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Isolation and identification of *Chlorpyrifos* degrading bacteria and its Growth response

Akshada Shelar, Manasi Gurav, Nivedita Joshi, Bandu Pawar and Amol Jadhav*
Department of Applied Microbiology, Yashwantrao Chavan Institute of Science, Satara, M.S., India
**Corresponding author: Amol Jadhav(Email: amolsjadhav12@gmail.com)*

ABSTRACT
Organic fertilizers with high effectiveness boost crop yield without sacrificing soil quality. Different crops and animals are raised on organic farms. The use of pesticides aids in the battle against pests and stops their growth, but it has a serious drawback in that it is poisonous and harmful to human health. Consumption of such hazardous foods causes serious illnesses, including cancer and life-threatening breathing issues. Studying pesticide-degrading organisms is crucial to find solutions to these issues. Pseudomonas mendosina is the one organism that was isolated using enrichment and isolation techniques on a nutrient agar medium with various amounts of the insecticide chlorpyrifos (JVWH01000383). We provide nutrient broth and minimal salt media for the growth curve investigation. The methodology of the study is based on the ability of a certain type of microbe to break down pesticides and lower the amount of their dangerous component in soil. The accessibility of pesticides, options for their breakdown, and bioremediation methods are all brought up as issues. Our research aims to identify microbes that degrade pesticide chlorpyrifos.
Keywords: *Pesticide, bioremediation, chlorpyrifos, insecticide.*

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INTRODUCTION

Agriculture has always been the most important sector because of its contribution to food, its main source of raw materials, and its big role in a nation's revenue. In order to maintain the huge amount of agricultural production of high-yielding seeds and proper fertilizers, huge amount ofquality pesticides have also been (1). Pesticides play an important role in crop production. Pesticides are used to control various insects and to control mosquitoes and termites in daily life. These pesticides have become a significant health hazard (1).Ingestion and accumulation of these toxic compounds can pose serious toxic risks to both environmental and human organisms (2). Pesticides affect the biological processes of living organisms. Pesticides affect not only pests but also insects, mold, fungi, weeds, and noxious plants (3). Insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators, and other compounds are included in the term "pesticides" (4).In 1965, Dow Chemical Company USA first made chlorpyrifos available (5). On a number of crops around the world, it is used to control a wide range of sucking and chewing insects and mites (4). Chlorpyrifos has the following chemical components: [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl-phosphothioate] (6). Chlorpyrifos shares a P-O-C connection with other organophosphorus insecticides including parathion and diazinon (7). Chlorpyrifos is non-polar and may dissolve in organic solvents, which allows it to linger in soil and water for a very long time. Chlorpyrifos can also survive in solid form for extended periods of time due to its sluggish rate of degradation, posing a serious threat to the ecosystem (5). Numerous studies have shown that a wide range of microorganisms is capable of totally mineralizing many aliphatic, aromatic, and heterocyclic chemicals as well as destroying insecticides (1). Because they are more affordable and have the ability to target specific organic pollutants for destruction, microbes are crucial for pesticide bioremediation. The biodegradation of pesticides is caused by the common phenomenon of biotransformation, which is often necessary for the survival of microorganisms (2).In order to examine how organisms, respond to variations in pH, turbidity, and growth conditions as well as to establish their user-fullness in biodegradation, the goal of this experiment is to isolate, describe, and discover chlorpyrifos-degrading bacteria.

MATERIAL AND METHODS-

Insecticide utilized: An organophosphate was used as the insecticide in this experiment.

This experiment makes use of the organophosphate pesticide chlorpyrifos. It is chosen because of its vast and widespread application in agriculture.

Collection of soil sample:

The soil was collected from a field of cauliflower in Vyajwadi Satara,Maharashtra. The soil was collected aseptically in polythene bags.

Isolation and enrichment of microorganisms:

For the Isolation of pesticide-degrading bacteria, minimal salt medium (MSM) media was prepared. 1 gm of soil sample was added to the 100 ml MSM medium. The sample was incubated on a rotary shaker at 150 rpm for 7 days at room temperature. After 7 days of enrichment, the inoculums was streaked on a nutrient agar plate. The plate was incubated for 24hours at room temperature with various concentrations of chlorpyrifos. After 24 hours colonies were observed on plates. After the isolates were transferred to the nutrient agar (NA) medium (which does not contain chlorpyrifos), those with high chlorpyrifos concentrations were selected for additional research.

Biochemical Identification: All the isolates were cultured on a Nutrient Agar plate. Based on Gram staining, size, and shape, microscopic analysis was performed. Catalase, oxidase, urea, Indole MR test, VP test, citrate, nitrate, gelatin activity, starch activity, pigment formation in water, and chloroform responses of isolates were utilized to characterize them. By using 16S rRNA sequencing, the pesticide-degrading bacteria were identified.

Growth kinetics:

Isolated organisms were inoculated in the NA broth, NA broth with 200 ppm chlorpyrifos, MS medium, and MS medium with chlorpyrifos without the addition of carbon sources for the growth kinetics investigations. A colorimeter was then used to measure the growth in each broth every hour for the following 24 hours. A graph was made using the readings.

16S rRNA sequencing:

16S rRNA sequencing provides the genus and species of identification of isolates that cannot be confirmed by the biochemical test.

RESULTS:

Isolation morphological characterization:

Isolation of chlorpyrifos degrading bacteria on nutrient agar medium containing a high concentration of chlorpyrifos (figure 1). Then an isolated colony (A2) is taken, and this is transferred onto a nutrient agar medium for screening.

Figure1: Isolation of chlorpyrifos degrading bacteria (A2)

Colony Characters: Colony characteristics and Grams nature were studied of isolated microorganisms (table 1 and figure 2)

Table 1: Colony characteristics of A2

Size	Shape	Color	Margin	Elevation	Consistency	Opacity	Gram Nature	Motility
1mm	Circular	Bluish Green	Entire	Flat	Moist	Opaque	Gram Negative short rod	Motile

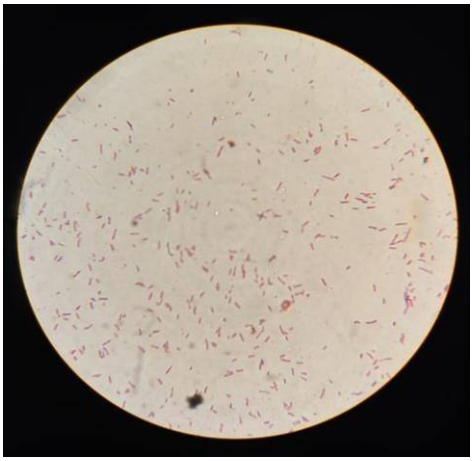

Figure 2: Gram Staining of A2

Biochemical Characterization:

The biochemical characteristics of colony A2wereperformedfor further identification and confirmation (table 2).

Table2: Biochemical tests of A2 isolates

Test	SampleX1
Catalase Test	+
Oxidase Test	+
Urease Test	_
Indole Test	_
MR test	_
VP Test	_
Citrate	+
Nitrate	+
Gelatin Activity	+
Starch Activity	_
Pigment production in water	+
Growthat 37^0C	+
Growthat45^0C	+
Sucrose	_

3.4 Growth Kinetics:

The bacterial strain *Pseudomonas mendocina* shows maximum growth on the minimal medium with pesticide, where the other carbon sources are absent. The bacteria use chlorpyrifos as their carbon and energy source and grow rapidly (table 3 and figure 3).

Table3: Growth kinetics of A2

PlaneNutrient Agar	NA+Pesticides	Planeminimal	Minimal+Pesticides
0.00	0.03	0.02	0.12
0.01	0.04	0.03	0.14
0.01	0.06	0.03	0.17
0.02	0.06	0.04	0.20
0.03	0.07	0.04	0.21
0.05	0.11	0.4	0.21
0.06	0.13	0.05	0.24
0.08	0.15	0.06	0.25
0.10	0.17	0.06	0.25
0.11	0.18	0.07	0.26
0.15	0.27	0.07	0.27
0.22	0.28	0.07	0.30

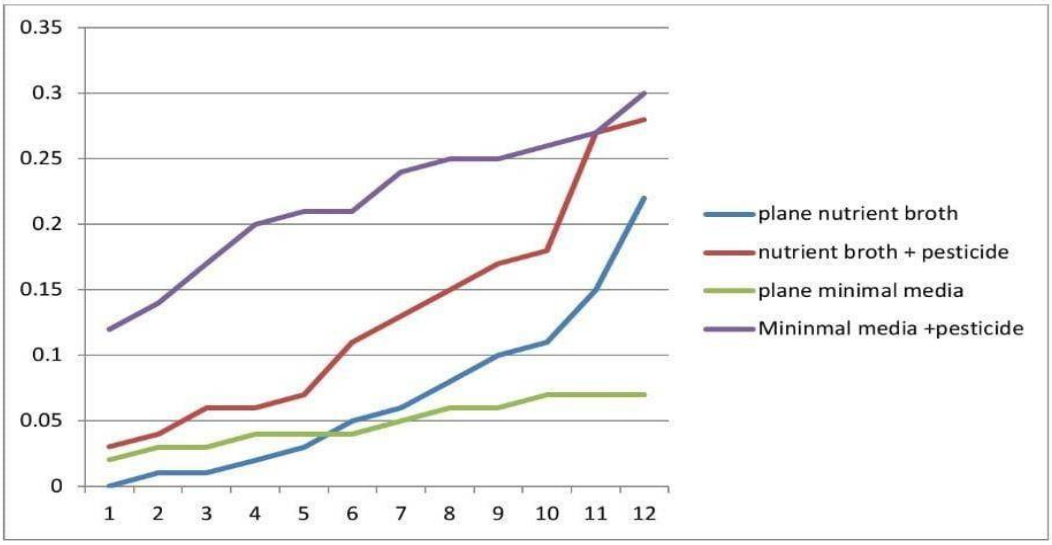

Figure3: Growth kinetics of A2 in different mediums containing 200ppm of chlorpyrifos.

16S rRNA sequencing:

Identification of isolates A2 was identified confirmed by using 16S rRNA sequencing. The isolate A2 was identified as *Pseudomonas mendocina*(fig.4).

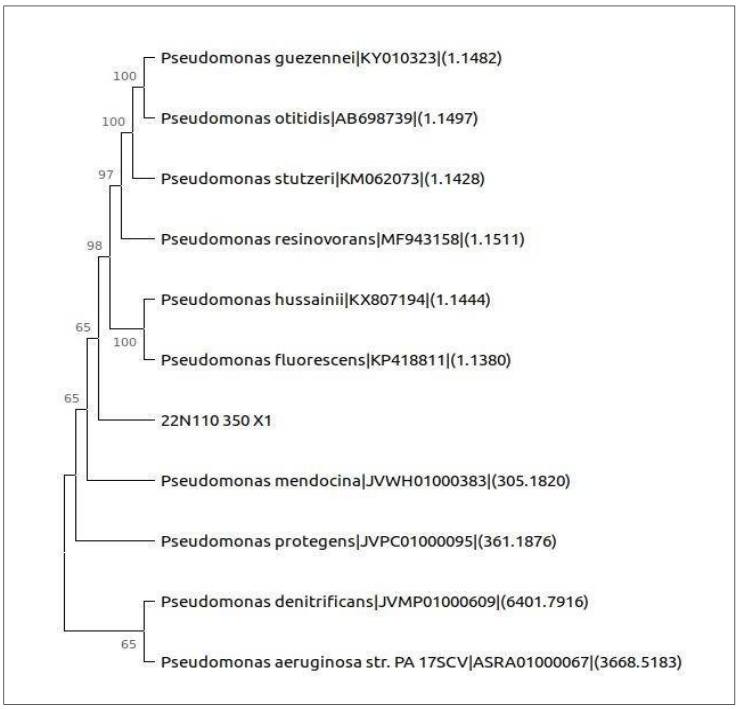

Figure4: Phylogenetic tree A2 based on 16S rRNA genes sequences how in the position of isolated bacteria strain (*Pseudomonas mendocina)* from the soil.

DISCUSSION:

The bacteria that break down chlorpyrifos was identified as *Pseudomonas mendocina* using biochemical tests and 16S rRNA sequencing. In order to achieve this, a soil sample was taken from a field of cauliflower in Vyajwadi, Maharashtra. Chlorpyrifos can be broken down to the fullest extent by this organism. The organisms employed chlorpyrifos as a source of carbon and energy for growth. The current study set out to locate and examine the bacteria responsible for breaking down chlorpyrifos. The ability to break down chlorpyrifos is greatest in bacteria. In agriculture, chlorpyrifos is widely utilized, and this use results in the most pollution. Thus, this bacterium assists in the bioremediation of chlorpyrifos and contributes to the reduction of pollution.

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