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Soil Bacteria in Chlorpyrifos Biodegradation

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Abstract: *The Chlorpyrifos is a broad spectrum organophosphate insecticide used against various crop pests and household pests. The excessive usage of this moderately hazardous pesticide adds up residues to the environment and become toxic to human and animal health. Biodegradation by the microbes is a viable and ecologically safe option to remediate Chlorpyrifos residues from the contaminated environment. A few microbes have the ability to degrade this pesticide for carbon and energy source. The soil bacterial species under Bacillus and Pseudomonas genera such as B. cereus, B. pumilus, B. subtilis, P. putida, P. stutzeri, P. aeruginosa, etc. have high Chlorpyrifos biodegradation efficiency. Alcaligenes sp, Agrobacterium sp., Enterobacter sp., Klebsiella sp., Serratia sp., Stenotrophomonas sp., Sphingomonas sp., Ralstonia sp., Flavobacterium sp, etc. isolated from soil also reported for having potential for degradation of Chlorpyrifos. The bacteria as isolate or in consortium degraded the pesticide by its enzymatic activities depending on environmental factors. The regular application of the pesticide in the agricultural soil results in increase of resistant bacteria with high degradation potential. The genes from these indigenous bacterial strains act as the gene pools for the development of recombinant microbes with pesticide mineralization property. These soil bacteria have been exploited for the insitu bioremediation and clean up of Chlorpyrifos contaminated soil.*

1. INTRODUCTION

The organophosphoruspesticides (OPs) occupies about 38 percent of total pesticide consumption in the world [1]. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate)is one among the widely used OP pesticides. Chlorpyrifos is widely used for the control of various indoors and outdoors pests. Chlorpyrifos is used to control arthropod pests belong to Coleoptera, Diptera,Homoptera and Lepidoptera in soil, on foliage and on animals. It is used against chewing and sucking crop pests such as leaf worms, armyworms, scale insects, aphids, cotton bollworm,cutworms, corn rootworms, leafhoppers and mites in cereals like rice, wheat, corn, sugarcane, cotton, fruits, vegetables and fodder crops. It is an effective control measure for house hold pests like cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is also used as vector control for public health as mosquito larvicide and adulticide [2-7]. It is a broad spectrum insecticide which is moderately hazardous as per WHO classification and neurotoxin which affects central nervous system. The half life of 10 -120 days depending on the environmental, soil factors and low water solubility increases its toxicity [8]. The residues of the contact pesticide affect the environment and human health [9]. It acts as neurotoxin by inhibiting the acetylcholine esterase activity. There is an urgent need to remediate the residues for maintaining the health of contaminated sites. The biodegradation is the most advantageous method for chlorpyrifos detoxification than physical or chemical methods. This paper focused on the role of bacteria in Chlorpyrifos decontamination.

2. BACTERIAL BIODEGRADATION OF CHLORPYRIFOS

The biodegradation by the microbes is aneffective and environmentally friendly method for the clean up of the sites contaminated with the chemical. The bacteria, fungi and actinomycetes were reported to have the chlorpyrifos degradation ability but the bacterial biodegradation is predominant in the environment. The first organophosphorus (OP) compound-degrading bacterial strain was isolated from a paddy field in the Philippines in 1973.

The Pseudomonas is a group of Gram-negative, aerobic gammaproteobacteria capable of degrading various xenobiotic compounds. The degradation of Chlorpyrifos by Pseudomonas isolated from different soil were reported by different researchers (Table 1)

Table 1: Chlorpyrifos Degradation by Pseudomonas sp.

Bacterial Strain	Initial concentration (mg L-1)	Degradation (%)	Reference
Pseudomonas sp.	500	91	[10]
Pseudomonas aeruginosa	50	80	[11]
Pseudomonas aeruginosa NCIM 2074	75	52	[12]
Psuedomonas putida MAS-1	140	100	[13]
Pseudomonas putida	20	76	[14]

Pseudomonas aeruginosa, *Pseudomonas nitroreducens* and *Pseudomonas putida* with chlorpyrifos degrading ability was isolated by [13]. *Pseudomonas aeruginosa* was capable of tolerating 2000 mgL^{-1}.

The *Bacillus* are Gram positive bacteria belong to the order *Bacillales* isolated from different soil samples also found to have high Chlorpyrifos degradation ability [15]. The Table 2 lists a few Bacillus sp. Involved in chlorpyrifos degradation.

Table 2: Degradation of Chlorpyrifos by Bacillus sp.

Bacterial Strain	Initial concentration (mg L-1)	Degradation (%)	Reference
Bacillus cereus	100	78.85	[16]
Bacillus licheniformis ZHU-1	100	99	[17]
Bacillus subtilisY242	150	95.12	[18]
Bacillus thuringiensis BRC-HZM2	200	88.9	[19]
Bacillus pumilus C2A1	300	90	[20]

Bacillus latersprorus DSP also reported with degradation ability [21].

Coliform species like Klebsiella ,Enterobacter, Serratia also have the ability to mineralize Chlorpyrifos. The enhancement in the number of *Klebsiellia sp.* was observed in Chlorpyrifos applied soil than other microbes [22]. *Klebsiella sp*. isolated from the paddy field and cotton soil was isolated by KaviKarunya and Reetha [23-24].

Alcaligenes faecalis DSP3 was degraded 76.2% of Chlorpyrifos in 18 days of incubation [25]. *Alcaligenes*sp.JAS 1 isolated from paddy field soil degraded 300 mg/l of chlorpyrifos within 48 h and in 24 h with nutrients [26]. Chishti and Arshad [27] stated the growth linked biodegradation of chlorpyrifos by Agrobacterium and Enterobacter sp. isolated from soil. Enterobacter asburiae isolated from an Australian soil by Singh et al. [28] also showed higher chlorpyrifos mineralization. *Arthrobacter* sp. isolated from methyl parathion-enriched soil also had the chlorpyrifos degradation ability [29].

Acinetobacter calcoaceticus isolated from rhizosphere of chives contaminated with chlorpyrifos capable of degrading 60 per cent of 100 mg/L chlorpyrifos [30]. Nagavardhanam and Vishnuvardhan [31-32] reported *Kocuria* sp. isolated from agricultural soil degraded 75 per cent of 3.84 g/L of chlorpyrifos. The opd gene cloned from Kocuria sp. also had effective chlorpyrifos degradation ability. Flavobacterium sp., Micrococcus sp., Mesorhizobium sp. HN3, Stenotrophomonas sp., Paracoccus sp. TRP, Sphingomonas sp. DSP-2, Cellulomonas fimi, Ralstonia sp. also capable of degrading Chlorpyrifos [7,25,29, 33-38].

The bacterial degradation of Chlorpyrifos was improved by consortia than the isolate. Lakshmi et al. [11] reported Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp., and Serratia marscecens isolated from the consortia had 84, 84, 81, and 80 percent degradation of chlorpyrifos (50 mg/L) in liquid medium after 20 days and 92, 60, 56, and 37% in soil after 30 days respectively. Bacterial consortium consisting *Bacillus* and *Pseudomonas* spp. isolated from pesticide contaminated soil of Uttarakhand degraded 99.13% and 98.5% respectively within 30 days [39]. The bacterial consortium isolated from agriculture soil consists of Pseudomonas putida, Klebsiella sp., Pseudomonas stutzeri and Pseudomonas aeruginosa degraded 500 mg L-1 chlorpyrifos at neutral pH and temperature 37^{o}C [40].

3. FACTORS AFFECTING BACTERIAL BIODEGRADATION

The biodegradation of chlorpyrifos depends on different biotic and abiotic factors. It includes potential of microbes, inoculums density, pesticide formulation and bioavailability of the pesticide. The abiotic factors such as pH, temperature,oxygen content, moisture,nutrients and different concentrations of the pesticide affect the biodegradation efficiency of the bacteria [41].

The biodegradation of chlorpyrifos by bacteria was low at acidic pH and at alkaline condition. The optimum conditions improve the efficiency of biodegradation. Most of the bacteria prefer pH at neutral range and mesophilic temperature for bacterial growth for effective degradation (Table 3).

Table 3: Optimum temperature and pH for Chlorpyrifos degrading bacteria

Bacterial strain	Temperature (°C)	pH	Reference
Bacillus cereus	30	7	[16]
Bacillus thuringiensis BRC-HZM2	30	7	[19]
Bacillus licheniformis ZHU-1	35	7.5	[17]
Bacillus pumilus C2A1	30	8.5	[20]
Pseudomonas spp.	30	7	[42]
Klebsiella sp.	35	6	[23-24]
Alcaligenes faecalis DSP3	30	7	[25]
Enterobacter asburiae	35	7	[28]
Mesorhizobium sp. HN3	37	7	[37]

4. MECHANISM IN CHLORPYRIFOS BIODEGRADATION BY BACTERIA

The bacterial biodegradation of Chlorpyrifos depends on two mechanisms namely Catabolism or Cometabolism. The Chlorpyrifos is completely mineralized by the bacteria and used up for energy and growth in catabolism process while partial degradation without utilizing for energy and nutrients occur in co-metabolism [43]. The hydrolase enzymes are actively involved in the mineralization by breaking P-O-C linkage inphosphoester bonds of Chlorpyrifos. The parathion hydrolase isolated from mixed microbial culture was first reported for Chlorpyrifos hydrolysis by Munnecke et al. [44].The Chlorpyrifos mineralization provides carbon or phosphorus and or energy to the bacterial growth. The metabolites of Chlorpyrifos are diethyl thiophosphate (DETP) and 3, 5, 6-trichloro-2-pyridinol (TCP). Further enzymatic activities of TCP yield Carbon dioxide, chloride and polar metabolites. The genes and enzymes involved in bacterial degradation of chlorpyrifos are being utilized in genetic engineering for developing capability in other bacterial strains.

5. CONCLUSION

Soil with repeated application of pesticides acts as a genetic resource of chlorpyrifos degrading bacteria. Further improvements in the bioremediation process with optimization will help in the remediation of Chlorpyrifos contaminated sites by bacterial isolate or consortia.

6. ACKNOWLEDGEMENT

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