dichlorovos (2, 2 dichlorovinyl dimethyl phosphate).are routinely employed insecticide. It is widely used to control household as well as agricultural pests. In view of its toxicity and persistency it is important to remove them from the environment. For the removal of such compounds from environment ,the conventional methods like landfills, chemical treatment, incineration and recycling are employed but these methods are costly and time consuming and causes formation of toxin intermediates (Dua et al,2002,Richinis .et .al,1997).Therefore it is essential to develops ecofriendly and economically feasible methods for pesticide detoxification. The most reliable and cost effective technique for pesticide removal is bioremediation, which successfully applied to bioremidiate soil contaminated with OP pesticides. The isolation of many bacteria capable of OP degradation have been carried out from soil around the world. (Zhongli et.al2001; Chang et al; 2005; Horne et al; 2002.).Some bacterial species detoxify OP by enzymatic reactions has been reported. (Chen-Good speed et.al; 2001, Kim et al; 2005.).The considerable attention has been given towards the isolation of indigenous bacteria having ability to degrade OP compounds. (Richinis et al; 1997, Mulchaldini; et.al, 1999.) So the ultimate goal of this study was to isolate and characterize potent bacteria strain capable of degrading the dichlorovos.

2. MATERIALS AND METHODS

2.1. Chemicals

The analytical grade dichlorovos (2,2 dichlorovinyl dimethyl phosphate,95% pure)was obtained from insecticide residue testing laboratory ,Pune and all other chemicals used in current study (analytical grade and are of higher purity)were purchased from local distributor,pune ,India.

2.2 Isolation, Characterization and Identification of microorganism:

The bacterial strain capable of dichlorovos degradation was isolated from the agriculture soil, which was previously exposed to dichlorovos since 5 years, using enrichment techniques.For isolation of bacteria, three different variants of minimal salt medium (MSM) were used such as phosphate limiting (P-L-MSM), Carbon limiting(C-L-MSM) and full strength MSM. The 250 ml conical flask containing 100 ml MSM were prepared in duplicate supplemented with 2 gm. pesticide contaminated soil sample and 100 µg l-1 of dichlorovos. Then the flasks were incubated at 30 0C in static as well as in shaking condition for 7 days at 120rpm in rotary shaker incubator. After 7th day

of incubation, the loopful suspension from each enrichment was streaked on agar plates with respective MSM containing 100 μg l-1of dichlorovos, followed by their incubation at 30 0C for 4 days .Morphologically distinct colonies from each plate were selected and used for further study. The bacterial isolate was maintained on nutrient agar plate.(composition:gm/lit;5,Nacl,10 bacteriological peptone,1,beef extract;2,yeast extract and agar)and stoared at 4 0C as a master stock culture.Characterization of isolate was carried out by morphological characterization, microscopic observation and biochemical tests (according Bergey's manual of systematic Bacteriology.)

2.3 Phylogenetic analysis

The nucleotide sequence of Pseudomonas stutzeri smk was blasted using NCBI server (http://blast.ncbi.nlm.nih.gov.in / Blast.cgi) and homologous species were used for phylogenetic analysis. The evolutionary history was inferred using neighbor-joining method (Saitou N. and Nei M.1987).The optimal tree with sum of branch length-148.4461 was shown. The evolutionary distance were computed using maximum composite likelihood method (Tamura K., Nei M., and Kumar S. 2004) and are in the units of the number of base substitutions per site .The analysis involved 75 nucleotides sequences .All positions containing gaps and missing data were eliminated. There total of 784 positions in the final data set. The evolutionary analysis was conducted using MEGA6 (3)

3. RESULTS AND DISCUSSION

3.1 Isolation, Identification and phylogenetic analysis of the isolate.

3.1.1 Isolation and morphological characterization.

The bacterium that degrades diclorovos and clothainidine pesticide was isolated from pesticide contaminated agriculture soil by enrichment culture techniques. The microscopic analysis of isolated bacterial cells, growth characteristics and biochemical analysis were carried out.

Table 1. Colony characteristics, Morphological characteristics of Clothainidine and Dichlorvos degrading isolate grown on nutrient agar at 30°C for 24 h

Size	Shape	Colour	Elevation	Surface texture	Consistency	Opacity
Moderate	Uneven	white creamy	Raised	smooth	sticky	Opaque

Table 2. Biochemical characteristics of clothainidine and Dichlorvos degrading isolate grown on nutrient agar at 30°C for 24 h.

Biochemical test	Result
Indole production	-
Urea hydrolysis	-
Catalase	+
MR test	-
VP test	-
Casein utilization	-
CO2 test	-
Gram staining	-
Motility test	+
Sugar utilization	
Glucose	+
Maltose	+
L-Arabinose	+
Fructose	+
Galactose	+
Sucrose	-
Mannose	-
Amino acid and other chemicals.	
Arginine	-
L-alanine	+

3.2 Phylogenetic characterization.

The isolate was further identified by 16S r RNA gene sequence analysis. The nucleotide sequence was submitted to Genbank, KT 281608 is the unique accession number provided by Genbank to *P. stutzeri smk* 16s rRNA nucleotide sequence. The Figure1 illustrates the phylogenetic position of *P. stutzeri* smk with other species of this genus that were existing in the GenBank database. The number in parentheses denotes the accession number of different species. The homology assay results indicated that the *P. stutzeri* smk showed maximum similarity the other species of the *P. stutzeri* strain VITD-0304 (KT364747.1) and *P. stutzeri* strain BOD-3 (JN565980.1) in the phylogenetic branch.
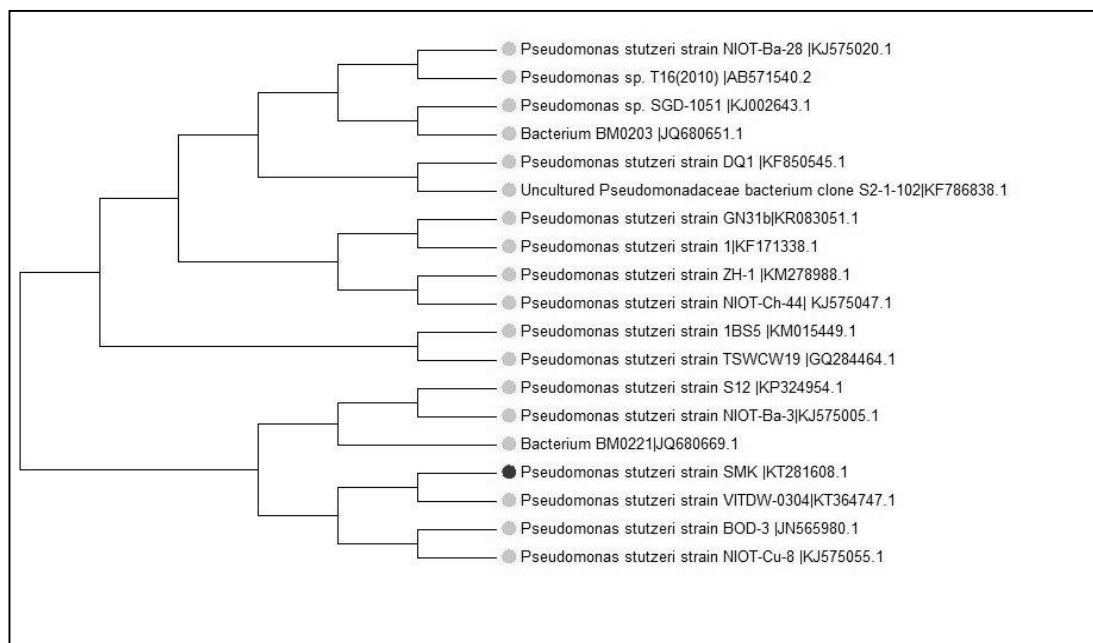
Fig 1: Phylogenetic analysis of 16S r RNA gene sequence of *Pseudomonas stutzeri* smk

DISCUSSION

Dichlorovos (DDVP, 2, 2-dichlorovynil dimethyl phosphate) is an organophosphorous pesticide with high solubility in water a (16 mg/ml at 25◦C, Merck Index, 1976), 9th Ed.)   DDVP acts by inhibiting acetylcholine esterase, an enzyme that is very important in the nervous system of all vertebrates and some invertebrates. Since the mutagenicity of DDVP was suspected in *Salmonella species* (W. Wild), some studies have been done on the degradation of this compound in the photochemical treatment (E. Evghenidou, T. Oncescu et al.) and in the soil by some soil microorganisms [J. Leveglia, H. Tse et al.]. It has been shown that DDVP is decomposed to dimethyl phosphate and dichloroacetaldehyde and subsequently broken down into dichloroacetic acid, 2, 2-dichloroethanol, and ethyl dichloroacetate [M.T. Lieberman et al.]. DDVP is highly toxic by inhalation, dermal absorption and ingestion [W.J. Hayes et al]. Effects of DDVP on prokaryotic and eukaryotic microorganisms have been reviewed by health organizations [IPCS-INCHEM]. The microbial degradation of organic pollutant can be either completely degraded into harmless compounds in mineralization or partial as intermediate metabolites in cometabolism. (Smith G.N, Racke K. D,). The prime aim of present study was to evaluate the bio potential of isolated bacterial culture for the biodegradation of the dichlorovos pesticide. Till date number of bacterial species have been reported for dichlorovos degradation. These includes *Proteus vulgaris, Vibrio sp., Serratia sp.* and *Acinetobacter sp.* (S. E. Agarrayet.al),

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

1. Theodoridis G,Barron E J; Suarez D P;Zhang Y L; Ding P; Lyga J W;Whiteside M P; Zawacki F J (2005) Pesticidal (dihalopropenyl) phenylalkyl substituted benzodioxolane and benzodioxole derivatives.United States Patent Application publications Pub.No:US 2005/0171356 Al.

2. Mohammed,M.S.(2009).Degradation of methomyl by the novel bacterial strain Stenotrophomonas maltophilia MI. Electronic J. Biotechnol. 12 (4): 1- 6.

3. Kang, D.G., Choi, S.S. and Cha, H.J. (2006). Enhanced biodegradation of toxic organophosphate compounds using recombinant Escherichia coli with sec pathway-driven periplasmic secretion of organophosphorus hydrolase. Biotechnol. Prog. 22: 406-410.

4. Bakry, N.M., El-Rashidy, A.H., Eldefrawi, A.T. and Eldefrawi, M.E. (2006). Direct actions of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholinic receptors. J. Biochem. Toxicol. 3: 235-259.

5. Grimsley, J., Rastogi, V. and Wild, J. (1998). Biological detoxification of organophosphorus neurotoxins. In: Bioremediation Principles and Practice-Biodegradation Technology Developments (S. Sikdar and R. Irvine, Eds). Technomic Publishers, New York. pp 557-613.

6. Miller, W.R and Sharpe, R.M. (1998). Environmental estrogens and human reproductive cancers. Endocr. Relat. Cancer, 5: 69-96.

7. Dua, M., Singh A., Sethunathan, N. and Johri, A.K. (2002). Biotechnology and bioremediation successes and limitations. Appl. Microbiol. Biotechnol. 58: 143-152.

8. Richins, R.D., Kanera, I., Mulchandani, A. and Chen, W. (1997).Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. Nature Biotechnol. 15: 984-987.

9. Zhongli, C., Shunpeng, L and Guoping, F. (2001). Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl. Environ. Microbiol. 59: 4922-4925.

10. Chang, T.C., Chang, H.C., Yu, F.W., Lenie, D., Mario, V and Chung, T.T. (2005). Species-level identification of isolates of the Acinetobacter calcoaceticus Acinetobacter baumanni complex

by sequence analysis of the 165- 23S rRNA gene spacer region. J. Clinical Microbiol. 43(4): 1632-1639. Chaudhry

11. Horne, I., Harcourt, R.L., Sutherland, T.D., Russel, R.J. and Oakeshott, J.G. (2002). Isolation of a Pseudomonas monteili strain with a novel phosphotriesterase. FEMS Microbiol. Lett. 206: 51-55

12. Chen-Goodspeed, M., Sogorb, M.A., Wu, F., Hong, S.B and Raushel, F.M. (2001). Structural determinants of the substrate and stereochemical specificity of phosphotriesterase. Biochemistry 40: 1325-1331

13. Richins, R.D., Kanera, I., Mulchandani, A. and Chen, W. (1997). Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. Nature Biotechnol. 15: 984-987

14. Mulchaldini, P., Mulchaldini, A., Kaneva, I. and Chen, W. (1999). Biosensor for direct determination of organophosphate nerve agents. I. Potentiometric enzyme electrode. Biosens. Bioelectron 14: 77-85.

15. Saitou N. and Nei M. (**1987**). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425

16. Tamura K., Nei M., and Kumar S. (**2004**). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) **101**:11030-11035

17. W. Wild: "Mutagenicity studies on organophosphorous insecticides", Mutat. Res., Vol. 32, (1975), pp. 133–150.

18. E. Evghenidou, K. Fytianos and I. Poulios: "Semiconductor-sensitized photodegradation of dichlorvos in water using TiO2 and ZnO as catalyst", Appl. Catal. B: Environ. Vol. 30, (2005), pp. 259–269.

19. T. Oncescu and P. Oancea: "On The Photolysis of Dichlorvos", Rev. Roum. Chim., Vol. 50, (2005), pp. 999–1007.

20. J. Leveglia and P.A. Dahm: "Degradation of organophosphorous and carbamate insecticides in the soil and by soil microorganisms", Annu. Rev. Entomol., Vol. 22, (1977), pp. 483–515.

21. EPA - US: "Phase I Comments for dichlorovos", (1999), pp. 1–62.

22. W.J. Hayes: Pesticides Studied in Man, Williams and Wilkins, Baltimore, MD., 1982

23. W.J. Hayes and E.R. Laws (Eds.): "Classes of Pesticides", Handbook of Pesticide Toxicology, Academic Press, Inc., NY, 1990.

24. IPCS-INCHEM, http://www.inchem.org/documents/jmpr/jmpmono/v93pr05.html.