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Original Research Article

Screening, Characterization and Identification of Soil Isolates for degradation of Organophosphorus group of pesticides (Dimethoate and Parathion)

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Organo-phosphorus pesticide, Screening, BLAST, FASTA

A B S T R A C T

Organophosphorus pesticide is a group of pesticides which inhibit the enzyme cholinesterase and hence cause neurological disorders in insects, humans and pest. This group includes Dimethoate, Parathion pesticides which are commonly used in agriculture. Present study includes degradation of these pesticides using soil isolates, so that an adverse effect of these pesticides on humans will be decreased. In this study, primary screening and secondary screening was carried out to isolate the organisms from the soil which can degrade these pesticides. In primary screening, soil samples were collected from the fields where these pesticides are commonly used. Ninety six isolates were obtained on Minimal medium which contains pesticide as a sole source of carbon. These isolates include both bacteria as well as fungi. In secondary screening, different pesticide concentrations were tested, four isolates could efficiently degrade maximum up to 19gm% of pesticide. Biochemical tests and online gene based tools BLAST and FASTA were used for identification of bacterial isolate.

Introduction

Pesticide is a substance or mixture of substances intended for preventing destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal foodstuff, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (Quin, 2000).

Pesticides can be classified by target organism, chemical structure, and physical state. Pesticides can also be classed as

inorganic, synthetic, or biological (biopesticides), although the distinction can sometimes blur. Biopesticides include microbial pesticides and biochemical pesticides. Plant-derived pesticides, or "botanicals", have been developing quickly. These include the rotenoids, nicotinoids, and a fourth group that includes strychnine, pyrethroid and scilliroside (Stoytcheva, 2011).

Pesticides can be classified based upon their biological mechanism function or application method. Most pesticides work by poisoning pests. A systemic pesticide moves inside a plant following absorption by the plant. With insecticides and most fungicides, this movement is usually upward (through the xylem) and outward. Increased efficiency may be a result. Systemic insecticides, which poison pollen and nectar in the flower may kill bees and other needed pollinators. Subclasses of pesticides include herbicides, insecticides, fungicides, rodenticides, pediculicides and biocides (Stoytcheva, 2011).

Most organophosphorus pesticides are used in agriculture and they are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid (Volkova and Godovikov, 1963). Organophosphorus pesticides are highly toxic and easily absorbed through the skin. Poisoning may also occur through the mouth. There are many effects when inhaled. The first effects are usually respiratory oriented and may include bloody or runny nose, coughing, chest disorder, difficult or short breath. These may include vomiting, Diarrhea, abdominal cramps, headache, eye pain & blurred vision. Severe poisoning will affect the central nervous system lack of co-ordination & eventually paralysis of the body extremities & respiratory muscles. (Cavanagh, 1969; Thundiyil *et al.*, 2008).

Despite of their high toxicity, Dimethoate and Parathion pesticides are still extensively used all over the world for its broad spectrum of action. Dimethoate and Parathion inhibit the enzyme choline esterase which is required for normal functioning neurotransmitter and hence cause severe neurological disorder in humans (Hassal, 1990).

Due to its toxicity, it is important to remove Dimethoate and Parathion from environment. A variety of physical & chemical methods are available to treat soil, contaminated with hazardous material. But many of these physico- chemical treatments do not actually destroy hazardous compounds so the role of microorganisms in bioremediation is important because of their ability to degrade hazardous compound into harmless ones. For microbial degradation, the target pesticide will be utilized as the sole source of carbon and energy for micro-organism.

Materials and Methods

Sample Collection: Soil samples were collected from the fields of five different locations in Maharashtra namely, Theur, Lonikalbhor, Shirval, Urulikanchan. Dimethoate and Parathion pesticides are commonly used in the fields of these areas.

Pesticide used: Dimethoate (Anu Products Pvt. Ltd.) and Parathion.

Primary screening: Soil samples collected are diluted in sterile distilled water upto10^{-9}. Minimal medium with 1gm% of respective pesticide was used for spread plate technique. Plates were incubated at 37^0C for 48–72 hours.

Secondary screening: Individual isolate obtained at primary screening was

inoculated on minimal medium with different pesticide concentration (1gm% to 30gm%). Plates were incubated at 37^0C for 48–72 hours.

Identification of bacterial isolates: Based on colony characters and biochemical tests bacterial isolates were identified (Williams and Wilkins, 1974). Gene sequence of isolated bacteria was obtained from Geneome Bio Pvt. Ltd., Pune. BLAST and FASTA, these online tools were used for complete identification (Hubbard *et al.*, 2002; Johnson *et al.*, 2008).

Results and Discussion

At primary screening 96 isolates were isolated which could grow on the Minimal medium with 1gm% of pesticide. These isolates include both fungal and bacterial isolates.

At secondary screening four isolates were obtained which could degrade 19gm % of pesticide. These isolates include both fungal and bacterial isolates. Above this concentration no growth was observed. Bacterial isolates (Figure 1) could grow efficiently at 48 hours of incubation at 37^0C.

The bacterial isolate give positive results for nitrate reduction, glucose fermentation, and catalase test. Negative results were recorded for indole test and oxidase test. Hence, referring to Bergey's Manual of Determinative Bacteriology 8th Edition, the isolated organism may belong to *Pseudomonas spp.*

Figure.1 At secondary screening, different concentrations of pesticides were tested. (a) Shows the growth of bacterial isolate on minimal media containing Dimethoate. (b) Shows the growth of bacterial isolate on minimal media containing Parathion

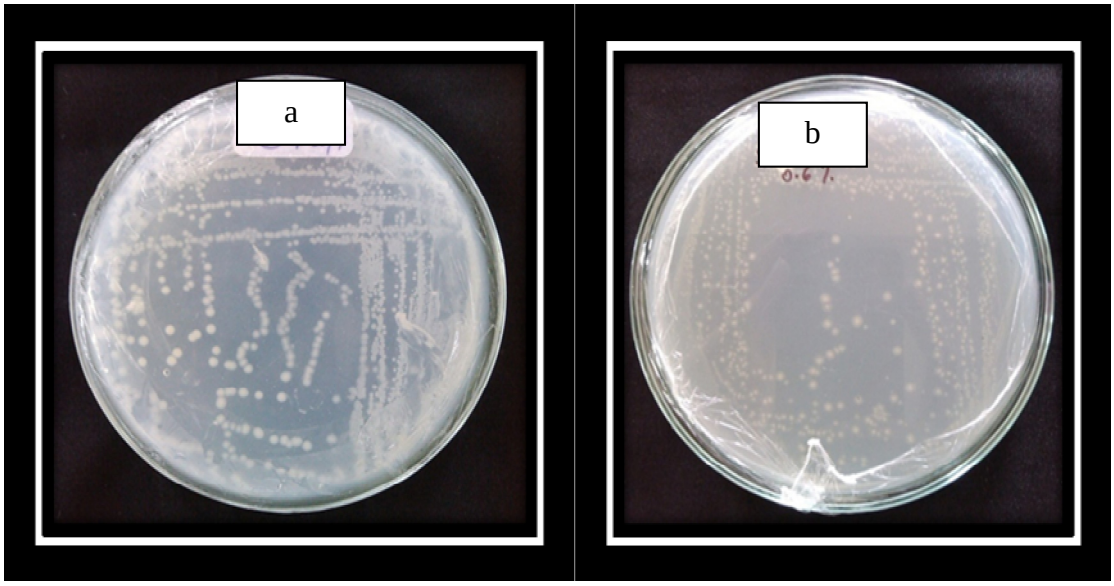


Figure.2 BLAST distance tree results for the isolate

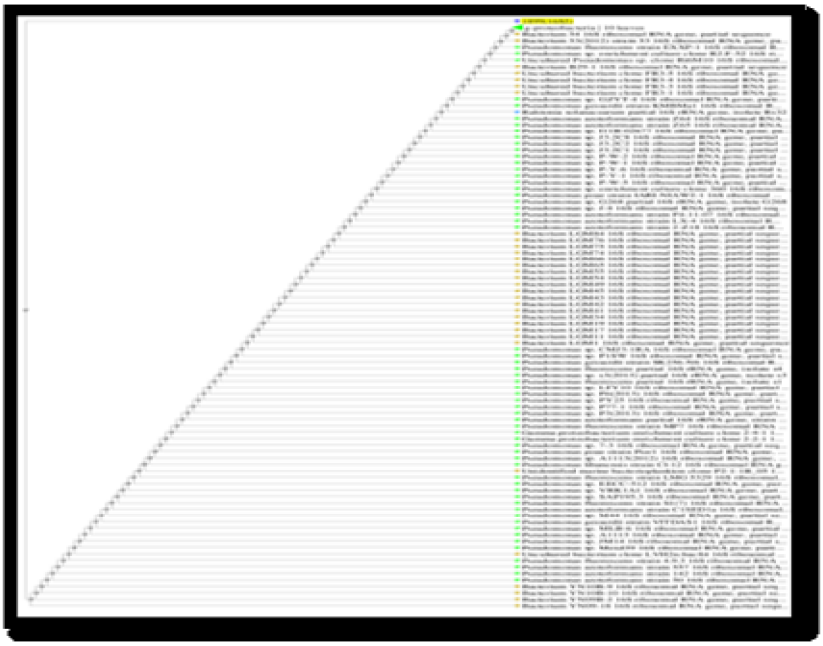
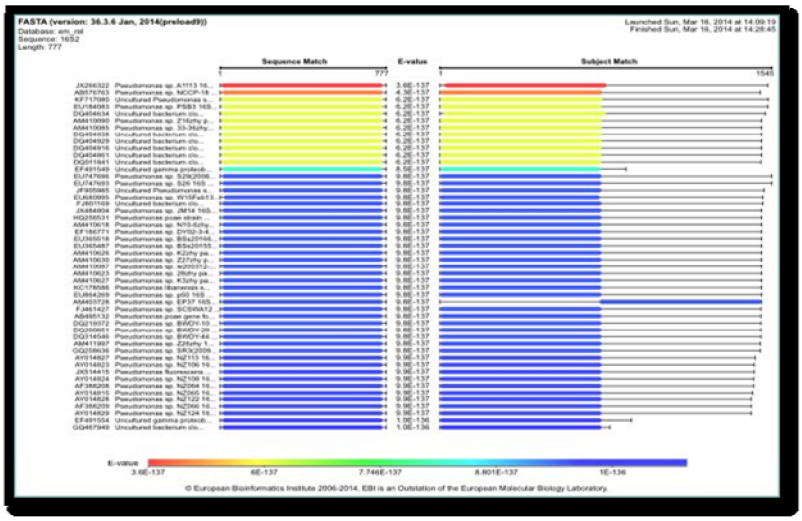


Figure.3 FASTA result shows that the E-value is minimum for *Pseudomonas spp.*A1113. Hence, the gene sequence obtained matches maximum with *Pseudomonas spp.*A1113



According to BLAST, the gene sequence matches with the sequence of *Pseudomonas spp* (Figure 2) and FASTA results showed organism is *Pseudomonas spp.*A1113 (Figure 3).

Dimethoate and Parathion both are organophosphorus pesticide which has approximate half-life as long as 206 days at 25^0C if not degraded (Hassal, 1990). Bacterial isolates can degrade various organophosphorus pesticides. (Siddaramappa *et al.*, 1973; Kanekar *et al.*, 2004).On the basis of the results presented here, we also propose that microbes can degrade organophosphorus pesticides efficiently. Isolates obtained can degrade these pesticides more rapidly. *Pseudomonas spp.*A1113 uses Dimethoate and Parathion as sole source of carbon and hence grows on the minimal medium and can be used for decontamination of pesticide polluted areas.

In present study, biological degradation of these pesticides has been carried out using soil isolates which include both fungal and bacterial isolates. Bacterial isolate identified as *Pseudomonas spp.*A1113 can biologically degrade these pesticides maximum up to 19gm%. Hence, *Pseudomonas spp.*A1113 can be used to degrade this pesticide, which would serve the ecofriendly and cheap way of degradation.

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