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POTENTIAL OF SOME SOIL BACTERIA FOR DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES - A REVIEW

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ABSTRACT

Organophosphate pesticides are commonly used in agriculture for pest control. The short and long-term exposures to Organophosphates induce inhibition of acetyl cholinesterase (AchE) activity, thus leading to nervous impairment in humans. This review records various aspects viz., organophosphates in soil, organophosphates toxicity, biodegradation of organophosphorus pesticides, bacterial degradation, enzymatic degradation of organophosphorus pesticides. The literature shows that there has been variation in *Bacillus*, *Arthrobacter*, *Xanthomonas* and *Actinomyces* population which indicates that OP pesticides had either stimulatory or inhibitory effects on different microbial groups. The bacterial species used for degradation are viz., *Pseudomonas putida*, *Acinetobacter rhizosphaerae Pseudomonas diminuta* MG and *Flavobacterium* ATCC 27551, *Nocardiodes simplex Stenotrophomonas malthophilia, Proteus vulgaris, Vibrio metschinkouii, Serratia ficaria, Serratia spp.,Yersinia enterocolitica.Bacillus amyloliquefaciens, Bacillus licheniformis, Pseudomonas species, Staphylococcus species.* Inspite of this many catabolic plasmids have been found in species of *Pseudomonas, Alcaligenes, Actinobacter, Flavobacterium, Klebsiella, Moraxella* and *Arthrobacter, which* have degradative potential. These kind of ecofriendly bacteria can be used to encourage and motivate the farmers to use natural biological pesticides

KEYWORDS: ***Organophosphorus Pesticides, Soil, Bacterial Degradation, Enzymatic Degradation***

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INTRODUCTION

India being an agriculture based country with more than 60-70 percent of its population dependent on agriculture, in recent years, plant protection has become one of the essential inputs in crop production. Initially, chemical pesticides were applied for cereal crops and now these are increasingly used due to increase in pest and disease attack on other crops as well. There has been a considerable qualitative and quantitative change in pesticide use in the last few years worldwide and in India as well. Among all the pesticides, organochlorine insecticides enjoy a unique status because of their high insecticidal efficacy and successfully controlling a number of diseases, such as malaria and typhoid. But, these were banned or restricted after the 1960s in most of the technologically advanced countries due to their long-term persistence in the environment, susceptibility to biomagnifications and toxicity to higher animals and had been replaced by organophosphorus pesticides (OP). Although, these newly introduced OP pesticides are highly toxic to man and animals even then are used to control a wide variety of insect pests, weeds and disease transmitting vectors because these are considered as biodegradable pesticides. Moreover, OP pesticides are widely used because they are cheaper than the newer alternatives. Indian farmers and growers regularly use organophosphates in high quantities. Among OP pesticides, monocrotophos, quinalphos and chlorpyriphos list on top in India. Ideally, a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced. The rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played havoc with human and other life forms. Increased use of chemical pesticides has resulted in contaminating environment and the long term implications on the society are found multidimensional. Now, the farmers are addicted to using agrochemicals indiscriminately and excessively to make the situation from bad to worse not only in India but also in other parts of the world as well. [1-2] OP pesticides are regularly detected at low levels in a range of food and food products. The foods most likely to contain residues of high-risk pesticides are apples, pears, peaches, grapes, green beans, tomatoes, peas, strawberries, peppers, melons, lettuce, and various juices. [3] It has now been well established that the major means of degradation of all pesticides is microbial. The data also indicates that the soil bacteria may be more important in degradation of certain pesticides. [4-8] Soil bacteria with the ability to degrade several pesticides have been isolated from soil. Biodegradation is a metabolic process that involves the transformation of an organic compound into its metabolite(s). When this metabolite is broken down into its inorganic components such as water and carbon dioxide or methane etc., the process is referred to as biomineralization. Thus, biodegradability represents the susceptibility of substances to be altered by microbial processes. The alteration may occur by intra or extracellular enzymatic attack that is essential for growth of the microorganisms. The attacked substances are used as a source of carbon, energy, nitrogen, or other nutrients or as final electron acceptor. Co-metabolic reactions occur when enzymatic attack is not necessarily beneficial to the microorganism, i.e., a physiologically useful primary substrate induces production of enzymes that fortuitously alter the molecular structure of another compound. [9] Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. [10-11] Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders. [12] Fungi generally biotransform pesticides by introducing minor structural changes to the molecule, rendering it nontoxic; the bio transformed pesticide is released into the soil, where it is susceptible to further degradation by bacteria. [13] Biodegradation may occur under either aerobic or anaerobic conditions. Under aerobic conditions, oxygen is used as a final electron acceptor; and under anaerobic conditions, microorganisms use compounds such as nitrate (NO_3), sulfate (SO_4^{2-}) or iron (Fe^{3+}) as the final electron acceptor instead of oxygen. The rate of biodegradation depends on environmental factors, numbers and types of microorganisms present, and the chemical structure of the target compound. [9] A review of Briceño *et al* shows that the microbial degradation of a pesticide in soil is strongly affected by its physical, chemical and biological characteristics, climatic factors such as pH, temperature, humidity, organic matter etc. [14] Moreover, addition of organic amendment and nutrients affect mainly the adsorption, movement, and biodegradation of pesticides. Bacteria play role in degradation of metamitron, chlorpyrifos, atrazine, iprodione pesticides include *Rhodococcus sp*., *Flavobacterium sp*., *Pseudomonas sp*. and *Arthrobacter sp*. respectively have been identified. Two more strains of *Pseudomonas putida* are reported to utilize diclofop methyl as a source of carbon and energy. [15-19] Majority of studies concerning the organophosphorus degradation have been done but they are far and wide .So in this review an attempt has been done to collect information on organophosphorus pesticide degrading bacteria that were able to degrade monocrotophos in agricultural soil.

ORGANOPHOSPHATE (OP) PESTICIDES

OP pesticides were first developed in Germany by, a chemist Gerhard Schrader in 19th century.during World War II in the form of tetraethyl pyrophosphate and are esters of phosphoric acid which include aliphatic, phenyl and heterocyclic derivatives and have one of the basic building blocks as a part of their complex chemical structure. Currently, organophosphorus group are among the various groups of pesticides that are being used the world over, which is a major and the most widely used group in both domestic and industrial settings. [20]

ORGANOPHOSPHATES IN SOIL

Agnihotri *et al* studied the influence of insecticides on soil microorganisms and their biochemical activities such as nitrification, ammonification, respiration, nitrogen fixation etc. [21] Jain et al, conducted research on the effect of monocrotophos pesticides on fungal strain *Aspergillus niger* in soil of Banasthali region. [22] It was

observed that *Aspergillus niger* has the capability of degrading 90% monocrotophos under optimal conditions within 10 days. Lakshmikantha conducted research on the effect of foliar insecticides fenvalerate, quinalphos and endosulfan on soil microorganisms and their biochemical processes in soils of Gulbarga. [23] Insecticides at normal recommended rate did not affect the major groups of soil microflora *viz*., bacteria, fungi, actinomycetes, free living nitrogen fixers and P-solubilizers, while four times more than the recommended concentration exerted high depressive effects followed by two times the recommended concentration. Urease and dehydrogenase activities were found sensitive to increased concentration. Except amonification, all other biochemical activities were found sensitive to external application of insecticides. Pandey and Singh observed short term inhibitory effects on the total bacterial population after chlorpyriphos and quinalphos application which were recovered within 60 days after seed treatment and by 45 days of soil treatment. [24] The fungal population was significantly enhanced after chlorpyriphos treatment. Singh *et al* also observed in a study on effects of chlorpyriphos, fenamiphos and chlorothalonil alone and in combination on soil microbial activity, that the measured soil microbial parameters especially the enzyme activities and total microbial biomass were stable in the pesticide free control soils throughout 90 days of inoculation period, but they were all adversely affected in the presence of added pesticides. [25] DeLorenzo and Serrano analyzed the toxicity of three pesticides, atrazine, chlorpyriphos and chlorothalonil individually and in two mixtures (atrazine and chlorpyriphos, atrazine and chlorothalonil) to the marine phytoplankton *Dunaliella tertiolecta*. [26] At higher concentration of more than or equal to 400 µg/*l*, chlorpyriphos elicited a significant effect on growth rate of *D. tertiolecta*, while atrazine and chlorpyriphos in mixture displayed additive toxicity. Menon *et al* reported that the arginine ammonification activity of rhizosphere microorganisms were inhibited by chlorpyriphos and its metabolites 3, 5, 6-trichloro-2-pyridinol and 3, 5, 6-trichloro-2-methoxypyridine (TMP) in both loamy sand and sandy loam soils after seed treatment with chlorpyriphos (5 g ai/kg seed). [27] There was also stimulation of rhizospheric N mineralization by parent compound and inhibition by metabolites, whereas it was the reverse in the non-rhizospheric soil. The interaction effects of monocrotophos, quinalphos and cypermethrin when applied either singly or in combination at 0, 5, 10 and 25 µg/ml on microbial population and dehydrogenase activity was studied by Gundi *et al.* [28] These insecticides significantly enhanced the proliferation of bacteria and fungi, and soil dehydrogenase activity even at the highest level of 25µg/ml. Also, the antagonistic activities were more pronounced towards soil microflora and dehydrogenase activity when the two insecticides monocrotophos or quinalphos plus cypermethrin were present together in the soil at higher level (25+25 µg/ml), whereas synergistic or additive responses occurred at lower level with the same combination of insecticides in soil. Sardar and Kole studied metabolism of chlorpyriphos in relation to its effect on the availability of some plant nutrients in soil and observed that there was significant decrease in available N and P content in the soil treated with chlorpyriphos @ 100 kg a.i./ha. [29] The inhibitory effect on available N was attributable to TMP (3, 5, 6-trichloro-2-methoxypyridine) and for phosphorus, it was due to presence of 3, 5, 6-trichloro-2-pyridinol and TMP rather than chlorpyriphos. Yang *et al* developed a rapid and sensitive sample pretreatment technique for the determination of organophosphorus pesticides (OPPs) in soil samples by using dispersive liquid-liquid microextraction (DLLME) combined with gas chromatography-flame photometric detection. [30] Linearities for the three target OPPs namely ethoprophos, chlorpyriphos, and profenofos were procured by five points in the concentration range of 2.5-1500 µg/kg, under optimum conditions. Each target analyte recoveries were observed in the range between 87.9% and 108.0%, 87.4% and 108.0%, and 86.7% and 107.2%, respectively.

ORGANOPHOSPHATES TOXICITY

Toxicity of Organophosphates to plants, animals and humans has been well documented. The short and long-term exposures to Organophosphates induce inhibition of acetyl cholinesterase (AchE) activity, thus leading to nervous impairment in humans and other vertebrates. [31] Most of the ill-health following exposure to organophosphorus compounds has been attributed to the inhibition of cholinesterases. However, the current literature has justifiably challenged this view as the inhibition of cholinesterases by itself cannot account for the wide range of disorders that have been reported following organophosphorus poisoning. It is becoming apparent that, although inhibition of cholinesterases plays a key role in the toxicology of organophosphates, individual susceptibility, the inhibition of other enzyme systems and the direct effects of organophosphates on tissues are also important. [32] Five OP pesticides, (Monocrotophos, Omethoate, Parathion-methyl, phoxim and dichlorvos) were examined for their effects on mammalian cell lines to determine their potential impact on physiological functions in vivo. [33] Boobis *et al* and Bouvier *et al* found that exposure to organophosphates (OPs) is also possible via intentional or unintentional contamination of food sources. [34-35] Although no clinical effects of chronic, low-level organophosphates (OPs) exposure from a food source have been shown, advancements in risk assessment and preparedness are ongoing. Organophosphates can be absorbed cutaneously, ingested, inhaled, or injected. Although most patients rapidly become symptomatic, the onset and severity of symptoms depend on the specific compound, amount, route of exposure, and rate of metabolic degradation. [36] Pesticide poisonings are among the most common modes of poisoning fatalities. In countries such as India and Nicaragua, organophosphates are easily accessible and, therefore, a source of both intentional and unintentional poisonings. The incidence of international organophosphate-related human exposures appears to be underestimated. [37] Organophosphates may affect children or other at-risk populations differently. The increased susceptibility has not been elucidated but may involve delayed or persistent effects. More work in this area is underway and should help identify the true risk potential. [38]

BIODEGRADATION OF ORGANOPHOSPHORUS PESTICIDES

Pesticides absorbed in the soil get acted upon by various physical, chemical, and biological forces. Of the three, biological forces plays major role in the degradation of pesticides. Several soil microorganisms have the ability convert pesticides into simpler non-toxic compounds. The process of degradation of pesticides by converting it into non-toxic compounds is known as "biodegradation". The processes by which pesticides are transformed or degraded in environmental compartments and the factors that modulate the kinetics are critical from both pest control efficacy and non target organism toxicity points. Both abiotic and microbiological transformation of pesticides have been reported and are found to contribute significantly to its degradation. Pesticides in soil and water can be biodegraded and is the primary mechanism of pesticide breakdown and detoxification in many soils. [39] Conventional approaches (e.g. landfilling, recycling, pyrolysis and incineration) for the remediation of contaminated sites are inefficient, costly and may lead to the formation of several toxic intermediates. [40] Thus, biological decontamination methods are preferable to conventional approaches because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates. [41-42] Microbial degradation of organophosphorus pesticides is of particular interest because of their high mammalian toxicity and widespread use. Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation. Some organophosphates such as parathion, it has been relatively easy to isolate degrading bacteria, where two different strains, *Flavobacterium* ATCC 27551 and *Pseudomonas diminuta Gm*, have been isolated from soils in the Philippines and USA, respectively. [43-44]

Rangaswamy and Venkateswarlu studied the degradation of selected insecticides, Quinalphos and monocrotophos by *Azospirillum lipoferum* and *Bacillus sp*. isolated from black soil following enrichment culture technique. [45] By the end of 7 days, about 40 per cent of monocrotophos supplemented to mineral salts medium was degraded by *A. lipoferum* and *Bacillus sp*. Nearly 56 percent and 76 per cent of quinalphos was degraded by *A. lipoferum* and *Bacillus sp*. respectively. Megharaj *et al* studied the biodegradation of methyl parathion (1000 ppm) by soil isolates of microalgae and cyanobacteria. [46] Two species of microalgae, *Chlorella vulgaris* and *Scenedesmus bijugatus* and four of cyanobacteria *Nostoc linckia*, *Nostoc muscorum*, *Oscillatoria animalis* and *Phormidium foveolarum* were isolated from the enrichments and hydrolysed the insecticide to undetectable levels by 20-30 days. Biodegradation and utilization of organophosphorus pesticides, monocrotophos and malathion (50 ppm) by *Aulosira fertilissima* ARM68 and *Nostoc muscorum* ARM 221 was reported by Subramanian *et al.* [47] Methyl parathion (O, O-dimethyl-O-(p-nitro-phenylphosphorothioate)) is one of the most used organophosphorus pesticides. This product is widely used throughout the world and its residues are regularly detected in a range of fruits and vegetables. Investigation of microbial degradation is useful for developing insecticide degradation strategies using microorganisms. Bacteria with the ability to degrade methyl parathion have been isolated worldwide. [48-49] Multiplex tendencies characterize pesticide applications in farming. A number of pesticide mixtures, especially pyrethroid and organophosphorus pesticide mixtures have been formulated as an improvement over individual pesticides. [50]Singh and Subhas studied the biodegradation of monocrotophos by two bacterial isolates isolated from cotton soil. [51] *Pseudomonas aeruginosa* F10B was found to degrade 98.95 per cent of the technical monocrotophos while *C. michiganense sub. sp. insidiosum* SBL11 degraded 87 per cent of the technical monocrotophos in shake flask culture in 24 hrs of incubation at 37°C. Oretiz- Hernandez *et al* reported that *Flavobacterium sp.* ATCC 27551 strain bearing organophosphate degradation gene hydrolysed the OP pesticides at the bond between phosphorus and the hetero atom producing phosphoric acid and other metabolites. [52] Sharungbam *et al* reported the degradation of quinalphos by bacteria isolated from soil. [53] It was observed that 11 isolates degraded up to 92 per cent of the insecticide at higher concentrations (8 ppm and 12 ppm) on the 10th day of incubation. Jilani and Altaf Khan isolated bacterial strain *Pseudomonas sp.* from soil. [54] They observed that *Pseudomonas* strain was able to grow in nutrient medium containing malathion (35-220 mg LG), methamidophos (80-320 mg LG), cartap (60-120 mg LG), and cypermethrin (40-125 mg LG), pesticide. However, the optimum concentration which supports normal bacterial growth during 24 hours was found to be 120 mg LG malathion, 160 mg LG methamidophos, 80 mg LG cartap and 60 mg LG cypermethrin. When compared with the control test, a significant increase in bacterial population was noted at low concentration of each pesticide, however, at high concentration lag phase increased but no zone of inhibition observed. These data indicate that the isolated *Pseudomonas* strain can be used as a microorganism for the bioremediation of pesticide contaminated soil or water.

BACTERIAL DEGRADATION

Complete biodegradation of a pesticide involves the oxidation of parent compound to form carbon dioxide and water. This process provides both carbon and energy for the growth and reproduction of microbes. Each degradation step is catalyzed by specific enzyme produced by a degrading cell or enzyme found external to the cell. Degradation of pesticide by either external or internal enzyme will stop at any step if an appropriate enzyme is not present. Absence of an appropriate enzyme is one of the common reason for persistence of any pesticide. If an appropriate microorganism is absent in soil or if biodegrading microbial population has been reduced due to toxicity of pesticide in that case a specific microorganism can be added or introduced in soil to enhance the activity of the existing population. [55] The use of bacteria for the degradation and detoxification of numerous toxic chemicals such as pesticides is an effective tool to decontaminate the polluted sites. Isolation of indigenous bacteria capable of metabolizing pesticides provides environmentally friendly means of *in situ* detoxification. [56] Degradation by microbes depends not only on the presence of degradative enzymes, but also on a wide range of environmental parameters. Temperature, pH, water

potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factor for pesticide degrading microorganisms, which requires further exploration in relation to total microbial population and their biochemical activities. [55] Some pesticides are readily degraded by microorganisms; others have proven to be recalcitrant. [57-59] In a study, a bacterial consortium which degrades tetrachlorvinphos (phosphoric acid, 2-chloro- 1-(2,4,5-trichlorophenyl) ethenyl dimethyl ester) was isolated from agricultural soil from Mexico. This consortium being composed of six pure strains were characterized based on their morphological and biochemical characteristics. The strains were presumptively identified as *Stenotrophomonas malthophilia, Proteus vulgaris, Vibrio metschinkouii, Serratia ficaria, Serratia spp.* and *Yersinia enterocolitica*. The consortium and the six bacteria were assessed in order to discover their ability to degrade tetrachlorvinphos (TCV) in mineral medium and in rich medium. Growth curve experiments showed that the bacterial consortium was able to grow in mineral medium containing TCV as the only carbon source. [60] Kumari *et al* isolated *Bacillus species*. [61] The isolated organism was added to the soil supplemented with malathion & incubated for 4 days at 37^{o}C on shaker at 200rpm. After 4 days of incubation the phosphate levels are estimated by Fiske-Subbaraw method. The phosphate levels are increased due to degradation of malathion. Baishya and Sharma conducted a study to isolate bacteria having Organophosphorus insecticides degrading ability from soil of some selected agro ecosystems of Dimoria region of Kamrup, Assam which is having a history of repeated pesticide applications. [62] The isolation of two pesticide viz. Malathion and Quinalphos, degrading bacteria was carried out using Mineral Salts Medium (MSM) and the isolated strains were identified as *Bacillus amyloliquefaciens, Pseudomonas species, Staphylococcus species* and *Bacillus licheniformis* based on staining techniques and plating on selective media. Deng *et al* isolated a bacterial strain from sludge collected at the drain outlet of a chlorpyrifos manufacture plant being capable of degrading *O*,*O*-dialkyl phosphorothioate and *O*,*O*-dialkyl phosphate insecticides, designated as G1.Physiological and biochemical characteristics and 16S rDNA gene sequence analysis suggested that strain G1 belongs to the genus *Stenotrophomonas*. [63] At an initial concentration of 50 mg/L, strain G1 degraded 100% of methyl parathion, methyl paraoxon, diazinon, and phoxim, 95% of parathion, 63% of chlorpyrifos, 38% of profenofos, and 34% of triazophos in 24 h. In a study soil sample collected from Agriculture University Gwalior, Madhya Pradesh, India which is having a history of repeated pesticide application found that bacterium capable of degrading Malathion and Dichlorvos were *Staphylococcus* sp. *Micrococcus* sp. *Entrobacte* sp. *Bordetella* sp. *Pseudomonas* sp. and *Klebsella sp*. The growth of all six pesticide degrading isolates was assessed in Mineral Salt Medium (MSM). The maximum growth rate by the isolates *Pseudomonas* sp. AUG12 were 1.564 and 1.435 for Malathion and Dichlorvos respectively after 140 h Plate assay revealed that *Pseudomonas* sp. AUG12 could grow with high concentration of Malathion (1900 mg/lit) and Dichlorvos (1500 mg/lit).[64] Maleeka *et al* isolated a total 44 isolates of dimethoate degrading bacteria from dimethoate contaminated soils from Tamil Nadu. [65] Among the 44 isolates 33 isolates were *Bacillus*, 7 isolates were *Enterobacter*, 3 isolates were *Pseudomonas* and 1 isolate was *Aeromonas*. *Bacillus pumilus* predominantly degraded dimethoate which has a higher growth rate of OD range 0.36 at 6 hours. The efficacy of the three isolated strains: *Xanthomonas* sp. 4R3-M1, *Pseudomonas* sp. 4H1-M3, and *Rhizobium* sp. 4H1-M1 was investigated for biodegradation of CP and its primary metabolic product, 3,5,6-trichloro-2-pyridinol (TCP). The results indicate that all three bacterial strains almost completely metabolized CP (10 mg/L) and TCP, occurring as a metabolic degradation product, in mineral salt media as a sole source of carbon and nitrogen. The isolated bacterial strains *Xanthomonas* sp. 4R3-M1 and *Pseudomonas* sp. 4H1- M3 could also degrade TCP (10 mg/L) as a sole carbon and nitrogen source, when provided externally. Thus, these bacterial strains may be effective in practical application of bioremediation of both CP and TCP. [66]

ENZYMATIC DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES

Microorganisms degrading xenobiotic chemicals are equipped with elaborate enzyme systems. Biodegradation of organophosphates involves activities of phosphatase, esterase, hydrolase and oxygenase enzymes. The most significant step in detoxifying organophosphate compounds is hydrolysis since that makes the compounds more vulnerable to further degradation. [67] The enzyme responsible for catalyzing this reaction is referred as an esterase or phosphotriesterase. Research has found a wide range of microorganisms possessing the organophosphate hydrolase enzyme. [68-73] The most well-known examples of natural isolates able to degrade organophosphates are *Pseudomonas diminuta* MG and *Flavobacterium* ATCC 27551. They have been shown to possess the organophosphate hydrolase (OPH) enzyme. Mulbry found *Nocardiodes simplex* NRRL B-24074 expressing OPH activity in a microbial consortium he isolated from cattle dip waste for coumaphos degradation. [72] Many pesticide degrading genes harboring in soil bacteria have been reported on plasmids. [74] These genes encoding for enzymes capable of degradation have been studied well, these plasmids are known as catabolic plasmids; the organism, containing them have the ability to degrade certain compounds. Many catabolic plasmids have been found in species of *Pseudomonas, Alcaligenes, Actinobacter, Flavobacterium, Klebsiella, Moraxella* and *Arthrobacter*. [40]Cho *et* al observed that the effectiveness of degradation by OPH varied dramatically ranging from highly efficient with paraoxon to relatively slow with methyl parathion. [75] A solid phase top agar method based on detection of the yellow product Pnitrophenol was developed for the rapid pre-screening of potential variants with improved hydrolysis of methyl parathion. One variant 22AII hydrolysed methyl parathion 25 folds faster than did the wild strain. Organophosphorus hydrolase (OPH), isolated from both *Flavobacterium* sp. ATCC 27551 and *Pseudomonas diminuta* MG is capable of hydrolyzing a wide range of oxon and thion OPs. [69,76] However, OPH has already been shown to lack any hydrolytic activity toward numerous dimethyl

Ops. [73] The *mpd* gene encoding an organophosphate degrading protein was isolated from a methyl parathion (MP) degrading *Plesiomonas* sp. The methyl parathion hydrolase gene (*mpd*) and enhanced green fluorescent protein gene (*egfp*) was successfully co-expressed using pETDuet vector in *Escherichia coli* BL21 (DE3). The co-expression of methyl parathion hydrolase (MPH) and enhanced green fluorescent protein (EGFP) were confirmed by determining MPH activity and fluorescence intensity. The recombinant protein MPH showed high enzymatic degradative activity of several widely used OP residues on vegetables. Subsequently, a dual-species consortium comprising engineered *E. coli* and a natural *p*-nitrophenol (PNP) degrader *Ochrobactrum* sp. strain LL-1 for complete mineralization of dimethyl OPs was studied. The dual-species consortium possesses the enormous potential to be utilized for complete mineralization of PNP substituted OPs in a laboratory-scale bioreactor. These studies demonstrated that MP could be degraded via the MP → PNP → hydroquinone → Krebs cycle by the dual species consortium. The data confirm that the mineralization process of MP is initiated by hydrolysis leading to the generation of PNP and dimethylthiophosphoric acid, and PNP degradation, then, proceeds through the formation of hydroquinone. The accumulation of PNP in suspended culture was prevented.[77] Two bacteria identified as *Pseudomonas putida* and *Acinetobacter rhizosphaerae*, able to rapidly degrade the organophosphate fenamiphos, were isolated. Denaturating gradient gel electrophoresis analysis revealed that the two isolates were dominant members of the enrichment culture. Clone libraries further showed that bacteria belonging to α-, β-, γ-*Proteobacteria* and *Bacteroidetes* were also present in the final enrichment, but were not isolated. Both strains hydrolyzed FEN to fenamiphos phenol and ethyl hydrogen isopropylphosphoramidate (IPEPAA), which was further transformed.[78] The two strains were using FEN as C and N source. Cross-feeding studies with other pesticides showed that *P. putida* degraded OPs with a P–O–C linkage. Thus, both bacteria were able to hydrolyze FEN, without prior formation of FSO or FSO2, to FEN-OH which was further transformed only by *P. putida*, suggesting elimination of environmentally relevant metabolites. In addition, *P. putida* was the first wild-type bacterial isolate able to degrade OPs. All the above characteristics of P. putida and its demonstrated ability to remove aged residues of FEN highlight its high bioremediation potential[78]. Herein, it was shown that the construction of genetically engineered microorganism (GEM) and the dual-species consortium has the potential to be used in the degradations of different kinds of pesticides. These studies show the benefits of bioremediation in multiple pesticide contaminated environments and mineralization of toxic intermediates in the environment, which can lead to complete bioremediation of contaminated sites that have an adverse effect. Yang *et al* performed an experiment with genetically engineered microorganisms. [79] In this work, an organophosphates (OP) degradation gene (mpd) and an organochlorine (OC) degradation gene (linA) were simultaneously introduced into *Escherichia coli* by using two compatible plasmids, resulting in strains with both OP degradation and OC degradation capabilities. The engineered *Escherichia coli* degraded OPs as well as OCs rapidly.

CONCLUSION

Present covered details about organophosphorus pesticide degrading bacteria that were able to degrade organophosphates in agricultural soil. Some of the important bacterial isolates having potential are *Paenibacillus koreensis*, *Corynebacterium jeikeium* and *Bacillus licheniformis Pseudomonas, Alcaligenes, Actinobacter, Flavobacterium, Klebsiella, Moraxella* and *Arthrobacter* respectively, which needs more attention or further exploration. The study shows that the many isolated bacterium can be used as a biological agent for the insitu bioremediation of organophosphorous contaminated soil. Normally study have been done only on the morphological, cultural and biochemical characterization of isolated bacteria. So further characterization like 16s rRNA analysis and sequencing of many bacteria which have organophosphate degrading gene may be essential part to continue. Inspite of applying the chemical methods for degrading pesticides, these ecofriendly bacteria can be used to encourage and motivate the farmers to use natural biological pesticides.

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CONFLICT OF INTERESTS

Conflict of interest declared none.

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