The Internet Journal of Microbiology 2007 : Volume 4 Number 1

Microbial Degradation of Organophosphorous Pesticide: Chlorpyrifos (Mini-Review)

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Citation: R. Kumar Bhagobaty, S. Ram Joshi & A. Malik : Microbial Degradation of Organophosphorous Pesticide: Chlorpyrifos (Mini-Review) . *The Internet Journal of Microbiology*. 2007 Volume 4 Number 1

Keywords: Biodegradation | Chlorpyrifos | Microbial | Organophosphorus | Pesticide

Abstract

Chlorpyrifos is one of the world's most widely used organophosphorus pesticides in agriculture. Exposure to chlorpyrifos and its metabolites have been related to a variety of nerve disorders in humans. Microbial degradation is considered to be an efficient and cost effective method for decontamination of toxic organophosphorus pesticides from the environment. Chlorpyrifos previously shown to be immune to enhanced biodegradation has now been proved to undergo enhanced microbe mediated decay into less harmful and non-toxic metabolites, under a set of favourable abiotic conditions. Recently, research activity in this area has shown that a diverse range of microorganisms is responsible for chlorpyrifos degradation. This article therefore aims at giving an overview of the present status of research in biodegradation of chlorpyrifos.

Introduction

Chlorpyrifos, a pesticide that can easily enter the human food chain has more victims to its credit than carcinogenic air pollutants such as polycyclic aromatic hydrocarbons (PAHS). A study conducted by researchers from US based Columbia University found a strong link between prenatal exposure to chlorpyrifos and low birth weight and smaller head size of infants. Several studies correlate the smaller size of the head with lower Intelligence quotient (IQ) and poor functioning. The researchers studied 263 pregnant females, who lived in areas exposed to almost the same level of pollution. The researchers used blood plasma levels to estimate the amount of chlorpyrifos. The use of chlorpyrifos has been vastly restricted in US and some European countries, even for agricultural purposes. However, it is still widely used in developing countries like India, where in the year 2000, it was the fourth highest consumed pesticide after monocrotophos, acephate and endosulfan. (Ansaruddin, P.A. 2003)

Chlorpyrifos is used both for agricultural pest control and in households as a termiticide. Commercially, it is available in different brand names like Dursban, Lorsban, Agromil, Dhanwan, Dorson, Dhanwan, Omexan to name a few. Chlorpyrifos interferes with the normal functioning of the central nervous system, including the brain. It works basically

the same way in humans as it does in insects. In fact, chlorpyrifos (Dursban) belongs to a group of chemicals known as organophosphates, which were first developed by the Germans in the 1930s and were later used to kill people in concentration camps during World War-II. More recently, members of the Japanese cult Aum Shinriko used a related organophosphate compound Sarin in trying to exterminate commuters on Tokyo metro. The US government is investigating whether substantial use of organophosphates to control desert pests during the Gulf War caused the neurological ailment known as the Gulf War Syndrome. (David, C. 2000)

Chlorpyrifos shows a wide spectrum of biological activity and is used to control range and forage insect pests as well as soil dwelling grubs, rootworms, borers and subterranean termites. It is available in a variety of formulations, such as granules, wettable powder, dustable powder and emulsifiable concentrate. (Swati & Singh 2002)

Extensive use of chlorpyrifos contaminates air, ground water, rivers, lakes, rainwater and fog water. The contamination has been found up to about 24 kilometers from the site of application. Symptoms of acute poisoning include headache, nausea, muscle twitching and convulsions and in some extreme cases even death. Human birth defects have also been associated with exposure to chlorpyrifos and its products. It also affects the male reproductive system. Chlorpyrifos is toxic to a variety of beneficial arthropods including bees, ladybird beetles and parasitic wasps. It kills fishes at concentrations as low as a few parts per trillion. Birds are also susceptible with effects ranging from reduced weight of nestlings, deformities and death. In plants there have been reports of delayed seedling emergence, fruit deformities and abnormal cell division upon prolonged exposure to chlorpyrifos. (NCAP, 2000)

Unlike in the case of other organophosphates, however, there have been no reports of enhanced degradation of chlorpyrifos since its first use in 1965 until, recently when Singh et al. 2003, isolated six chlorpyrifos degrading bacteria from an Australian soil showing enhanced degradation of chlorpyrifos. Yang et al. 2005 isolated Alcaligenes faecalis DSP3, which is capable of degrading both chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCP). Although the microbial degradation of chlorpyrifos has been investigated, the existing papers lack information on the genetic and enzymatic aspects involved in its degradation.

This article therefore, aims at throwing a chronological light on the research efforts undertaken worldwide to isolate and identify chlorpyrifos degrading microbes and the genes responsible for its enhanced biodegradation.

Microbiological transformation of chlorpyrifos and its metabolites

In general, microorganisms demonstrate considerable capacity for the metabolism of many pesticides. Although they are capable of catalyzing similar metabolic reactions as mammals and plants, they possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds. There are two major types of microbial degradation of organic chemicals. The first, termed catabolism is a type of degradation in which the organic chemical or a portion thereof is completely degraded (e.g. mineralized) and the energy or nutrient gained contributes to cell growth. The second, incidental metabolism or cometabolism, involves the partial degradation of an organic chemical with no net benefit to the organism, the compound being merely caught up in some metabolic pathway during the normal metabolic activities of the microorganisms (Racke 1993).Studies conducted in soil have generally reported significantly longer dissipation half–lives under sterilized versus natural conditions and led to the conclusion that microbial activities are important in the degradation of chlorpyrifos in soil (Miles et al. 1984). Evidence from soil degradation studies indicates that cleavage and mineralization of the heterocyclic ring occurs in soil due to the activities of microorganisms (Racke & Coats 1990). However, the singularly most important microbial role in the chlorpyrifos degradation pathway may be the further metabolism and mineralization of 3, 5, 6-trichloro-2-pyrinidinol (TCP) and 3, 5, 6-trichloro-2-methoxypyridine (TMP) metabolites (Racke 1993).

Microbial enzymes have been shown to hydrolyze chlorpyrifos under controlled conditions. Munnecke and his co-worker in 1975, first reported the ability of parathion hydrolase, an organophosphorus ester-hydrolyzing enzyme isolated from a mixed microbial culture, to hydrolyze chlorpyrifos.

Jones and Hastings (1981) reported the metabolism of 50-ppm chlorpyrifos in cultures of several forest fungi (Trichoderma harzianum, Penicillium vermiculatum, and Mucor sp.).

After 28 days, chlorpyrifos and its metabolite TCP were present in all cultures at levels of 2-5 % and 1-14% of applied, respectively. Ivashina (1986) studied chlorpyrifos degradation by several microbial cultures maintained in liquid media containing 10 ppm chlorpyrifos. Dissipation was more rapid in a sucrose-supplemented media containing Trichoderma sp. and glucose supplemented media containing Bacillus sp. than in control media containing no microorganisms. Chlorpyrifos disappeared from the microbial cultures in a linear fashion over a 2-week period. Lal and Lal (1987) observed some degree of degradation by the yeast Saccharomyces cervisiae. Only half the initial chlorpyrifos was recovered 12 h after the cultures were inoculated with 1-10 ppm. The possible metabolism by two lactic acid bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus) was reported by Shaker et al. (1988). The synthetic culture medium, in which the bacteria were grown initially contained 7.4 ppm, but displayed a 72-83% loss in chlorpyrifos after 96 h. Havens and Rase (1991) circulated a 0.25 % aqueous (EC) solution of chlorpyrifos through a packed column containing immobilized parathion hydrolase enzyme obtained from Pseudomonas diminuta. Approximately 25 % of the initial dose was degraded after 3 h of constant recirculation through the column. Strains of Aspergillus flavus and Aspergillus niger isolated from agricultural soil with previous history of chlorpyrifos use were, also reported to biomineralise chlorpyrifos in liquid culture medium (Swati & Singh 2002). Yu et al. (2006) isolated and characterized a fungal strain capable of degrading chlorpyrifos.18S rDNA gene analysis revealed that the showed that the fungal strain had a high level of homology (99%) to those from other Verticillium species. They found that the degradation of chlorpyrifos in by the fungal strain in mineral salt medium increased almost linearly with increasing concentrations of chlorpyrifos (r^2 =0.9999), suggesting that the degradation is subjected to pseudo-first order kinetics. With the first order kinetic function, the DT_{50} of chlorpyrifos at concentrations of 1, 10, and 100 mg/l, were calculated to be 2.03, 2.93, and 3.49 days, respectively. In the controls the hydrolysis percentages of chlorpyrifos were found to be less than 5%. They further used the cell free extracts of the strain to detoxify chlorpyrifos in vegetables and reported that the cell free extracts of the fungus can used for enhanced degradation in vegetables.

Some evidence also indicates that, the metabolites of chlorpyrifos are also degraded and mineralized by soil microorganisms. Several researchers have noted the extensive mineralization of TCP and TMP to carbon dioxide in soil. Racke et al. (1988) reported that

approximately 65-85 % of TCP applied (5ppm) to several soils was mineralized within 14 days. The initially accelerating rate of mineralization observed in these soils was indicative of microbial enzyme induction or adaptation. Racke and Robbins (1991) probed a suite of soils for evidence of the presence of TCP-catabolizing microorganisms. Of the 25 soils investigated, only two displayed significant degradation of TCP within 21 days of inoculation into mineral slats medium containing 5-ppm TCP as the sole carbon source. Isolation a pure culture of bacteria capable of using 3, 5, 6-trichloro-2-pyridinol (TCP) as the sole source of carbon and energy under aerobic conditions was reported for the first time by Feng and his co-workers in 1998. The bacterium was identified as a Pseudomonas sp. and designated as ATCC 700113. The TCP degradation yielded $CO_{2,}$ chloride and some unidentified polar metabolites. They further reported that the degradation of the parent compound, TCP, by the Pseudomonas sp. appeared to involve a reductive de-chlorination mechanism.

Bacterial degradation of chlorpyrifos

Chlorpyrifos is characterized by the same P-O-C linkage as in other organophosphate pesticides, such as diazinon {(Sethunathan 1971), (Sethunathan & Pathak 1972)} , parathion (Sethunathan& Yoshida 1973), methyl parathion and fenitrothion (Mishra et al. 1992).Guha et al. (1997) reported the involvement of plasmids in degradation of malathion and chlorpyrifos by Micrococcus sp. isolated from soil. Mallick et al. (1999) reported the rapid degradation of chlorpyrifos, added to a mineral salt medium or applied to the soil, as a sole carbon source, by the Flavobacterium sp. ATCC 27551 isolated from diazinon retreated rice fields (Sethunathan& Yoshida 1973) and an Arthrobacter sp. isolated from a flooded soil retreated with methyl parathion (Mishra et al. 1992).Huang et al. (2000) studied the degradation of chlorpyrifos in poultry and cow-derived effluents and reported that chlorpyrifos was degraded by aerobic microbial processes in animal–derived lagoon effluents. Further, analysis of the microbial community involved in the degradation process by denaturing gradient gel electrophoresis(DGGE) of PCR amplified 16sRNA genes showed that a single band became dominant in effluents during chlorpyrifos degradation, thereby indicating the role of a single aerobic-bacterial population in the degradation of chlorpyrifos.

Singh et al. (2003) studied the effects of soil pH on the biodegradation of chlorpyrifos in

United Kingdom and Australian soils and reported that the dissipation of chlorpyrifos in United Kingdom soils varying in pH from 4.7 to 8.4 was mediated by the cometabolic activities of the soil microorganisms. A robust bacterial population that utilized chlorpyrifos as a source of carbon was detected in an Australian soil and the enhanced ability to degrade chlorpyrifos was successfully transferred to the United Kingdom soils. Only soils with a pH of ≥ 6.7 were able to maintain this degrading ability 90 days after inoculation. Transfer and proliferation of degrading microorganisms from the Australian soil to the UK soils was monitored by molecular fingerprinting of bacterial 16sRNA genes by PCR- denaturing gradient gel electrophoresis (DGGE).

Recently Singh et al. (2004) reported the enhanced degradation of chlorpyrifos by an Enterobacter strain B-14, and found that the strain responsible for enhanced biodegradation of chlorpyrifos showed greatest similarity to Enterobacter asburiae based on 16s rRNA studies of the bacterium. This strain was shown to utilize chlorpyrifos as a sole source of carbon and phosphorus and hydrolyzed chlorpyrifos to diethylthiophosphoric acid (DETP) and 3, 5, 6-trichloro-2- pyrinidol. Further studies by them with B-14 revealed that the strain possessed a novel phosphotriesterase enzyme system, as the gene coding for this enzyme had a different sequence from the widely studied organophosphate degradative gene (opd). The authors also reported that addition of the strain B-14 to chlorpyrifos contaminated soils resulted in higher degradation rate than that observed in non-inoculated soils.

Yang et al. (2005) isolated Alcaligenes faecalis DSP3, which is capable of degrading both chlorpyrifos and TCP.More, recently Yang et al. (2006) were successful in cloning the mpd gene from a chlorpyrifos – degrading bacterium and using it for bioremediation of contaminated soil. Six chlorpyrifos –degrading bacteria were isolated using chlorpyrifos as the sole source of carbon by enrichment procedure. Their strain YC-1 showed the highest degrading capability and was putatively identified as the genus Stenotrophomonas. The strain YC-1 degraded 100 mg/l of chlorpyrifos within 24 hour to DETP and TCP. DETP was utilized as a source of carbon and phosphorus, but it did not degrade TCP. Upon addition of the strain YC-1 to fumigated and non-fumigated soils resulted in a more rapid rate of chlorpyrifos degradation than that of uninoculated controls. 100 mg / kg of chlorpyrifos was degraded completely within 15 days.

Degradation of chlorpyrifos in control non-fumigated soils (without inoculation) was lower with less than 24% of the applied concentration degraded in 15 -days incubation studies. The rate of degradation in inoculated soils increased with increasing soil P^H from 4.3 to 7.0 but there was no significant difference in degradation rate in soil with P^H 7.0-8.4. The degradation rate of chlorpyrifos in acidic soils was found to be slower in acidic soils than in neutral and alkaline soils.

Presence of plasmid was not detected in strain YC-1 by the alkali lysis method, which indicated that the mpd gene was located on the chromosome. The mpd gene was subsequently cloned in E.coli DH5α cells and subsequently expressed in E.coli BL21 (DE3). Sequence BLAST result showed that the gene was 99% similar to mpd gene of Plesimonas sp. M6 (GenBank accession no. AF338729), 99% similar to mpd gene of Pseudomonas putida (GenBank accession no. AY029773) and 99 % similar to mpd gene of Ochrobactrum sp. MP-4 (GenBank accession no. AY627036) at the nucleotide level.

Conclusion

Isolation and characterization of pesticide degrading microorganisms is crucial for enhancing our understanding of the variety of mechanisms and biodegradative pathways relating to their enhanced degradation in the environment. Chlorpyrifos, which was previously thought to be immune to enhanced biodegradation, has now been shown to undergo enhanced biodegradation by bacterial and fungal species. Bioremediation technologies are in the process of development for this toxic compound and related nerve agents using organophosphorus hydrolase enzyme. Future, studies on the genes responsible for enhanced biodegradation will enable us to elucidate the exact degradative pathway involved in its microbial biodegradation.

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