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APPOSITENESS OF BACTERIAL ENDOPHYTES IN THE BIODEGRADATION OF CHLORPYRIFOS

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Abstract: In recent times deaths and deformities caused due to organophosphate poisoning has emerged as a very serious problem all across the globe with approximately 3 million poisoning and 200000 deaths annually. Chlorpyrifos, one of the most extensively used insecticides is also neurotoxic upon prolonged exposure as it inhibits the normal activity of the enzyme acetylcholine esterase needed for proper nervous transmission. Initially, it was not expected to be toxic to plants but there have been numerous reports citing that it has adversely affected a lot of plants like alfalfa, clover, *Arabidopsis thaliana* and *Pinus halepensis.* Damage caused to the environment and health by the concomitant use of this insecticide makes it imperative to develop strategies and techniques to carry out their elimination in a safe, efficient and economical manner. Bioremediation is a cost effective approach that uses microbes to remove pollutants. Various techniques and strategies of bioremediation *e.g.* phytoremediation enhanced by endophytic microorganisms, rhizoremediation have been employed in recent times to remove hazardous waste from the biosphere. Here, in this review we have discussed the different aspects of bioremediation and as to how the endophytic bacteria are naturally genetically tailored to metabolize and degrade such xenobiotic compounds.
Keywords: Bioremediation, Chlorpyrifos, Endophytes, Organophosphorous compounds, Biofertilizer.
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INTRODUCTION

Endophytes are defined as microorganisms (fungi, bacteria) that colonize living, internal tissues of plants without causing any immediate, negative effects. The term endophyte was first introduced in 1886 by De Bary for microorganisms (fungi, yeast, and bacteria) colonizing internal plant tissues (De Bary, 1884). In 1887, Victor Gallipe postulated that soil microorganisms can penetrate healthy plant tissues; therefore, recognition of colonization mechanisms is so valuable. Hirsch and Braun (1992) described endobionts as a group of microorganisms colonizing tissues without any visible consequences of infection (latent pathogens). One of the recent definitions of endophytes was proposed (Posada and Vega, 2005) who used this term to describe all organisms inhabiting different internal parts of plants, including seeds. There are approximately 300,000 plant species living on the Earth, and each individual plant can be the host to one or even more kinds of endophytes (Petrini, 1991; Strobel and Daisy, 2003; Huang *et al*., 2007). They may be isolated from roots, stems, leaves, and inflorescences of weeds, fruit plants, and important vegetables (Bulgari *et al*., 2012; Bhore *et al*., 2010; Munif *et al*., 2012). Endophytic bacteria have been isolated from monocotyledonous plants *e.g.* Liliaceae, grass, zea, rice, and orchids (Gangwar and Kaur, 2009; Kelemu *et al*., 2011; Lin *et al*., 2012; Miyamoto *et al*., 2004; Peng *et al*., 2006; Rogers *et al*., 2012), as well as dicotyledonous plants, for instance oak (Basha *et al*., 2012; Ma

et al., 2013). Some endophytes have been characterized from different tree species, for example oak, pear tree, *Sorbus aucuparia*, and *Betula verrucosa* (Krid *et al*., 2010; Scortichini and Loreti, 2007). The existence of endophytes has also been confirmed in beets, corn, bananas, tomatoes, and rice roots (Brown *et al*., 1999; Cao *et al*., 2005; Altalhi, 2009; Pereira *et al*., 1999). These organisms, classified as *Bacillus* sp., *Enterobacter* sp., and *Sporosarcina aquimarina* (Rylo sona Janarthine *et al*., 2011), have been found in roots of some coastal mangrove pioneer plants (*Avicennia marina*). Endophytes can be classified into three main categories of plant-inhabiting life strategies (Hardoim*et al*., 2008). Obligate endophytes are unable to proliferate outside of plants and are likely transmitted via seed rather than originating from the rhizosphere (Hardoim *et al*., 2008). Facultative endophytes are free living in soil but will colonize plants when the opportunity arises, through coordinated infection (Hardoim *et al*., 2008). Most endophytes relating to plant growth promotion belong to this group. The third group, the passive endophytes, does not actively seek to colonize the plant, but do so as a result of stochastic events, such as open wounds along the root hairs. This passive life strategy may cause the endophyte to be less competitive since the cellular machinery required for plant colonization is lacking (Verma*et al*., 2004; Rosenblueth and Martínez-Romero, 2006; Hardoim *et al*., 2008), and therefore may be less appropriate as plant growth promoters.

COLONIZATION OF PLANTS BY ENDOPHYTES

The interaction between plants and microorganisms in the soil is well recognized. Hiltner in 1904 (Hartmann *et al*., 2008) first observed that microorganisms were more abundant in the soil surrounding the plant roots than in soil remote from the root and called this area the rhizosphere. Plant roots exude many organic compounds that stimulate microbial growth and can have a major impact on the composition of the rhizosphere microbiome (Lemanceau *et al*., 1995; Grayston *et al*., 1998; Miethling *et al*., 2000). Recently, research focus has been redirected on the composition of the rhizosphere microbiome, examining the impact it can have on plant growth and health (Berg and Smalla, 2009; Mendes *et al*., 2011; Berendsen *et al*., 2012). The microbiome within plant roots can differ significantly from that within the rhizosphere, suggesting plants impact the microbial communities found inside their roots (Germida *et al*., 1998; Gottel *et al*., 2011). Extensive research has been done on the potential of root endophytes as plant inoculants for plant growth promotion (Thakore, 2006). The three main mechanisms that drive endophyte community structure:

i. Soil factors that determine survival.
ii. Plant factors that determine colonization and compatibility.
iii. Microbial factors that determine the ability of the endophyte to survive and compete within the root.

Endophytic bacteria show a tremendous diversity not only in plant hosts, but also in bacterial taxa (Bacon and Hinton, 2006; Hardoim *et al*., 2008; Vendan *et al*., 2010). Some hosts are reported to have several endophytes, and the latter may have a wide host range. Therefore, several different species of endophytes can be isolated from a single plant. It is said that the diversity of endophytic communities in the endosphere is regulated by stochastic events, which are influenced by deterministic processes of colonization in turn (Battin *et al*., 2007).It should be added that the microenvironment of soil has an influence on the colonization of plant endophytes by diverse bacteria and their community composition (Hardoim *et al*., 2008). It has been postulated that the early step in the colonization of a plant may depend on absorption of soil aggregates, biodiversity of plants and their physiology, as well as microbial prevalence (Hardoim *et al*., 2008). The main factors that may regulate microbial colonization include the plant genotype, the growth stage, the physiological status, the type of plant tissues, some soil environmental conditions, as well as some agricultural practices (Conrath *et al*., 2006; Singh *et al*., 2009). Moreover, the microbial metabolic pathways of colonization may play

an important role as determinants of endophyte diversity. For example, the rate of motile bacteria isolated from the interior part of roots was approximately five fold higher than that of bacteria in the soil tightly adhering to the roots (Czaban *et al*., 2007). It has been proved that the ability of soil bacteria to approach plant roots is induced by chemotaxis and the efficiency in microcolony formation. These are thekey factors that determine the success of bacteria to become endophytic (Bacilio-Jiménez *et al*., 2003). The process of plant colonization by endophytic microorganisms is a complex phenomenon. It includes recognition of the host, spore germination, penetration, colonization, and maintenance of endophytes in the host cells (Van Antwerpen *et al*., 2002). They can be contained in seeds and vegetative planting material, since they originate from the surrounding natural environment such as the rhizosphere and phyllosphere. The processes of colonization depend on several biotic and abioticfactors. It has been shown that they include physical and biological characteristics of the host plant, temperature, humidity conditions, and seasonal fluctuations of other cohabiting microorganisms (Quadt-Hallman *et al*., 1997).

ROLE OF ENDOPHYTIC MICROORGANISMS IN BIOREMEDIATION

The collaboration between the plant and endophytes can play a key role in the degradation of hazardous contaminants in therhizosphere. Bacterial endophytes might function more effectively than bacteria added to the soil because they participate in a process known as bioaugmentation (Newman and Reynol, 2005). Large numbers of bacterial strains isolated from grapevine (*Vitis vinifera L*.) plants were resistant to lead, mercury, nickel, zinc, and manganese (Altalhi, 2009). In their study, the authors Guo *et al*. (2010) showed that the endophytic bacterium *Bacillus* sp. reduced cadmium to approximately 94% in the presence of industrially used metabolic inhibitors N,N'-dicyclohexylcarbodiimide (specific ATPase inhibitor, DCC) or 2,4-dinitrophenol (DNP). Similarly, inoculation with endophytic bacteria, *Serratia nematodiphila* LRE07, alleviated growth inhibition in *Solanum nigrum L*. in the presence of cadmium (Wan *et al*., 2012). Ma *et al*. (2011) isolated Ni-resistant endophytic bacteriafrom tissues of *Alyssum serpyllifolium* growing in serpentine soils in Braganca in the northeast part of Portugal. Inoculation of *Brassica juncea* seeds with this strain significantly increased the plant biomass. Bioremediation of heavy metals involving endophytic bacteria L14 (EB L14) isolated from acadmium hyper accumulator *Solanum nigrum L.* has been described by Chen *et al*. (2012).The endophytic microbial community may also assist in phytoremediation of petroleum. Preference for petroleum degrading bacteria in the root interior has been illustrated with an example of plants growing in petroleum-contaminated soil (Siciliano *et al*., 2001). Aken and coworkers (2004) have indicated that *Methylobacterium populum* sp. nov. strain BJ001 isolated from poplar trees is able to degrade energetic compounds such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro- 1,3,5-triazine (HMX), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Mineralization of about 60 %of RDX to carbon dioxide was observed within 2 months time.

The bioremediation potential during degradation of xenobiotic compounds by three strains of *Pseudomonas* sp. isolated from xylem sap of poplar trees was tested by Germaine *et al*. (2004). Oliveira *et al*. (2012) have isolated three strains from cerrado plants exhibiting the capacityfor degradation of different fractions of petroleum,diesel oil, and gasoline. Over the recent years, much more attention has been focusedon the application of endophytic bacteria for phytoremediation. *Burkholderia cepacia* L.S.2.4 bacteria genetically modified by introduction of a *pTOM* toluene degradation plasmid of *B. cepacia* G4, a natural endophyte of yellow lupine, were used for phytoremediation of toluene (Barac *et al*., 2004). The recombinant strain induced strong (upto 50–70 %) degradation of toluene. Germaine *et al* in 2009 described inoculation of the pea (*Pisum sativum*) with a genetically modified bacterial endophyte that naturally possessed the ability to degrade 2, 4-

dichlorophenoxyacetic acid. The results showed that the plants inoculated with *Pseudomonas putida* VM1441 (pNAH7) had a higher degradation capacity of up to 40 % for 2,4-dichlorophenoxyaceticacid from the soil (Germaine *et al*., 2009). The first *in situ* inoculation of poplar trees growing on a trichloroethylene (TCE)-contaminated site with TCE-degrading strain *P. putida* W619-TCE was done by (Weyens *et al*., 2009). This kind of inoculation resulted in a 90 % reduction of TCE evapotranspiration under the field conditions. This promising result was obtained after introduction of *P. putida* W619-TCE to poplar trees, as a root endophyte. Probably, the TCE metabolic activity in the members of the poplar's endogenous endophytic population was obtained by further horizontal gene transfer (Weyens *et al*., 2009). In subsequent studies, Weyens *et al*. (2010) used engineered endophytes for improving phytoremediation of environments contaminated by organic pollutants and toxic metals. The yellow lupine was inoculated with *B. cepacia* VM1468 possessing (a) the *pTOM-Bu61*plasmid coding for constitutive trichloroethylene degradation and (b) the ncc-nre Ni resistance/sequestration. Inoculation with *B. cepacia* M1468 into plants resulted in a decrease in Ni and TCE phytotoxicity, which was reflected by a 30 % increase in root biomass and up to a 50 % decrease in the activities of enzymes involved in antioxidative defense in the roots. In addition, the decreasing trend in TCE evapotranspiration showed about a fivefold higher Ni uptake observed after inoculation of plants (Weyens *et al*., 2010).

ORGANOPHOSPHOROUS PESTICIDES

Organophosphate pesticides account for about 38% of the total pesticides used worldwide (Singh and Walker, 2006). A considerable amount of the pesticide either accumulates in the soil or enters into water bodies after application. Unfortunately, less than 0.1% of the total applied pesticide reaches the target and the rest remains in the environment (Pimentel, 1995). Chlorpyrifos [O, O-diethyl O-(3,5,6-trichloro-2-pyridinyl) -phosphorothioate] is one of the most widely used organophosphate pesticides. It was first developed by the Germans in the 1930s and first introduced in 1965 in the USA as a home and garden insecticide by Dow Chemical's (Worthing, 1979). Humans are exposed to OPs via ingested food and drink and by breathing polluted air (WHO, 2001).

Chlorpyrifos: Chlorpyrifos is a non-systemic insecticide, which is effective against a wide range of insect pests of economically important crops (Fang *et al*., 2006). It enters into an insect body by contact and ingestion, and is also absorbed through the gut, skin and pulmonary membranes (Simon *et al*., 1998). Usually, it affects the nervous system of the target insects by inhibiting the activity of acetylcholinesterase by phosphorylation, both at the synapse of neurons and in the plasma (Hui *et al*., 2010). As a result, acetylcholine is accumulated at the neuron synapse which causes the death of the target insect. Chlorpyrifos residues were detected up to eight years after application for termite treatment in 16 houses in North Carolina (Wright *et al*., 1994). Table 1 describes the physiochemical properties of chlorpyrifos. Living organisms are exposed to pesticide residues in soil and water, resulting in a risk to the ecological imbalance (Kulshrestha and Kumari, 2011). There are also some reports on chlorpyrifos residues in the food chain (Aysal *et al*., 2004; Chandra *et al*., 2010). Several ecosystems across the world have been reported to be contaminated as a result of indiscriminate use of organophosphate pesticides, causing poisoning of millions of people and over 200,000 deaths annually (Cisar and Snyder, 2000; Singh *et al*., 2009). Moreover, serious damage to non-target species, such as endocrine disruption, birth defects, low birth weights, reduced head circumference, nervous system disorders and immune system abnormalities, has also been reported (Furlong *et al*., 2006; Rauh *et al*., 2011). Oxidative stress in animals is also induced by exposure to chlorpyrifos (Giordano *et al*., 2007). In addition, it is found to be associated with bladder cancer and chromosomal damage (Lee *et al*., 2004). Similarly, hyperglycaemia has been observed

in a number of animals as a result of chlorpyrifos acute and sub-chronic exposures (Abdollahi *et al*., 2004).

The remediation of chlorpyrifos-contaminated sites to mitigate the hazardous effects of such toxic chemicals is required. A number of methods, including chemical treatment, volatilization, photodecomposition and incineration, can be applied for the detoxification of chlorpyrifos (Racke, 1993; Muhammad, 2010; Gao *et al*., 2012). However, most of them are not applicable for diffused contamination at low concentration because they are expensive, inefficient and not always environmental friendly. Biotic degradation is one of the most viable options for the remediation of chlorpyrifos in soil and water. Several researchers have focused on the microbial degradation which has been reported as a primary mechanism of pesticide dissipation from the soil and water environment (Awad *et al*., 2011; Massiha *et al*., 2011). In some early studies, chlorpyrifos was reported to be resistant to biodegradation due to accumulation of the antimicrobial degradation products in soil (Serdar *et al*., 1982; Racke *et al*., 1990). Later, several studies have revealed that many microorganisms are capable of degrading chlorpyrifos efficiently (Singh *et al*., 2004, 2006; Zhu *et al*., 2010; Kulshrestha and Kumari, 2011; Liu *et al*., 2012).

Fate of Chlorpyrifos in the environment: The fate of chlorpyrifos is affected not only by its own physicochemical properties (Table 1), but also by characteristics of the soil, management practices and environmental conditions (Halimah *et al*., 2010). Pesticides are distributed in the solid, liquid and gaseous phases in the vadose zone after their application depending upon the constant of adsorption, desorption and volatilization (Marino *et al*., 2002). The applied chlorpyrifos binds to plants, soil particles or sediments (Gebremariam *et al*., 2012). After a certain period of time its major fraction is either volatilized, hydrolyzed or biodegraded. Volatilization from soil depends on a number of factors such as concentration, temperature and soil properties. In 1993, Racke, reported 2.6% and 9.3% volatilization of the applied chlorpyrifos from sand and a silt loam soil, respectively, within 30 days of its application. Whang *et al* in 1993 observed that one-half of the applied chlorpyrifos was volatilized from no-till surface soils during a period of 26 days. However volatilization from foliage was more pronounced with 80% loss within 24-48 hours compared to 25% loss from soil surfaces. Usually, chlorpyrifos reacts with photochemically-produced hydroxyl radicals in the atmosphere and degrades to chlorpyrifos-oxon with an estimated half-life of 4.2 h. Residual chlorpyrifos is considered to be critical as it can last for long periods of time in the environment depending on the initial concentration of pesticide and the biodegradation rate (Surekha *et al*., 2008; Nawaz *et al*., 2011).

Biodegradation of Chlorpyrifos: Biotic degradation is a common process for the removal of organic pollutants because of its low cost and less collateral destruction of indigenous organisms. Several species of bacteria have been reported to degrade organophosphate pesticides in liquid media and soil (Table 2). Various studies have illustrated that pesticide-contaminated soils can be decontaminated by inoculation with specifically adapted microorganisms (Diez, 2010; Abo-Amer, 2011; Massiha *et al*., 2011). The soils with previous exposure to chlorpyrifos contain a variety of microorganisms carrying organophosphate degrading enzyme(s) (Bhagobaty and Malik, 2008; Sasikala *et al*., 2012).

Table 1. Physiochemical properties of Chlorpyrifos

Characteristics of Chlorpyrifos		References
Chemical name	O, O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate	Simon *et al*. (1998)
Chemical formula	$C_9H_{11}Cl_3NO_3PS$	Simon *et al*. (1998)
Molecular weight	350.6 a.m.u.	Simon *et al*. (1998)

Physical appearance	White crystalline solid	Worthing (1979)
Melting point	42 - 43.5 ˚C	Worthing (1979)
Vapour pressure	1.8 X 10^{-5} mm Hg at 25 ˚C	Worthing (1979)
Henry's law constant	2.9 X 10^{-6} atm m^{-3} mole at 25 ˚C	PBT Profiler
Solubility	Water 0.002 g/L at 25 ˚C 0.0014 g/L at 25 ˚C Methanol 450 g/L at 25 ˚C Acetone >400 g/L at 20 ˚C Dichloromethane >400 g/L at 20 ˚C Ethyl acetate >400 g/L at 20 ˚C Toluene >400 g/L at 20 ˚C n-Hexane >400 g/L at 20 ˚C	Kidd and James (1991) Racke (1993), DowAgro Sciences (2003) Worthing (1979)
Partitioning coefficient	Log K_{ow} 4.96 - 5.11 3.78 soil slurry Log K_{oc} 3.78	Suntio *et al*. (1988) Swann *et al*. (1983) Suntio *et al*. (1988)
Half life	pH 4.5, 25 ˚C 77 days pH 6.0, 25 ˚C 49 days pH 7.0, 15 ˚C 100 days pH 8.0, 25 ˚C 19 days	Chapman and Cole (1982) Chapman and Cole (1982) McCall *et al*. (1983) Chapman and Cole (1982)

Table 2. Bacterial Species reported to degrade OP insecticides in Liquid media and Soil

S.No.	Microorganisms	Mode of Degradation	References
1.	*Alcaligenes faecalis*	Catabolic	Yang *et al*. (2005)
2.	*Bacillus cereus*	Catabolic	Liu *et al*. (2012)
3.	*Bacillus licheniformis* ZHU-1	Catabolic	Zhu *et al*. (2010)
5.	*Enterobacter* sp.	Catabolic	Singh *et al*. (2003)
6.	*Klebsiella* sp.	Catabolic	Ghanem *et al*. (2007)
7.	*Paracoccus* sp. TRP	Catabolic	Xu *et al*. (2008)
8.	*Pseudomonas aeruginosa*	Catabolic	Lakshmi *et al*. (2008)
9.	*Pseudomonas stutzeri* (B-CP5)	Catabolic	Awad *et al*. (2011)
10.	*Serratia* sp.	Catabolic	Xu *et al*. (2007)
11.	*Sphingomonas* sp.	Catabolic	Li *et al*. (2007)
12.	*Stenotrophomonas* sp.	Catabolic	Yang *et al*. (2006)
13.	*Synechocystis* sp. strain PUPCCC 64	Catabolic	Sing *et al*. (2011)
14.	*Pseudomonas diminuta*	Co-metabolic	Serdar *et al*. (1982)
15.	*Micrococcus* sp.	Co-metabolic	Guha *et al*. (1997)
16.	*Flavobacterium* sp. TCC27551	Co-metabolic	Mallick *et al*. (1999)
17	*Bacillus pumilus* C2A1	Co-metabolic	Anwar *et al*. (2009)

The contaminated sites are considered as an excellent source for the isolation of the pesticide-degrading microbial community. By using enrichment culture techniques, several microbial species capable of utilizing chlorpyrifos as the sole source of C have been isolated either from pesticide-contaminated soil, sludge or waste water (Ghanem *et al*., 2007; Latifi *et al*., 2012; Liu *et al*., 2012; Savitha and Raman, 2012). A reasonably good number of studies reveal that microorganisms have potential application in the bioremediation of chlorpyrifos-contaminated soils (Singh *et al*., 2003, 2004; Yang *et al*., 2005; Li *et al*., 2007, 2008; Lakshmi *et al*., 2008; Zhu *et al*., 2010). Singh *et al*. (2003) first reported enhanced biodegradation of chlorpyrifos in the soils of Australia and then this enhanced ability was successfully transferred to the five soils in the UK. The soils with a pH of >6.7 were able to maintain this degrading ability for 90 days after inoculation. They found that the isolate showing 16S rRNA sequence similarity to *Pseudomonas* strain was likely to be involved in biodegradation of chlorpyrifos in soil. The following year, they reported that the addition of strain *Enterobacter* B-14 to soil treated with 35 mg of chlorpyrifos kg^{-1} having a low indigenous population resulted in a greater degradation than non-inoculated soil (Singh *et al*., 2004). The addition of bacterial strains *Bacillus licheniformis* ZHU-1 to soils treated with

chlorpyrifos showed almost complete degradation in 12-14 days (Zhu *et al*., 2010). However, Mohan *et al*. 2004 carried out degradation of a chlorpyrifos-contaminated soil using native mixed microflora in slurry bioreactor at 3000 mg/g, 6000 mg/g and 12,000 mg/g and found that 91%, 82% and 14% of chlorpyrifos was respectively degraded after 72 h. Using a soil slurry medium, Kumar (2011) reported that a mixed bacterial culture (GCC134) was more effective and resulted in 85% degradation of chlorpyrifos compared to 77% degradation by mono-cultures in 30 days. The degradation of chlorpyrifos by microorganisms is also facilitated by the plant roots in rhizosphere soil. Korade and Fulekar (2009) tested the potential of ryegrass for rhizosphere bioremediation of chlorpyrifos in mycorrhizal soil. In pot-culture experiment, chlorpyrifos added at an initial concentration of 10 mg/kg soil was observed to be degraded completely within seven days where the remaining amended concentrations (25-100 mg/kg) decreased rapidly under the influence of ryegrass mycorrhizosphere as the incubation progressed to 28 days. The microorganism surviving in the rhizospheric soil spiked at the highest concentration (100 mg/kg) was identified as *Pseudomonas nitroreducens* PS-2. In bioaugmentation experiments, the percentage dissipation of chlorpyrifos by strain PS-2 was 100% in the inoculated rhizospheric soil as compared to 76.24, 90.36 and 90.80% in the non-inoculated soil for initial concentrations of 25, 50 and 100 mg/kg at the 14th, 21st and 28th day intervals, respectively. Dubey and Fulekar (2012) performed a comprehensive study to evaluate the potential of *Pennisetum pedicellatum* plants to assist rhizosphere associated degrading strains for chlorpyrifos remediation. Time-course pot experiments were conducted in a greenhouse with *P. pedicellatum* grown in soil amended with chlorpyrifos at concentrations ranging from 10 to 100 mg kg^{-1} for 60 days. A novel strain *Stenotrophomonas maltophilia* MHF ENV20 isolated from the remediated rhizosphere soil showed better survival and degraded 100, 50 and 33.3% chlorpyrifos within 48, 72 and 120 h at 50, 100 and 150 mg/kg pesticide concentrations, respectively. These findings indicate that rhizosphere remediation is an effective bioremediation technique to remove chlorpyrifos residues from soil.

GENES AND ENZYMES RESPONSIBLE FOR THE DEGRADATION OF CHLORPYRIFOS

Chlorpyrifos is degraded by a variety of microorganisms. These microorganisms are capable of producing pesticide-degrading enzymes such as organophosphorus hydrolase (OPH) (Gao *et al*., 2012), phosphotriesterase (PTE) (Theriot and Grunden, 2011), acid organophosphorus anhydrolase (OPAA) (Cheng *et al*., 1993) and methyl parathion hydrolase (MPH) (Chino-Flores *et al*., 2012). The biochemistry of organophosphate pesticide degradation by most of the microorganisms appears to be identical, where OPH or PTE catalyzes the first step of the degradation (Singh and Walker, 2006). Microbial OPH cleaves PeO (chlorpyriphos), PeF (mipafox) and/or PeS bonds (demeton-S) of organophosphate pesticides (Ang *et al*., 2005). Depending on the microorganisms and environmental conditions, cleavage of chlorpyrifos yields two major metabolites such as TCP and DETP (Bootharaju and Pradeep, 2012), while some other metabolites, including desethyl chlorpyrifos, chlorpyrifosoxon, desethyl chlorpyrifos-oxon and 3,5,6-trichloro-2 methoxy pyrimidine are produced in very minute quantities. The TCP is considered as mobile and persistent in the soil (Kim and Ahn, 2009). It can be further degraded to 3, 5, 6-trichloro-2- methoxypyridine (TMP) and carbon dioxide (Racke, 1993). *Pseudomonas* sp. was reported to mineralize TCP in a liquid medium (Feng *et al*., 1997). The TCP was mineralized by *Pseudomonas* sp. via reductive dechlorination pathway (Feng *et al*., 1998), however, several microbial spp. capable of degrading hydroxypyridine, which is analogous to TCP, have been reported (Kaiser *et al*., 1996). Singh and Walker (2006) proposed a pathway for the degradation of chlorpyrifos by microorganisms, showing details of each biodegradation step. Cain *et al*. (1974) reported

that first 2- or 3-hydroxypyridine is oxidized to 2, 5-dihydroxypyridine and then the production of maleamic acid occurs through ring cleavage. Oxygen atoms that are used to transform 4-hydroxypyridine via 3, 4-dihydroxypyridine are derived from water molecules by hydroxypyridine hydrolase (Watson *et al*., 1974). It is very likely that TCP is metabolized by various microorganisms in a similar manner to hydroxypyridine mineralization. The presence of TCP in microbial metabolites (extracts) indicates that microorganisms can degrade the chlorpyrifos pesticide intra cellularly as well as extra cellularly. Rapid depletion of chlorpyrifos from a culture medium may be due to extracellular degradation of chlorpyrifos. Chungjatupornchai and Fa-Aroonsawat (2008) isolated a gene for organophosphorus hydrolase from *Flavobacterium* sp. and expressed it in *Synechococcus* PCC 7942. They showed that this enzyme was located both on the surface as well as intra-cellularly. In addition, phosphatase plays an important role in the biodegradation of chlorpyrifos which is known as an extracellular enzyme (Madhuri and Rangaswamy, 2002; Thengodkar and Sivakami, 2010). The OPH is believed to be an ideal enzyme for the degradation of organophosphate pesticides because of its broad substrate profile and ability to hydrolyze compounds at a rate approaching to the diffusion limits (Dumas *et al*., 1989). The molecular mass of OPH purified from different microorganisms varies substantially. Molecular mass of OPH (60 kDA) purified from *Penicillium lilacinum* (Liu *et al*., 2004) was 1.6 times greater than that of fungus *Cladosporium cladosporioides* (38.3 kDA) (Gao *et al*., 2012). The molecular mass of OPH purified from bacterial spp. *Alteromonas* sp. JD 6.5 and *Alteromonas undina* MG was 60 kDA and 53 kDA, respectively (Cheng *et al*., 1993), whereas the molecular mass of MPH purified from *Pseudomonas sp.* WBC-3 was found to be 33.5 kDA (Cui *et al*., 2001). Similarly, an enzyme OPAA also known for the detoxification of organophosphate compounds was isolated and purified. The OPAA isolated from *Alteromonas undinawas* composed of a single polypeptide with a molecular weight 53 kDa compared to the OPAA containing 517 amino acids with a molecular weight of 60 kDa of *Alteromonas* sp. JD 6.5 (Cheng *et al*., 1993). However, the OPAA from *Alteromonas haloplanktis* contained 440 amino acids with a molecular weight 50 kDa (Cheng *et al*., 1997). This difference in mass of OPAAs of two *Alteromonas* sp. was found due to the presence of an extended Cterminal region in the JD 6.5 enzyme (DeFrank and White, 2002). It showed low catalytic activity against PeO, but high activity against PeF bonds.

The OPH enzymes are encoded by the *opd* (organophosphate degradation) and *mpd* (methyl parathion hydrolase) genes, and are members of the amidohydrolase super family (Seibert and Raushel, 2005). The crystallographic structure of OPH reveals that it is a homo-dimer with equal active sites at the C-terminus of each monomer (Benning *et al*., 1994). It has (b/a)8-barrel structural fold that catalyzes the hydrolysis of amide or ester functional groups at C and P centers (Seibert and Raushel, 2005). Singh *et al*. (2004) reported a novel PTE enzyme system (isolated from *Enterobacter* sp. strain B-14)-encoding gene that had a different sequence from the widely studied organophosphate-degrading *opd* gene. Yang *et al*. (2003) discovered an *opdA* enzyme from *A. radiobacter* mP230 that degraded a broad range of organophosphates. It was very similar to OPH first isolated from *Pseudomonas diminuta* MG. Despite a high level of sequence identity, OPH and *opdA* exhibited different substrate specificities. Singh *et al*. (2006) isolated OPH encoding gene *opd* from geographically different regions and taxonomically different species. The *opd* genes isolated from *Bacillus diminuta* and *Flavobacterium* sp. ATCC 27551 were located on non-homologous plasmids that possess 100% similarity in DNA sequences. On the basis of these observations, the authors concluded that horizontal gene transfer (HGT) could be involved in *opd* gene distribution and HGT might be aided by mobile genetic elements or transposons. Very recently, Chino-Flores *et al*. (2012) isolated a novel gene *opdE* (753 bp encoding a protein of 25 kDa) from *Enterobacter* sp. which showed no similarity to

any previously isolated genes reported to degrade organophosphates. The *mpd* gene isolated from *Stenotrophomonas sp.* and *Sphingomonas sp.* strain Dsp-2p was also reported to degrade chlorpyrifos (Yang *et al*., 2006; Li *et al*., 2007).

Table 3. Genes and Enzymes involved in the Biodegradation of OP Insecticides

S.No.	Gene	Encoded enzyme	Insecticides	Reference
1.	*mpd* *opd*	Organophosphate Hydrolase (OPH) Phosphotriesterase enzyme	Chlorpyrifos	Chen *et al*., 2012 Singh *et al*., 2004
2.	*Opd*	Organophosphate Hydrolase (OPH)	Coumaphos	Serder *et al*., 1989 Somara and Siddavattam, 1995
3.	*opaA*	Organophosphorus Acid Anhydrolase (OPAA)	Sarin, soman, and O-cyclohexyl methylphosphonofluoridate	Cheng *et al*., 1996
4.	*opdA*	Organophosphate-Degrading Enzyme (OPDA)	Sarin and soman	Horne *et al*., 2002b
5.	*hocA*	Phosphotriesterase	Oxon and thion organophosphorus ompounds	Horne *et al*., 2002c
6.	*adpB*	Adenosine-Di-Phosphatase (ADPase)	OP compounds	Mulbry, 1992
7.	*pdeA*	Phosphodiesterase	Organophosphate xenobiotics as pesticides and chemical warfare agents	Tehara and Keasling, 2003
8.	*pepA*	Aminopeptidase (AMPP)	OP compounds	Jao *et al*., 2004
9.	*phn*	Phosphonatase	Glyphosate	Chen *et al*., 1990 Parker *et al*., 1999
10.	*glp A&B*	Carbon-phosphorus lyase (C-P Lyase)	Glyphosate	Penaloza-Vazquez *et al*., 1995

ROLE OF ENDOPHYTES IN PLANT GROWTH PROMOTION

Apart from the role of bacterial endophytes in bioremediation they also facilitate plant growth via three interrelated mechanisms: phytostimulation, biofertilization, and biocontrol (Bloemberg and Lugtenberg, 2001).

Phytostimulation: Phytostimulation is the direct promotion of plant growth through the production of phytohormones (Bloomberg and Lugtenberg, 2001). The most highly studied example of phytostimulation involves lowering plant hormone ethylene levels by the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Several endophytes that release ACC deaminase have been shown to increase plant growth, including *Arthrobacter* spp. and *Bacillus* spp. in pepper plants (*Capsicum annuum*) (Sziderics *et al*., 2007), as well as *Pseudomonas putida* and *Rhodococcus* spp. in peas (*Pisum sativum*) (Belimov *et al*., 2001). The mechanism of plant growth promotion is unknown, however, ACC deaminase production may reduce abiotic stress by balancing plant ethylene-level production, because elevated ethylene levels inhibit cell division, DNA synthesis, and root/shoot growth (Burg, 1973). The production of other plant hormones including indole-3-acetic acid, jasmonates, and abscisic acid by bacterial strains may also stimulate plant growth (Patten and Glick, 2002 ; Forchetti *et al*., 2007).

Biofertilization: The promotion of plant growth by increasing the accessibility or supply of major nutrients is termed biofertilization (Bashan, 1998). A well-studied form of biofertilization is nitrogen fixation, which is the conversion of atmospheric nitrogen to ammonia (Bloemberg and Lugtenberg, 2001). Several PGPBEs have been studied extensively for their ability to fix nitrogen including *Azospirillum spp.*(Hill and Crossman, 1983), *Pantoea agglomerans* (Verma *et al*., 2001), and *Azoarcus* spp. (Hurek *et al*., 2002). Some PGPBEs can increase phosphorus availability to the plant through phosphorus solubilization. The release of low molecular weight acids can allow the chelation of the metal cation attached to phosphorus, making it more accessible to plants (Kpomblekou-A and Tabatabai, 2003). Forchetti *et al*. (2007) isolated, characterized,

and quantified the phosphate solubilization abilities of endophytes in sunflower (*Helianthusannuus*), identifying *Achromobacter xiloxidans* and *Bacillus pumilus* as having the highest chelating capabilities. Yazdani and Bahmanyar (2009) showed that the use of PGPBEs in fertilizer treatments for corn (*Zeamays*) reduced the need for phosphorus application by 50% without significant loss in grain yield.

Biocontrol: The promotion of plant growth through protection from phytopathogens is known as biocontrol. Several mechanisms may be involved, including the production of siderophores or antibiotics. Siderophores, such as pyochelin and salicylic acid, chelate iron and can indirectly contribute to disease control by competing with phytopathogens for trace metals (Duffy and Défago, 1999). Antimicrobial metabolites produced by PGPBEs, such as 2, 4-diacetylphloroglucinol (DAPG), can enhance disease suppression in plants. For example, eggplant wilt caused by *Ralstonia solanacearum* was reduced by 70% after seeds were inoculated with DAPG-producing endophytic isolates (Ramesh *et al*., 2008).

CONCLUSION

Expanding agricultural land is difficult because this possibility is limited by a number of important constraints such as competing with urban growth and scarcity of fresh water. Therefore, improvement of agricultural productivity and reducing the mass damage caused to agricultural lands and human health by the persistent use of chemical pesticides and fertilizers will be the key approach for reducing the global food insecurity over the coming decades. Endophytes since they harbor inside and are in a close proximity with the plants have the potential to become preferred substitutions for some of the routinely used conventional synthetic products. Hence, these bio-preparations can substantially contribute to the sustainable production of environmentally friendly and low chemical residue products. Endophytes not only appear promising to increase crop yields but also remove contaminants, inhibit pathogens, and produce fixed nitrogen or novel substances. Their importance has still not been comprehensively defined. Therefore the challenge lies in the fact that more knowledge and awareness is needed to attain a better understanding of endophyte ecology and their molecular interactions and their potential role in sustainable agriculture.

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