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Review Paper

ADVANCES IN MICROBIAL BIODEGRADATION OF CHLORPYRIFOS

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ABSTRACT

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) an organophosphate pesticide is one of the most commonly used insecticide, acaricide and termiticide in agriculture, households and public health. The exposure of this moderately hazardous pesticide creates health concerns due to choline esterase inhibition, neurotoxicity, psychological and immunological effects. The microbial bioremediation of chlorpyrifos is a viable option for cleaning up the contaminated sites with its eco-friendliness, high efficiency and cost-effectiveness. 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., *Trichoderma harzianum,* etc. and cyanobacteria like *Anabaena* sp., *Aulosira fertilissima, Phormidium valderianum* for chlorpyrifos degradation. The microbes capable of producing biosurfactants increase biodegradation efficiency of chlorpyrifos by improving bioavailability of the xenobiotic compound by developing critical micellar concentration. The complete detoxification of the pesticide occurs with those microbes that also degrade its metabolites and avoid its accumulation in the environment. The cloning of mpd gene from chlorpyrifos degrading bacterial strains to *Escherichia coli* helps in developing its biodegradation capability. This paper focuses on the advancements in chlorpyrifos biodegradation for the efficient onsite remediation of the contaminated environment.

Key Words : Chlorpyrifos, Biodegradation, Metabolites, Biosurfactants, Mpd gene

INTRODUCTION

Organophosphorus pesticides are one of the major groups of pesticides accounting 38 per cent of total global pesticide consumption that replaced organochlorines to a greater extent against crop loss by pest attack and improving crop yield. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is one of the broad spectrum organophosphate pesticide used against various agriculture and household pests. It is used for crop protection in cotton, rice, sugarcane, peanuts, tobacco, vegetables, fruits and ornamental plants against sucking, chewing and boring insects. It also has acaricidal and termiticidal properties. Chlorpyrifos was the fourth largest consumed organophosphate pesticide next to monocrotophos, acephate and endosulfan.[1]

According to WHO classification, chlorpyrifos belongs to class II pesticides with moderate toxicity. [2] The excessive and injudicious application of pesticides led to environmental deterioration and human health concerns. [3] The Chlorpyrifos enter in to the human body via inhalation, ingestion and through skin contact and inhibits acetylcholine esterase enzyme in the central nervous system. [4-6] The exposure of this OP compound during pregnancy led to children with birth defects. [7,8] The ingestion of this compound can also cause liver toxicity, immunological disorders and its long term accumulation in body tissues, proteins, fats and bones create other health problems. [9] The chlorpyrifos residues in the soil and water environment resulted in biodiversity loss, alter its quality and productivity. The microbial

bioremediation play an important role in chlorpyrifos degradation in the environment.

AIMS AND OBJECTIVES

This paper focused on the advancements in chlorpyrifos biodegradation and clear understanding of its mechanism for the efficient onsite remediation of the contaminated environment.

DISCUSSION

Fate of chlorpyrifos in the Environment

The half life of chlorpyrifos ($C_9H_{11}C_{13}NO_3PS$) in soil varies from 10 to 120 days depending on factors like pH, temperature and moisture. [10] The chlorpyrifos undergoes degradation by physical, chemical and biological means. The complete degradation of it is not possible due to its structural complexity and its metabolites are represented in **Fig 1**. The step 1 indicates rapid metabolism of chlorpyrifos to chlorpyrifos oxon by oxidative desulfuration by mixed-function oxidases. The step 2 is the conversion of chlorpyrifos directly to TCP and diethyl thiophosphate. The chlorpyrifos oxon can also be deactivated to diethylphosphate and TCP by hydrolysis as in step 4. The hydrolysis of chlorpyrifos to monethyl 3, 5, 6 – trichloro – 2 - pyridinyl phosphorothioate as in step is a minor reaction pathway.

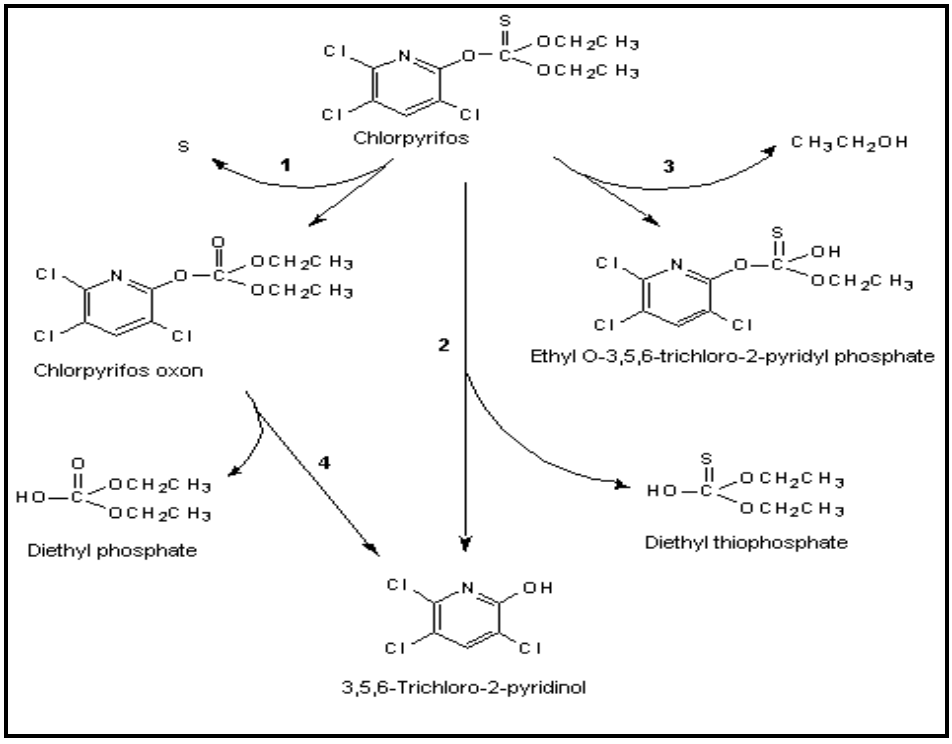

Fig. 1 : Metabolic pathway for chlorpyrifos [15]

The toxicity of one of its degradation product for 3,5,6-trichloro-2-pyridinol (TCP) increases with half life of 65 and 360 days in soil. [11-14]

Biodegradation of chlorpyrifos

The microbial degradation of organic pollutant can be either completely degraded into harmless compounds in mineralization or partial as intermediate metabolites in cometabolism.[15,16] The microbial bioremediation of chlorpyrifos in soil and aquatic environments plays an Important role in its degradation due to hydrolytic and oxidative enzymes. It is a viable option for cleaning up the contaminated sites with its eco-friendliness, high efficiency and cost-effectiveness. The biodegradation of organophosphates by bacteria were reported by several researchers as follows :

Pseudomonas aeruginosa, P. putida [12,13,14,17] *Bacillus pumilus, Bacillus cereus,* [5,12,14,17] *Serratia marcescens,* [12,17] *Klebsiella* sp.,[10,12,17,18] *Alcaligenes* sp., *Alcaligenes faecalis* , [10, 18, 19] *Flavobacterium* sp., [12, 20] *Enterobacter* strain B-

14, [21-23] *Agrobacterium* sp., [14, 23] *Arthrobacter* sp., [12, 14] *Stenotrophomonas* sp., *Ralstonia* sp., *Sphingomonas* sp. Dsp-2 that had chlorpyrifos degradation ability was also isolated by researchers. [13,24,25]

Sabdono and Radgasa [26] isolated coral bacterium *Bacillus firmus* BY6 associated with *Acropora* sp. capable for utilizing chlorpyrifos as carbon source. Briceno et al. [27] reported actinobacteria of genus *Streptomyces* with degradation potential of 99.2%.

The chlorpyrifos degrading fungi were *Phanerochaete chrysosporium, Aspergillus terreus, A. niger, A. flavus, Fusarium, Verticillium* sp. *DSP, Trichoderma harzianum* [28-33] *Anabaena* sp., *Aulosira fertilissima, Phormidium valderianum, Synechocystis* sp. etc. were cyanobacteria reported with chlorpyriphos degradation potential. [34-36]

The chlorpyrifos-degradation of *Cupriavidus taiwanensis* Lux-X1 was reported by Zhu et al. [37] Zhao et al. [38] identified chlorpyrifos-degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus. Pseudomonas stutzeri* improved complete degradation of chlorpyrifos in two days under bench scale stirred tank bioreactor in contrast to degradation in less than a month at optimal conditions under batch and fed-batch operating modes . [39]

Enzymatic activity in chlorpyrifos biodegradation

Organophosphorus hydrolase (OPH; EC 3.1.8.1) is one of the important hydrolytic enzymes in detoxification technology that hydrolyze various OP pesticides containing P–O, P–F and P–S bonds. [6,40] The OPH enzymes, including O-Phenylenediamine Dihydrochloride (OPD), Methyl Parathion Hydrolase (MPH) Mevalonate Pyrophosphate Decarboxylase (MPD) etc, was identified for the hydrolysis for specific classes of organophosphorus compounds. [41] The OPH was first isolated from *Pseudomonas diminuta* MG [42] has the ability to hydrolyse a wide range of OP compounds. Cho et al. [43,44] reported enhanced hydrolysis of chlorpyrifos with substrate specificity, using DNA shuffling.

The Methyl Parathion Hydrolase (MPH, E.C.3.1.8.1) that differ from OPH is also capable to hydrolyse a broad spectrum of OP compounds and was first isolated from *Plesiomonas* sp. strain M6. [45] MPH was found as a crucial enzyme for chlorpyrifos degradation. [46] Several organophosphorus compounds can be hydrolyzed by the mpd-encoded MPH with structural homology to parathion-methyl enabled Chlorpyrifos degrading enzymes.

Harishankar et al. [47] reported the intestinal bacteria *Lactobacillus lactis, L. fe-rmentum* and *Escherichia coli* were able to grow even at higher concentration of chlorpyrifos (>1400 µg/mL). The degradation of chlorpyrifos induced Organophosphorous Phosphatase (OPP) production and concentration were 28 times higher in the extracellular than inside the cells. The chlorpyrifos degradation efficiency for *L. fermentum*, L. *lactis* and E. coli were reported to 70 per cent, 61 per cent and 16 per cent with 3,5,6-trichloro-2-pyridinol (TCP), chlorpyrifos oxon and chlorpyrifos-oxon and diethyl-phosphate as end products respectively.

Gao et al. [48] purified and characterized a novel chlorpyrifos hydrolase from the fungi C*ladosporium cladosporioides* Hu-01.

Isolation of OPD gene with chlorpyrifos degradation potential

The organophosphate-degrading genes (opd gene) were isolated from species that were capable to degrade chlorpyrifos. [10,49,50] Most of them were plasmid based or located on the chromosome. The opd gene from *Agrobacterium radiobacter* was located on the chromosome. [49]

Singh et al.[22] identified a novel phosphor-triesterase enzyme from the coding of gene differ from organophosphate degradative gene (opd) in a *Enterobacter* strain B-14. Gao et al. [48] purified a novel chlorpyrifos hydrolase from cell extract of a fungus *Cladosporium cladosporioides* Hu-01 (collection number: CCTCC M 20711) with P-O and P-S bond. An amino acid sequence MEPDGELSALTQGANS, which shared no similarity with any reported organophosphate-hydrolyzing enzymes was also identified.

Improvements in chlorpyrifos biodegradation

The different approaches like biosurfactants, complete detoxification, nutrient supplementation, immobilization, etc. and genetic engineering enhanced the degradation capability of chlorpyrifos degrading microbes and helped to improve its detoxification.

Biosurfactants

Surfactants are surface active amphipathic

molecules that improve the portioning of hydrophobic compounds to aqueous phase forming emulsions at or above critical miscellar concentration that improve the bioavailability to potential degraders. [51] The environmental friendly biosurfactants are of interest due to its high efficiency at low concentration, stability at wide pH and temperature and biocompatibility. Glycolipids, phospholipids, lipopeptides, lipopolysacchrides, sophorolipids, coryne-mycolic acids, trehalose and sucrose lipids and particulate biosurfactants were the different types of biosurfactants produced by genera *Pseudomonas, Candida, Corynebacterium, Nocardia, Rhodococcus, Bacillus, Acinitobacter, Arthrobacter* etc. that help in pesticide biodegradation.

The *Pseudomonas* strain ChlD capable of producing a biosurfactant rhamnolipid and addition of partially purified biosurfactant in 0.1 g/l resulted in more than 98 per cent degradation of 10 ppm of chlorpyrifos in 120 h in comparison to 84 percent in its absence. The higher concentration of biosurfactant addition led to miscelleration and inhibits degradation efficiency.[52] *Nocardia mediterranei* enhanced the rate of biodegradation in organophosphates like Chlorpyrifos, methyl parathion and dichlorvos from 20 per cent to 45 per cent by production of biosurfactant trehalolipid (trehalose 6 6' dimycolate), a glycolipid compound specific to organophosphates.[53]

Complete detoxification

The intermediate metabolite of chlorpyrifos 3,5,6-trichloro-2-pyridinol (TCP) is toxic for the growth of chlorpyrifos degrading microbes. The microbes capable of complete detoxification of the pesticide also degrade its metabolites and avoid its accumulation in the environment. Many workers isolated microbes capable of remediating chlorpyrifos and TCP. *Alcaligenes faecalis* DSP3 and *Sphingobacterium* sp. JAS3, *Ganoderma* sp. JAS4 and *Gordonia* sp JAAS1 were a few microbes that mineralize the chlorpyrifos.[18,33,54,55] The co-culturing of *Serratia* sp. a potential chlorpyrifos degrader and *Trichosporon* sp. a TCP mineralizing strain isolated from activated sludge was capable of mineralizing 50 mg chlorpyrifos l^{-1} within 18 h at 30°C and pH 8 using a total inocula of 0.15 g biomass l^{-1}. [56] Li et al. [25] isolated *Sphingomonas* strain Dsp-2 hydrolyzed chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP) by the gene encoding the chlorpyrifos hydrolytic enzyme with its greater hydrolytic efficiency than the wild-type mpd from *Plesiomonas* sp. M6.

Nutrient supplementation

Harish et al. [32] reported efficiency of chloropyrifos degradation by *Trichoderma harzianum* was increased by 10-20% with the supplementation of 0.1% dextrose to the mineral media. Silambarasan and Abraham [19] observed chlorpyrifos degrading *Alcaligenes* sp. JAS1 strain isolated from paddy field soil inoculated in soil with nutrients amendment undergone complete degradation of pesticide in 24 h in comparison to 48 h without nutrients.

Immobilization

The chlorpyrifos removal was improved from 40.17 per cent to 71.05 per cent with the immobilization of cells in mixed culture of *Streptomyces* sp. [57] Vijayalakshmi and Usha [58] studied degradation of chlorpyrifos by free cells and calcium-alginate immobilized cells of *Pseudomonas putida* isolated from an agricultural soil. Ca-alginate immobilized cells of *Pseudomonas putida* had higher degradation efficiency of 96 % for chlorpyrifos at 2% concentration at pH 7, temperature 35°C, 10 ml of inoculum size, shaking speed of 150 rpm and in presence of 200 mg/l glucose and 300 mg/l yeast extract in comparison to 76% degradation by free cells. The immobilized cells improved degradation of 10% chlorpyrifos concentration to 63% over 43 % by free cells.

Genetic engineering

The genetically engineered bacteria with high detoxification potential were developed by gene engineering and enzyme engineering.

Niu et al. [59] reported optimum pH of 7 and inoculum volume of 50 ml/kg on chlorpyrifos residues degradation by mutagenic bacteria DX1 in soil. Kapoor and Rajagopal [60] studied the degradation of organophosphate pesticides by recombinant organophosphorus hydrolase.

Caoa et al. [61] cloned a novel 6012 bp gene cluster from TCP-degrading strain P2 responsible for dehalogenation of 3,5,6-trichloro-2-pyridinol (TCP). The gene cluster consisted a monooxygenase gene (*tcpA1*), a flavin reductase gene (*tcpB1*), *tcpR1*, *orf1* and *orf2*. TcpA1 and TcpB1 worked together to catalyze the

dehalogenation of three chlorine of TCP, and generated a more readily biodegradable product of 3, 6-dihydroxypyridine-2,5-dione. The gene cluster consisted a monooxygenase gene (*tcpA1*), a flavin reductase gene (*tcpB1*), *tcpR1*, *orf1* and *orf2*. TcpA1 and TcpB1 worked together to catalyze the dehalogenation of three chlorine of TCP and generated a more readily biodegradable product of 3,6-dihydroxypyridine-2,5-dione.

Li et al. [24] cloned gene clusters from *Ralstonia* sp. T6 involved in 3,5,6-trichloro-2-pyridinol degradation. The *tcpRXA* genes constitute a gene cluster consisting $FADH_2$-dependent monooxygenase gene *tcpA*, LysR family transcriptional regulator (TcpR) and flavin reductase (TcpX). T6-Δ*tcpA*-com, the complementation strain for the mutant strain T6-Δ*tcpA*, recovered the ability to degrade TCP, and the strain *E. coli* DH10B-*tcpRXA*, which expressed the *tcpRXA* gene cluster, had the ability to transform TCP to the green intermediate metabolite 3, 6-dihydroxy pyridine-2,5-dione (DHPD).

The cloning of mpd gene from chlorpyrifos degrading bacterial strains to *Escherichia coli* helps in developing its biodegradation capability. Wang et al. [62] cloned *Escherichia coli* with opd gene that degrade chlorpyrifos co-metabolically. Yang et al. [63]cloned the mpd gene from chlorpyrifos– degrading bacterium *Stenotrophomonas* isolated using chlorpyrifos as the sole source of carbon by enrichment method that degraded 100 mg/l of chlorpyrifos within 24 hour to DETP and TCP. The thermostability and acidic stability of MPH have been improved by site-directed mutation. [64-74]

Yang et al. [46] engineered *P. putida* JS444 with altered specificity of MPH enhance the degradation of chlorpyrifos. Methyl Parathion Hydrolase (MPH) has been displayed on the surface of microorganisms for the first time using only N- and C-terminal domains of the Ice Nucleation Protein (INPNC) from *Pseudomonas syringae* INA5 as an anchoring motif. A shuttle vector pINCM coding for INPNC–MPH was constructed and used to target MPH onto the surface of a *Pseudomonas putida* JS444.

CONCLUSION

The bacteria, fungi and cyanobacteria with its hydrolytic enzyme activity had the remediation potential for chlorpyrifos. The biosurfactant production, mineralization ability, immobilization of cells, nutrient supplementation, etc. enhanced the activity of microbial strains in chlorpyrifos degradation. The genetic engineering by means of mutation, cloning, recombinant technology in genes and enzymes involved in chlorpyrifos degradation also improved the detoxification process of chlorpyrifos by microbes. All the achieved advancements in the biodegradation of chlorpyrifos help in the in-situ detoxification of contaminated sites.

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REFERENCES

1. Ansaruddin P.A. and Vijayalakshmi K., The womb is not safe anymore, *Indeg. Agr. News*., **2**(1), 3 **(2003)**.
2. Kumar S., Fareedullah M. D., Sudhakar Y., Venkateswarlu B. and Kumar A., Current review on organophosphorus poisoning, *Arch. Appl. Sci. Res*., **2**(1), 199-215 **(2010).**
3. Atif Randhawa M., Muhammad Anjum F., Ahmed A. and Saqib Randhawa M., Field incurred chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol residues in fresh and processed vegetables, *Food Chem.*, **103**(1), 1016–1023, **(2007).**
4. Abou - Donia M. B., Organophosphorus ester-induced chronic neurotoxicity, *Arch. Environ. Heal. Int. J.*, **58**(8), 484-497, **(2003).**
5. Anwar S., Liaquat F., Khan Q. M., Khalid Z.M. and Iqbal S., Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6- trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1, *J. Haz. Mat.*, **168**, 400–405, **(2009).**
6. Latifi A.M., Khodi S., Mirzaei M., Miresmaeili M. and Babavalian, H., Isolation and characterization of five chlorpyrifos degrading bacteria, *Afr. J. Biotechnol*., **11**(1), 3140–3146, **(2012)**.
7. Bhagobaty R.K., Joshi S.R. and Malik A., Microbial degradation of organo-

phosphorous pesticide : Chlorpyrifos (mini-review), *Int. J. Microbiol.* , **4** (1), 1-12, **(2006).**

8. Rauh V.A., Perera F.P., Horton M.K., Whyatt R.M., Bansal R., Hao X.J., Liu J., Barr D.B., Slotkin T.A. and Peterson B.S., Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc. Nat. Acad. Sci.* USA, **109**(2), 7871–7876, **(2012).**
9. Environmental risk assessment, *Review of chlorpyrifos poisoning data*, US ERA, 1-46, **(2002)**.
10. Singh B.K. and Walker A., Microbial degradation of organophosphorus compo-unds, *FEMS Microbiol. Rev.*, **30**(1),428–471, **(2006).**
11. Lakshmi C.V., Kumar M. and Khanna S., Biotransformation of chlorpyrifos and bioremediation of contaminated soil, *Int. Biodeterior. Biodegr.*, **62**(2), 204–209, **(2008).**
12. Lakshmi C.V., Kumar M. and Khanna S., Biodegradation of chlorpyrifos in soil by enriched cultures, *Curr. Microbiol.*, **58**(1), 35–38, **(2009).**
13. Pino N. and Pen˜uela G., Simultaneous degradation of the pesticides methyl parathion and chlorpyrifos by an isolated bacterial consortium from a contaminated site, *Int. Biodeterior. Biodegrad.*, **65**(2) , 827–831, **(2011).**
14. Maya K., Singh R.S., Upadhyay S.N. and Dubey S.K., Kinetic analysis reveals bacterial efficacy for biodegradation of chlorpyrifos and its hydrolyzing metabolite TCP, *Proc. Biochem.*, **46**(2), 2130–2136, **(2011).**
15. Smith G.N., Watson B.S. and Fischer F.S., Investigations on dursban insecticide. metabolism of [36Cl] O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothiate in rats, *Agr. Food Chem.*, **15**(2), 132-138, **(1967).**
16. Racke K. D., Environmental fate of chlorpyrifos, *Rev. Environ. Contam. Toxicol.*, **131**(1), 1-151, **(1993).**
17. Ghanem I., Orfi M. and Shamma M., Biodegradation of chlorpyrifos by *Klebsiella* sp. isolated from an activated sludge sample of waste water treatment plant in Damascus, *Folia Microbiol.*, **52**(1), 423–427, **(2007).**
18. Yang L., Zhao Y.H., Zhang B.X., Yang C.H. and Zhang X., Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium, FEMS *Microbiol. Le*tt., **251**(1),67–73, **(2005).**
19. Silambarasan S. and Abraham J., Kinetic studies on enhancement of degradation of chlorpyrifos and its hydrolyzing metabolite TCP by a newly isolated *Alcaligenes* sp. *JAS1, J. Taiwan Inst. Chem. Eng.,* **44**(3), 438–445, **(2013).**
20. Ning J., Gang G., Bai Z., Hu Q., Qi H., Ma A., Zhuan X. and Zhuang G., In situ enhanced bioremediation of dichlorvos by a phyllosphere *Flavobacterium* strain, *Front. Environ. Sci. Engineer.* in China, **6**(1), 231-237, (**2012).**
21. Singh B. K., Walker A., Morgan J.A.W. and Wright D.J., Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium, *Appl. Environ. Microbiol.*, **69** (2), 5198–5206, **(2003).**
22. Singh B.K., Walker A., Morgan J.A.W. and Wright D.J., Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils, *Appl. Environ. Microbiol.*, **70**(2), 4855–4863, **(2004).**
23. Chishti Z. and Arshad M., Growth linked biodegradation of chlorpyrifos by *Agrobacterium* and *Enterobacter* sp., *Int. J. Agric. Biol.*, **15**(2), 19-26, (**2012).**
24. Li J., Huang Y., Hou Y., Li X., Cao H. and Cui Z., Novel gene clusters and metabolic pathway involved in 3,5,6-Trichloro-2-Pyridinol degradation by *Ralstonia* sp. T6. *Appl. Env. Microbiol.*, doi:10.1128/-AEM.01817-1, **(2013).**
25. Li, X., Jian He and Shunpeng Li;. Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2 and cloning of the mpd gene, *Res. Microbiol.*, **158**(2), 143-149, **(2007)**.
26. Sabdono A. and Radgasa., Phylogenetic diversity of organophosphorus pesticide degrading coral bacteria from mid-west coast of Indonesia, *Biotech.*, **7** (4), 694-701, **(2008).**

27. Briceño G., Fuentes M.S., Palma G., Jorquera M.A., Amoroso M.J. and Diez M.C., Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil, *Int. Biodeterior. Biodegrad.*, **73**(2),1–7, (**2012).**
28. Bumpus J. A., Kakar, S. N. and Coleman R. D., Fungal degradation of organo-phosphorous insecticides, *Appl. Biochem. Biotechnol.*, **39**(2),715-726, **(1993).**
29. Swati and Singh D.K., Utilization of chlorpyrifos by *Aspergillus niger* and A. flavus as carbon and phosphorus source, *17th World Cong. of Soil Sci.*, 14 - 21 Aug 2001, Bangkok, Thailand, **(2002).**
30. Yu Y.L., Fang H., Wang X., Wu X.M., Shan M. and Yu J.Q. Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables, *Biodegrad.*, **17**(2), 487-494, **(2006).**
31. Fang H.,. Xiang Y. Q., Hao Y. J., Chu X. Q., Pan X. D., Yu J. Q. and Yu Y. L., Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi, *Int. Biodeter. Biodegrad.*, **61**(2),294-303, **(2008).**
32. Harish R., Supreeth M. and Chauhan J.B., Biodegradation of organophosphate pesticide by soil fungi, *Adv. Bio Tech.*, **12** (9), 4-8, **(2013).**
33. Silambarasan S. and Abraham J., Efficacy of *Ganoderma* sp. JAS4 in bioremediation of chlorpyrifos and its hydrolyzing metabolite TCP from agricultural soil, *J. Bas. Microbiol.*,**1**(2), 1–12, **(2013).**
34. Palanisami S., Prabaharan D. and Uma L., Fate of few pesticide metabolizing enzymes in marine cyanobacterium *Phormidium valderianum* BDU 20041 in perspective with chlorpyifos exposure, Pest. *Physiol. Biochem.*, **94**(2)**,** 68–72, **(2009).**
35. Park B. and Lee S., Biotransformation of aldrin and chlorpyrifos-methyl by *Anabaena* sp. PCC 7120, *Korea J. Environ. Agr.*, **29**(2), 184-188**, (2010).**
36. Singh D.P., Khattar J.I.S., Nadda J., Singh Y., Garg A., Kaur N. and Gulati A., Chlorpyrifos degradation by the cyanobacterium *Synechocystis* sp. strain PUPCCC 64, *Environ. Sci. Poll. Res.*, **18**(2), 1351–1359, **(2011).**
37. Zhu B. 1., Cao, Y., Wang D., Tang X., Hua R., Shi T. and Sun L., Survival and chlorpyrifos-degradation of strain *Cupriavidus taiwanensis* Lux-X1 in different type soils, *J. Food, Agr. Environ.*,**11** (2), 873-876, **(2013).**
38. Zhao L., Wang F. and Zhao J., Identification and functional characteristics of chlorpyrifos-degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus*, *J. Bas. Microbiol.*, Article first published online :. DOI : 10.1002 /jobm.201200639, **(2013).**
39. Moscoso F., Teijiz I., Deive, F. J. and Sanroma´n M. A., Approaching chlorpyrifos bioelimination at bench scale bioreactor, *Bioproc. Biosyst. Eng.*, **36**(2)**,** 1303–1309, **(2013).**
40. Fulekar M. H., *Environmental Biotechnology*. CRC Press, Enfield, 197-215, **(2010).**
41. Zheng Y., Long L., Fan Y., Gan J., Fang J. and Jin W., A review on the detoxification of organophosphorus compounds by microorganisms, *Afri. J. Microbiol. Res.*, **7**(20), 2127-2134, **(2013).**
42. Serdar C. M. and Gibson D. T. Enzymatic hydrolysis of organophosphates : Cloning and expression of a parathion hydrolase gene from *Pseudomonas diminuta*, *Biotechnol.*, **3**(2), 567–571, (**1985).**
43. Cho C.M., Mulchandani A. and Chen W., Altering the substrate specificity of organophosphorus hydrolase for enhanced hydrolysis of chlorpyrifos, *Appl. Envron. Microbiol.*, **70**(1), 4681-4685, **(2004).**
44. Cho Y.A., Lee H. S., Park E. Y., Lee Y. T., Hammock B. D., Ahn K. C. and Lee J.K., Development of an ELISA for the organo phosphorous insecticide chlorpyrifos. *Bull. Korea. Chem. Soc.*, **23**(2)**,** 481-487, **(2002).**
45. Cui Z., Li S. and Fu G., Isolation of methyl-parathion-degrading strain M6 and cloning of the methyl-parathion hydrolase gene, *Appl. Environ. Microbiol.*, **67**(11), 4922-4925, **(2001).**
46. Yang C., Cai N., Dong M., Jiang H., Li J., Qiao C., Mulchandani A. and Chen W., Surface display of MPH on *Pseudomonas*

putida JS444 using ice nucleation protein and its application in detoxification of organophosphates, *Biotechnol. Bioengg*., **99**(1), 224-230, **(2008).**

47. Harishankar M. K., Sasikala C. and Ramya M., Efficiency of the intestinal bacteria in the degradation of the toxicpesticide, chlorpyrifos. *Biotech.*, **3**(1),137–142, **(2013).**
48. Gao Y., Chen S., Hu M., Hu Q. and Luo J., Purification and characterization of a novel chlorpyrifos hydrolase from *Cladosporium cladosporioides* Hu-01. PLoS ONE **7**(6), e38137. Doi:10.1371/journal.pone.0038 137, **(2012).**
49. Horne I., Sutherland T. D., Harcourt R. L., Russell, R. J. and Oakeshott J. G., Identification of an OPD (Organophosphate Degradation) gene in an *Agrobacterium* isolate, *Appl. Environ. Microbiol*, **68**(1) ,3371-3376, **(2002).**
50. Zhang R., Cui Z., Jaindong J., Jain H., Xiangyang G. and Sphunpeng L., Diversity of organophosphorus pesticide degrading bacteria in a polluted soil and conservation of their organophosphorus hydrolase genes, *Can. J. Microbiol*., **51**(2), 337–343, **(2005).**
51. Noordman W.H. and Janssen D.B. Rhamnolipid stimulates uptake of hydrophobic compounds by *Pseudomonas aeruginosa*, *Appl. Environ. Microbiol*., **68**(2), 4502-4508**, (2002).**
52. Singh P.B., Sharma S., Saini H.S. and Chadha B.S., Biosurfactant production by *Pseudomonas* sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos, *Let. Appl. Microbiol*., **49**(2), 378–383, **(2009).**
53. Sukirtha T.H. and Usharani M.V., Production and qualitative analysis of biosurfactant and biodegradation of the organophosphate by *Nocardia mediterranie*, *J. Bioremed. Biodeg*., **4**(1),198, doi: 10.4172/2155-6199.1000198, **(2013).**
54. Abraham J. and Silambarasan S. ,Biodegradation of chlorpyrifos and its hydrolyzing metabolite 3,5,6-trichloro-2-pyridinol by *Sphingobacterium* sp. JAS3, *Proc. Biochem*., **48**(2), 1559–1564, **(2013).**
55. Abraham J., Shanker A. and Silambarasan S., Role of *Gordonia* sp. JAAS1 in biodegradation of chlorpyrifos and its hydrolysing metabolite 3,5,6-trichloro-2-pyridinol, *Let. Appl. Microbiol*., **57** (6), 510–516, **(2013).**
56. Xu G., Li Y., Zheng W., Peng X., Li W. and Yan Y., Mineralization of chlorpyrifos by co-culture of *Serratia* and *Trichosporon* sp., *Biotechnol. Lett.*, **29**(2), (2),1469–1473, **(2007).**
57. Fuentes M.S., Briceño G.E., Saez J.M., Benimeli C.S., Diez M.C. and Amoroso M.J., Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized Streptomyces strains, *Biomed. Res. Int.*, 2013:392573. doi: 10.1155/2013/392573, **(2013).**
58. Vijayalakshmi P. and Usha M.S., Degradation of chlorpyrifos by free cells and calcium-alginate immobilized cells of *Pseudomonas putida*, *Adv. Appl. Sci. Res.*, **3** (5),2796-2800, **(2012).**
59. Niu M., Wei J. and Liang C., Study on chlorpyrifos residues degradation by mutagenic bacteria DX1 in soil, *Adv. Mat. Res*., **726-731**(1), 3992-3995, **(2013).**
60. Kapoor M. and Rajagopal R., Enzymatic bioremediation of organophosphorus insecticides by recombinant organo-phosphorous hydrolase, *Int. Biodeterior. Biodegrad*, **65**(2), 896-901, **(2011)**.
61. Caoa L., Xub J., Wua G., Lia M., Jianga J., Hea J., Lia S. and Honga Q., Identification of two combined genes responsible for dechlorination of 3,5,6-trichloro-2-pyridinol (TCP) in *Cupriavidus pauculus* P2, *J. Haz. Mat*., **260** (15), 700–706, **(2013).**
62. Wang A. A., Mulchandani A. and Chen W., Specific adhesion to cellulose and hydrolysis of organophosphate nerve agents by a genetically engineered *Escherichia coli* strain with a surface-expressed cellulose-binding domain and organophosphorus hydrolase, *Appl. Environ. Microbiol.*, **68**(2),1684–1689, **(2002).**
63. Yang C., Liu N., Guo X. and Qiao C., Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil, FEMS *Microbiol. Lett* ., **265**(2), 118-125, **(2006).**
64. Tian J., Wang P., Gao S., Chu X., Wu N. and Fan Y., Biotechnology research

institute, chinese academy enhanced thermostability of methyl parathion hydrolase from *Ochrobactrum* sp. M231 by rational engineering of a glycine to proline mutation, *FEBS J.*, **277**(2),4901–4908 **(2010).**

65. Huang F.L. and Huang K., Methylphenidate improves the behavioral and cognitive deficits of neurogranin knockout mice, *Genes Brain Behav.*, **11**(7), 794–805, **(2012).**
66. Das D. and Kaur R., Biodegradation of phenol by various indigenous micro-organism, *J. Environ. Res. Develop.*, **7**(2A), 1009-1015, **(2012)**.
67. Pandey K. M., Debbarma Ajay, Das Hirakjyoti, Roy Aamitava and Nath Writuparna, Environmental impact assessment and management : Protecting ecology of NE India, *J. Environ. Res. Develop.*, **7**(4), 1454-1466, **(2013).**
68. Vardhanan Shibu, Haridas Ajit and Manilal V. B., Closed setting : A green technology for controlling coir setting pollution of backwaters, *J. Environ. Res. Develop.*, **7**(4A), 1523-1530, **(2013)**.
69. Mehta S.A., et al., Optimization of physiological parameters for pectinase production from soil isolates and its applications in fruit juice clarification, *J. Environ. Res. Develop.*, **7**(4A), 1539-1546, **(2013)**.
70. Kaur R. and Pramanik K., Biodegradation of tiger prawn shell by lactic acid fermentation, *J. Environ. Res. Develop.*, **7**(2A), 936-945, **(2012)**.
71. Parmar, et al., Biodegradation of resorcinol by *Pseudomonas* sp. isolated from industrially contaminated soil sample, *J. Environ. Res. Develop.*, **6**(4), 1066-1071, **(2012)**.
72. U.G. Sawaikar, et al., Effect of effluent recycling and shock load on the biodegradation of organic solvents in UASB reactors, *J. Environ. Res. Develop.*, **6**(3), 554-561, **(2012)**.
73. Derle Shilpa G., et al., Eco-friendly biodegradation of reactive yellow 145 by newly isolated *Bacillus boroniphilus* from industrial effluent, *J. Environ. Res. Develop.*, **7**(1A), 303-311, **(2012)**.
74. Tewari Chandra Prakash, Biodegrdation of pentachlorophenopl PCP by consortium of *Flavobacterium* sp. in tannery effluent, *J. Environ. Res. Develop.*, **7**(2A), 876-882, **(2012)**.

I think the environment should be put in the category of our national security. Defense of our resources is just as important as defense abroad. Otherwise what is there to defend?

Robert Redford