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Isolation and identification of novel antibiotics from soil bacteria: A promising approach to combating antibiotic resistance

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Abstract- The rising global threat of antibiotic resistance underscores the urgent need to discover new antimicrobial agents. This study focuses on isolating and characterizing unique strains of soil bacteria associated with solanaceous plants in Ranchi, India, with the aim of identifying novel antibiotic compounds. A diverse range of solanaceous plants, such as tomatoes, potatoes, and peppers, have been traditionally known to host microorganisms with unique metabolic capabilities. These plant-associated bacteria often exhibit promising bioactive properties, including the production of antibiotics that may prove effective against multi-drug-resistant pathogens. In this research, soil samples were collected from various solanaceous plant rhizospheres in the Ranchi region. These samples were processed through selective isolation techniques to obtain pure bacterial cultures. The isolated strains were then studied for various morphological and biochemical tests to evaluate their antibiotic-producing potential. Initial screening revealed a rich diversity of soil bacteria, which include different genera, each with its own unique characteristics and classifications. Subsequently, the crude extracts were subjected to bioassays against a panel of pathogenic bacteria, including drug-resistant strains, to assess their antibiotic activity. The results demonstrated the presence of promising antibiotic-producing strains with broad-spectrum activity This study's findings offer potential leads for the development of novel antibiotics from soil bacteria associated with solanaceous plants in Ranchi. Such antibiotics hold promise in combatting the global health challenge posed by antibiotic-resistant pathogens, emphasizing the importance of harnessing the microbial diversity within our soil ecosystems to address this critical issue.

Key words: soil microbes, bacterial strains, drug discovery, bioactive compounds, novel antibiotics

INTRODUCTION

The emergence and proliferation of antibiotic-resistant pathogens pose a significant global health threat, necessitating the continuous search for new antibiotics to combat infectious diseases. Historically, soil has proven to be a rich source of diverse microorganisms, including bacteria capable of producing bioactive compounds with antimicrobial properties. Among the various niches within soil ecosystems, the rhizospheres of plants have been recognized as particularly fertile ground for the isolation of novel antibiotic-producing bacteria.[1,2]

Solanaceous plants, belonging to the Solanaceae family, encompass a wide array of economically important crops such as tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*), and peppers (*Capsicum* spp.). These plants have long been known to host a diverse microbial community in their root zones, offering a unique ecological niche for microorganisms.[3] This association between solanaceous plants and their rhizospheric bacteria has garnered attention due to the potential for uncovering

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microorganisms with unique metabolic capabilities, including the production of antibiotics.[4]

The soil in the Ranchi region of India, characterized by its diverse flora and agroecosystems, holds particular promise as a hotspot for the isolation of novel antibiotic-producing bacteria. Previous studies have highlighted the significance of Indian soil as a reservoir of antibiotic-producing microorganisms.[5] Furthermore, the agrarian nature of Ranchi and the prevalence of solanaceous crops in the region make it an attractive target for the exploration of plant-microbe interactions and the potential discovery of novel antibiotics.[6]

This research aims to isolate and characterize soil bacteria associated with solanaceous plants in Ranchi, with a specific focus on identifying unique strains capable of producing novel antibiotic compounds. By leveraging the rich microbial diversity within the rhizospheres of solanaceous plants, this study seeks to contribute to the ongoing efforts to combat antibiotic resistance. The exploration of soil microbiota for antibiotic discovery is rooted in the historical successes of soil-derived antibiotics. For instance, the discovery of Streptomycin, derived from soil bacteria, marked a breakthrough in treating tuberculosis and other bacterial infections.[7] The rationale behind focusing on Solanaceous plants in Ranchi arises from the unique interactions between plant roots and soil bacteria, which can result in the production of secondary metabolites, including antibiotics.[8] Ranchi, with its diverse climatic conditions and rich biodiversity, presents an ideal setting for such microbial investigations.[9] This research aligns with the growing trend of exploring novel environments for antibiotic-producing microorganisms, a strategy that is gaining traction in response to the diminishing returns from traditional sources.[10] The study's approach combines cultivation-dependent and cultivation-independent techniques, addressing the challenge that many soil microbes are recalcitrant to laboratory cultivation.[11] Advanced molecular techniques, such as metagenomic analysis, are employed to circumvent these limitations and uncover the full spectrum of microbial diversity present in the soil samples.[12]

Furthermore, the study considers the ecological and evolutionary aspects of antibiotic production, acknowledging that antibiotics may serve as signalling molecules in microbial communities, not just as antimicrobial agents.[13] This perspective provides a broader understanding of the role of antibiotics in nature and their potential applications. The potential outcomes of this research extend beyond the discovery of novel antibiotics. They encompass a deeper understanding of soil microbial ecology, particularly in the context of Solanaceous plantations in Ranchi. Such knowledge is invaluable for both ecological studies and biotechnological applications.[14] Ultimately, this research aims to contribute to the global effort to combat antibiotic resistance, a challenge that poses a significant threat to public health worldwide.[15]

MATERIALS & METHODS

Survey and Collection of Soil Samples:

Location Selection: Chose three distinct crop fields from Palandu in Ranchi, Jharkhand focusing on regions with varying soil types and vegetation, particularly around Solanaceous plants. Sample Collection, transportation and storage: Soil was collected from three different crop fields i.e., tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.) and eggplant (*Solanum melongena* L.). Clean spatula was used to dig up the soil at a particular depth i.e., 2-inch to 6-inch from the roots of the crop in to a clean airtight bag and was safely transferred to the laboratory and kept in the refrigerator under controlled conditions. Stored the samples at 4^{o}C until processing.[16,17]

Serial Dilution and Isolation of Pure Culture:

To isolate individual bacterial colonies, soil samples were serially diluted in sterile saline. 1g of dried soil was added to 10ml of saline as a stock solution, then diluted sequentially to achieve 10^{-1}, 10^{-2}, and 10^{-3} concentrations.[18] The diluted samples were plated on Nutrient Agar, prepared by autoclaving the media and Petri plates, followed by drying.[19] 0.5ml of each dilution was spread onto the agar, and the plates were incubated at 37°C for 24 hours to allow colony formation. Distinct colonies were then isolated and sub-cultured using the quadrant streaking method.

Staining characterization:

Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria. A bacterial colony was transferred to a clean slide, air-dried, and heat-fixed. The smear was stained sequentially with crystal violet, Gram's iodine, and safranin, with acetone used as a decolourizer. After each step, the slide was rinsed with distilled water. The stained smear was then examined under a microscope to observe the Gram reaction of the bacterial cells.[20,21]

Identification of Bacteria by Morphological and Biochemical Tests:

Morphological Analysis: Examined the bacterial colonies for size, shape, colour, and other characteristics under a microscope.[22] Biochemical Tests: Some tests were performed as described by The Bergey's manual such as indole test, citrate utilization test, catalase test, and oxidase test to further identify the bacterial strains.[23]

Test microorganisms for determination of antimicrobial activity and screening:

The preserved bacteria were grown in nutrient broth medium, incubated for three days. After three days the broth was centrifuged at 10,000 rpm for 20 minutes to remove the cells. The supernatant containing the active metabolites was collected.[24] The crude metabolites of all the bacteria were tested for their antibacterial activity against themselves using agar well diffusion method.[25]

RESULTS

The soil sample were collected from rhizosphere of three different types of Solanaceous plant fields - Tomato, Potato, and Eggplant- from Palandu in Ranchi, Jharkhand.

Colonies were identified on agar plates, and those with well-defined margins were subculture onto fresh medium plates. Following an incubation period, colonial morphology was observed as shown in Fig 1. Totally 45 bacterial strains with potential antibiotic production were isolated.

The crude metabolites of all the 45 bacterial isolates were tested for antibacterial activity which exhibited zone of inhibition against one or other target organisms as shown in Fig 2. Among them best 5 bacteria, named by codes are TB-0122, TB-0322, PB-0522, PB- 0422 and BB-0522 showed maximum zone of inhibition against the target pathogen strains as compared to others (shown in Table 1). From these samples, a total of five bacterial strains were isolated. These strains underwent gram staining, and biochemical characterization tests, with the results detailed in Table 2 and Table 3. Morphological features were cross-referenced with Bergey's Manual of Systematic Bacteriology for accurate identification.

The selected isolated culture strains of bacteria were identified as TB-0122 (*Pseudomonas aeruginosa*), PB-0522 (*E. coli*), PB-0422 (*Klebsiella pneumoniae*), BB-0522 (*Staphylococcus aureus*), TB-0322 (*Acinetobacter baumannii*). The bacterial cultures were examined for their ability to produce antibiotics by employing the agar well diffusion technique. The presence of inhibition zones was noted when testing them against themselves i.e., *P. aeruginosa, E.coli, K.pneumoniae, S.aureus, A.baumannii.*

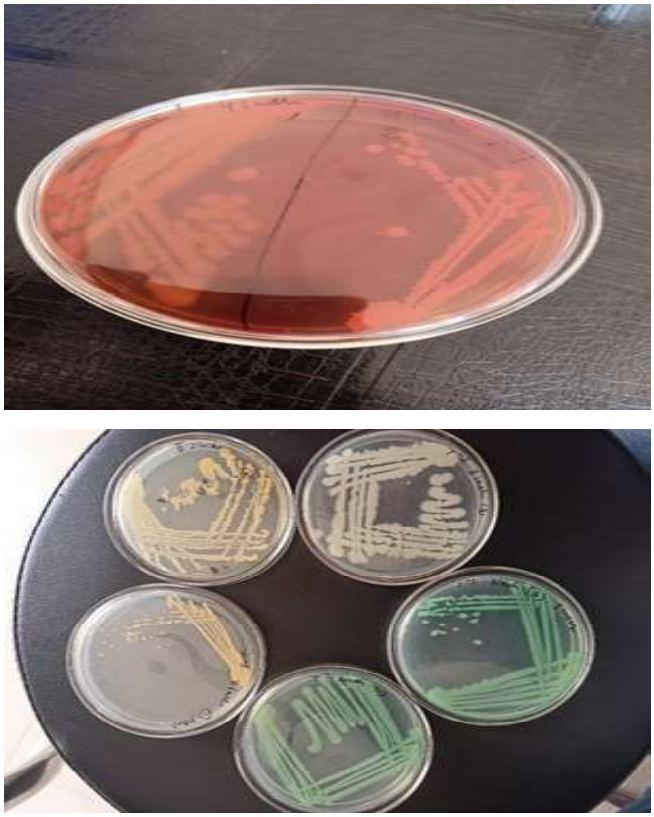

Fig 1: Showing isolated microbes

Table 1: Showing colony morphology and Gram staining of bacterial isolates

CHARACTERISTICS	TB-0122	TB-0322	PB-0522	PB-0422	BB-0522
1.) Shape of colony	Rod-shaped	Rod-shaped	Rod-shaped	Rod shaped	Spherical shaped
2.) Surface texture	Smooth	Smooth	Smooth	Smooth	Smooth
3.) Pigmentation	Sticky	Mucoid, non-pigmented	Mucoid	Mucoid	Buttery to gummy
4.) Gram staining	Gram-negative	Gram-negative	Gram-negative	Gram negative	Gram positive
5.) Colour of colony	Greenish colour	Pale yellow to greyish white	Little pink	Whitish cream	yellow

NOTE: -Name of the isolates are coded in which, 1st alphabet represents the crop name (like T for Tomato, P for Potato, and B for Brinjal); 2nd alphabet represents the Bacteria (B); next 2 digits represents plate number; and last 2 digits represents year of isolation (22 of 2022).

Table 2: Showing biochemical test results for the identification of bacterial isolates

BIOCHEMICAL TESTS	TB-0122	TB-0322	PB-0522	PB-0422	BB-0522
1) Methyl-red test	(-)	(-)	(+)	(-)	(+)
2) Citrate utilization test	(-)	(+)	(-)	(+)	(+)
3) Indole ring test	(-)	(-)	(+)	(-)	(-)
4) Catalase test	(+)	(+)	(+)	(+)	(+)
5) Voges and Proskauer test	(-)	(-)	(-)	(-)	(+)

Table 3: Showing Antagonistic activity of bacterial isolates against test pathogens

SL.NO	CODE NO. OF THE ISOLATES			TEST PATHOGENS		
		E.Coli	Klebsiella	Peudomonas	Acinetobacter	S. aureus
1.	TB-0122	10mm	11mm	--------	8mm	12mm
2.	TB-0322	10mm	10mm	8mm	--------	12mm
3.	PB-0522	-------	-------	8mm	10mm	11mm
4.	PB- 0422	-------	-------	1mm	8mm	10mm
5.	BB-0522	10mm	11mm	12mm	10mm	-------

DISCUSSION

The rhizosphere soil from Tomato, Potato, and Eggplant fields in Ranchi, Jharkhand, showed distinct soil characteristics and microbial diversity, each contributing to varying degrees of fertility and microbial activity. Understanding these differences is key to optimizing crop yields and sustainability in Solanaceous cultivation.

The study involved serial dilution and isolation of bacterial strains from these soils, revealing valuable insights into microbial diversity and antibiotic production potential. Identifying these Gram-negative and Gram-positive bacteria highlights their potential roles in agriculture, medicine, and biotechnology. Further research is needed to explore the functional roles of specific microbial communities in these soils.

Characteristics of Identified Bacterial Strains:

***Pseudomonas aeruginosa* (TB-0122):** A Gram-negative, rod-shaped bacterium, *Pseudomonas aeruginosa* is known for its metabolic versatility, biofilm formation, and antibiotic production. While common in soil, it can also be an opportunistic pathogen in humans. *E. coli* (PB-0522): *Escherichia coli* is a Gram-negative, facultative anaerobe, often found in the intestines of warm-blooded organisms. Though mostly harmless, certain strains can cause severe illness. It's widely used in research due to its rapid growth and well-characterized genetics.

***Klebsiella pneumoniae* (PB-0422):** This Gram-negative bacterium is found in soil and water but can also cause infections like pneumonia and UTIs in humans. Some strains are antibiotic-resistant, posing public health risks. *Staphylococcus aureus* (BB-0522): A Gram-positive bacterium, *Staphylococcus aureus* is part of the normal skin and nasal flora but can cause infections ranging from mild to severe. The rise of antibiotic-resistant strains like MRSA makes it a critical focus of study. *Acinetobacter baumannii* (TB-0322): *Acinetobacter baumannii* is a Gram-negative bacterium found in soil and water, known for its role as a nosocomial pathogen with high antibiotic resistance.

The identification of these bacteria from agricultural soils highlights the complex interactions between soil microbiota, human health, and environmental sustainability. While some strains are pathogenic, they also hold potential in antibiotic production, bioremediation, and agriculture. Further research is essential to understand their roles, especially concerning antibiotic resistance. Morphological and biochemical characteristics of these isolates provide valuable insights into their taxonomy, physiology, and potential functions in soil ecosystems, aiding in their identification and classification.

Morphological Characteristics: The morphological and biochemical characterization of the isolated bacterial strains from soil samples highlights their diversity and potential ecological roles. The rod-shaped morphology of TB-0122, TB-0322, PB-0522, and PB-0422 is typical of Gram-negative bacteria, while BB-0522's spherical shape is characteristic of Gram-positive cocci like Staphylococcus aureus.[26] The smooth surface texture across all isolates indicates their adaptability to various soil conditions[27], and the pigmentation in TB-0122, PB-0522, and PB-0422 suggests the production of protective compounds. Biochemical tests, such as positive results in the Methyl Red and Citrate Utilization tests, further differentiate these strains, providing insights into their metabolic capabilities and roles in nutrient cycling and plant-microbe interactions.[28] The assessment of antibiotic production through agar well diffusion confirms their potential applications in agriculture and medicine.

Agar Well Diffusion Technique Results: The isolated bacterial strains were tested for their antibiotic production potential against standard reference strains, including *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Acinetobacter baumannii*.[29,30] The presence of inhibition zones in the agar well diffusion assays indicated that the isolates produced antibacterial compounds with inhibitory activity against these reference strains.[24] These findings underscore the

ecological significance of soil bacteria in antibiotic production and their potential applications in agriculture, medicine, and environmental remediation. Further research is needed to characterize these compounds and explore their therapeutic potential.

CONCLUSION

The comprehensive study involving the isolation, identification, morphological characterization, biochemical testing, and antibiotic production assessment of bacterial strains isolated from agricultural soil samples has provided valuable insights into the microbial diversity, functional capabilities, and potential ecological roles of soil bacteria associated with different crop fields.

The isolation process yielded five distinct bacterial strains, namely TB-0122 (*Pseudomonas aeruginosa*), TB-0322 (*Acinetobacter baumannii*), PB-0522 (*E. coli*), PB-0422 (*Klebsiella pneumoniae*), and BB-0522 (*Staphylococcus aureus*), which were further characterized based on their morphological features, Gram staining properties, and biochemical profiles. Morphological observations revealed the diverse shapes and surface textures of the bacterial isolates, ranging from rod-shaped to spherical forms, with variations in pigmentation and colony colours. Gram staining confirmed the Gram-negative nature of most isolates, except for BB-0522, which was identified as a Gram-positive bacterium.

Biochemical tests provided detailed insights into the metabolic capabilities and physiological characteristics of the bacterial strains, including their ability to ferment glucose, utilize citrate as a carbon source, produce indole, and exhibit catalase activity. These tests further facilitated the identification and classification of the bacterial isolates, aligning with their respective taxonomic classifications.

Lastly, the agar well diffusion technique revealed the antibiotic production potential of the isolated bacterial cultures against a panel of reference bacterial strains, demonstrating their ability to produce antibacterial compounds or metabolites with inhibitory activity against closely related and distinct bacterial species.

In summary, the integrated analysis of isolation methods, morphological characterization, Gram staining, biochemical testing, and antibiotic production assessment has provided a comprehensive understanding of the microbial diversity, functional capabilities, and potential ecological significance of soil bacteria associated with agricultural crop fields in the studied region. These findings emphasize the ecological importance of soil microbiota in maintaining soil health, nutrient cycling, plant-microbe interactions, and their potential applications in agriculture, environmental remediation, biotechnology, and healthcare sectors. Further research and exploration are warranted to elucidate the molecular mechanisms, biosynthetic pathways, therapeutic potential, and ecological impacts of the identified bacterial isolates, fostering innovation, sustainability, and interdisciplinary collaborations in microbiology, agriculture, and environmental sciences.

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