

## Isolation and Characterization of Microorganisms that Degrade Dimethoate

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### Abstract

Microorganisms have the ability to degrade a large number of pollutants found in the environment. Microorganisms such as bacteria can degrade toxic compounds and chemicals in an environment friendly way. Dimethoate is an organophosphate pesticide. It is a broad-spectrum insecticide. In the present study, dimethoate degrading microorganisms have been isolated from various soil samples. The different isolates that were obtained were characterized by various biochemical tests. Standardization of degradation was performed. Leuco crystal violet reagent was used as an indicator and colorimetric analysis was done at 595 nm. In total, 8 isolates were got. 3 belonged to *Bacillus* sp. and 3 were identified as *Staphylococcus aureus*. 2 isolates were identified as *Aspergillus* sp. HPLC analysis was carried out for the organism that showed the best degradation results.

**Keywords:** Microorganisms; Bacteria; Degradation; Pollutants

### Introduction

Organic synthetic pesticides are used in the environment as a control measure against the pests and the parasites to have a secure agricultural and industrial produce. Despite their usefulness in the increase in the yield of food production, the extensive use of pesticides during production, processing, storage, transport or marketing of agricultural commodities can lead to environmental pollution. Persistence of pesticides in the soil can vary from few hours to many years as in case of Organophosphorous (OP) pesticides. The waste generated during pesticide manufacturing is very complex, containing chemical compounds used for manufacturing and the residuals generated during manufacturing/formulation process [1]. The World Health Organization (WHO) data [2] show that only 2-3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests, while the rest remains in the soil [1]. Therefore, the surface soil containing residual pesticides causes toxicity in the surrounding environment. No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects [2]. Deaths and chronic diseases occurring due to pesticide poisoning are about 1 million per year globally [3]. There was evidence on the risk of cancer of workers occupationally exposed to herbicides or dioxins (since dioxins contaminated the herbicide mixtures used in Vietnam, and of the Vietnamese population [4]). In India, the first case of poisoning was reported in 1958 in Kerala where more than a hundred people died due to consumption of wheat contaminated with parathion [5]. India is the tenth largest consumer of pesticides, the first being China and the twelfth largest manufacturer of pesticides globally. Pesticides reach rivers and oceans as runoffs from the fields. More than 90 percent of water and fish samples from all streams contained one, or more often, several pesticides in a study done by US Geological Survey (USGS) on major river basins in the mid 90's [6]. In a survey in India, 58% of drinking water samples drawn from various hand pumps and wells around Bhopal were

contaminated with Organo Chlorine pesticides above the EPA standards [7]. Dimethoate is an organophosphorous pesticide that is used as an insecticide and an aricide. It has been listed as toxic under the GHS (globally harmonized system of classification and labelling of chemicals) classification. It is toxic if swallowed and in contact with skin. The major objective of the following study is to isolate and characterize microorganisms degrading dimethoate from environmental samples and the standardization of degradation by the organisms. Transformation has been carried out using the plasmid of *Bacillus* and *Staphylococcus*. *E. coli* DH5 $\alpha$  was used as the host culture.

### Materials and Methods

#### Collection of sample

The samples used in the study were collected from various industrial areas of Chennai, Tamil nadu, India. The following nine samples were used in the study (Table 1).

S.No.	Sample	Geographical Area
1	Farmland soil	Avadi, Chennai, Tamil Nadu
2	Tyre factory soil	Avadi
3	Home dust	Ashok Nagar, Chennai, Tamil Nadu

Table 1: Sample collection data.

#### Pesticide used in the study

The pesticide used in the study was dimethoate. It is an organophosphorous pesticide. The pesticide was purchased from a local pesticide seller in T nagar, Chennai (Figure 1).

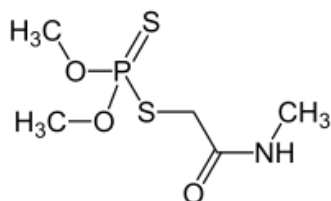


Figure 1: Structure of dimethoate.

### Isolation of microbial consortia

Primary screening was done to isolate the microorganisms that are able to tolerate dimethoate pesticide and use it as a source of energy. For this purpose minimal salt medium was used. The samples were sprinkled in minimal salt medium broth in test tubes in different concentrations of pesticide starting from 0.5 to 4%. Growth of organisms was observed under the microscope when gram staining of broth was performed. The organisms were transferred from broth to MSM plates with corresponding pesticide concentration using spread plate method and allowed to grow for a few days. Growth was observed in plates with 0.5 to 2% pesticide concentration. Organisms growing in 2% concentration were taken for further studies.

### Biochemical characterization

Biochemical tests were performed for all the isolates using Bergey's Manual of Systemic Bacteriology for biochemical characterization.

Biochemical test	Farmland soil	Tyre factory soil	Home dust	Home dust	Home dust	Home dust
	SS1	SS2	SS3 a	SS3 b	SS3 c	SS3 d
Oxidase test	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+
Citrate utilization test	+	+	+	+	+	+
Methyl red test	+	+	+	+	+	+
Voges proskauer test	+	+	+	+	+	+
Triple sugar ion test	+	+	+	+	+	+
Urease test	-	+	-	-	-	-
Indole test	+	+	+	+	+	+
Coagulase test	-	-	-	+	+	+
Identification	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Staphylococcus aureus</i>	<i>Bacillus aureus</i>	<i>Staphylococcus aureus</i>

Table 2: Biochemical test results.

From tyre factory sample, 2 types of fungi were obtained. Under microscopic observation, they were both found to be *Aspergillus sp.*

### Standardization of degradation

Degradation tests were carried out at varying concentration, pH, temperatures and static and non-static cultures. The varying concentrations of pesticides used were 0.5%, 1%, 2%, 3% and 4%. The different pH used were 2, 4, 6 and 8. Three different temperatures were used. They were 14°C, 34°C and 45°C. Colorimetric analysis was done at 595 nm to check the amount of pesticide degraded.

### Transformation studies

The plasmids were isolated from bacterial strains and they were transformed into host culture. *E. coli* DH5a strain was used as the host culture. For isolation of plasmid DNA alkaline lysis method was used. Competent cells were prepared using 0.1% calcium chloride solution. Blue white screening method was used for checking transformation which involved the use of ampicillin, X-Gal and IPTG.

### Results

#### Isolation of microbial consortia

In total 6 isolates of bacteria were obtained from the 3 different environmental samples. Five isolates were gram positive rods or cocci. The bacteria got from ice cream factory effluent were gram negative cocci. All the bacterial cultures were pure cultures.

#### Biochemical characterization

The biochemical tests showed that four isolates were *Bacillus sp.* while two isolates were *Staphylococcus sp.* (Table 2).

### Standardisation of degradation

Standard graph was drawn to study degradation in various physical conditions (Figure 2). The linear regression equation of the standard graph obtained in the study was  $y=0.005+0.5264x$ .

### Degradation test at varying concentrations

The OD (Optical Density) values for each of the samples were extrapolated on the standard graph in order to get the concentrations in mg/100 ml. Out of the five, organism 4 (*Staphylococcus aureus*) gave the best degradation results. Organism 2 (*Bacillus* sp.) also gave good degradation results comparable with that of organism 4 (*Staphylococcus aureus*). Organism 5 (*Staphylococcus aureus*) showed the least degradation activity. The amount of residual dimethoate after 7 days were 0, 4, 17, 28 and 29  $\mu\text{g/mL}$ ; the rate constants were 0.775, 0.305, 0.225, 0.167 and 0.127 each per day, and the efficiency of dimethoate degradation were 100%, 96%, 83%, 72% and 71%, for *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Proteus mirabilis* and *Bacillus pumilus* respectively [8].

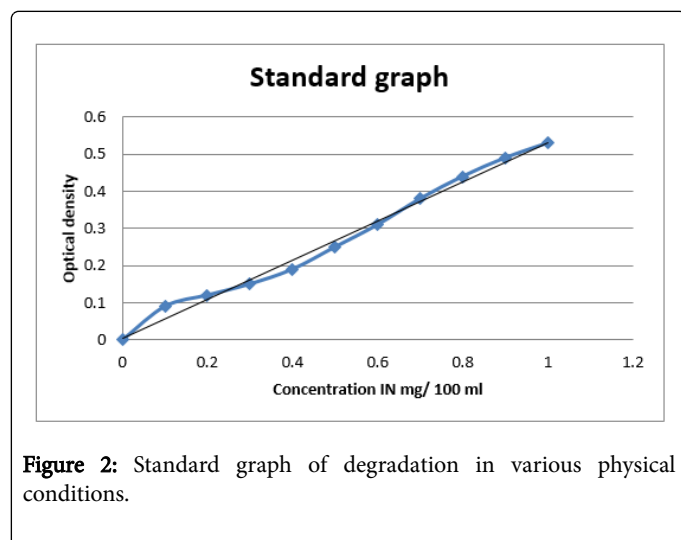


Figure 2: Standard graph of degradation in various physical conditions.

### Degradation at varying pH

At pH 2, very good results of degradation are shown by Organism 1 (*Bacillus* sp.), organism 2 (*Bacillus* sp.) and organism 3 (*Staphylococcus aureus*) while organisms 3 and 5 (*Staphylococcus aureus*) show least degradation.

At pH - 4, moderate degradation is observed in organism 1 (*Bacillus* sp.) and organism 5 (*Staphylococcus aureus*). Least degradation is observed in organism 5 (*Staphylococcus aureus*). The other 2 showed good degradation activity.

At pH - 6, maximum degradation is observed in organism 4 (*Staphylococcus aureus*) and 1 (*Bacillus* sp.). However, maximum activity is seen within the first 24 hours and then it seems to have slowed down.

At, pH - 8, organisms 1 (*Bacillus* sp.), 3 and 5 (*Staphylococcus aureus*) show good degradation.

Both *Bacillus* sp. and *Staphylococcus aureus* show better activity in an alkaline pH.

### Degradation at varying temperature

Out of the five, organism 4 (*Staphylococcus aureus*) gave the best degradation results. All the organisms showed best degradation at 40°C.

### Degradation test in static and non-static broth culture

*Staphylococcus aureus* was selected to check its degradation in two different conditions, shaking and non-shaking broth culture, since it gave best degradation results in varying concentrations, pH and temperature. The degradation is slightly more in shaking than in non-shaking flask.

### Transformation

**Plasmid isolation:** The presence of plasmid was detected in bacteria from Ambattur and Vedanthangal lake water. Alkaline lysis method was used for the extraction of the plasmids. The plasmid was run on agarose gel along with ladder to see the approximate size of the plasmid. The size of the plasmids varied from 1 kb to 3 kb.

**Transformation:** Transformation was done using DH 5 $\alpha$  as host culture and transformed colonies with plasmids from the two different organisms were transferred to different plates and left for incubation for 24 hours. After incubation, growth of blue and white colonies was observed.

The white colonies indicate the transformed colonies. The blue colonies indicate the untransformed colonies. The transformation mixture was plated onto LB plates with pesticide concentration of 2%. It was found that competent cells of DH 5 $\alpha$  were successfully transformed.

### High performance liquid chromatography

The 13th day sample inoculated with organism 4 (*Staphylococcus aureus*) was analysed by HPLC test (Figure 3).

### Discussion

Dimethoate has been labeled as hazardous 13<sup>th</sup> day sample inoculated with organism 4 (*Staphylococcus aureus*) under the GHS (globally harmonized system of classification and labelling of chemicals) classification. The present study focuses on the isolation of microorganisms that degrade dimethoate from two different soil samples. The isolated microorganisms have been characterized and identified as *Bacillus* sp., *Staphylococcus* sp. and *Aspergillus* sp. A study on the degradation capabilities of the five different organisms has been carried out and degradation has been standardized for them. Leuco crystal violet reagent has been used to check degradation. It has been observed that the amount of pesticide in each sample is less after 312 hours when compared to 24 hour old samples after inoculation. Organism SS3 c showed the best degradation potential and HPLC (High Performance Liquid Chromatography) analysis was carried out for the sample degraded by the organism. Dimethoate adsorption was measured on eight soil types (pH from 8.0-8.45; OM from 0.73-2.95%; clay content from 5.9-14.9%) in which resulting isotherms followed an L-shape [9]. Some pesticides hydrolyze very rapidly. The hydrolysis rate can be rapid in the pH range of 8 to 9. For every pH point increase, the rate of hydrolysis will increase by approximately 10 times [10]. Within the pH range of 7-11, dimethoate degradation depends on the alkalinity of the medium rather than the time of storage. At pH 4.2 degradation is largely dependent on the length of time the dimethoate solution has been stored [11]. The half-life of dimethoate in clay loam soil was determined to be 10 and 5 days at 10°C and 20°C, respectively. This confirmed a faster decrease in the pesticide due to the increased temperature [9]. HPLC analysis was carried out to check degradation. Bioremediation was carried out on a small scale using

varying combinations of the organisms that were isolated in the study. When all the organisms were present, degradation was maximum. The overall aim is to achieve a natural method to eliminate dimethoate from the environment in an environment friendly manner.

## Conclusion

The present study suggests that the isolated organisms are able to utilize dimethoate as energy source since no other nutrients were available or were present in limited quantity. Hence, they degrade dimethoate. In the study, *Bacillus* sp., *Staphylococcus aureus* and *Aspergillus* sp. are found to flourish well in presence of dimethoate.

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