Isolation and Characterization of Endosulfan-Degrading Bacteria from Agricultural Soils

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Abstract

Pesticides are chemical substances that are meant to kill pests. No doubt pesticide play a pivotal role in enhancing agricultural yields and safeguarding crops against pest infestations; however, their toxic nature poses substantial threats to human health, the environment and the broader ecosystem. The persistence of pesticides in the food chain further exacerbates these concerns. While traditional physical and chemical methods for pesticide removal exist, they often fall short due to limitations in efficacy and cost-effectiveness. This study investigates a viable approach to bioremediation as a means of reducing pesticide pollution, a process that leverages the remarkable pesticide-degrading capabilities of specific bacterial genera. In this investigation, three bacterial genera– Acinetobacter, Flavobacterium, and Bacillus– were identified as potent agents for pesticide degradation. Bioremediation emerges as a compelling strategy due to its inherent eco-friendliness, cost-effectiveness, and efficiency in detoxifying pesticide-contaminated environments.

Keywords: Acinetobacter, Bacillus, Bioremediation, Flavobacterium, Pesticides

Introduction

Agriculture has always been the most important sector of India. Ensuring food security for more than 1 billion Indians in the face of declining cultivable land resources involves the employment of high yielding varieties of seeds, balanced fertilizer applications, and the prudent application of quality pesticides (Naphade et al.,2012). Pesticides are chemical compounds used to eliminate pests. They are chemical or biological agents that weaken, incapacitate, and kill pests. Based on the types of targeted pests, the pesticides can be divided into several groups, namely insecticides, herbicides, rodenticides, bactericides, fungicides, and larvicides (Raffa et al.,2021). Pesticides importance in crop yield improvement is generally acknowledged and accepted. However, they continue to pose a hazard to the environment since excessive pesticide application results in the buildup of massive amounts of pesticides in soil and water, damaging the food chain

and drinking water (Rahman et al.,2018). Due to the problems of pesticide contamination, for clean-up of pesticide contaminated soil, develop technologies that guarantee their elimination. Currently bioremediation is one of the most environmentally safe and cost-effective method for decontamination and detoxification of pesticide contaminated environment.

Endosulfan is an organochlorine insecticide in the polycyclic chlorinated hydrocarbon family. It has been widely utilised in agriculture, horticulture, and forestry for more than 30 years (Daniel et al.,1986). Endosulfan is composed of two stereoisomers [alfa- and beta-endosulfan (ratio, 3:7). Thiodan, Cyclodan, Thimol, Thifar, and Malix are trademarks that have been registered (Peñuela and Barceló,1998). Endosulfan contamination and persistence in soil and aquatic habitats cause it to accumulate in phytoplankton, zooplankton, fish, and vegetable cells (Li et al.,2009). Endosulfan remains in soil and water for 3-6 months or longer (Goswami and Singh,2009). According to many research, a large variety of microorganisms are capable of decomposing pesticides (Rani et al.,2008).

A diverse group of bacteria, including members of the genera *Alcaligenes, Flavobacterium, Pseudomonas* and *Rhodococcus,* metabolize pesticides (Aislabie and Lloyd-Jones,1995). The isolation of indigenous bacteria capable of metabolising certain pesticides has received a lot of attention and is seen as an efficient method for pesticide bioremediation (Rahman et al.,2018). The present investigation was aimed to isolate and identify pesticides degrading bacteria from soil samples in Samastipur district, Bihar (India).

Methodology of the Study
Collection of soil sample

Soil samples from different farmlands near Samastipur (longitude 85.8044, latitude 25.71526) district (Bihar) area will be collected in duplicates. In each case one sample will be collected from soil contaminated with pesticides and another from pesticide free soil. After collection soil samples will be collected in sample collecting jar and will be transferred aseptically to the laboratory.
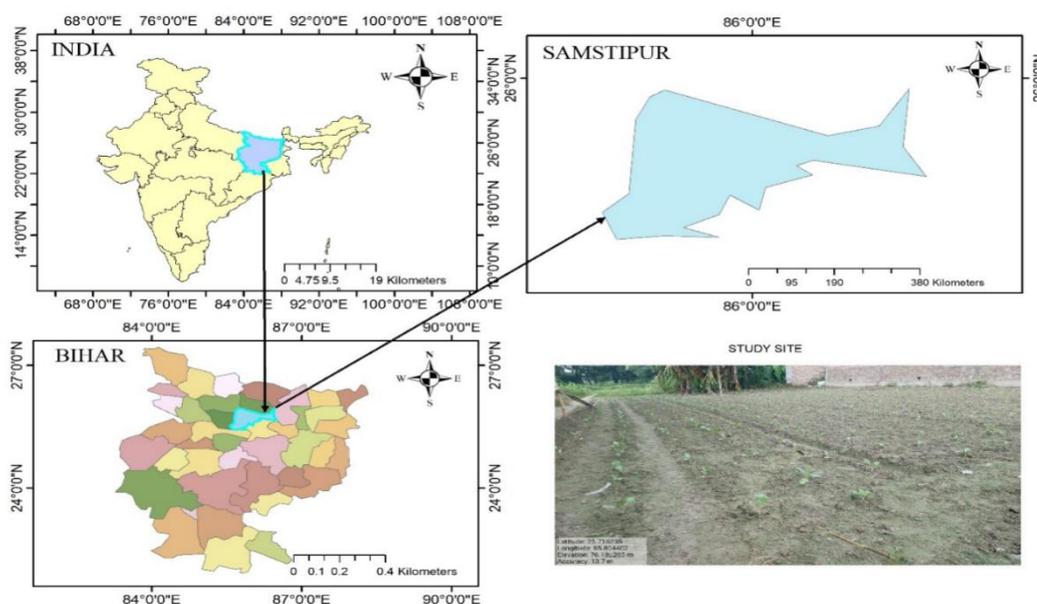
Fig. 1: Map of the study site Samastipur, Bihar, India.

Chemical and media

Pesticide (Endosulfan) dissolved in acetonitrile to make a stock solution was filter sterilized and kept in refrigerator for use. Bushnell has agar media (p^H 7.0) was used for the isolation and cultivation of Endosulfan degrading bacterial strains.

BUSHNELL HAAS (BH) MEDIUM COMPOSITION

$MgSO_4$	0.2g/l
$CaCl_2$	0.02g/l
KH_2PO_4	1g/l
K_2HPO_4	1g/l
$(NH_4)_2SO_4$	1g/l
$FeCl_3$	0.05g/l

Analysis of physio-chemical parameter

Standard procedures were used to analyse the physicochemical parameters of the soil samples, including pH, temperature, and moisture content, as well as phosphate, magnesium, and chloride (Radhika and Kannahi,2014).

Isolation of Endosulfan degrading bacteria

Minimal media containing pesticide as a carbon source will be used to isolate pesticide(Endosulfan) degrading bacteria. Microorganisms are generally found in nature (air, soil and water) as mixed populations. The bacterial species were isolated from Endosulfan contaminated soil of samastipur district (Bihar), by serial dilution method.

Different bacterial colonies were observed in Bushnell Haas agar medium. This colonies were identified by Gram's staining and biochemical tests. Pesticides will be used at different concentration of 0.1%, 0.5% and 1% in Bushnell Haas Medium as a selective enrichment agent for isolation of bacteria.

Results and Discussion

In this study, the following three bacterial genera were able to degrade endosulfan: Acinetobacter, Flavobacterium, and Bacillus.

### Physico-chemical characteristics of soil sample analysis

The physic-chemical characteristics of soil sample the p^H (7.6), Temperature (27°C), moisture content (86%), Phosphate (30.6±0.0mg), Magnesium (13.0±0.5mg) and Chloride (10.5±0.6mg) (Table1).

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Identification and growth conditions of isolates

The identification characteristics of these isolated organisms growth were observed by measuring absorbance at 600nm in Acinetobacter (0.55), Flavobacterium (0.61) and Bacillus(1.25) respectively (Table 2).
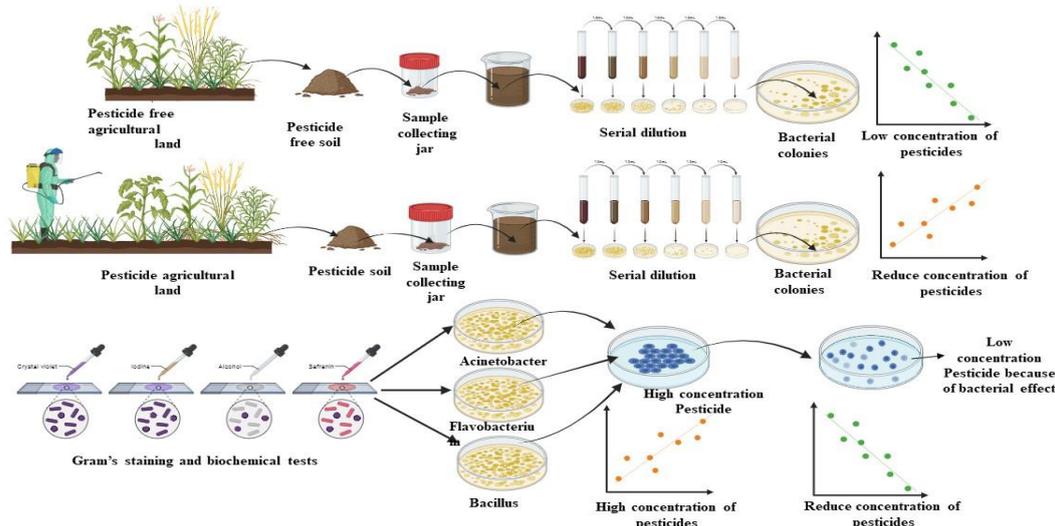
Fig 2: Experimental Workflow: Isolation and Degradation of Endosulfan by Soil Bacteria

Endosulfan utilizing bacteria

In this result of Endosulfan degradation of Acinetobacter, Flavobacterium, and Bacillus. In Acinetobacter, maximum zone of accumulate in 0.1%, 0.5%,1.0% at 2.17±0.40,1.93±0.3 and 0.77±0.20 respectively, followed by Flavobacterium maximum zone of accumulate in 0.1%,0.5% and 1.0% at 2.07±0.3, 2.12±0.8, 0.66±0.14 and Bacillus maximum zone of accumulate in 0.1%, 0.5% and 1.0% at 2.28±0.50, 1.97±0.4, 0.87±0.10(Table 3).

Table 1: soil sample analysis

Sl. No.	Soil analysis	Endosulfan degrading soil
1	p^H	7.6
2	Temperature ^0C	27^0C
3	Moisture content	86%
4	Phosphate(mg)	30.6±0.0mg
5	Magnesium(mg)	13.0±0.5mg
6	Chloride(mg)	10.5±0.6mg

Values are Mean ±standard deviation

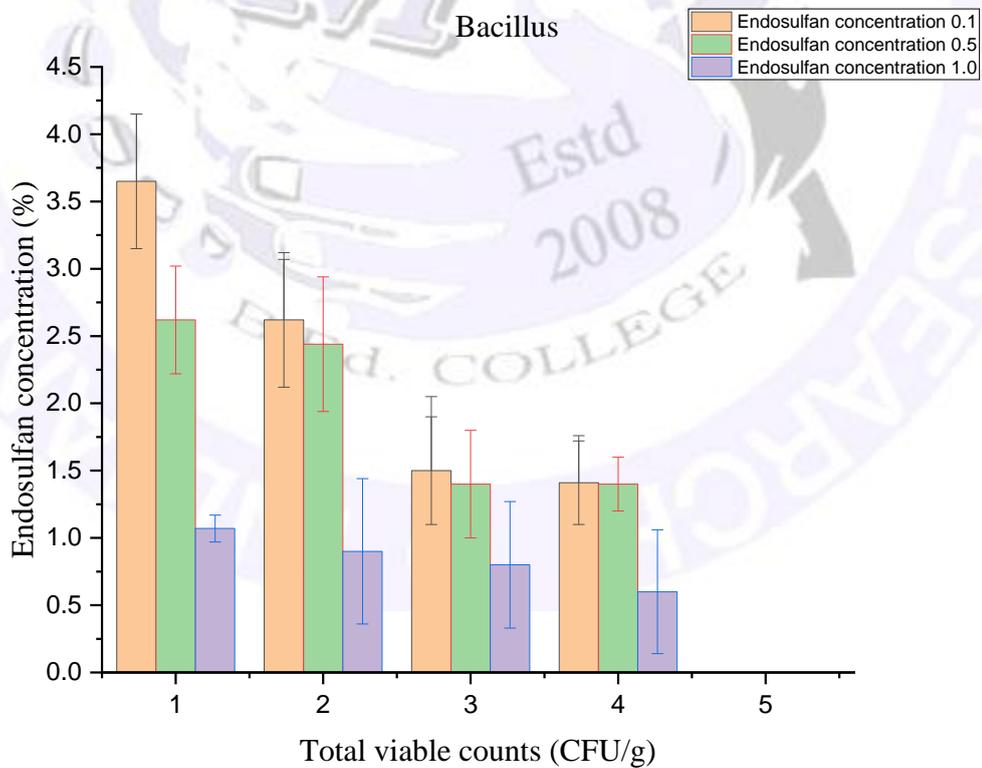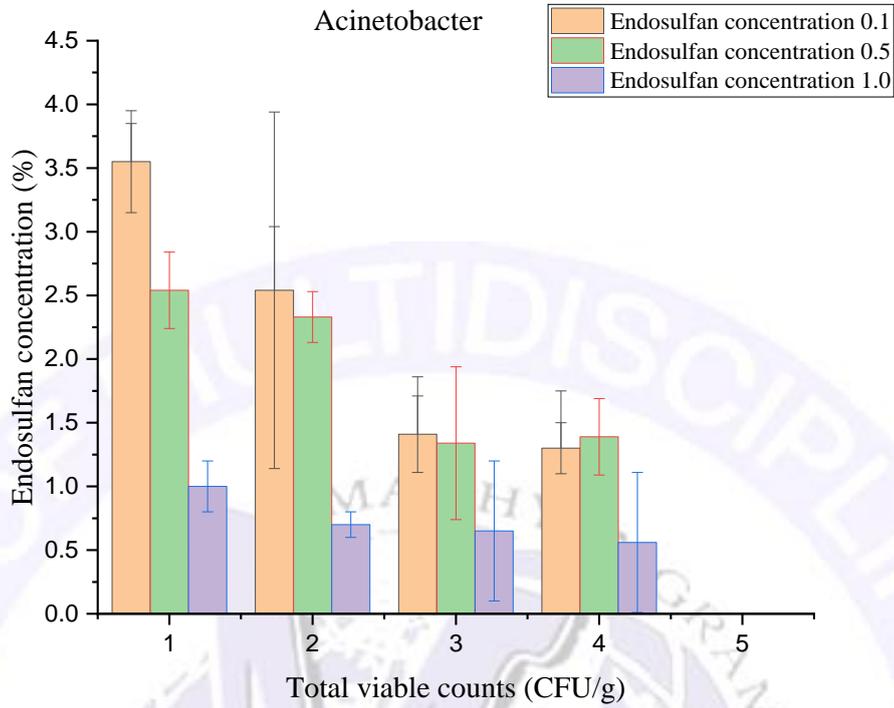**Table 2:** Analysis of Endosulfan degrading organisms

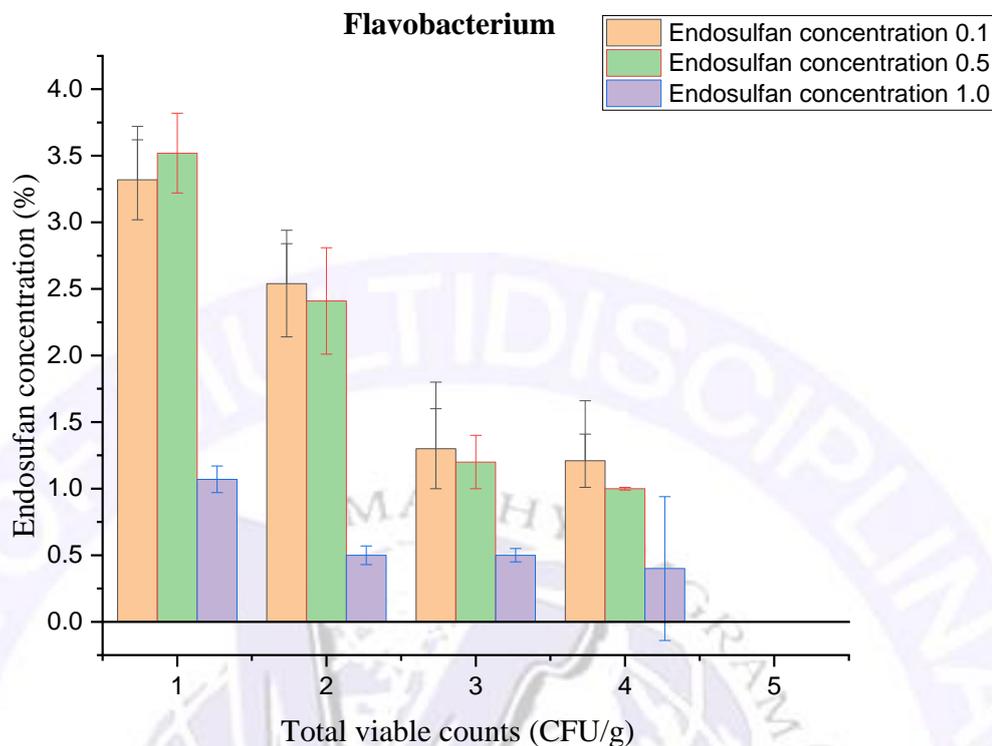| Sl. No.	Organisms	OD value at 600nm
1	Acinetobacter	0.55±0.35
2	Flavobacterium	0.61±0.30
3	Bacillus	1.25± 0.5

Values are Mean±standard deviation

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

Sl. No.	Organisms	Dilution	Total viable counts (CFU/g)	Endosulfan resistance bacterial counts(CFU/g)		
				Endosulfan concentration		
				0.1%	0.5%	1.0%
1	Acinetobacter	10^{-4} 10^{-5} 10^{-6} 10^{-7}	5.50±0.30 4.25±0.50 3.76±0.45 3.54±0.45	3.55±0.40 2.54±1.40 1.41±0.30 1.30±0.20	2.54±0.3 2.33±0.2 1.34±0.6 1.39±0.3	1.00±0.20 0.70±0.10 0.65±0.55 0.56±0.55
2	Flavobacterium	10^{-4} 10^{-5} 10^{-6} 10^{-7}	5.50±0.40 4.60±0.30 3.95±0.50 3.67±0.45	3.32±0.30 2.54±0.40 1.30±0.30 1.21±0.20	3.52±0.30 2.41±0.40 1.20±0.20 1.00±0.01	1.07±0.10 0.50±0.07 0.50±0.05 0.40±0.54
3	Bacillus	10^{-4} 10^{-5} 10^{-6} 10^{-7}	5.60±0.50 4.32±0.45 3.94±0.55 3.32±0.35	3.65±0.50 2.62±0.50 1.50±0.40 1.41±0.31	2.62±0.4 2.44±0.5 1.40±0.4 1.40±0.2	1.07±0.10 0.90±0.54 0.80±0.47 0.60±0.46

Values are Mean ±standard deviation

Fig. 3: This graph illustrates the total bacterial population and the endosulfan resistance of three bacterial genera (Acinetobacter, Flavobacterium, and Bacillus) at different dilution levels and endosulfan concentrations (0.1%, 0.5%, and 1.0%) in contaminated soil samples. The data presents the colony-forming units per gram (CFU/g) for both total viable counts and endosulfan-resistant bacterial counts.

Conclusion

Acinetobacter, Flavobacterium, and Bacillus were isolated from farmland soil sample collected from Samastipur district, Bihar (India). It can utilize Endosulfan as sole source of carbon, nitrogen and energy. Results of this study showed that endosulfan-degrading bacteria are widely distributed across various regions of the farmlands in Samastipur district, Bihar.

In conclusion, our results indicated that Acinetobacter, Flavobacterium, and Bacillus could be a good choice for the bioremediation of Endosulfan contaminated soil.

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