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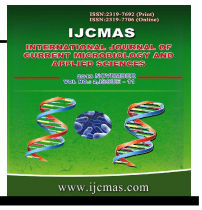

Original Research Article

Isolation of Microorganism for Bioremediation of Monocrotophos Pesticide

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A B S T R A C T

Microorganisms plays a major role for saving our environments by degrading xenobiotic compounds, chemicals wastes, which are toxic either in their native form or modified to be toxic. Isolation of microbial strain able to degrade chemical compounds was started usually from polluted sources, such as soil. The persistence of Monocrotophos (MCP) an extensively used Organophosphorous pesticide in environment and its toxic effect on biota necessitate its removal. In the present study a soil fungus and bacteria capable of utilizing or breaking down MCP was isolated from a site contaminated by the same pesticide. The isolated microbes were inoculated into the medium with 1% MCP. The two fungal and bacterial sp. were found to shown tolerance against 1% MCP and of which one was identified as *Aspergillus* sp. by lactophenol cotton blue staining method. These microorganisms show bioremediation in environment.

Keywords

Xenobiotic; Organo-phosphorous Pesticide; Tolerance; Bioremediation

Introduction

The pollution of the environment with man-made organic compound has become such an evident issue that it needs no further introduction. Microorganisms play a major role in the breakdown and mineralization of these pollutants (Alexander, 1981).

Bioremediation processes is a effective methods that stimulate the biodegradation incontaminated soils (McLaughlin, 2001; Swannell *et al*.,1996). For the remediation of both the physiochemical and biological method can be used but because of certain disadvantage like economic, long duration and also secondary pollution the researchers were focusing towards the biological method (Atlas, 1995; Hoff, 1993; Swannell *et al*., 1996).

Microorganisms used to perform the function of bioremediation are known as bioremediators. Technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of

bioremediation technologies are phytoremediation, bioventing, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation. (Fragoeiro, 2005).

Biodegradation and bioremediation are matching process to an extent that both of these are based on the conversion or metabolism of pesticide by microorganisms. The difference between these two is that the biodegradation is the natural process where as bioremediation is the technology. Temperature, pH,water potential,nutrients and the amount of pesticides or metabolites in soil may also act as limiting factor for pesticide degrading microorganism which require further exploration in relation to total microbial population and their biochemical activities (Fogarty and Tuovinen, 1990).

Pesticides are the chemical substances that kill pests. In the context of soil, pests are fungi, bacteria insects, worms, and nematodes etc. that cause damage to field crops. Thus, in broad sense pesticides are insecticides, fungicides, bactericides, herbicides and nematicides that are used to control or inhibit plant diseases and insect pests. Although wide-scale application of pesticides is an essential part of augmenting crop yields; excessive use of these chemicals leads to the microbial imbalance, environmental pollution and health hazards. An ideal pesticide should have the ability to destroy target pest quickly and should be able to degrade non-toxic substances as quickly as possible.(Vaccari *et al*, 2006).

In this experiment we use monocrotophos (MCP) pesticide which is an organophosphorous compound. Organophophorous compounds alone make up for 70 percent of the pesticides used worldwide. The global problems of pest resistence, resurgence and pesticide residues in crop and soil associated with the excessive use of pesticides necessitate employing a variety of detoxification methods. Monocrotophos (MCP), an organophophorous insecticide used in agricultural operations persists as soil residue and seeps into ground water. Natural degradation of MCP takes place over a period of 12-16 days and the process could be expedited through bioremediation.(S.Sam Manohar Das & S. Anitha,2007).

Among the several microbes capable of degrading MCP, fungal isolates were highly effective. The main objective of this experiment is "To isolate the microbes for bioremediation and their degradation tolerancy on monocrotophos (pesticide)." The three main objects of this experiments are Collection of soil sample, Media preparation and Isolation, identification and screening of microbes for bioremediation of pesticide.

Materials and Methods

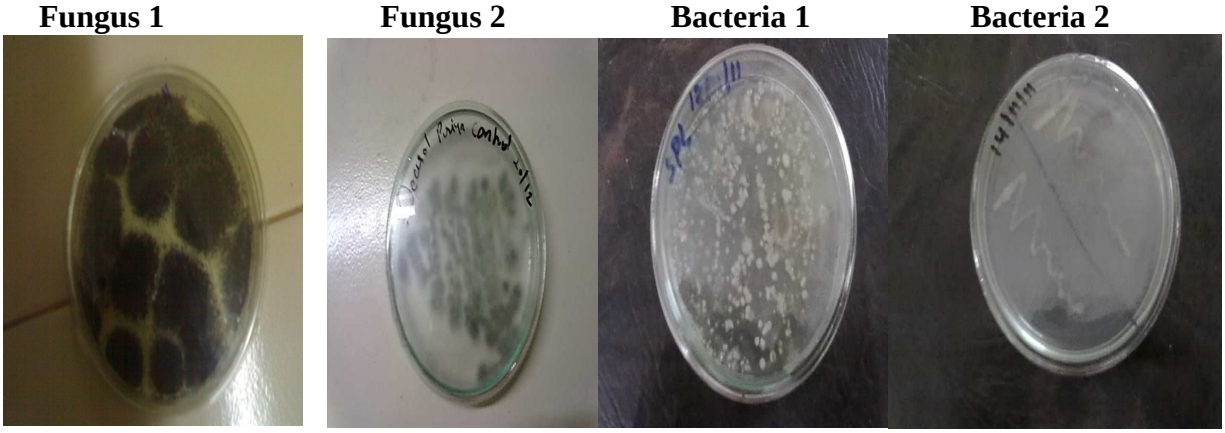
Collection of soil samples

Soil samples (about 250 g) were collected in 500 ml sterile polythene bags from agricultural field, garbaged area and industrial area. The collected soil samples were brought to the laboratory for microbial isolation.

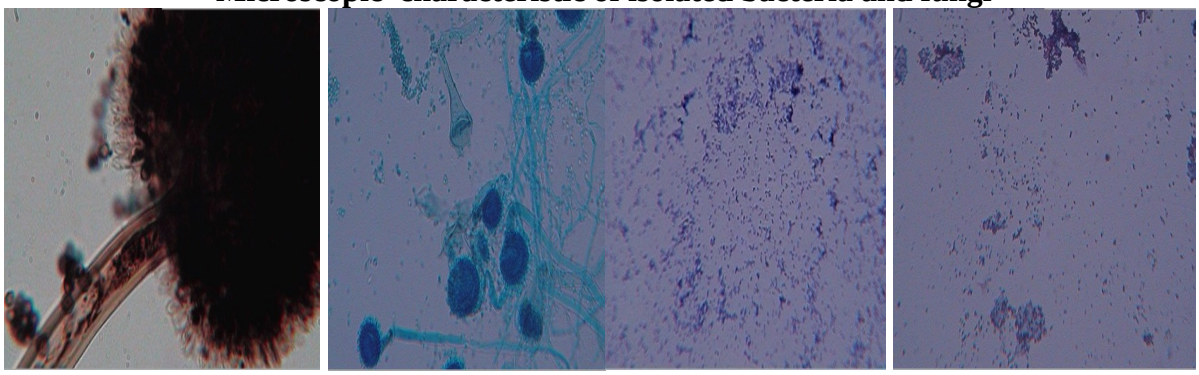
Isolation, Identification and Screening of fungus

The stock solution of soil was prepared by mixing 1 gm of soil in 10 ml distilled water and the solution was allowed to stand for 30 seconds. After all the soil debris had settled down, the supernatant

Figure.1 Observation and culture characteristics of bacteria and fungi



Microscopic characteristic of isolated bacteria and fungi



was decanted into a sterile test tube and serially diluted. Dilutions below 10^{-6} were inoculated into nutrient broth for bacteria and Czapek dox broth for fungus containing different concentrations of MCP (0.3, 0.5, 0.7 and 1%). Incubate these broth in suitable conditions as per needed. After 3-4 days of inoculation the bacterial and fungal strains were plated onto a NAM and Czapek dox plate respectively containing a respective concentration of MCP. NAM plate was incubated overnight at 37°C and Czapek plate were incubated for 4-6 days at 25°C. After these incubation periods two fungal and bacterial strains were observed on plate, of which one was identified as *Aspergillus* sp. by lactophenol cotton blue staining method. The bacterial sp. were identified as Gram positive monococcus and diplococcus by Gram staining method.

Results and Discussion

In the present work we have isolated two bacteria & two fungal species which shows tolerancy against 1% MCP and are identified as:-

Microscopic characteristics of isolated microbes are Fungus 1 - Aseptate mycelium, Spores have spines. Fungus 2 - Aseptate mycellium, Asci within ascocarp. Bacteria 1 - Stained purple colour and round in shape, Bacterias are found in pairs. Bacteria 2 - Stained purple colour, Single round shaped structure.

Identification Fungal Species By Lactophenol Cotton Blue Staining

Fungus 1 *Aspergillus niger* and Fungus 2 – *Aspergillus* Sp. were identified.

Identification of Bacterial Sp. By Gram Staining

Bacteria 1 – Diplococcus, Gram Positive and Bacteria 2 – Monococcus , Gram Positive were identified.

These two bacterial and two fungal sp. thus can be used in bioremediation process to remediate the monocrotophos pesticide. Sam Manohar Das and Anitha had studied the tolerance against 0.5% MCP by inoculating the fungus *Aspergillus niger* but in the present investigation the tolerance against 1% MCP were studied on both the bacterial and fungal species. Further work which can be proceed is to study the bioremediation rate from these isolated microbes on increasing concentration of MCP.

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