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Isolation and Characterization of Microorganisms from Industrial Effluents That Degrade Dimethoate

Shivangi Singh, Priya R Iyer

PG and Research Department of Biotechnology
Women`s Christian College, Chennai, India

Corresponding Author: **Priya R Iyer,** PG and Research Department of Biotechnology, Women`s Christian College, Chennai, India.

Abstract: *Microorganisms like bacteria and fungi are capable of degrading a wide range of industrial effluents and environmental pollutants. Use of microorganisms to degrade toxic chemicals is a natural and environment friendly method. Dimethoate is an organophosphorous pesticide. In the following study, dimethoate degrading microorganisms have been isolated from various industrial effluents. The isolated organisms have been characterized by various biochemical tests. Standardisation of degradation was performed using Leuco crystal violet reagent as indicator of degradation and colorimetric analysis was performed at 595 nm. 6 different effluent samples were used. 8 different isolates were got. 4 isolates belonged genus Bacillus, 2 belonged to genus Staphylococcus and 2 organisms were fungi belonging to the genus Aspergillus.*

1. INTRODUCTION

The use of organic synthetic pesticides became a widespread practice ever since the 1960'S, in order to better prevent, control and destroy pests. They are used in the environment as a control measure against the pests and the parasites to have a secure agricultural and industrial produce. Persistence of pesticides in the soil can vary from a few hours to many years as in case of Organophosphorous (OP) pesticides. 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 led to environmental pollution (Mulligan, 2005). The World Health Organization (WHO) data 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 (EPA, 2005). Therefore, the surface soil containing residual pesticides causes toxicity in the surrounding environment. Pesticides have resulted in serious health implications to humans and the environment. There is now overwhelming evidence of the hazards that these chemicals cause humans, other animals and the environment (Forget, 1993; Jayaratnam, 1981). Indiscriminate use of chemicals might work for a few years, but after awhile, there aren't enough beneficial soil organisms to hold onto the nutrients" (Savonen, 1997). Dimethoate is an organophosphorous pesticide. It is a broad spectrum pesticide has been used to control a wide range of insects including ticks, mites, flies and aphids. Dimethoate has been found to be of great agricultural value as a systemic insecticide. It combines contact and systemic action and is effective against a wide range of insects (D. M. Sanderson and E. F. Edson). Dimethoate can be applied to agricultural crops and ornamentals, in addition to urban areas for landscape maintenance and pest control. Dimethoate has been listed as toxic under the GHS(globally harmonized system of classification and labelling of chemicals) classification. It causes damage to organs through prolonged or repeated exposure. Dimethoate poisoning is associated with the neuromuscular transmission block in both animals and humans(Hayes and Laws, 1991; DeBleecker et al., 1993; Dongren et al., 1999). Dimethoate is toxic to aquatic life with long lasting effects. The major objective of the following study is to isolate and characterize microorganisms degrading dimethoate from industrial effluents and the standardization of degradation by the organisms. Transformation has been carried out using the plasmid of *Bacillus* and *Staphylococcus* and using *E.coli* DH5α as the host culture.

2. MATERIALS AND METHODS

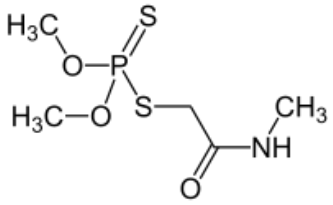
Collection of Sample

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.

S.NO.		SAMPLE	GEOGRAPHICAL AREA
1	ES 1	Tannery effluent	Adyar (Central Leather Research Institute)
2	ES 2	Ice factory effluent	Royapuram
3	ES 3	Bakery effluent	Ashok Nagar
4	ES 4	Ice cream factory effluent	Red hills
5	ES 5	Ambattur lake water	Ambattur
6	ES 6	Vedanthangal lake water	Vedanthangal

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.



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 Manuel of Systemic Bacteriology for biochemical characterization.

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^{o} C, 34^{o} C and 45^{o} 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 DH5 α 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.

3. RESULT

Isolation of Microbial Consortia

In total 6 isolates of bacteria were obtained from the 6 different effluent 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.

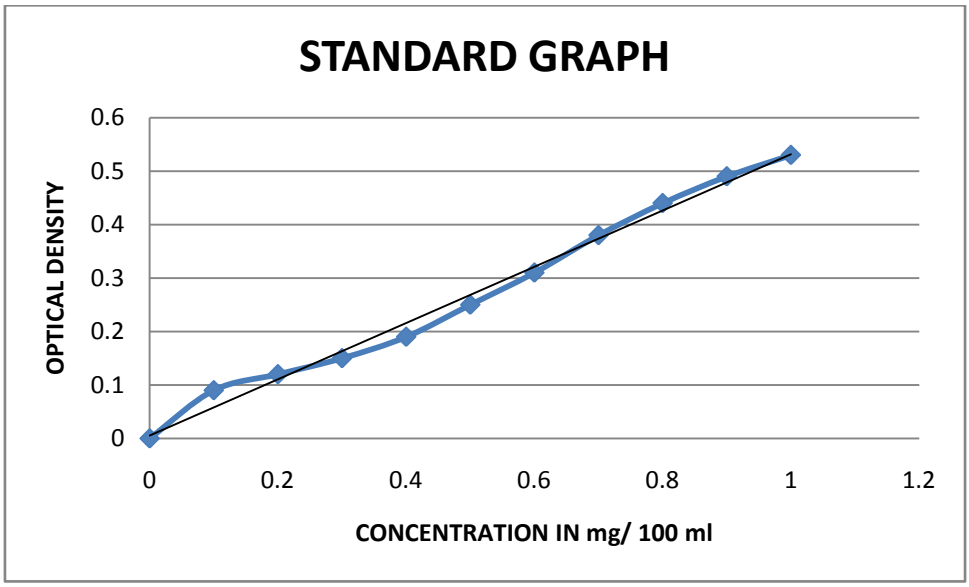
Biochemical Characterization

The biochemical tests showed that four isolates were *Bacills sp*. while two isolates were *Staphylococcus sp.*

	Tannery effluent bacteria	Ice factory effluent bacteria	Bakery effluent bacteria	Ice cream factory effluent bacteria	Ambattur lake water bacteria	Vedanthangal lake water bacteria
Oxidase test	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+
Citrate utilization test	+	+	+	+	+	+
Methyl red test	+	+	+	+	+	+
Voges proskauer test	+	+	+	+	+	+
Triple sugar ion test	+	+	+	+	+	+
Urease test	+	+	+	+	+	+
Indole test	+	+	+	+	+	+
Coagulase test	+	+	+	-	+	-
Identification	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Staphylococcus sp.*	*Bacillus sp.*	*Staphylococcus sp.*

Standardisation of Degradation

Standard graph was drawn to study degradation in various physical conditions. 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 µg/mL; therate constants were 0.775, 0.305, 0.225, 0.167and 0.127 each per day, and the efficiency of dimethoate degradation were 100%, 96%, 83%,72% and 71%, for Bacillus *licheniformis*, *Pseudomonas aeruginosa*, *Aeromonashydrophila*, *Proteus mirabilis* and *Bacillus pumilusrespectively (*Manisha Deb Mandal et al (2011).

Degradation at Varying pH:

The amount of pesticide in each sample is less after 312 hours when compared to 24 hour old samples after inoculation.

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 (*Staphyloccusaureus*). 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.

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 (April Van Scoy).Within the pH range of 7–11, dimethoate degradation depends on the alkalinity of the medium rather than the time of storage. (I.O.D. El Beit, 1978).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 (Howard, 2008).

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 ^{o} C.

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 (April Van Scoy).

Degradation Test in Static and Non Static Broth Culture:

The degradation is slightly more in shaking than in non-shaking flask. Flasks containing media contaminated with pesticide were incubated on a rotary bath shaker at room temperature and at 200 rpm. In 6 hour intervals, sample was collected. The pH of the media decreased drastically with simultaneous degradation of dimethoate. The pH decreased from 7.2 to 3.2 after 15 days of incubation (Ahmed Abdel-Mageed and FatmaAly El-Nakieb, 2008).

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 1-3 kb.

- *Transformation:*

Transformation was done using DH 5 α 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 α were successfully transformed.

High Performance Liquid Chromatography:

The 13th day sample inoculated with organism *Staphylococcus aureus* was analysed by HPLC test. The retention time was found to be 5 minutes. A simple and analytical method for the determination of dimethoate was developed by C. PavanKumar and B. M. Gurupadayya. From the chromatogram retention time was found to be 4.75 min, the correlation coefficient (r2) was 0.9967. The limit of detection (LOD) was calculated and found to be 0.11 µg and limit of quantification (LOQ) was found to be 0.33 µg (C. PavanKumar and B. M. Gurupadayya, 2013).

4. 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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