Microbial Biodegradation of Cypermethrin in Agricultural Soils of Uttar Pradesh: Characterization and Plasmid-Associated Degradative Potential

Sazia Siddiqui (*First Author*)
Department of Biosciences
Integral University
Lucknow, 226026, India
shaziamicro88@gmail.com

Aisha Kamal (*Corresponding Author*)
Department of Bioengineering,
Integral University,
Lucknow, 226026, India
aishakamal04@gmail.com

Abstract**—** *Cypermethrin's continued usage in agriculture has contaminated the environment, negatively affecting the health of the soil microbiota and ecosystem as a whole. In this work, native soil bacteria that can break down Cypermethrin were isolated, characterised, and their potential for biodegradation was investigated. The bacteria were gathered from Uttar Pradesh's agricultural soils that were contaminated by pesticides. Using enrichment procedures, three bacterial strains were identified: Escherichia coli, Pseudomonas aeruginosa, and Pseudomonas fluorescens. Cultural, morphological, biochemical, and plasmid profiling investigations were used to confirm their identity. The isolates' ability for environmental detoxification was indicated by Pseudomonas aeruginosa maximum growth in media with Cypermethrin concentrations as high as 180 mg/L. Plasmid profiling identified genetic components with molecular weights ranging from 2.5 kb to 5.0 kb that was involved in the breakdown of Cypermethrin. These results show how plasmid-bearing bacteria may be used to bioremediate locations contaminated by pyrethroids*

Keywords**— *Cypermethrin, Biodegradation, Plasmid profiling, Pseudomonas aeruginosa, Agricultural soil, Bioremediation, Soil microbiota***

I. INTRODUCTION

Modern agriculture makes considerable use of synthetic pyrethroids, such cypermethrin, because of their great insecticidal activity and minimal toxicity to mammals. However, because of their bioaccumulative and persistent nature, environmental concerns have been highlighted [1]. Cypermethrin has the ability to attach itself to soil particles and be active for long periods of time, which can contaminate groundwater, surface water and disturbed healthy soil microbial communities [2]. Overuse of these substances has led to negative impacts on non-target organisms, microbial resistance, and decreased soil fertility [3].

The amazing metabolic diversity and adaptability of soil-dwelling bacteria enable them to flourish in polluted environments [4]. To break down complicated and harmful substances like Cypermethrin, certain bacteria create specialised enzyme pathways, frequently mediated by plasmids. Plasmids may contain genes that code for hydrolases, monooxygenases, and esterases, which would aid in the detoxification of xenobiotics [5].

Developing environmentally friendly remediation techniques can be guided by an understanding of the methods by which native microbes break down pesticides. The current investigation aims to isolate bacterial strains that break down Cypermethrin from pesticide-stressed soils, ascertain how quickly these strains develop when exposed to Cypermethrin, and examine the role of plasmids in degradation. These understandings are essential for improving environmentally friendly farming methods and reducing the hazards associated with pesticides.

II. MATERIALS AND METHODS

Soil Sample Collection

Agricultural soils from Uttar Pradesh fields that have a documented history of pesticide use was chosen. Samples of soil were taken using sterile instruments between 0 and 15 cm below the surface and then placed in sterile polythene bags. Each sample was labelled with the date of collection and the name of the site. Native microbial communities were maintained by processing the samples within 48 hours and preserving them at 4°C.

Enrichment and Isolation of Cypermethrin-Degrading Bacteria

In order to identify bacteria that break down Cypermethrin, 10 g of each soil sample was added to 100 mL of Bushnell-Haas broth that included 40 mg/L of Cypermethrin as the sole carbon source. Using a rotary shaker set to 150 rpm, the flasks were incubated for seven days at 30°C. Next, serial dilutions were plated on NA that had been treated with 40 mg/L of cypermethrin. Subcultures of distinct colonies exhibiting growth were made for purity and additional research.

Morphological and Biochemical Characterization

The process of morphological identification involved analysing the shape, elevation, texture, and colouration of the colony. Gram staining was used to characterize the bacterial cell. The biochemical tests included nitrate reduction, citrate utilisation, oxidase, indole, methyl red, Voges-Proskauer, and catalase was performed to ascertain species identity. These

findings were contrasted with accepted reference data from Bergey's Manual [6].

Determination of Pesticide Tolerance Range

Bacterial isolates was cultured in NB supplemented with cypermethrin at progressively higher concentrations (30, 60, 90, 120, 150, and 180 mg/L) in order to evaluate pesticide tolerance. Using a UV–Vis spectrophotometer, optical density (OD600) was measured at 24-hour intervals for 72 hours while the cultures were incubated at 37°C. To determine the best growth circumstances and inhibition thresholds, growth curves were generated [7].

Plasmid Isolation and Electrophoresis

Using the alkaline lysis procedure, plasmid DNA was isolated from log-phase cultures. Electrophoresis of the extracts was performed on 0.8% agarose gels that contained ethidium bromide. To measure plasmid size, a 1 kb molecular weight ladder was employed [8]. The bands were observed under ultraviolet light and recorded for future reference.

III. RESULTS AND DISCUSSION

Bacterial Identification

The three major bacterial isolates that consistently grew in Cypermethrin-enriched media were determined to be *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*. All of the isolates were motile rods that were Gram-negative. In contrast to *E. coli*, which was indole-positive and methyl red-positive, *Pseudomonas species* were oxidase-positive and used citrate. These findings validated the strains' identities by matching bergey's manual. *Pseudomonas species* are known for their ability to withstand stress, particularly in habitats that are contaminated with organic contaminants. Plasmid-encoded enzymes help them be metabolically flexible, which increases their ability to break down xenobiotics [9].

Growth Response to Cypermethrin

The growth response changed as the concentration of Cypermethrin increased. *Pseudomonas aeruginosa* had a high tolerance, growing optimally at 120 mg/L and remaining viable at 180 mg/L. *P. fluorescens* grew moderately between 60 and 90 mg/L, but at higher doses, it drastically decreased. Beyond 60 mg/L, *E. coli* only demonstrated growth. The enhanced efficacy of *P. aeruginosa* points to an active detoxification process that may be connected to particular plasmid-borne catabolic pathways. The fact that it can use Cypermethrin as a carbon source highlights how useful it is for bioremediation. *P. aeruginosa* has been demonstrated to mineralise pyrethroids and organophosphates effectively in other investigations, which our study supports (shown in table 1 and fig 1).

Table 1: Incidence of bacterial species degrading Cypermethrin

Total no. of samples	No. of isolates	*P. fluorescence*	*P. aeruginosa*	*E. coli*
15	10(66.6%)	5 (50%)	2(20%)	3(30%)

$\chi^2_{cal} = 1.392 > \chi^2_{tab} = 1.386$; S (Significant)

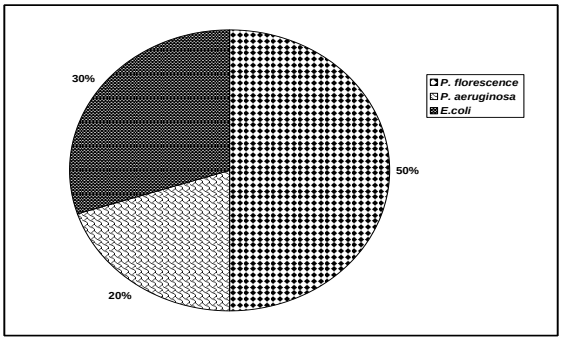

Fig 1. Incidence of bacterial species degrading Cypermethrin

Guale [10] found that the degrading bacteria, which are primarily from the genus *Pseudomonas*, are rod-shaped, gram negative, highly oxidative, and metabolically diverse. They can break down oils, pesticides, and aromatic hydrocarbons. Cypermethrin (50 mg/l) was transformed by pure culture of *Pseudomonas flourescence* in the presence of tween 80 under aerobic circumstances with a half-life of fewer than five days [11]. According to Jilani & Khan [12] *Pseudomonas species* can lower technical grade Cypermethrin levels from 60 mg/l to 6 mg/l. in 20 days. Degradation was achieved by treating pesticide waste in an aerobic environment with microorganisms present.

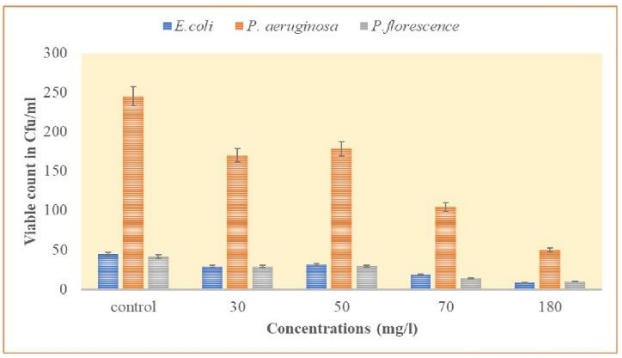

Fig 2. Growth response of Cypermethrin degrading bacterium at different concentration and time at 24 hours.

Figure 2 shows the growth response of bacteria that break down Cypermethrin at several concentrations and times throughout a 24-hour period, as well as a control group that does not use Cypermethrin. The graphical depiction of control displays *P. aeruginosa* maximal growth. It also exhibits the highest growth at concentrations of 30 mg/l, 50 mg/l, and 70 mg/l, with *E. coli* and *P. fluorescence* following closely behind at 70 mg/l. At concentrations of 30 mg/l and 50 mg/l, *E. Coli* exhibits growth rates that are nearly identical to *P. fluorescence*. However at 70 mg/l, the growth rate rises. The data displays the bacterium's rate of breakdown at various concentration levels. The growth rate decreases as pesticide concentrations rise, according to the graph.

Several researchers showed that the growth kinetics provides an evidence of mineralization potential of organism [13]. Growth experiment conducted with *Pseudomonas aeruginosa* showed that it is able to grow in the presence of Cypermethrin at 0.1% and 1%. It was noted that after

incubation at 37 ^{0}C, plating on nutrient agar medium from the solution of nutrient broth inoculated with *Pseudomonas aeruginosa*, cypermethrin showed a higher number of viable count at low concentration whereas at high concentration the number of organisms decreased when compared with the control.

Plasmid Profiling and Molecular Insights

The plasmids of *P. aeruginosa* (~5.0 kb), *E. coli* (~3.8 kb), and *P. fluorescens* (~2.5 kb) were found to be of varying sizes according to the electrophoresis results (fig 3). The idea that plasmid-encoded degrading capabilities might exist is supported by the existence of these plasmids. Genes expressed by plasmids usually contain enzymes like esterases and Cypermethrin hydrolases. Cypermethrin ester linkages are hydrolysed by enzymes, resulting in the production of less hazardous metabolites [14]. The genetic blueprint controlling pesticide breakdown may be revealed by further sequencing of these plasmids.

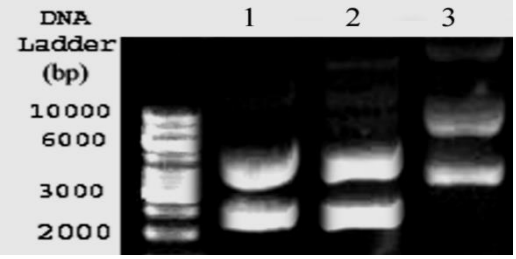

Fig 3. Sizes of plasmid DNA isolated from bacterium. Lane 1- Ps.fluorescens, Lane 2- E..coli, Lane 3- P. aeruginosa.

IV. CONCLUSION

This work shows how native bacterial strains can break down Cypermethrin from pesticide-contaminated soils were successfully isolated and characterised. The isolates with the biggest plasmid and the highest pesticide resistance and growth were *Pseudomonas aeruginosa*, suggesting a significant potential for biodegradation. Plasmids indicate a genetic basis for this metabolic activity, which has consequences for the creation of sophisticated bioremediation techniques. To determine the long-term efficacy and scalability of employing these strains in situ for the rehabilitation of contaminated agricultural fields, more genomic research and field testing are advised.

V. ACKNOWLEDGEMENT

The authors are grateful to Integral University, Lucknow, and DST-FIST funding (SR/FST/LS-1/2017/13(C), for providing research facilities to the department and are also indebted to the Department of Doctoral Studies, Integral University, Lucknow, for providing suggestions during the research.

REFERENCES

[1] R. Kaur and J. Singh, “Toxicity, monitoring, and biodegradation of cypermethrin insecticide: A review”. Nature Environment and Pollution Technology, 2021, 20(5), pp. 1997-2005.

[2] A. Borowik, W. Jadwiga, Z. Magdalena, and K. Jan, "The impact of permethrin and cypermethrin on plants, soil enzyme activity, and microbial communities." International journal of molecular sciences 24, no. 3 (2023): 2892.

[3] O.I. Ogidi and M.A. Udeme, "Impacts of Chemical Use in Agricultural Practices: Perspectives of Soil Microorganisms and Vegetation." In One Health Implications of Agrochemicals and their Sustainable Alternatives, pp. 765-792. Singapore: Springer Nature Singapore, 2023.

[4] S. Giri, P. Himani, S. Kajal, and K. Mukesh, "Microbial Contributions to Crop Adaptation: Innovation for Climate-Resilient Agriculture." In Cutting Edge Technologies for Developing Future Crop Plants, pp. 349-364. Singapore: Springer Nature Singapore, 2025.

[5] N. Shweta, S. Sripada, and S. Keshavkant, "Mechanisms, types, effectors, and methods of bioremediation: the universal solution." In Microbial Ecology of Wastewater Treatment Plants, pp. 41-72. Elsevier, 2021.

[6] N. Sharma, S. Nisha, S. Shakshi, S. Pushpinder, and D. Bindu, "Identification, morphological, biochemical, and genetic characterization of microorganisms." In Basic biotechniques for bioprocess and bioentrepreneurship, Academic Press, 2023, pp. 47-84.

[7] D. Bhatt, "Exploring the Fate of Boscalid Fungicide in Diverse Soils: Adsorption-Desorption Behavior, Kinetics and Microbial Degradation." 2024.

[8] M.F.U. Rehman, "Structural studies of the DNA partitioning protein IncC from the plasmid RK2." PhD diss., University of Birmingham, 2018.

[9] R.S. Kumar, S. Deeksha, K.B. Subir, and K.T. Prabodh, "Biodegradation of environmental pollutant through pathways engineering and genetically modified organisms approaches." In Microorganisms for sustainable environment and health, Elsevier, 2020, pp. 137-165.

[10] M. Guale, “Isolation and characterization of hydrocarbon degrading bacteria from hydrocarbon contaminated sites: microbial bioremediation perspective” PhD diss 2020.

[11] I. Kansal, K. Arushi, Swati, and R. Singh, "Cypermethrin toxicity in the environment: analytical insight into detection methods and microbial degradation pathways." Journal of Applied Microbiology 134, no. 6 (2023): lxad105.

[12] S. Jilani and M. A. Khan, "Treatment of Toxic Organics in Industrial Wastewater using Activated Sludge Process." International Journal of Environmental Research 8, no. 3 (2014): 719-726.

[13] W. Qin, C.Y. Wang, Y.X. Ma, M.J. Shen, J. Li, K. Jiao and L.N. Niu, “Microbe-mediated extracellular and intracellular mineralization: environmental, industrial, and biotechnological applications”. Advanced Materials, 32(22), 1907833, 2020.

[14] Y. Xiao, Q. Lu, X. Yi, G. Zhong, and J. Liu, “Synergistic degradation of pyrethroids by the quorum sensing-regulated carboxylesterase of Bacillus subtilis BSF01”. Frontiers in Bioengineering and Biotechnology, 8, 889, 2020.