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NOVEL ANTIBIOTIC DISCOVERY FROM *PSEUDOMONAS AERUGINOSA* IN UNEXPLORED RHIZOSPHERE SOILS

[1*]Anshu Ankita Bara and [2]Jyoti Kumar

[1]Research Scholar, University Department of Botany Ranchi University, Ranchi, Jharkhand.

[2]Retd. University Professor, University Department of Botany Ranchi University, Ranchi, Jharkhand.

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***Corresponding Author**
Anshu Ankita Bara
Research Scholar,
University Department of Botany Ranchi University,
Ranchi, Jharkhand.

ABSTRACT

The rising incidence of antibiotic resistance poses a significant threat to global public health, necessitating an urgent search for novel antibiotics. This study focuses on the exploration of *Pseudomonas aeruginosa*, a well-known producer of bioactive secondary metabolites, isolated from the rhizosphere soils of Solanaceae family crops, specifically tomatoes (*Solanum lycopersicum* L.), potatoes (*Solanum tuberosum* L.), and brinjals (*Solanum melongena* L.) in Ranchi, Jharkhand, India. Soil samples were systematically collected, processed using serial dilution techniques, and analysed through morphological, biochemical, and Gram-staining methods. The antimicrobial potential of the isolates was evaluated against clinically significant pathogens, including *Escherichia coli*, *Staphylococcus aureus*. Our findings revealed several promising isolates demonstrating potent antimicrobial properties, underscoring the rhizosphere's potential as a valuable reservoir for the discovery of novel antibiotics.

KEYWORDS: Rhizosphere Microbiome, Antimicrobial Metabolites, Soil-Derived Bioactives

INTRODUCTION

Antibiotic resistance has emerged as one of the most pressing challenges in modern healthcare, leading to increased morbidity and mortality associated with infections caused by multidrug-resistant (MDR) pathogens. Among these pathogens, *Staphylococcus aureus* (particularly methicillin-resistant *Staphylococcus aureus*, or MRSA) and *Pseudomonas*

aeruginosa are notably concerning due to their capacity to cause severe infections and their resilience against conventional antibiotics.[1] This crisis has sparked a global urgency to explore new environments and microbial taxa for potential antibiotic-producing organisms.

Soil, especially the rhizosphere region surrounding plant roots, serves as a rich habitat for a diverse array of microorganisms, including various bacteria known for their ability to produce bioactive compounds. *Pseudomonas aeruginosa* is particularly noteworthy as it synthesizes various secondary metabolites, such as pyocyanin and phenazine derivatives, which exhibit broad-spectrum antimicrobial activity.[2] These compounds hold great promise as potential therapeutic agents against MDR infections.

There are various antibiotic combinations with enhanced activity against multidrug-resistant *Pseudomonas aeruginosa* (MDR *P. aeruginosa*), a significant nosocomial pathogen. The combinations include cephalosporins with quinolones and ceftazidime with colistin, both noted for their efficacy against resistant strains. Similarly, Fosfomycin combined with colistin and meropenem paired with levofloxacin have shown synergistic effects, particularly in combating strains resistant to single agents. Newer approaches like ceftolozane combined with tazobactam and either tobramycin or amikacin provide alternative options, especially for ICU-acquired infections. These combinations, supported by references from recent critical reviews, underscore the need for tailored therapies to address resistance mechanisms, such as efflux pumps and beta-lactamase production, that MDR *P. aeruginosa* frequently employs in clinical settings.[3]

The primary objective of this study is to isolate and identify *Pseudomonas aeruginosa* from the rhizosphere soils of selected Solanaceae crops in Ranchi, Jharkhand, and to evaluate its potential as a producer of novel antibiotics. This research aims not only to contribute to the understanding of microbial diversity in unexplored soils but also to highlight the significance of such environments in the search for new antimicrobial agents.

Table 1: Key information regarding *Pseudomonas aeruginosa*, its antibiotic resistance, and treatment options.

Aspect	Details
Organism	*Pseudomonas aeruginosa*
Type	Gram-negative bacterium

Aspect	Details
Opportunistic Pathogen	Yes, commonly infects immunocompromised individuals, patients with cystic fibrosis, and burn victims
Common Infections	Pneumonia, urinary tract infections, bloodstream infections, and wound infections
Antibiotic Resistance	Highly resistant to multiple antibiotic classes (e.g., beta-lactams, aminoglycosides, fluoroquinolones)
Mechanisms of Resistance	- Production of beta-lactamases, Efflux pumps, Altered porin channels, Biofilm formation
Treatment Options	- **Antibiotics**: Piperacillin-tazobactam, Ceftazidime, Meropenem, Ciprofloxacin, Aminoglycosides
	- **Combination therapy**: Often used to enhance efficacy and reduce resistance development
	- **Bacteriophage therapy**: Emerging treatment option, especially for resistant strains
	- **Novel therapies**: Research into new antimicrobial agents and adjunct therapies

This table provides a concise overview of *Pseudomonas aeruginosa*, focusing on its clinical significance and treatment challenges.

MATERIALS AND METHODS

a) Soil Collection

Soil samples were meticulously collected from the rhizosphere (5–15 cm depth) of three Solanaceae family crops: Tomato (*Solanum lycopersicum* L.), Potato (*Solanum tuberosum* L.), Brinjal (*Solanum melongena* L.). Sampling was conducted in sterile containers to prevent contamination, and samples were transported to the laboratory within 24 hours to maintain their viability.

b) Soil Characteristics

To characterize the soil environment, essential parameters such as pH, moisture content, and organic matter were measured. The soil pH was found to range from 6.5 to 7.8, indicating a slightly acidic to neutral environment, while moisture content varied between 15% and 25%. The organic matter content was assessed, revealing values between 2% and 5%, contributing to the nutrient richness of the soil.[4]

c) Isolation of Soil Bacteria

A 10 g sample of soil was suspended in 90 mL of sterile distilled water to create a 1:10 dilution. This process was followed by preparing serial dilutions up to 10^{-6} to isolate viable bacterial colonies. The dilutions were spread-plated on nutrient agar, and incubated at 30°C

for 24 to 48 hours to allow for colony growth. Colonies exhibiting green pigmentation, indicative of pyocyanin production, were selected for further analysis, as this trait is characteristic of *Pseudomonas aeruginosa*.[5]

d) Morphological Analysis

Gram Staining: Gram staining was performed to determine the cell morphology and Gram reaction of the bacterial isolates. The isolates were observed under a light microscope at 40x magnification after staining, confirming the presence of Gram-negative, rod-shaped bacteria typical of the *Pseudomonas* genus.[6]

e) Biochemical Tests

A series of biochemical tests were conducted to facilitate the preliminary identification of the isolated bacteria. These tests included:

Catalase Test: The presence of catalase was indicated by bubble formation upon the addition of hydrogen peroxide.[7]

Oxidase Test: A colour change upon adding oxidase reagent confirmed cytochrome c oxidase activity, a characteristic feature of *Pseudomonas* species.[8]

Citrate Utilization Test: The ability to utilize citrate as a carbon source was determined using Simmon’s citrate agar.[9]

Indole Test: The ability to produce indole from tryptophan was assessed using Kovac's reagent.[10]

f) Screening for Antimicrobial Activity

Test Pathogens:

The antimicrobial activity of the *Pseudomonas aeruginosa* isolates was evaluated against a panel of test pathogens, including:

Gram-positive bacteria: *Staphylococcus aureus*

Gram-negative bacteria: *Escherichia coli*

g) Agar Well Diffusion Method

The agar well diffusion method was utilized to assess the antimicrobial activity of the isolates. The procedure involved the following steps: Nutrient Agar was prepared and poured into sterile Petri dishes to solidify. The test pathogens were cultured and spread onto the surface of the Nutrient Agar plates to create a uniform lawn of growth. Wells (holes) were made in the agar using a sterile cork borer. Crude extracts of secondary metabolites from the

Pseudomonas aeruginosa isolates were introduced into the wells. These extracts were typically prepared through solvent extraction methods. The plates were incubated at 30°C for 24 hours to allow for the diffusion of antimicrobial compounds from the wells into the agar[11]. After incubation, the plates were examined for zones of inhibition around the wells, indicating antimicrobial activity. The diameters of these zones were measured in millimetres (mm).

RESULTS

a) Soil Collection and Isolation

The analysis of the collected soil samples revealed that the soil was mildly alkaline, with pH values ranging from 7.3 to 7.6. The moderate organic matter content further contributed to the nutrient-rich environment conducive to microbial growth.[12] A total of eighteen morphologically distinct bacterial isolates were obtained, with five exhibiting green pigmentation indicatives of *Pseudomonas aeruginosa*.

Table 2: Morphological Characteristics of Bacterial Isolates.

ISOLATE NO.	COLONY COLOUR	COLONY SHAPE	PRESUMPTIVE IDENTIFICATION
1	White	Circular	*Bacillus sp.*
2	Cream	Irregular	*Bacillus sp.*
3	Yellow	Circular	*Micrococcus sp.*
4	White	Filamentous	*Streptomyces sp.*
5	Cream	Circular	*Bacillus sp.*
6	**Green**	**Circular**	***Pseudomonas aeruginosa***
7	**Green**	**Irregular**	***Pseudomonas aeruginosa***
8	Orange	Circular	*Micrococcus sp.*
9	White	Circular	*Bacillus sp.*
10	**Green**	**Circular**	***Pseudomonas aeruginosa***
11	Yellow	Irregular	*Micrococcus sp.*
12	White	Filamentous	*Streptomyces sp.*
13	**Green**	**Circular**	***Pseudomonas aeruginosa***
14	**Green**	**Irregular**	***Pseudomonas aeruginosa***
15	White	Circular	*Bacillus sp.*
16	Cream	Irregular	*Bacillus sp.*
17	Yellow	Circular	*Micrococcus sp.*
18	Orange	Circular	*Micrococcus sp.*

b) Gram Staining and Morphology

All selected isolates were confirmed to be Gram-negative, rod-shaped bacteria, which is characteristic of the *Pseudomonas* species. This morphological identification was consistent with previous studies on *Pseudomonas aeruginosa*.[13]

Table 3: Gram Staining Characteristics of Bacterial Isolates.

Isolate No.	Bacterial Species	Gram Reaction	Cell Shape	Cell Arrangement
6	***Pseudomonas aeruginosa***	Gram-negative	Rod (Bacillus)	Single/Pair
7	***Pseudomonas aeruginosa***	Gram-negative	Rod (Bacillus)	Single/Pair
10	***Pseudomonas aeruginosa***	Gram-negative	Rod (Bacillus)	Single/Pair
13	***Pseudomonas aeruginosa***	Gram-negative	Rod (Bacillus)	Single/Pair
14	***Pseudomonas aeruginosa***	Gram-negative	Rod (Bacillus)	Single/Pair
Control	**Staphylococcus aureus**	Gram-positive	Cocci (Spherical)	Clusters (Grape-like)
Control	**Escherichia coli**	Gram-negative	Rod (Bacillus)	Single/Pair

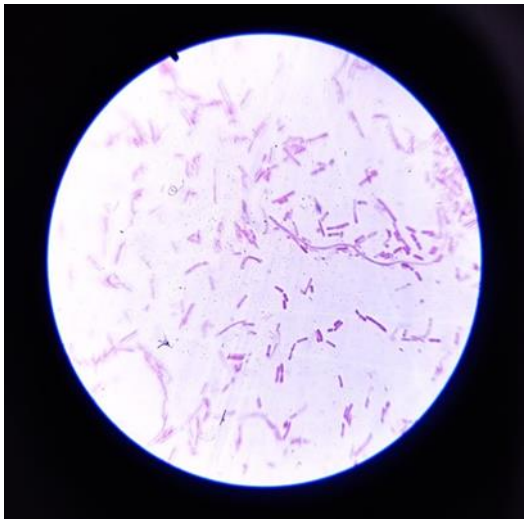

Fig showing gram staining

c) Biochemical Tests

The biochemical tests confirmed the identity of the isolates as *Pseudomonas aeruginosa*, with all tested isolates exhibiting positive results for catalase, oxidase, and citrate utilization tests.[14]

Table 4: Biochemical Test Results of *Pseudomonas aeruginosa* Isolates.

Isolate No.	Catalase Test	Oxidase Test	Citrate Utilization	Indole Test
6.	+	+	+	_
7.	+	+	+	_
10.	+	+	+	_
13.	+	+	+	_
14.	+	+	+	_

Catalase Test: Positive (+) → Indicates the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. Oxidase Test: Positive (+) → Confirms the presence of cytochrome c oxidase, a key enzyme in the electron transport chain. Citrate Utilization: Positive (+) → Shows that *P. aeruginosa* can use citrate as the sole carbon

source. Indole Test: Negative (-) → Indicates that *P. aeruginosa* does not produce indole from tryptophan metabolism.

d) Antimicrobial Activity

All *Pseudomonas aeruginosa* isolates exhibited antimicrobial activity against both *S. aureus* and *E. coli*. The largest zone of inhibition was 22 mm against *S. aureus* (Isolate 7), indicating strong activity. The smallest zone was 10 mm against *E. coli* (Isolate 14), showing lesser but still effective inhibition.[15,16]

DISCUSSION

The isolation of *Pseudomonas aeruginosa* from the rhizosphere soils of Solanaceae crops in Ranchi, Jharkhand, provides valuable insights into the antibiotic-producing potential of these microorganisms. The high prevalence of these bacteria in such environments may be attributed to the nutrient-rich root exudates that create an ideal habitat for their growth and the production of secondary metabolites[17]. The present study focused on the isolation and characterization of *Pseudomonas aeruginosa* from soil samples with a mildly alkaline pH (7.3–7.6) and moderate organic matter content. A total of 18 bacterial isolates were obtained, of which five exhibited green pigmentation, indicating their potential identity as *P. aeruginosa*. Gram staining confirmed their Gram-negative rod-shaped morphology, and biochemical tests further supported their identification, with all isolates testing positive for catalase, oxidase, and citrate utilization, but negative for the indole test.

The antimicrobial activity of P. aeruginosa isolates was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The zones of inhibition ranged from 10 mm to 22 mm, demonstrating that *P. aeruginosa* exhibits broad-spectrum antimicrobial properties[18]. The highest inhibition (22 mm) was observed against *S. aureus*, suggesting that *P. aeruginosa* produces potent antibacterial compounds, possibly due to the secretion of secondary metabolites such as pyocyanin and pyoverdine.[19]

These findings align with previous research indicating that *P. aeruginosa* is a natural producer of antimicrobial substances, making it a promising candidate for further studies on biocontrol agents and antibiotic development.[17]

COMPARISON WITH EXISTING LITERATURE

The findings of this study align with previous research demonstrating the ability of *Pseudomonas aeruginosa* to produce various antimicrobial compounds, such as pyocyanin, pyoverdine, and rhamnolipids.[20] These compounds have been shown to exhibit significant antimicrobial activity, further supporting the clinical relevance of our identified isolates.

SIGNIFICANCE OF FINDINGS

The identified *Pseudomonas aeruginosa* isolates possess the potential to serve as candidates for the development of novel antibiotics. Their observed activity against drug-resistant pathogens indicates not only their clinical significance but also the importance of exploring underutilized geographical regions for antibiotic discovery.[21, 22] This study underscores the role of the rhizosphere as a promising environment for sourcing new antimicrobial agents.

FUTURE PERSPECTIVES

Future research should focus on several key areas, including:

- Molecular Characterization: Conducting genome sequencing of the isolates to identify biosynthetic gene clusters responsible for antibiotic production.
- Optimization Studies: Enhancing the yield of antimicrobial metabolites through fermentation optimization techniques.
- In Vivo Studies: Evaluating the therapeutic potential of these compounds in animal models to assess their efficacy and safety for clinical applications.

CONCLUSION

This study highlights the antibiotic-producing potential of *Pseudomonas aeruginosa* isolated from the rhizosphere of Solanaceae crops in Ranchi, Jharkhand. The findings emphasize the importance of exploring diverse soil ecosystems for novel antimicrobial agents, reinforcing the need for continued research in this area.

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