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Isolation and Identification of Pesticides Degrading Bacteria from Farmland Soil

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Abstract

Chemicals used as pesticides are designed to kill pests. Pesticides undoubtedly play a crucial role in raising agricultural yields and protecting crops from pest infestations, but their toxicity poses serious risks to the environment, human health, and the larger ecosystem. Cypermethrin is a pyrethroid pesticide, is use since 1980s in agricultural fields to check pest infestation in different crops for enhanced food production. Excessive use of cypermethrin results in soil contamination, affecting soil micro flora. Because of this contamination's toxicity and long-term endurance, the environment is seriously threatened. A potential solution for the remediation of Cypermethrin contaminated environments. For the purpose to better understand natural remediation processes and maybe use these bacteria for environmentally friendly and sustainable farming practices, we isolated and identified cypermethrin-degrading bacteria from agricultural soil in this study. Following the application of cypermethrin to contaminated agricultural fields, soil samples were collected, and enrichment cultures were used to isolate the bacteria. Our results show that the soil analyzed included a variety of bacterial communities that were able to break down cypermethrin. The study has identified several bacterial genera. Three potent bacteria responsible for pesticide breakdown have been identified: *B. subtilis, P. aeruginosa*, and *E. coli*. Due to its inherent eco-friendliness, affordability, and effectiveness in detoxifying pesticide-contaminated ecosystems, bioremediation seems as a tempting method.

***Key words*:** Pesticide, *E. coli, P. aeruginosa, B. subtilis,* Bioremediation

A pyrethroid pesticide, cypermethrin [(+/-)-α-cyano-3-phenoxybenzyl (+/-)-cis, trans-3(2, 2dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate] is used to control insects. According to Cassida [1], there are four main generations of pyrethroids, and cypermethrin is a member of the fourth generation. Cypermethrin undoubtedly increased agricultural product productivity while shielding crops from insects. According to reports, using cypermethrin extensively can have negative effects on the activity and population of beneficial soil microflora [2]. Depending on the physicochemical characteristics of the soil, cypermethrin's half-life in the environment can range from 14.6 to 76.2 days [3]. Cypermethrin has an impact on the ATPase system and voltage-dependent sodium channel in neuronal membranes.It connects directly to nuclear DNA, causing destabilization and DNA unwinding [4]. Cypermethrin, a fourth-generation pyrethroid pesticide, has significantly boosted agricultural productivity by protecting crops from insects. However, its extensive use poses risks to beneficial soil microflora and the environment due to its toxicity and persistence.

On the basis of the facts of toxicity and persistency of this pesticide, it is required to develop some methods to eliminate cypermethrin from the soil. The role that bacteria play in breaking down pollutants and the need for bioremediation solutions are closely related since bioremediation relies on microorganisms, particularly bacteria, to naturally break down and detoxify a variety of contaminants found in the environment. According to Ansar *et al*. [5], pollution of the air, land, and water is a major worldwide issue that is prevalent. Excavation and incineration are two costly and environmentally damaging traditional techniques of cleaning up contaminated places. For the removal of contaminated regions, bioremediation provides an economical and sustainable solution. Pesticides are metabolized by an array of different bacteria, comprising representatives of the genera Flavobacterium, Pseudomonas, Rhodococcus, and Alcaligenes [6]. An effective technique for pesticide bioremediation is the isolation of native bacteria that can metabolize specific pesticides [7]. This approach has generated a lot of their choice. "Pollutant-degrading bacteria" or "biodegraders" are common terms used to describe these microorganisms. Microorganisms, such as bacteria, convert contaminants into less complex and non-toxic molecules through a process known as biodegradation [8]. Bacteria accomplish this by converting complicated contaminants into smaller compounds that fit more easily into their cellular processes through enzymatic activities. Since they may change over time to become more adept at breaking down particular contaminants, bacteria are incredibly adaptive. According to Escher *et al*. [9], a capacity to adapt is crucial for handling the complex and varied mixes of pollutants

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present in contaminated environment. Bacteria can target a wide range of pollutants, including organic compounds, hydrocarbons, heavy metals, and pesticides. Therefore, they are versatile tools for remediating various types of pollution. In some cases, multiple bacterial species can work together in a synergistic manner to degrade complex pollutants. This can enhance the efficiency of bioremediation processes. They do not involve the transportation of contaminated materials and do not generate additional waste. Bioremediation strategies leverage the natural abilities of bacteria and other microorganisms to clean up polluted environments efficiently and cost-effectively. The present investigation was aimed to isolate and identify pesticides degrading bacteria from agriculture contaminated soil.

MATERIALS AND METHODS

Soil

The agricultural areas located in Bihar's Samastipur district, with coordinates of 25.71526 latitude and 85.8044 longitude, were the site of the soil sampling. The fact that local farmers frequently use cypermethrin as a pesticide on their farmed crops makes these fields especially fascinating. The procedure for gathering soil samples was carried out with careful consideration, ensuring the accuracy and reliability of results by collecting each sample twice. When the soil samples were collected from various regions of the district, they were carefully placed into sealed jars or containers to preserve their integrity on the way to the lab for further testing and analysis.

Chemicals and media

To make a stock solution, the pesticide Cypermethrin was dissolved in acetonitrile. To ensure the purity of this solution, any impurities were carefully filtered out and sterilized. After sterilization, the Cypermethrin stock solution was carefully refrigerated to maintain its integrity for future use. A specialized growth medium named Bushnell agar was used for the isolation and culture of bacterial strains that could break down cypermethrin. In order to provide the best conditions for bacterial development, this medium was adjusted to a neutral pH of 7.0. The method of isolating and cultivating bacterial cultures that degrade Cypermethrin was carried out meticulously and precisely using this pH-adjusted Bushnell agar.

Analysis of physico-chemical parameter

According to chemical analysis, representative samples of the initial soil were taken, air-dried, and crushed to a one-millimeter thickness. To determine the gravimetric soil moisture content, a 10g sample of soil was dried at 105 °C for a whole night [10]. A percentage (%) was used to show the moisture content. A glass electrode pH meter and electrical conductivity meter (pH HI1131) was used to measure the pH (soil: deionized water = 1:2.5 w/v) of the sample. Standard procedures were used to analyze the phosphate, magnesium, and chloride [11].

Isolation of cypermethrin degrading bacteria

To identify bacteria that degraded pesticide (Cypermethrin), minimal media containing pesticide as a carbon source was utilized. Through the use of serial dilution, the bacterial species were isolated from soil contaminated with cypermethrin. In Bushnell Haas agar media, many bacterial colonies were observed. Biochemical analysis and Gram's staining were employed to identify these colonies. As a selective enrichment agent for bacterial isolation, pesticides were added to Bushnell Haas Medium at various concentrations of 0.1%, 0.5%, and 1%.

RESULTS AND DISCUSSION

Analysis of physico-chemical parameter

Numerous physicochemical characteristics of the soil sample under investigation have been meticulously tested and recorded. These characteristics provide important insights into the composition and condition of the soil. The soil sample's pH is measured to be 7.6. One of the most important factors affecting the availability of nutrients and microbial activity in the soil is its pH value, which represents the acidity or alkalinity of the soil. The temperature of the soil is recorded at 28 degrees Celsius (28°C). Soil temperature is a significant factor affecting biological activity, nutrient cycling, and plant growth in the soil ecosystem. The moisture content of the soil is relatively high, at 87%. This measurement shows how much water is in the soil, which is essential for sustaining various soil organisms and plant life. There are 30.6 milligrams of phosphate per unit of measurement in the soil sample. A sign of the fertility of the soil, phosphate is an element essential to plant growth. It is discovered that the soil has 13.0 milligrams of magnesium per unit [12]. Another essential mineral for plants, magnesium is involved in a number of soil metabolic activities. With a measurement uncertainty of ±0.5 milligrams, the concentration of chloride in the soil is 10.5 milligrams per unit. Chloride concentrations are important to determine the salinity of the soil and can affect plant health. (Table 1) provides an extensive summary of the qualities of the soil sample by summarizing these physicochemical properties. Making conclusions about soil management and improvement techniques and determining if the soil is suitable for certain agricultural or environmental uses require an understanding of this information [13].

Table 1 Soil analysis

Soil analysis	Pesticide degrading soil
pH	7.6
Temperature °C	28 °C
Moisture content	87%
Phosphate(mg)	30.6 ± 0.0mg
Magnesium(mg)	13.0 ± 0.6mg
Chloride(mg)	10.5 ± 0.5mg

Values are mean ± Standard deviation

Isolation of cypermethrin degrading bacteria

Soil samples obtained from an agricultural area contaminated with pesticides were used to identify bacterial species. A serial dilution procedure was carried out to isolate these bacteria, which required dilution of the soil sample several times to obtain individual bacterial colonies. The isolated bacterial colonies were then cultured on Bushnell Haas agar medium, where they became separate colonies. Then, in order to identify them, these colonies performed several kinds of analytical tests. The first step was to perform Gram's staining, a method that classifies bacteria into two groups: Gram-positive and Gram-negative, according to the properties of their cell walls. Different species of bacteria were made easier to distinguish using this staining method. The bacteria underwent a series of biochemical tests after being stained with Gram's reagent. Several experiments were conducted as part of these evaluation in order to identify particular metabolic traits and chemical processes that the bacteria exhibited. Using particular nutrients, producing particular enzymes, or fermenting sugars are a few examples of these reactions. Our accurate

identification and classification of the bacterial species found in the pesticide-contaminated soil was achieved by the analysis of the test outcomes [14].

Identification and growth conditions of isolates

In this study, we examined carefully the unique characteristics linked to the development of isolated microorganisms. We used a technique that measured light absorbance at a wavelength of 600 nanometers (nm) to do this. In microbiology, this specific wavelength is frequently used to evaluate the density and growth of bacterial cultures. In this investigation, we concentrated on the bacteria *E. coli*, *P. aeruginosa* and *B. subtilis*. We measured the absorbance values at 600 nm for each of these organisms to evaluate their growth characteristics. The results indicated distinct absorbance values corresponding to every microbe, as shown in Table 2. The absorbance value of *E. coli* at 600 nm was found to be 0.55, demonstrating the extent of its growth in the specified conditions. Comparatively, *P. aeruginosa* exhibited a slightly greater absorbance value of 0.61 at the same wavelength, indicating that under the experimental conditions, its growth was a little more robust than *E. coli*. On the other hand, of the three bacteria, B. subtilis had the greatest absorbance value, measuring 1.25 at 600 nm. With a significantly higher absorbance value, *B. subtilis* seems to possess developed the most out of all the separated organisms, indicating that the population was robust and growing in the experimental setting. The absorbance measurements at 600nm provided valuable insights into the growth characteristics of *E. coli*, *P. aeruginosa* and *B. subtilis*, allowing us to distinguish the varying degrees of growth exhibited by these isolated microorganisms in our study [15].

Table 2 Analysis of cypermethrin degrading organisms

Organisms	OD value at 600nm
Escherichia coli	0.55 ± 0.35
Pseudomonas auroginosa	0.61 ± 0.30
Bacillus subtilis	1.25 ± 0.5

Values are mean ± Standard deviation

Cypermethrin utilizing bacteria

In this result of cypermethrin degradation of *Escherichia coli*, *Pseudomonas auroginosa* and *Bacillus subtilis*. In *Escherichia coli*, maximum zone of accumulate in 0.1%, 0.5%,1.0% at 2.18±0.40,1.94±0.3 and 0.77±0.20 respectively, followed by *P. aeruginosa* maximum zone of accumulate in 0.1%,0.5% and 1.0% at 2.08±0.3, 2.13±0.8, 0.66±0.14 and *B. subtilis* maximum zone of accumulate in 0.1%, 0.5% and 1.0% at 2.27±0.50, 1.96±0.4, 0.88±0.10 (Table 3).

Table 3 Total bacterial population and degradation of Cypermethrin at concentrations of (0.01 and 0.1%) in contaminated soil

Organisms	Dilution	Total viable counts (CFU/g)	Cypermethrin resistance bacterial counts (CFU/g)		
			Cypermethrin concentration		
			0.1%	0.5%	1.0%
Escherichia coli	10^{-4}	5.60 ± 0.30	3.55 ± 0.40	2.54 ± 0.3	1.00 ± 0.20
	10^{-5}	4.35 ± 0.50	2.54 ± 1.40	2.33 ± 0.2	0.70 ± 0.10
	10^{-6}	3.86 ± 0.45	1.41 ± 0.30	1.34 ± 0.6	0.65 ± 0.55
	10^{-7}	3.44 ± 0.45	1.30 ± 0.20	1.39 ± 0.3	0.56 ± 0.55
Pseudomonas auroginosa	10^{-4}	5.50 ± 0.40	3.32 ± 0.30	3.52 ± 0.30	1.07 ± 0.10
	10^{-5}	4.60 ± 0.30	2.54 ± 0.40	2.41 ± 0.40	0.50 ± 0.07
	10^{-6}	3.95 ± 0.50	1.30 ± 0.30	1.20 ± 0.20	0.50 ± 0.05
	10^{-7}	3.67 ± 0.45	1.21 ± 0.20	1.00 ± 0.01	0.40 ± 0.54
Bacillus subtilis	10^{-4}	5.60 ± 0.50	3.65 ± 0.50	2.62 ± 0.4	1.07 ± 0.10
	10^{-5}	4.32 ± 0.45	2.62 ± 0.50	2.44 ± 0.5	0.90 ± 0.54
	10^{-6}	3.94 ± 0.55	1.50 ± 0.40	1.40 ± 0.4	0.80 ± 0.47
	10^{-7}	3.32 ± 0.35	1.41 ± 0.31	1.40 ± 0.2	0.60 ± 0.46

Values are mean ± Standard deviation

Table 4 Inhibiting the growth pattern of bacteria using cypermethrin

Bacteria	Cypermethrin concentration		
	0.01%	0.5%	1%
Escherichia coli	+	+	+
Pseudomonas auroginosa	+	-	-
Bacillus subtilis	+	+	-

Cypermethrin is a pyrethroid pesticide whose persistence in the environment has caused considerable environmental and health concerns. The chemical's persistent presence in soil and water habitats endangers both human health and the planet's biodiversity. Identifying and investigating Cypermethrin-degrading bacteria has emerged as a critical part of environmental remediation in order to address this issue. Cypermethrin degradation is a complex process and microbes, particularly bacteria, play a critical role in breaking down this chemical. Understanding cypermethrin-degrading bacterium identification is critical for various reasons. Identifying these bacteria (*Escherichia coli*, *Pseudomonas auroginosa* and *Bacillus sutilis*), first and foremost, can lead to the development of bioremediation techniques. Bioremediation, which uses microorganisms metabolic ability to detoxify contaminants, provides an environmentally acceptable and cost-effective solution to cypermethrin pollution [16]. Knowing the specific bacteria involved allows us to tailor bioremediation efforts, enhancing their efficiency and reducing the harmful effects of cypermethrin. The identification of cypermethrin-degrading bacteria holds the promise of reducing the environmental burden of chemicals. The identification of cypermethrin-degrading bacteria is a significant advancement in mitigating the environmental impact of this widely used pesticide. By using these bacteria's power, we can accelerate the degradation of cypermethrin residues and significantly aid in the preservation and cleansing of the environment. Identifying these microorganisms has the potential to improve our ability to degrade things through genetic and biochemical methods. Through genetic engineering and synthetic biology, scientists can modify bacteria to improve the enzyme pathways

responsible for the breakdown of cypermethrin. This technique has the potential to lead to more efficient and targeted bioremediation strategies, boosting the effectiveness of cypermethrin removal from the environment [17].

CONCLUSION

The results of the research provide strong evidence to support utilizing *Escherichia coli*, *Pseudomonas auroginosa*, and *Bacillus subtilis* for the bioremediation of soil contaminated with cypermethrin. Their extensive distribution over the farming districts highlights their potential as useful instruments in environmental cleanup initiatives. These bacteria demonstrate resilience and efficiency when utilizing cypermethrin as a carbon, nitrogen, and energy source. We may be able to provide a long-lasting and practical solution to the serious problem of cypermethrin contaminants in agricultural soils by utilizing the natural capacities of these microbes.

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