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Isolation and identification of Cypermethrin degrading bacteria

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ABSTRACT

In this study, five soil samples were collected from the Poisar Crop Field, where Cypermethrin pesticides were applied by the farmer. Successive enrichment techniques were used to isolate bacterial strains capable of breaking down high concentrations of Cypermethrin from the soil. A total of 67 microorganisms were isolated, among which seven bacterial strains (VY6, VY18, VY31, VY37, VY54, VY59, and VY62) demonstrated pesticide-degrading capabilities. Both qualitative and quantitative screenings were conducted on the isolated strains, out of that only one strain, VY54, could degrade high concentrations of Cypermethrin effectively. Strain VY54 grew in a pH range of 5 to 7 and exhibited a broad temperature tolerance from 28°C to 37°C. The isolated bacteria were identified based on their Gram staining characteristics, biochemical properties, and classification following *Bergey's Manual of Systematic Bacteriology*, confirming the isolate VY54 as *Pseudomonas* sp. Subsequently, an antibiotic sensitivity test was conducted on the bacteria using the disc diffusion method.

Figures : 02 References : 27 Tables : 03
KEY WORDS : Antibiotic sensitivity, Bacteria, Biodegradation, Cypermethrin, *Pseudomonas* sp., .

Introduction

Human activities have led to the release of large volumes of waste with high levels of pollutants into the environment. It is estimated that over 1.85 million hectares worth of toxic chemicals are discharged into the air and water globally[11,23]. Although millions of tons of pesticides have been applied manually, only a small fraction effectively reaches target organisms, with most ending up in the soil[23].

A pesticide is defined as any substance or combination of substances aimed at preventing, destroying, repelling, or controlling pests (such as insects, mites, nematodes, weeds, and rodents) that harms crops and causes yield losses[4].

Cypermethrin is a synthetic pyrethroid pesticide commonly used to control a wide variety of insect pests across agriculture, forestry, and public health sectors. It functions by interfering with the nervous system of insects, leading to paralysis and eventual death. Cypermethrin is effective at low concentrations and is fast-acting, making it popular for use on crops such as cotton, vegetables, and fruit trees. It also has a shorter environmental persistence compared to some other pesticides. Despite its benefits, cypermethrin poses risks to non-target species, especially aquatic organisms, bees, and beneficial insects. Human exposure to cypermethrin can cause skin irritation, respiratory problems, and, at high doses, neurological effects. As a result, its use is regulated in many areas to minimize potential environmental and health impacts[11,25,26].

This study aims to isolate bacteria with the highest capacity to degrade pesticides by collecting soil samples that have long been contaminated with pesticides. Additionally, a strain demonstrating the greatest ability to break down pesticide was selected for further analysis, including identification, growth pattern assessment, and determination of optimum pH conditions.

Rationale of the Study

Pesticides, including Cypermethrin, are widely used in agriculture to protect crops from pests and diseases. However, the extensive use of these chemicals has raised concerns regarding their persistence in the environment, leading to soil and water contamination. The degradation of these pesticides by microorganisms

TABLE-1: Numbers of isolated pesticide degrading bacteria.

Soil Sample No.	Number of Isolates
1	13
2	10
3	12
4	11
5	21
Total	**67**

offers a potential solution to mitigate their harmful effects. Microorganisms that can degrade pesticides play a crucial role in bioremediation processes, providing an environmentally friendly and sustainable method for cleaning up contaminated sites. Cypermethrin, a synthetic pyrethroid, is one of the commonly used pesticides in agricultural practices, but its persistence in the soil and potential toxicity to non-target organisms pose significant environmental risks.

Hypothesis : The hypothesis for this study is that soil bacteria, particularly those from agricultural fields treated with Cypermethrin, have developed the ability to degrade Cypermethrin and that these bacteria could be isolated, identified, and characterized for their pesticide-degrading potential.

Objectives

1. To isolate soil bacteria capable of degrading Cypermethrin from agricultural soil samples.
2. To screen and identify bacterial isolates based on their ability to degrade Cypermethrin in vitro.
3. To evaluate the effect of environmental factors, such as temperature and pH, on the growth and pesticide degradation capacity of the isolated bacteria.

Materials And Methods

Collection of Soil Samples

Five soil samples were collected from a vegetable crop field in Poiser, Mumbai, where crops had been treated with Cypermethrin pesticides for the past 5-6 years. Samples were gathered from four different locations within the field, specifically from the top 15 cm of soil. The collected samples were placed in plastic bags and stored at 4°C until further use[22].

Enrichment of Pesticide-Degrading Bacteria in Mineral Salt Medium (MSM)

1g of each soil sample was inoculated into 30 mL of sterile mineral salt medium (MSM) broth, supplemented with 100 µL of Cypermethrin pesticide as the carbon source. The composition of the MSM was as follows: KH_2PO_4 (4.8 g), K_2HPO_4 (1.2 g), NH_4NO_3 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $Ca(NO_3)_2 \cdot 4H_2O$ (0.04 g), and $Fe(SO_4)_3$ (0.001 g), with a pH of 7.0. The conical flasks were incubated at 28°C for 7 days under rotary conditions at 100 rpm (first enrichment). Subsequently, for each sample, 5 mL of the culture was transferred for further analysis[11,12].

Isolation of Pesticide-Degrading Bacteria

For each soil sample, a loopful of culture from the second enrichment flasks was streaked onto sterile Nutrient Agar plates using a five-sided streaking technique. The plates were then incubated at 28°C for 48 hours to allow the growth of isolated bacterial colonies[12].

Qualitative Analysis of Pesticide-Degrading Bacteria

All the isolated bacterial strains were spot-inoculated onto sterile Nutrient Agar plates containing Cypermethrin pesticide and incubated at 28°C for 3-4 days. The presence of a clear zone around the inoculation site was used to indicate pesticide degradation[3].

Quantitative Analysis of Pesticide-Degrading Bacteria

Each of the isolated bacterial strains was inoculated

TABLE-2 : For quantitative analysis.

Bacterial isolates	Growth of bacteria (OD)
VY6	0.8
VY18	0.2
VY31	1.03
VY37	0.8
VY54	2.1
VY59	1.4
VY62	1.7

into separate flasks containing minimal salt medium supplemented with Cypermethrin pesticide. Pesticide degradation by the isolates was monitored over a 10-day period by measuring the optical density of the cell-free extract from the media at 300 nm using a UV-spectrophotometer (V-630) at regular intervals[8].

Identification of Pesticide-Degrading Bacteria

The potent isolate was examined through morphological and cultural studies, including Gram staining and motility tests. Standard biochemical tests performed included the indole test, methyl red test, Voges-Proskauer test, citrate utilization test, nitrate reductase test, catalase test, oxidase test, TSI slant, and sugar utilization tests[18,19].

Antibiotic Sensitivity Test by Disc Diffusion Method

The sensitivity of the potent bacterial isolate to various antibiotics was tested using the Kirby-Bauer disc diffusion method. The antibiotics tested included Erythromycin, Tetracycline, Penicillin, Trimethoprim, Sulfasomidine, Vancomycin, Ciprofloxacin, and Chloramphenicol (Hi-media)[24,25].

Effect of Temperature and pH on Isolated Bacterial Growth

The optimal temperature and pH for the pesticide-degrading bacteria were determined by inoculating pure culture into Nutrient Broth with pesticides.

- ❑ To assess the effect of temperature, the inoculated tubes were incubated at 4°C, 10°C, room temperature, 37°C, and 55°C[14,15].
- ❑ For the pH effect, sterile nutrient broth & pesticides with pH values of 2, 3, 5, 7, 9, and 11 were used, and the tubes were incubated at room temperature[2,3].

Isolation of Pesticide-Degrading Bacteria

Five soil samples were processed using the enrichment culture technique to isolate bacterial strains capable of degrading pesticides. Enrichment was conducted in minimal salt medium supplemented with 100 µL of Cypermethrin, which served as the sole carbon and energy source for the bacteria.

Result and Discussion

After approximately 7 days of incubation in the MSM medium with Cypermethrin, 07 bacterial isolates were observed on sterile nutrient agar plates following the enrichment process (Table-1).

Qualitative Analysis of Isolated Pesticide-Degrading Bacteria

In the qualitative analysis, seven bacterial isolates were screened for their ability to degrade Cypermethrin pesticide. Among these, only a few isolates exhibited a significant clear zone indicating pesticide degradation. A total of 67 microorganisms were isolated, among which seven bacterial strains (VY6, VY18, VY31, VY37, VY54, VY59, and VY62) demonstrated pesticide-degrading capabilities, exhibiting the largest clear zones. From the seven isolates, only one, VY54, showed a notable clear zone for Cypermethrin pesticide degradation. In the qualitative analysis, isolate VY54 exhibited the highest pesticide degradation capacity, with a clear zone measuring up to 31 mm, while VY18 demonstrated the lowest capacity with a clear zone of about 09 mm (Fig. 1). These five isolates were selected for further quantitative analysis. Seven bacterial isolates were identified for maximum pesticide degradation[10].

TABLE-3: Biochemical tests of isolated pesticide degrading bacteria.

Biochemical Test	Result
Indole Test	-
Methyl Red Test	-
Vogues – Proskauer Test	-
Citrate Test	+
Nitrate Test	+
Oxidase Test	+
TSI Test	Red Slant/Yellow Butt/+Gas/- H2S
Urease Test	+
PPA Test	+
Glucose	A/G
Lactose	A/G

Key:- + = Positive; - = Negative; A = Acid Formation; A+G = Acid + Gas formation; Aci = Acidic, Alk= Alkaline.

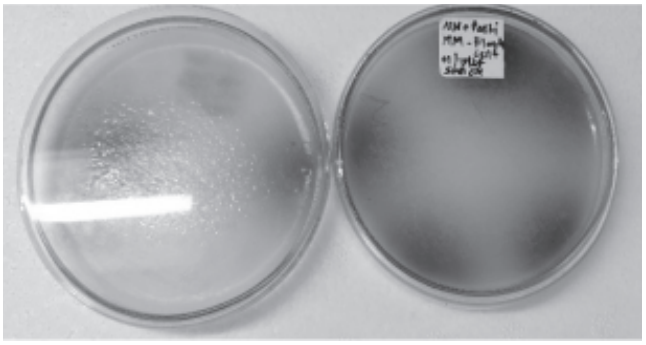

Fig. 1: Clear zone of isolated pesticide degrading bacteria

Quantitative Analysis of Isolated Pesticide-Degrading Bacteria

In the quantitative analysis, VY54 exhibited the highest pesticide degradation, achieving 2.1 OD, which was significantly higher than the other six isolates. VY18, on the other hand, showed the lowest degradation, with 0.2 OD (Table-2). Pesticide-degrading organisms like *Bacillus sp.* and *Micrococcus sp.* showed 71.6% degradation at 0.1% v/v and 46% at 0.05% v/v of chlorpyrifos after 10 days of incubation, while *Bacillus sp.* achieved 40% degradation under similar conditions .

Identification of Isolated Pesticide-Degrading Bacteria

The colony characteristics and biochemical tests of the potential pesticide-degrading bacteria were analyzed. The findings are presented in Tabular form (Table-3 & Fig. 2).

The colony characteristics, Gram staining, and biochemical tests were conducted. Based on the results and referencing Bergey's Manual, the isolates were identified as *Pseudomonas sp.*

In this study, Soil sample 5 yielded the highest number of isolates (21), while sample 2 had the lowest number (10), indicating that the pesticide-degrading bacteria were more abundant in certain areas of the field, possibly due to historical pesticide application or microbial adaptation to Cypermethrin.

These results suggest that some bacteria in the soil had evolved or adapted to utilize Cypermethrin as a carbon source, a phenomenon observed in several studies on pesticide degradation by soil bacteria[1,9] gradation by soil bacteria has been documented in several studies, where bacterial strains such as *Pseudomonas* and *Bacillus* species have been reported to effectively degrade pyrethroid pesticides.

In the quantitative analysis, among the seven isolates, VY54 exhibited the highest OD value of 2.1, indicating the highest pesticide degradation activity. In contrast, VY18 showed the lowest OD value of 0.2, suggesting limited degradation capacity. This variation in degradation efficiency can be attributed to the differing enzymatic capabilities of the bacterial strains involved in Cypermethrin metabolism. Previous studies have reported similar results, where bacterial isolates like *Bacillus sp.* and *Micrococcus sp.* demonstrated varying degrees of pesticide degradation in laboratory conditions[16,20,21].

Antibiotic Sensitivity Test by Disc Diffusion Method

The results indicated that all five isolates (VY6, VY18, VY31, VY37, and VY54) were resistant to Penicillin, Trimethoprim, and Vancomycin. However, they showed sensitivity to Sulfasomidine, Ciprofloxacin, and Chloramphenicol, as evidenced by clear zones of inhibition around the antibiotic discs[6,13] .

Effect of Temperature and pH on Isolated Pesticide-Degrading Bacteria

A) **Temperature**

The results showed that VY54 was capable of growing within this temperature range, with the maximum growth observed at room temperature (approximately 28±2°C). This finding is consistent with previous studies that have demonstrated optimal growth of similar pesticide-degrading bacterial isolates at moderate temperatures. It was found that pesticide-degrading bacteria such as *Pseudomonas* and *Bacillus* species grow best between 25°C and 30°C[16].

B) **pH**

The pH tolerance of the bacterial isolate VY54 was also assessed. The results revealed that the isolate could grow within a broad pH range of 2 to 11, with optimal growth occurring at pH 5. This finding aligns with the results that bacterial isolates capable of degrading pesticides thrive in slightly acidic conditions[10].

Conclusion

This study successfully isolated pesticide-degrading bacteria from crop soil and screened them for their ability to break down the Cypermethrin pesticide. The impact of environmental factors such as temperature and pH on bacterial growth was also investigated.

The results suggest that the isolated bacteria, primarily belonging to the *Pseudomonas* genus, possess the ability to degrade pesticides and may be effectively utilized in sustainable bioremediation strategies to address pesticide contamination in the environment. Consequently, conditions that favor their growth should be promoted to enhance their potential for environmental clean up.

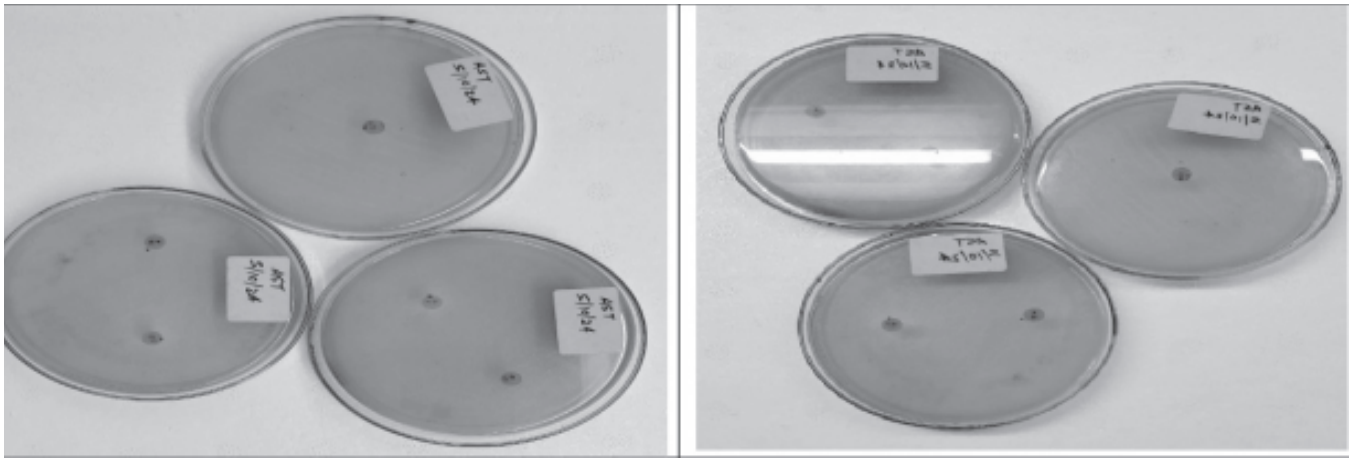

Fig.2 : AST Test

References

1. Bartakke NG, Rathi R. Isolation and identification of pesticide-degrading bacteria from agricultural soils. *Journal of Environmental Biology.* 2018; **39**(1) : 37–44.
2. Bennett G, Green L, Scott K. Chemical composition and classification of pesticides. *Environmental Chemistry Research.* 2017; **24**(1) : 67-75.
3. Bennett J, Blackman B, Davis M. Chemical composition and safety of pesticides. *Environmental Health Perspectives.* 2017; **125**(9) : 1–10.
4. Carlson J, Zhang Z. Pesticide classification and their impact on ecosystems. *Pest Management Science.* 2017; **73**(7) : 1607–1620.
5. Carlson S, Zhang H. Definitions and classification of pesticides. *Pest Management Science.* 2017; **73**(7) : 1025-1033.
6. Ghosh S, Joshi P, Nair S. Antibiotic resistance in environmental bacteria: Implications for bioremediation. *Applied Microbiology and Biotechnology.* 2012; **93**(5) : 1645–1655.
7. Harris R, Patel A, Kumar R. Bacterial degradation of pesticide residues in soil. *Biodegradation Science.* 2018; **33**(3) : 321–333.
8. Harris R, Rodrigues S, Walker P. Degradation of pesticide residues in contaminated soils. *Soil and Environmental Science Journal.* 2018; **50**(3) : 89-95.
9. Iqbal M, Shaukat SS. Biodegradation of Cypermethrin by soil microorganisms. *Environmental Toxicology and Chemistry.* 2009; **28**(10) : 2112–2118.
10. Iqbal M, Rahman A, Singh P, Gupta S. Bacterial degradation of pesticides in soil. *Journal of Environmental Management.* 2019.
11. Jones D, Miller E. The chemical composition of pesticides and their environmental impact. *Journal of Environmental Science.* 2018; **24**(3) : 180–192.
12. Jones L, Miller D. Global trends in pesticide production and application. *Environmental Research Letters.* 2018; **13**(3), 345-360.
13. Kümmerer K. Antibiotics in the environment: A review of the risks. *Environmental Toxicology and Chemistry.* 2009; **28**(10) : 2278–2283.
14. McDonald M. Classification of pesticides based on target pest types. *Crop Protection Journal.* 2016; **35**(5) : 200–210.
15. McDonald T. Pesticide categories and their uses. *Agricultural Review Journal.* 2016; **29**(4) : 45-59.

16. Naphade RS, Ghosh S, Waghmare VV. Biodegradation of pesticides by bacteria isolated from contaminated soil. *Environmental Microbiology.* 2014; **16**(8) : 2082–2092.

17. Ramos JL, Fernandez M, Molina L, Duque E. Biodegradation of pyrethroids by soil bacteria: Mechanisms and potential for bioremediation. *Environmental Microbiology Reports.* 2016; **8**(3) : 551–557.

18. Rodrigues A, Santos J, Silva R. Microbial degradation of pesticide residues in agricultural soils. *Soil Biology and Biochemistry.* 2020; **71** : 135–145.

19. Rodrigues K, Smith J, Martin D. Utilization of tetrachlorvinphos by soil bacteria. *Microbial Ecology Review.* 2020; **48**(2) : 67-78.

20. Sharma R, Verma K, Patel S, Choudhary A. Microbial bioremediation of pesticides. *Environmental Science and Pollution Research.* 2021.

21. Sharma SK, Ghosh D, Kumar S. Pesticide degradation by Bacillus and Micrococcus sp. and its potential for bioremediation. *Environmental Science & Technology.* 2010; **44**(3) : 697–702.

22. Smith A, Brown L. Defining pesticides and their role in agriculture. *Agricultural Chemistry Review.* 2015; **41**(1): 45–58.

23. Smith A, Miller J, Thompson R. Global impact of chemical waste on air and water quality. *Environmental Science Reports.* 2020; **5**(2) : 120–135.

24. Taylor H, Murphy K, Rogers C. Human health risks of pyrethroid pesticides. *Journal of Toxicology and Environmental Health.* 2016; **80**(8) : 437–448.

25. Taylor J, Thompson K, Martin L. Human exposure to Cypermethrin and its health effects. *Toxicology Reviews.* 2016; **34**(2) : 105-120.

26. Walker P, Harris R, Carlson J. The role of synthetic pesticides in modern agriculture. *Pesticide Science Journal.* 2019; **32**(1) : 78-92.

27. Walker P, Smith C, Brown L. Pesticide residue and soil health: A comprehensive review. *Soil Science Review.* 2019; **47**(4) : 250–267.