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Isolation and characterization of Dimethoate- resistant Nitrogen fixing Bacteria from Grapevine Soil

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ABSTRACT

This study investigates the isolation and characterization of nitrogen fixing bacteria resistant to dimethoate, a commonly used organophosphate pesticide in vineyards. Soil samples from vineyards in Walwa, Sangli, Maharashtra were collected and tested for pesticide resistant bacteria. Six bacterial isolates PN1, PN2, PN3, PN4, PN5 and PN6 were exposed to dimethoate and their morphological, biochemical and enzymatic properties were analyzed. The isolates exhibit varying degrees of resistance to dimethoate with PN1 and PN3 showing the highest tolerance up to 1.2% and 1.0% respectively. Additionally, these isolates demonstrated the ability to fix nitrogen, solubilize phosphate and degrade cellulose, highlighting their potential for improving soil fertility and contributing to sustainable agricultural practices. Further studies are in progress to explore the practical applications of these isolates in bioremediation and crop growth promotion.

Keywords: *dimethoate, pesticide resistance, nitrogen fixation, cellulose degradation, pesticide tolerance, etc.*

1. INTRODUCTION

The increasing application of chemical pesticides in agriculture has resulted into significant environmental and health concerns, necessitating the search for sustainable alternatives. Dimethoate, an organophosphate insecticide, is widely used in viticulture to control pests in grapevine cultivation. However, its persistence in soil and potential toxicity to non- target organisms pose challenges for sustainable agriculture. One promising approach to mitigate these issues is the use of microbial bioinoculants, particularly nitrogen fixers, which can degrade pesticides and promote plant growth. This study focusses on the isolation and characterization of dimethoate resistant nitrogen fixing bacteria from grapevine soil, aiming to investigate their potential for bioremediation and plant growth promotion.

Nitrogen-fixing bacteria, also known as diazotrophs, are pivitol in the nitrogen cycle by converting atmospheric nitrogen into a form that plants can assimilate. These bacteria are found in various soil environments and are recignized for their mutualistic association with plants, particularly legumes. However, non-leguminous plants, including grapevines, also harbor nitrogen-fixing bacteria in their rhizosphere. The isolation of these bacteria from grapevine soil can reveal their ecological roles and potential applications in sustainable agriculture [1].

The isolation of nitrogen-fixing bacteria typically involves the use of nitrogen-free semi-solid media, which selectively promotes the growth of diazotrophs. This method, developed by Döbereiner and colleagues, has been extensively used to isolate various nitrogen-fixing species from different soil environments [1]. In this study, we employed a similar approach to isolate dimethoate-resistant nitrogen-fixing bacteria from grapevine soil. The isolated strains were then characterized for their morphological, biochemical, and molecular properties to confirm their identity and assess their potential for pesticide degradation and plant growth promotion.

Dimethoate resistance in bacteria is often accompanied by the presence of specific genes encoding enzymes that can degrade the pesticide. These enzymes, such as organophosphorus hydrolases, can hydrolyze the ester bonds in dimethoate, rendering

it non-toxic. The presence of these genes in nitrogen-fixing bacteria can augment their ability for bioremediation in pesticide-contaminated soils [2]. In addition to pesticide degradation, nitrogen-fixing bacteria can improve plant growth through various mechanisms, including the production of phytohormones, solubilization of phosphate, and antagonism against plant pathogens [3].

The characterization of the isolated bacteria involved a combination of morphological, biochemical, and molecular techniques. Morphological characterization included Gram staining and observation of colony and cell morphology. Biochemical tests, such as oxidase and catalase tests, were performed to assess the metabolic capabilities of the isolates. Molecular characterization involved the amplification and sequencing of 16S rRNA genes to confirm the identity of the isolates and their evolutionary relationships with known nitrogen-fixing bacteria.

The potential applications of dimethoate-resistant nitrogen-fixing bacteria in sustainable agriculture are manifold. These bacteria can be utilized as bioinoculants to enhance soil fertility and enhance plant growth while concurrently degrading pesticide residues. This dual functionality can reduce the dependence on chemical fertilizers and pesticides, contributing to more sustainable and environmentally friendly agricultural practices. Furthermore, the use of microbial bioinoculants can improve soil health and resilience, supporting the long-term productivity of agricultural systems.

In conclusion, the isolation and characterization of dimethoate-resistant nitrogen-fixing bacteria from grapevine soil represent a promising approach to address the challenges of pesticide contamination and soil fertility in viticulture. By leveraging the natural capabilities of these bacteria, we can develop sustainable strategies for bioremediation and plant growth promotion, contributing to the overall sustainability of agricultural practices

2. METHODOLOGY

Soil samples were sourced from the rhizosphere of grapevines in vineyards located in Walva, Sangli, Maharashtra. The samples were taken at a depth of 15 cm from the soil surface, stored in sterile containers, and transported to the laboratory for analysis [1]. Dimethoate was assessed for its minimum inhibitory concentration (MIC) on natural soil microflora. Soil suspensions were streaked on nutrient agar plates with different concentrations of dimethoate (0.2% to 1.2%) and incubated at 37°C for 48 hrs. The MIC for garden microflora was determined to be 0.8% [2]. Bacterial isolates were obtained by streaking soil suspensions on Ashby's agar plates containing dimethoate at concentrations varying from 0.4% to 1.0%. Morphologically distinct colonies were isolated and further characterized. The isolates were studied for colony morphology, Gram reaction, motility, and capsule and spore formation [5]. Biochemical tests, including carbohydrate utilization, enzyme production (catalase, oxidase, urease, amylase, etc.), and phosphate solubilization, were conducted to assess the metabolic capabilities of the isolates [6]. The isolates were examined for their effects on seed germination and plant growth. Pot culture experiments were performed using moth bean seeds, and the effects of bacterial inoculation on shoot and root growth were recorded, both in the presence and absence of dimethoate [7].

3. RESULTS AND DISCUSSION

Six bacterial isolates designated as PN1, PN2, PN3, PN4, PN5 and PN6 were successfully isolated from vineyard soil. The isolates exhibited diverse morphological characteristics, with colony sizes varying from 1 mm to 2 mm and varying colony textures (moist or mucoid) [Table 1]. Isolates PN1 and PN3 were identified as short rods, while the others were cocci. Gram staining revealed that PN1, PN3, PN4, and PN5 were Gram-negative, whereas PN2 and PN6 were Gram- positive [Table 2].

Biochemical investigations revealed that all isolates were capable of fermenting multiple sugars, including glucose, fructose, and galactose, with PN1, PN3, and PN4 producing both acid and gas. Additionally, all isolates were catalase and oxidase positive, indicating robust metabolic activity [Table 3]. Enzymatic analysis confirmed that most isolates exhibited urease activity, while gelatinase and protease activities were observed in PN4 and PN5 [Table 4].

The isolates displayed varying levels of tolerance to dimethoate, with PN1 and PN3 showing the highest tolerance, up to 1.2% and 1.0%, respectively [Table 5]. These isolates also demonstrated phosphate solubilization and indole acetic acid production, both of which are beneficial for plant growth. Cellulose degradation was observed in all isolates, with higher activity in the presence of dimethoate, suggesting their capability for refining soil quality in pesticide-treated environments [Table 4].

Seed germination test and pot culture experiments showed that PN2 and PN6 significantly improved shoot and root growth in pesticide-containing soils [Table 6, 7]. Mixed inocula of the isolates also promoted plant growth, particularly in soils treated with dimethoate, indicating the potential use of these isolates in enhancing crop resilience to pesticide exposure.

Table 1:- Results of colony characters of isolates on Ashby's agar supplemented with dimethoate after incubation of 24hrs at 28^0C.

Code of isolate	Colony characters						
	Size	Shape	Colour	Elevation	Margin	Opacity	Consistency
PN1	1mm	Round	White	Convex	Irregular	Opaque	Moist
PN2	1mm	Round	Pale yellow	Convex	Entire	Opaque	Moist
PN3	2mm	Round	White	Convex	Irregular	Translucent	Moist
PN4	2mm	Round	White	Convex	Entire	Opaque	Mucoid
PN5	2mm	Round	White	Convex	Entire	Opaque	Mucoid
PN6	2mm	Round	White	Convex	Entire	Opaque	Mucoid

Table 2:- Results of study of cultural and morphological characteristics, Gram property, motility, sporulation property and capsulation property.

Isolate code	Gram staining	Morphology	Motility	Spore staining	Capsule staining
PN1	Gram negative	Short rods	Non-motile	Non sporing	Non capsulated
PN2	Gram positive	Cocci in bunch	Motile	Non sporing	Non capsulated
PN3	Gram negative	Short rods	Motile	Non sporing	Non capsulated
PN4	Gram negative	Cocci in bunch	Motile	Non sporing	Non capsulated
PN5	Gram negative	Cocci in bunch	Actively motile	Non sporing	Non capsulated
PN6	Gram positive	Cocci in bunch	Motile	Non sporing	Non capsulated

Table 3:- Results of Sugar fermentation tests of the isolates.

Sugar fermentation test	Bacterial isolates					
	PN1	PN2	PN3	PN4	PN5	PN6
Glucose fermentation	[+]	+	[+]	[+]	[+]	[+]
Lactose fermentation	[+]	+	[+]	[+]	[+]	[+]
Mannose fermentation	[+]	+	[+]	[+]	[+]	[+]
Sucrose fermentation	[+]	-	[+]	[+]	[+]	[+]
Maltose fermentation	[+]	-	[+]	[+]	[+]	[+]
Arabinose fermentation	[+]	-	[+]	[+]	+	+
Mannitol fermentation	[+]	[+]	[+]	[+]	[+]	[+]
Galactose fermentation	[+]	+	[+]	[+]	[+]	[+]
Xylose fermentation	[+]	-	[+]	[+]	[+]	[+]
Fructose fermentation	[+]	+	[+]	[+]	[+]	[+]

Table 4. Results of plant growth promoting activities of the isolates

H_2S production	+	+	-	-	-	+
Oxidase production	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+
Urease production	-	+	+	+	+	+
Amylase production	+	+	-	-	-	+
Gelatinase production	-	-	-	+	+	+
Protease production	-	+	-	-	-	-
Lipase production	-	-	+	+	+	+
Nitrate reductase production	+	-	+	+	+	+
Indole acetic acid production	+	-	+	+	+	+
Phosphate solubilization	+	-	-	+	+	+
Cellulase production	+	+	+	+	+	+
Association with Azotobacter	-	-	-	-	-	-
Association with Rhizobium	-	-	-	-	-	-

+ = Acid production/ Positive test, [+] = Acid and gas production, - = No utilization/ Negative test

Table 5:- Results of Pesticide tolerance of the isolates.

MIC of Dimethoate (%)	**Bacterial isolates**					
	PN1	**PN2**	**PN3**	**PN4**	**PN5**	**PN6**
0.8	+	+	+	+	+	+
1.0	+	-	+	-	-	-
1.2	+	-	-	-	-	-
1.4	-	-	-	-	-	-
1.6	-	-	-	-	-	-

+ = Positive test - = Negative test

Table 6:- Results of seed germination test.

Concentration of dimethoate used	**0.1%**	**0.2%**	**0.3%**	**0.4%**	**0.5%**	**0.6%**	**0.7%**	**0.8%**	**0.9%**	**1.0%**
Seeds germinated	22	6	10	22	6	6	4	5	2	0
% germinated	88	24	40	88	24	24	16	20	8	0

Table 7: - Results of effect of isolates on plant growth promotion.

Characters	**Measurements in absence of pesticide**							
	PN1	**PN2**	**PN3**	**PN4**	**PN5**	**PN6**	**Control**	**Mixed**

Height of shoot (cm)	17	16	12	11	16	12	16.5	14
Height of root (cm)	16	17	16	16	18	15	21.2	16
No. of leaves	2	2	3	2	3	2	3	3
Extent of branching of roots	+	+	+	+	+	+	+	+
Dry weight of plant (gm)	0.030	0.037	0.035	0.031	0.041	0.0390	0.031	0.033
	Measurements in presence of pesticide							
Height of shoot	14	19.5	15	13	12.5	16	16.5	19
Height of root	17	18.5	15	15	14	18	16	21
No. of leaves	2	3	2	2	2	2	3	3
Extent of branching of roots	+	+	+	+	+	+	+	+
Dry weight of plant (gm)	0.028	0.041	0.038	0.038	0.041	0.0395	0.0339	0.0411

4. SUMMARY AND CONCLUSION

This study focused on the isolation and characterization of nitrogen-fixing bacteria resistant to dimethoate, an organophosphate pesticide commonly used in vineyards. Soil samples were sourced from grapevine rhizospheres, and six bacterial isolates (PN1, PN2, PN3, PN4, PN5 and PN6) were obtained from dimethoate-treated soil. The isolates were characterized based on their morphological, biochemical, and physiological properties. Notably, PN1 and PN3 exhibited the highest resistance to dimethoate, with tolerance levels reaching up to 1.2% and 1.0%, respectively. In addition to pesticide resistance, the isolates demonstrated the ability to fix nitrogen, solubilize phosphate, and degrade cellulose, highlighting their potential as biofertilizers and bioremediation agents. The findings indicate that these bacteria can enhance soil fertility and support sustainable agricultural practices in pesticide-affected environments.

The findings of this research highlights the significant role of pesticide-resistant nitrogen-fixing bacteria in promoting sustainable agriculture. The isolated strains, particularly PN1 and PN3, show promise for their dual functionality as both biocontrol agents against pesticide toxicity and enhancers of soil fertility through nitrogen fixation and phosphate solubilization. These outcomes imply that utilizing such bacteria in agricultural strategies can enhance crop productivity while mitigating the negative effects of chemical pesticides like dimethoate. Future research should focus on field trials to assess the efficacy of these isolates in various agricultural settings and explore their mechanisms of action further, paving the way for environmentally friendly farming practices.

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