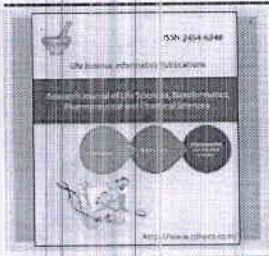

Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article

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

Satish G.Parte[1], Arun.S.Kharat[2] Ashokrao D.Mohekar[3].

1.Dept. of zoology M.J.S.College, Shrigonda; Ahmednagar,

2. Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad

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.

***Corresponding Author:** Satish G. Parte

Department of zoology M.J.S.College, Shrigonda, Ahmednagar

* Email Address: satishparte007@gmail.com

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

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

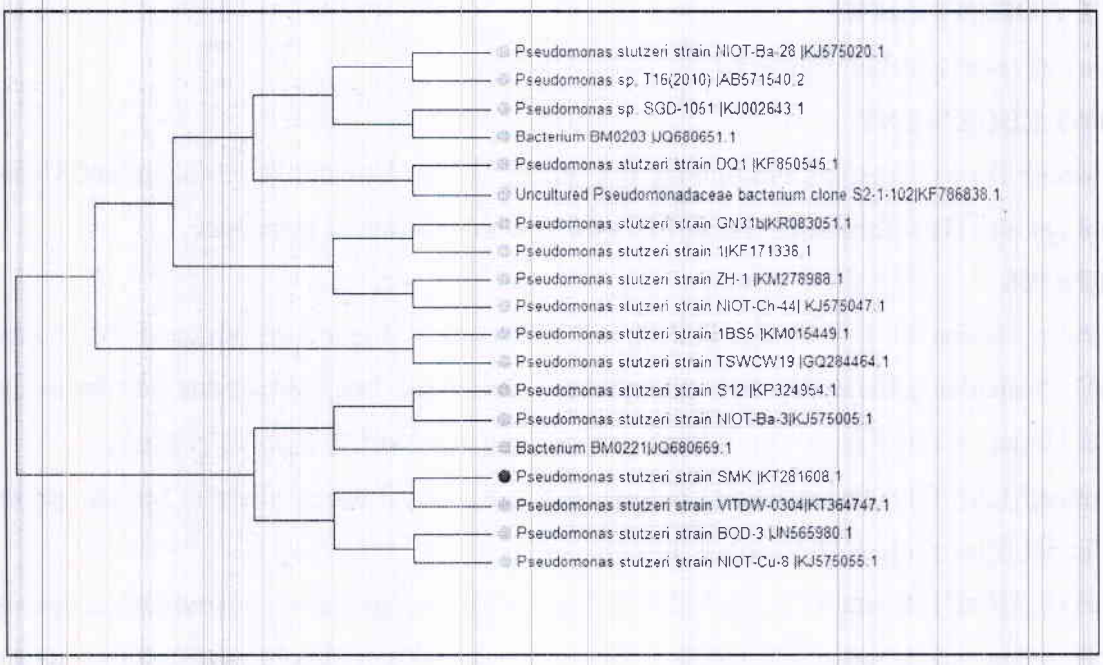

Fig 1: Phylogenetic analysis of 16S r RNA gene sequence of *Pseudomonas stutzeri* smk

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),

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.htm