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# Isolation and characterization of organophosphorus pesticide degrading bacterial isolates

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**ABSTRACT**

*General agricultural use of pesticides carries with it potential hazards to man and directly by exposure to toxic residues in food and indirectly to the environment. In an effort at developing active microbial strains that could be of relevant in bioremediation of pesticides contaminated soil, a feasibility study was conducted to isolate bacteria having Organophosphorus insecticides degrading abilityfrom soil of some selected agro ecosystems of Dimoria region of Kamrup, Assam which is having a history of repeated pesticide applications. 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 Bacilluslicheniformisbasedon staining techniques and plating on selective media.*

**Keywords:** *Bacterial isolates, Biodegradation, Pesticides, Malathion, Quinalphos*

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## INTRODUCTION

The wide application of organophosphorous (OP) insecticides such as Chloropyrifos, Ethion, Parathion, Malathion, Quinalphos, Dichlorvos, are employed for plant protection against insect pests. This organophosphorous pesticide is one of the major chemicals responsible for the contamination and deterioration of soil and groundwater, particularly in the closevicinities of agricultural fields [1]. Owing to their high toxicity and persistence in the environments, most of them are banned all over the world.

### Organophosphorus compounds

Currently, among the various groups of pesticides that are being used the world over,organophosphorus group forms a major and the most widely used group [2]. OP pesticides were first developed in Germany by Schrader in 1930 during World WarII in the form of tetraethyl pyrophosphate as a by-product of nerve gas development. OPs areacutely toxic and act by inhibiting acetylcholine esterase, an important enzyme in the nervoussystem [2]. On exposure to OPs, the enzyme is unable to function causingaccumulation of acetylcholine, which interferes with the transmission of nerve impulses at thenerve endings.

Organophosphorus insecticides are esters of phosphoric acid which include aliphatic,phenyl and heterocyclic derivatives and have one of the basic building blocks as a part oftheir complex chemical structure. Some of the main agricultural products are parathion,methyl parathion, chlorpyriphos, malathion, monocrotophos and dimethoate.Although organophosphates are biodegradable in nature, their residues are found inenvironment. Considering their toxicity, research on biodegradation of organophosphates isbeing carried out all over the world.

### Microbial degradation of organophosphorus pesticides

Insecticides and their degradation products generally get accumulated in the top soiland influence not only the population of various groups of soil microbes but also theirbiochemical activities like nitrification, ammonification, decomposition of organic matter andnitrogen fixation [3]. Microorganisms play an important role in

degradingsynthetic chemicals in soil [4]. They have the capacity to utilize virtually allnaturally and synthetically occurring compounds as their sole carbon and energy source.

The microbial cleavage of variousorganophosphorus insecticides was studied and two strains of ***Pseudomonas* sp**. isolated from diazinon andmalathion enrichments were found most versatile [5]. Degradation of malathion bybacterial strain ***Pseudomonas sp. N-3*** isolated from industrial effluents was found to degrade malathion up to 150 ppm only in the presence of ethanol (1% v/v) as a co-substrate[6]**.**

Thedegradation of selectedinsecticides, Quinalphos and monocrotophos by ***Azospirillumlipoferum***and ***Bacillus* sp**.isolated from black soil following enrichment culture technique was also reported [7]. By the end of 7 days, about 40per cent of monocrotophos supplemented to mineral salts medium was degraded by ***A.lipoferum***and ***Bacillus* sp**. Nearly 56 per cent and 76 per cent of quinalphos was degraded by*A. lipoferum*and *Bacillus* sp. respectively [7]. The degradation of quinalphos by bacteria isolated from soil was reported and 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 [8].

**Objectives of the study:**
A hundreds of pesticides of different chemical moieties are widely used for agricultural purpose. Soil receives large amounts of pesticides even from bulk handling, direct application at fields or accidental release which lead to occasional contamination of a wide range of water and terrestrial ecosystems, and accumulation of these compounds has many health hazards associated with it [6]. Hence the degradation process of pesticides in different ecosystems universally takes a large space of interest.

Keeping all these points in view, the present study was carried out to isolate and characterize organophosphorus degrading bacterial isolates from soil of some selected agro ecosystems of Dimoria region of Kamrup, Assam consisting the following objectives with a view of bioremediation.

1. Isolation of efficient **malathion** insecticides degrading bacteria.
2. Isolation of efficient **quinalphos** insecticides degrading bacteria.
3.Microscopic study of bacterial cultures.
4.Identification of bacterial isolates.

## MATERIALS AND METHODS

### 1. SAMPLING

#### 1.1 Selection of sampling station

For the purpose of this study samples were collected from some selected agricultural fields of Dimoria region of Kamrup District, Assam. In all study areas the same method was used to collect soil sample. In total 4 stations were selected. Two stations each from vegetable farms and **low land rice field** was selected. Three soil samples at 0-15 cm depth were collected randomly from each station to prepare a single composite sample of each station.

#### 1.2 Sampling procedure

In order to collect soil samples (0-15 cm depth) grasses, mosses, litter and other plant residues were removed from soil surface. Collection of soil samples was done by using an auger. In each case, a triangular block was cut with the help of the auger. Soils were collected in plastic bags, which were sealed and labelled properly. Three soil samples from a rooting depth of 15 cm were collected randomly from each sampling station and each sample composite was labelled as **S1**- Vegetable farm Composite sample 1; **S2**- Vegetable farm Composite sample 2; **S3**- rice farm Composite sample 1 and **S4**- rice farm Composite sample 2.

#### 1.3 Soil sample preparation

Preparation of soil samples is based on the **ISO 11464 method** (Soil quality- pre-treatment of samples for physic-chemical and biological analysis). Collected samples were brought to the laboratory for analysis. Before analysis, the samples were spread out thinly on a piece of hard paper for drying in air in a shade. The big lumps were broken down, and visible plant roots, pebbles and other undesirable matters were removed. After the soil become completely dry, and after homogenization, a portion of each sample was passed through a 2-mm mesh screen and preserved in clean sealed polythene bagsand stored in sealed polythene boxes to avoid air contamination at 4°C before microbial and biochemical analysis.

### 2. MICROBIOLOGICAL PROCEDURES

#### 2.1Pesticides used:

Pesticides used in this present study are**Malathion** and **Quinalphos**

**2.2 Isolation of Malathion degrading bacteria**:-
Pour plate technique was used for the isolation of pesticide degrading bacteria in Nutrient agar.

For isolation and selection of Malathion degrading bacteria, microbial colonies were isolated from collected soil samples. 5gms of eachsoil sample except control sample was mixed in 100 ml autoclaved water in 6 different conical flasks and kept at 100rpm for 24hrs at 37◦C.

A selective medium (M1) was prepared containing the following composition:

| M1 Media composition | Quantity (g) |
|---|---|
| Malathion (commercial grade 50%) | 0.5 |
| KH2PO4 | 0.1 |
| MgSO4 | 0.02 |
| NH4NO3 | 0.5 |
| Agar | 1.5 |

To this solution 15µl of a mineral solution (MS) containing the following composition was added.

| Mineral Solution composition | Quantity(g) |
|---|---|
| FeSO4 | 10 |
| CaCl2 | 10 |
| CuNO3 | 0.5 |
| MnCl2 | 0.4 |

From each processed samples plating was done on plates with **M1 media** and the inoculated plates were subsequently incubated at 37◦C for 48 hrs.

Colonies obtained were further cultured in nutrient medium (NM) containing the following composition.

| Nutrient medium (NM) composition | Quantity(g) |
|---|---|
| Yeast extract | 0.07 |
| Peptone | 0.05 |
| Glucose | 0.05 |
| K2HPO4 | 0.03 |
| MgSO4.7H2O | 0.007 |
| Malathion (commercial grade 50%) | 0.5 |

Serial transfer of microorganisms was madeby streaking and inoculating to nutrient medium containing Malathion.Selection of pure culture is done by repeating sub culturing for 4-6 times. The isolated strains were maintained on Nutrient agar and King's B agar slants and stored at 4°C.

**2.3Isolation of Quinalphos degrading bacteria**:-
The bacterial cultures capable of degrading Quinalphos were isolated from collected soil samples using enrichment technique, with some concentration of Quinalphos. Standard analytical grade solution of Quinalphos (25% E.C.) was purchased from the local market. 1gm of each soil sample was inoculated into 6 different 100ml Erlenmeyer flask containing 100ml of mineral salt medium(MSM) supplemented with 0.5ml concentration of Quinalphos in 300ml.

The composition of Mineral Salts Medium (MSM) is given below:

| MS Medium composition | Quantity(g) |
|---|---|
| NaNO3 | 0.3 |
| MgSO4 | 0.05 |
| KCl | 0.05 |
| K2HPO4 | 0.1 |
| KH2PO4 | 0.05 |
| FeSO4 | 0.001 |
| yeast extract | 0.05 |
| Glucose | 1.0 |

The flasks were incubated on a rotary shaker at 150 cycles per minute for 7 days at room temperature (25-30◦C). At daily intervals, one loop full of enrichment culture from the flask was streaked on nutrient agar plates supplemented with Quinolphos (5g) and incubated at room temperature for 24-48hrs. Nutrient agar media was prepared by adding 7g of agar and 5g quinalphos in 250ml water. Individual colonies of bacteria which varied in shape and colour were

picked up and were sub cultured onto nutrient agar plates containing same concentration of Quinalphos until pure culture was isolated. The isolated strain was maintained at 4◦C.

**2.4 Microscopic study of bacterial cultures:**

The bacterial isolates were studied for their various microscopic characters such as:

***2.4.1. Colony morphology:*** Study of colony morphology includes colour, size, margin, elevation etc.

***2.4.2. Gram's staining:*** Gram's staining of the cultures was performed by the method as described by ***Cappucino*.**

***2.4.3. Motility test*:** Motility test was performed by hanging drop method as described by ***Cappucino*.**

**2.5. Identification of bacterial isolates:**

Identification of bacterial isolates were carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining, Motility test etc. Gram staining reaction was performed to evaluate type of strain.

## RESULTS AND DISCUSSION

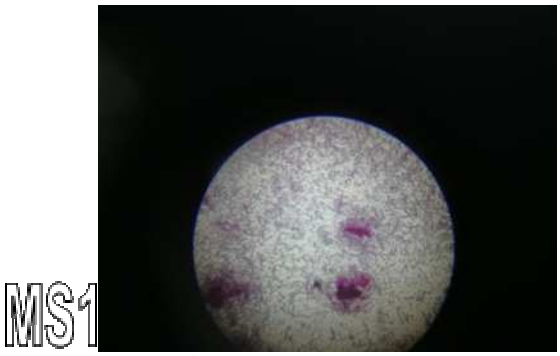
**1. Isolation of Pesticides Degrading Bacterial Strain**

A total of four bacterial cultures were isolated based on colony morphology. Two different colonies were observed on nutrient agar medium enriched with MALATHION from soil samples of Vegetable Farm Composite. The isolated strains were designated as **MS1** and **MS2**.

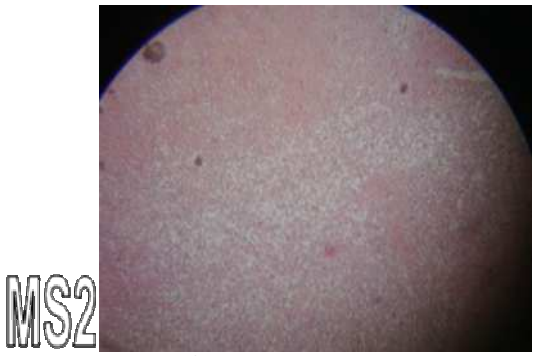
**MS1**- Pesticides Degrading Bacterial Isolate fromVegetable Farm 1 Composite sample.
**MS2** - Pesticides Degrading Bacterial Isolate from Vegetable Farm 2 Composite sample.

Two different colonies were observed on nutrient agar medium enriched with QUINALPHOS from soil samples of Rice Farm Composite and Vegetable Farm Composite and were designated as **QS1** and **QS2.**

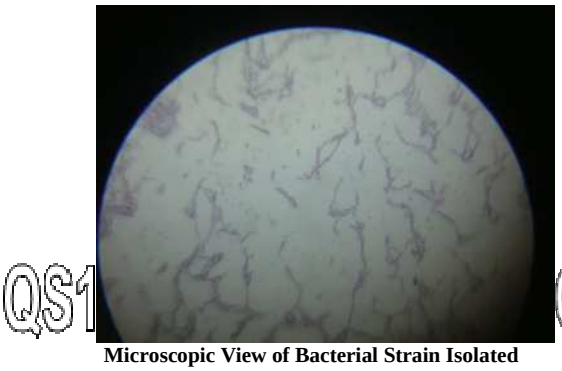
**QS1**- Pesticides Degrading Bacterial Isolate from Rice field 1 Composite sample.
**QS2**- Pesticides Degrading Bacterial Isolate from Vegetable Farm 2 Composite sample.



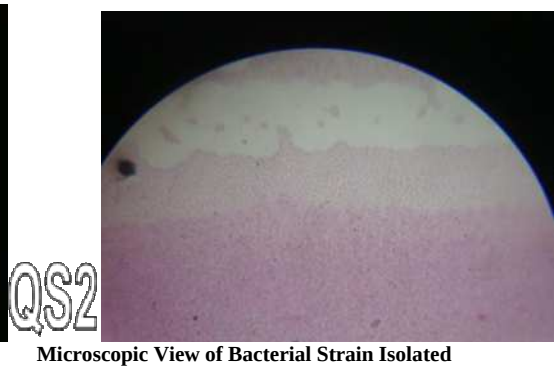

**Microscopic View of Bacterial Strain Isolated Farm 1 Composite Soil Sample.**




**Microscopic View of Bacterial Strain Isolated From Vegetable From Vegetable Farm 2 Composite Soil Sample.**




**Microscopic View of Bacterial Strain Isolated From Rice Field 1 Composite Soil Sample.**




**Microscopic View of Bacterial Strain Isolated From Vegetable Farm 2 Composite Soil Sample.**

**2. Microscopic Study ofthe Bacterial Strains**

***2.1 Colony morphology*:** the colony morphology of the four bacterial isolates was recorded in the table 1.

**Table 1: Morphology and characteristics of isolated bacterial strains**

| Sl. No. | Strain label | Colony colour | Size | Shape |
|---|---|---|---|---|
| 1 | MS1 | Creamy colour | 1.0mm | Irregular type |
| 2 | MS2 | Light yellow | 0.5mm | Flat & irregular |
| 3 | QS1 | White to cream | 1.5mm | Single round type |
| 4 | QS2 | Yellowish grey | 0.5mm | Round,granular |

***2.2 Gram's Staining:*** The result of the gram's reactions was recorded in the table 2.

**Table 2: Gram characters of the tested bacterial strains**

| Sl. No. | Strain label | Gram character |
|---|---|---|
| 1 | MS1 | Positive , Rod |
| 2 | MS2 | Negative , Rod |
| 3 | QS1 | Positive ,Coccus |
| 4 | QS2 | Positive , Rod |

***2.3 Motility test:*** The result of the motility test is recorded in the table 3:

**Table 3: Motility test of the tested bacterial strains**

| Sl. No. | Strain label | Motility |
|---|---|---|
| 1 | MS1 | Motile |
| 2 | MS2 | Unipolar motility |
| 3 | QS1 | Non-motile |
| 4 | QS2 | Motile |

Based on the morphological studies and preliminary tests like Gram staining, Motility test of the isolated colonies, the observed colonies may be identified as follows:

**Table: 4 Identification of the bacterial isolates**

| Strain label | Colony name |
|---|---|
| MS1 | *Bacillus amyloliquefaciens* (may be) |
| MS2 | *Pseudomonas species* (may be) |
| QS1 | *Staphylococcus species* (may be ) |
| QS2 | *Bacillus licheniformis* ( may be) |

## CONCLUSION

Pesticides constitute the key control strategy for pest management and have beenmaking significant contribution towards improving crop yields. Currently, among the variousgroups of pesticides that are being used world over, organophosphates form a major andmost widely used group, accounting for more than 36 per cent of the total world market[2]. Quinalphos, monocrotophos, chlorpyriphos, malathion, parathion aresome of the widely used organophosphorus pesticides.

The widespread use of these pesticides over the years has resulted in problemscaused by their interaction with the biological systems in the environment [9]. Considering the toxic effect of these pesticides, it is essential to remove these chemo pollutantsfrom the environment. Biological removal of chemo-pollutants becomes the methodof choice, since microorganisms can use a variety of xenobiotic compounds includingpesticides for their growth and mineralize and detoxify them.

With extensive use of pesticides, environment hazards has lead to several problems such as deterioration of soil quality, leaching, acidification, denitrifiction, air pollution, and reduced biodiversity, disrupting the ecosystem. To protect the environment, best remedy is to use the ecofriendly microbes to reduce the contamination.

In this context, the focus of the present study was to isolate, characterize most efficientmicroorganisms capable of degradation of malathion and quinalphos insecticide.Some bacterial strains isolated from theagricultural soil, namely *Bacillus amyloliquefaciens, Pseudomonas species, Staphylococcus species,and Bacilluslicheniformis*which werelabelled as MS1, MS2, QS1 and QS2, respectively, showedthe ability to degrade malathion and quinalphos

insecticides. Our results obtained suggest that the bacterial species, being isolated, can be used to decontaminate the area polluted.

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