Study on pesticide-degrading bacteria isolated from soil samples

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Abstract

Pesticide pollution poses a significant environmental threat, necessitating effective remediation strategies. This study investigates the biodegradation of pesticides by bacteria, focusing on the isolation, characterization, and application of pesticide-degrading microorganisms. Bacteria capable of degrading various pesticides, including organophosphates and organochlorines, were isolated from contaminated soil samples. These isolates were identified using molecular techniques, and their degradation capabilities were assessed through laboratory experiments. The research explored the metabolic pathways involved in pesticide degradation, identifying key enzymes and genes responsible for the breakdown of specific pesticides. Environmental factors influencing degradation rates, such as temperature, pH, and nutrient availability, were also examined. The findings revealed that bacterial degradation is a promising approach for the bioremediation of pesticide-contaminated sites. The study's outcomes provide insights into the mechanisms of pesticide degradation and the potential of using bacteria for environmental cleanup. Further research is needed to optimize the use of these microorganisms in field applications and to address the challenges associated with biodegradation.

1. Introduction

The study of pesticide-degrading bacteria is a critical area of research that addresses the growing concerns surrounding pesticide use in agriculture and its impact on the environment. Pesticides are widely used to enhance crop production by controlling pests, diseases, and weeds (Pranali Patil et.al, 2020). However, their extensive use has led to significant environmental challenges, including soil and water contamination, loss of biodiversity, and potential health risks to humans and wildlife. As a result, there is an urgent need to explore biological solutions to mitigate the adverse effects of pesticides, and this is where the role of bacteria comes into play (Md. Atikur Rahman et.al, 2018). Bacteria are incredibly diverse microorganisms that inhabit various environments, including soil, water, and even the human body. Some bacterial

species possess the unique ability to degrade complex organic compounds, including pesticides. These bacteria can metabolize pesticides into less harmful substances, thereby reducing their toxicity and facilitating their removal from the environment. Understanding the mechanisms by which these bacteria operate is essential for developing effective bioremediation strategies (Saravanan et. al,2020). The first step in studying pesticide-degrading bacteria involves the isolation of bacterial strains from environments contaminated with pesticides. Researchers typically collect soil or water samples from agricultural fields where pesticides have been applied. These samples are then cultured in laboratory settings to identify bacteria that can thrive in the presence of pesticides. This process often involves selective media that favor the growth of pesticide-degrading bacteria, allowing researchers to isolate specific strains for further study. Once bacterial strains are isolated, researchers conduct a series of experiments to evaluate their ability to degrade various pesticides. This includes exposing the bacteria to different concentrations of pesticides and monitoring the degradation process over time. Advanced analytical techniques, such as gas chromatography and mass spectrometry, are often employed to quantify the breakdown products and measure the efficiency of degradation.

2. Materials and Methods

2.1 Study Area:-

The soil sample was collected from the Palaud crop field, New Raipur, and GTB Agro Chemicals industrial area in Saddu, Raipur, Chhattisgarh, during the month of February. Chhattisgarh has a tropical savanna climate with hot, humid summers and mild, dry winters. Chhattisgarh's agricultural landscape is varied and boastful, with many crops. Rice, the staple food, dominates the fields, and pesticide use is common in agriculture. Chhattisgarh has a significant agricultural sector, and while the exact number of pesticide-using industries is not readily available, it's safe to say that there are many. However, the government is working to promote sustainable farming practices and reduce pesticide use.

2.2 Sample collection

The soil samples were collected from paddy field soil, Tomato field, Brinjal field, Green Beans field at the palaud in New Raipur, and GTB Agro chemicals industrial area soil at the Saddu, Kachna Rd, beside Brehm Kumari Ashram, Raipur, Chhattisgarh.

2.3 Analysis of Soil pH

The pH of the soil was tested in water suspension (1g og soil was dissolved in 50 ml of distilled water) and was stirred for 10 min with a glass rod, and the soil was kept for 30 min. Get ready the meter: switch on your pH meter and allow it to warm up for about 30 minutes. Wash the probe with distilled water and softly blot dry. Set the probe into the pH 7.00 solution and wait until the reading stabilizes. Manage the setting knob on the meter to match the pH 7.00 value. Set with pH 4.01 solution: wash the probe with distilled water and gently blot dry. Place the probe into the pH 4.01 solution and wait until the reading stabilizes. Regulate the setting knob on the meter to match the pH 4.01 value. Wash the probe with distilled water and gently blot dry. Now ready to test soil pH.

2.4 Isolation of bacteria from soil sample.

Weigh out 1g of soil and suspend it in sterile saline Water. Perform serial dilutions of the soil suspension to reduce the bacterial concentration. Place the diluted suspensions into nutrient agar plates. Use a disinfectant spreader to distribute the suspension evenly. Incubate the plates at a correct temperature of 37°C for 24 hours. A sterile loop is used to spread a small amount of the mixed culture onto an agar plate in a series of zig-zag patterns. As the loop is moved, the bacterial concentration decreases, allowing individual cells to form colonies. The plates were incubated at an appropriate 37°C temperature to permit bacterial growth. A single colony is selected from the plate, ideally one that appears well-isolated and distinct. This colony characterizes a population derived from a single bacterial cell.

2.5 Pesticide degradation test

Preparation of nutrient agar plates, and add 10 ml of pesticide into the agar at a specific concentration. Chlorpyphos 50% + Cypermethrin, Potassium Sulphate, Chlorpyriphos 20% EC, Profenofos 40% + Cypermethrin 4% EC, Thiamethoxam 25% pesticide used for pesticide degrading test. Bacterial colonies were picked and streaked aseptically on solid media plates, then incubated at optimal temperatures (37°C for 24 hr) for bacterial growth.

2.6 Morphological test

Gram staining is an important laboratory technique used to classify bacteria based on the features of their cell walls. Selecting a clean glass microscope slide. Using a sterile loop, obtain a small amount of the bacterial culture, gently touch the loop to the colony, and transfer it to

the slide. Spread the sample evenly across the slide to create a thin film. This step is crucial as a thick film can lead to inaccurate results. Allow the slide to air dry completely. Place the slide on a staining rack and cover the smear with crystal violet stain. Tolerating it to sit for about 1 minute. Crystal violet is the principal stain that will color all the bacteria. Softly rinse the slide with distilled water to remove extra crystal violet. Ensuing, shelter the smear with iodine solution for around 1 minute. Iodine performs as a penetrating agent, forming a complex with the crystal violet, which helps to retain the dye in the cells. Wash the slide gently with distilled water. This step is critical. Apply decolorizer (usually ethanol or acetone) to the slide for about 10-30 seconds. The timing can vary, so it's imperative to monitor the slide closely. The decolorizer will wash out the crystal violet from Gram-negative bacteria but will permit it in Gram-positive bacteria. - Directly wash the slide with distilled water to stop the decolorization process. Cover the smear with safranin stain for about 30 seconds. Safranin will stain the Gram-negative bacteria, open-handed them a pink color. Wash the slide again with distilled water and allow the slide to air dry completely. Once the slide is dry, place it under a microscope. Start with a lower magnification to locate the sample, then switch to a higher magnification (100x oil immersion) for detailed observation. Detect the color of the bacteria.

2.7 Biochemical test

2.7.1 Oxidase Test:

Oxidase test is a biochemical test used to identify the presence of cytochrome c oxidase, a key enzyme in the electron transport chain of aerobic respiration. This test is typically performed on bacterial cultures to determine their ability to utilize oxygen for energy production. Take 500mg of oxidase reagent, was liquify it in 50 ml of distilled water and directly put it in a dark bottle. Place a drop of the reagent onto a filter paper, then, using a sterile loop, transfer a small amount of the bacterial culture to the reagent. Observe the filter paper for the color change.

2.7.2 Catalase Test:

The catalase test is an ordinary microbiological test used to pick out bacteria that produce the enzyme catalase. Take a pure culture of the bacteria. Using a disinfected loop, transfer a small amount of the bacterial culture onto a clean glass slide. Carefully add a drop of 3% hydrogen peroxide (H_2O_2) solution to the bacterial sample. Observe the glass slide for the bubbles.

2.7.3 Motility test

Prepare a semi-solid medium (e.g., SIM medium). This medium is less viscous than agar, allowing motile bacteria to move through it. A small amount of the bacterial culture is inoculated into the medium using a straight wire or needle. One test tube is stored as an uninoculated relative control. The inoculation is completed by a needle into the middle of the medium. Inoculated tubes were incubated at the appropriate temperature, 37°C, for the bacteria to grow. After incubation, observe the tubes for growth.

2.7.4 Methyl Red (MR)Test

Prepare the medium: A specific medium (e.g., MR broth) is used. This medium contains glucose as the primary carbon source. A small amount of the bacterial culture is inoculated into the medium using an inoculated needle. One test tube is stored as an uninoculated comparative control. The inoculated tubes were incubated at the appropriate temperature for the bacteria to grow. After incubation, a few drops of methyl red (MR) reagent were added to one of the tubes. Observe the tubes for a change in colour for the MR test.

2.7.5 Voge-Proskauer (VP)Test

This test is used to differentiate between two types of bacteria based on their ability to ferment glucose and produce specific metabolic byproducts. Prepare a specific medium (e.g., VP broth) is used. This medium contains glucose as the primary carbon source. A small amount of the bacterial culture is inoculated into the medium using an inoculated needle. One test tube is stored as an uninoculated relative control. The inoculated tubes were incubated at the appropriate temperature for the bacteria to grow. Next incubation, 5 drops of alpha-naphthol and potassium hydroxide (KOH) were added to one of the tubes. Notice the tubes for a change in colour for the VP test.

2.7.6 Citrate utilization test

This test is used to determine if a bacterium can utilize citrate as its only source of carbon.50 ml Simmons citrate agar was prepared. This medium comprises citrate as the sole carbon source and a pH indicator (bromthymol blue). Pour the medium into the culture tubes and prepare a slant. A small amount of the bacterial culture is inoculated into the medium using an inoculated needle. One test tube is stored as an uninoculated relative control. The inoculated tubes were incubated at the appropriate temperature for the bacteria to grow. Next incubation, notice the slant culture for the growth and coloration of the medium.

2.7.7 Triple Sugar Iron test (TSI)

Prepare a TSI media.TSI agar is an alteration medium encompassing three sugars: lactose, sucrose, and glucose. It similarly encompasses ferrous sulfate, which reacts with hydrogen sulfide to form a dark precipitate. A minor amount of the bacterial culture is inoculated into the slant and butt of the TSI agar tube. One test tube is stored as an uninoculated relative regulator. The inoculated tubes were incubated at the appropriate temperature for the bacteria to grow. Notice the slant culture for the growth and color of the medium.

2.7.8 Indole Test:

Prepare a Peptone broth containing medium. Inoculate the medium with the bacterial culture. One test tube is stored as an uninoculated relative control. Incubate the culture at an acceptable temperature for the bacteria to grow (37°C). After incubation, add a few drops of Kovac's reagent to the culture. Notice the tubes for a change in colour for the indole test.

2.7.9. Urease test

The urease test is a bacteriological test used to identify bacteria that produce the enzyme urease. Urease is an enzyme that breaks down urea into ammonia and carbon dioxide. The ammonia formed by the reaction raises the pH of the medium, which can be marked by a color change indicator. 2.30g of urea media were used to make 50 ml of urea agar medium. After homogenizing the whole medium, it was autoclaved. Then urea solution was prepared, 5ml distilled water and 1g of urea were melted in it. Next, autoclaving the urea agar medium, the urea solution was added, and the conical flask was swirled normally to dissolve the urea solution. Prepare the urea agar slant. Using a disinfected inoculating loop, transfer a small amount of the bacterial culture to the surface of the urea agar slant. Incubate the inoculated slant at 37°C for 24-48 hours. Next incubation, detect the slant for a color change.

2.7.10. Sugar fermentation test

A biochemical test named the sugar fermentation test is performed to determine if bacteria can ferment sugars, a specific type of carbohydrate. This test might differentiate the bacterial groups. In only one sheet of Whatman paper, all the bacterial isolates are given at different places as shown in the figure. After 1-2 hours, if the color changes to yellow, it is positive, and if it remains the same, then it is negative.

3. Result

(a) (b) (c) (d) (e)

Fig 3.1 Sample collection area (a) GTB Agro chemicals industrial, (b) Paddy field, (c) Tomato field, (d) Brinjal field, (e) Green Beans field.

3.1 Soil pH

Different crop fields' soil generally has an optimum pH**.** This data is interpreted in Table 3.1

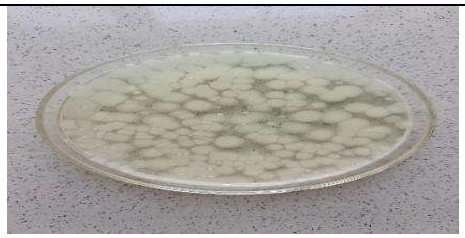
Table 3.1 List of soil pH levels

Name of the Soil area	pH
Industry	8.18
Paddy field	6.80
Tomato field	7.98
Brinjal field	7.15
Green bean field	6.60

3.2 Isolation of bacterial colonies

Nutrient agar is a broadly used, all-purpose growth medium for cultivating various bacteria, particularly bacteria. It's a solid medium. Nutrient agar is considered a non-selective medium because it doesn't contain any specific inhibitors or selective agents that would inhibit the growth of certain organisms. In this work,5 samples were found to have bacterial growth on all of the NAM medium plates. This data is interpreted in Table 3.2

Table 3.2 Bacterial growth on NAM medium

Sample No	Medium	No. of colonies	Bacteria Isolation (Mother plates)
Sample 1 Industry area	Nutrient medium	119	
Sample 2 Paddy field	Nutrient medium	79	
Sampla 3 Tomato field	Nutrient medium	263	
Sample 4 Brinjal field	Nutrient medium	434	
Sampla5 Green beans fried	Nutrient medium	203	

3.3 Morphological characterization of bacterial isolates

The suspected sample inoculated was observed with the growth of microbes. The colonies that form in the medium.

Table 3.3: Summarizes the morphology of the produced colonies.

Sample no	Medium	Shape	Size	Edge/margin	Color	Opacity	elevation	surface	Texture
Sample 1	Nutrient agar medium	irregular	<1mm	Entire	White, Yellow, Light white	opaque	convex	glistening	Shiny, viscid
Sample 2	Nutrient agar medium	Round	<1mm	Entire	white	opaque	convex	glistening	Shiny, viscid
Sample 3	Nutrient agar medium	Round	<1mm	Entire	White, Transparant Light pink	opaque	convex	glistening	Shiny, viscid
Sample 4	Nutrient agar medium	Round	<1mm	Entire	white	opaque	convex	glistening	Shiny, viscid
Sample 5	Nutrient agar medium	Round	<1mm	Entire	White,	opaque	convex	glistening	Shiny, viscid

3.4 Pesticide degradation by bacterial isolates

The presence of the pesticide in the test environment is essential for confirming the activity of the bacteria. It ensures that the observed growth and changes were directly linked to the ability of the bacteria to degrade the pesticide. 17 bacteria were used in the pesticide degradation method. This data is interpreted in Table 3.4

Table 3.4: List of pesticides degrading bacteria

Bacteria no.	Chlorpyphos50%+ Cypermethrin	Potassium Sulphate	Chlorpyriphos 20% E.C	Profenofos 40% + Cypermethrin 4% EC	Thiamethoxam 25%
R1	_	+	_	_	_
R2	_	+	_	_	_
R3	_	+	_	_	_
R4	_	+	_	_	_
R5	+	+	+	+	+
R6	+	+	+	+	+
R7	_	+	_	_	_
R8	_	+	_	_	+
R9	_	+	+	+	+
R10	_	+	+	+	+
R11	_	+	_	_	_
R12	_	+	_	+	+
R13	_	+	_	_	_
R14	_	+	_	+	+

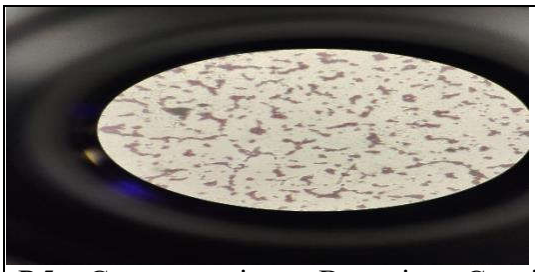
R15	_	+	_	_	_
R16	_	+	_	_	_
R17	_	+	+	+	_

3.5 Biochemical characterization of bacterial isolation

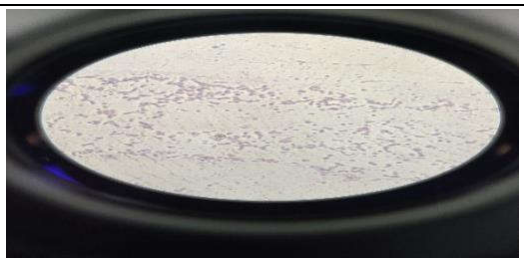
Only 8 bacteria were used for biochemical tests, as 10 types of biochemical tests were performed. Oxidase test, catalyst test, motility test, MR-VP test, citrate test, TSI test, indol test, urease test, sugar fermentation test. This data is interpreted in **Table 3.5**

Table 3.5: Biochemical characterization of bacterial isolation

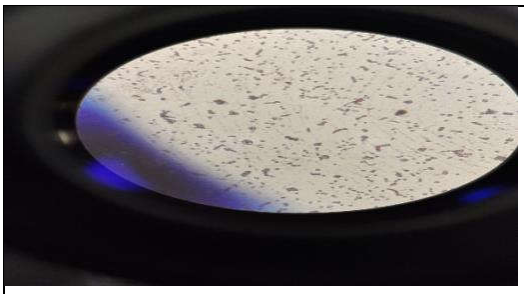
Biochemical test	bacterial isolation							
	R5	R6	R8	R9	R10	R12	R14	R15
Gram staining	_	_	_	_	_	_	_	_
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+
Vp test	_	_	_	_	_	_	_	_
MR test	+	+	+	+	+	+	+	+
Urease test	+	+	+	+	+	+	_	+
Citrate test	+	+	+	+	_	_	_	_
Sugar fermentation test	+	+	+	+	+	+	+	+
Indole Test	_	_	_	_	_	_	_	_
TSI test	+	+	_	_	+	+	_	+
Motility test	+	+	+	+	+	_	_	+



R5 Gram-negative, Bacteria, Cocci, *Pseudomonas spp*



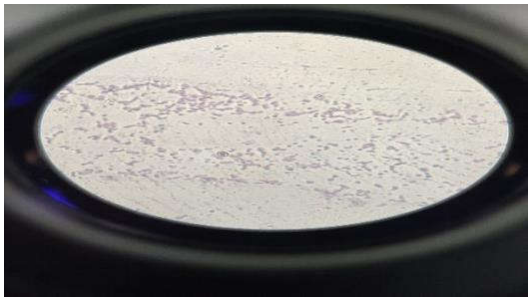
R6 Gram-negative, Bacteria, Cocci, *Neisseria spp*



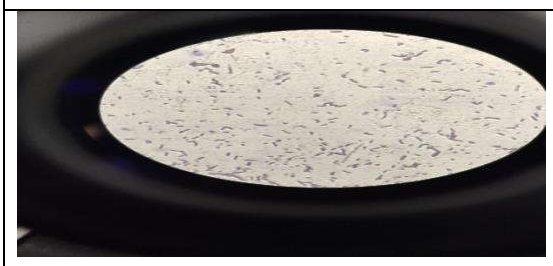
R8 Gram-negative Bacteria, Cocci, *Cryseobacterium spp*

R9 Gram-negative Bacteria, Cocci, *Alcaligene spp*

R10 Gram-negative Bacteria, Bacillus, *Burkholderia spp*



R12 Gram-negative Bacteria, Cocci, *Shigella spp*



R14 Gram-negative Bacteria, Bacillus, *Shigella spp*

R17 Gram-negative Bacteria, Bacillus *Salmonella spp*

Fig 3.2 Microscope examination of bacterial isolation

4. Conclusion

Pesticides have been integral to modern agriculture, ensuring crop protection and enhanced yields. However, their widespread use has resulted in environmental contamination, posing risks to ecosystems and human health. The exploration of pesticide-degrading bacteria offers a promising solution to mitigate these adverse effects. This conclusion synthesizes the findings and implications of research on these bacteria, emphasizing their potential in bioremediation and sustainable practices. Firstly, the identification of various bacteria capable of degrading specific pesticides highlights the diversity and adaptability of microbial life. Studies have

shown that certain bacteria can metabolize complex pesticide compounds, breaking them down into less harmful substances. This ability not only aids in detoxifying contaminated environments but also demonstrates the potential for utilizing these microorganisms in bioremediation strategies. By harnessing the natural processes of these bacteria, we can develop effective methods to clean up pesticide-laden soils and water bodies. Moreover, understanding the mechanisms of biodegradation is crucial for optimizing these processes. Research has revealed that enzymes produced by pesticide-degrading bacteria play a pivotal role in breaking down chemical bonds within pesticide molecules. By studying these enzymatic pathways, scientists can enhance the efficiency of biodegradation, potentially leading to the development of bioengineered strains with improved capabilities. This knowledge can pave the way for innovative bioremediation techniques that are both cost-effective and environmentally friendly. The impact of environmental factors on the activity of pesticide-degrading bacteria cannot be overlooked. Factors such as temperature, pH, and nutrient availability significantly influence microbial metabolism. Therefore, it is essential to tailor bioremediation approaches to specific environmental conditions to maximize the effectiveness of these bacteria. Future research should focus on field trials to assess the performance of these bacteria in real-world scenarios, taking into account the complexities of natural ecosystems. Furthermore, the integration of pesticide-degrading bacteria into agricultural practices presents an opportunity for sustainable farming. By applying these bacteria to soils, farmers can reduce pesticide residues, promote soil health, and enhance crop resilience. This biocontrol approach not only decreases reliance on chemical pesticides but also aligns with the principles of sustainable agriculture, fostering a healthier environment for future generations. the study of pesticide-degrading bacteria is a vital area of research with significant implications for environmental management and agricultural sustainability. As the global community grapples with the challenges of pesticide pollution, leveraging the capabilities of these microorganisms offers a pathway toward cleaner ecosystems and healthier agricultural practices. Continued research and collaboration among scientists, policymakers, and farmers will be essential to fully realize the potential of pesticide-degrading bacteria in addressing environmental concerns and promoting sustainable development. By prioritizing these efforts, we can work towards a future where agriculture and environmental health coexist harmoniously.

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