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Isolation and identification of endophytic bacteria associated with Rhizospheric soil of *Tecomella undulata* collected from Jodhpur, Rajasthan

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Abstract
This study focuses on the isolation and identification of endophytic bacteria associated with the rhizospheric soil of *Tecomella undulata* collected from Jodhpur, Rajasthan, India. Soil samples were collected in pre-sterilized polythene bags and subjected to surface sterilization before being plated on Nutrient Agar to isolate bacterial strains. Morphological characterization through Gram staining revealed that all isolates were Gram-negative rods. Various biochemical tests assessed metabolic capabilities, including protease, gelatinase, amylase, catalase, and indole production, indicating diverse functional potentials. Notably, 83.3% of the isolates demonstrated phosphate solubilization capabilities, enhancing phosphorus availability for plant growth. Siderophore production was observed in 11 isolates, facilitating iron uptake critical for plant health. The results suggest that these endophytic bacteria can significantly contribute to plant growth by improving nutrient availability and enhancing resilience against abiotic stresses. This study highlights the potential of endophytic bacteria in promoting the growth of *Tecomella undulata*, emphasizing their role in sustainable agricultural practices in arid regions.

Keywords: Endophytic bacteria, rhizospheric soil, Tecomella undulata, phosphate solubilization, siderophore production, plant growth

Introduction

The rhizosphere, the zone of soil surrounding plant roots, is a complex and dynamic environment that harbors a diverse community of microorganisms, including bacteria, fungi, and archaea. These microorganisms play crucial roles in plant health, growth, and nutrient acquisition, often acting as natural enhancers of soil fertility. Endophytic bacteria, which reside within plant tissues without causing harm, have garnered significant attention for their beneficial effects on plant physiology, including growth promotion, disease resistance, and stress tolerance.

Tecomella undulata, commonly known as the desert teak, is a prominent tree species in the arid and semi-arid regions of India, particularly in Rajasthan. This species is not only valued for its timber but also for its ecological importance in stabilizing soil and supporting local biodiversity. The unique adaptations of *T. undulata* to harsh environmental conditions make it an ideal candidate for studying the interactions between plants and their associated microbial communities.

Recent research has highlighted the potential of endophytic bacteria to enhance nutrient availability, promote plant growth, and mitigate the effects of abiotic stresses such as drought and salinity. However, the specific endophytic bacterial communities associated with *Tecomella undulata* in the rhizospheric soil of Jodhpur remain largely unexplored. This study aims to isolate and identify the endophytic bacteria associated with the rhizospheric soil of *T. undulata* and to characterize their plant growth-promoting properties.

Understanding the diversity and functional capabilities of these endophytic bacteria is critical for developing sustainable agricultural practices, particularly in arid regions where soil fertility is often limited. This research not only contributes to the fundamental knowledge of plant-microbe interactions but also has implications for enhancing the growth and resilience of *T. undulata*, thereby promoting its conservation and utilization in sustainable forestry and agriculture.

Materials and methods

Sample collection

Sampling of rhizospheric soil of *Tecomella undulata* was done from Jodhpur, Rajasthan state, India. The soil was collected in pre-sterilized polythene bags and were stored at 4^0C. Approximately, 250 g soil were collected. Collected samples were subjected for isolation of associated bacterial strains.

Surface sterilization and isolation

Collected soil sample was surface sterilized by "immersion in 90% (v/v) ethanol for one minute followed by 1% (v/v) NaOCl for 10 min and then washed six times with sterile distilled water. Sterilized samples were plated on Nutrient Agar (NA) medium and incubated for 24-48 hrs at 30°C. The colonies appeared under were picked up and diluted by dilution series and subsequently streaked on NA agar plates". The sterility check was done by incubating the water of final wash water in NA and no growth of any microorganism was observed, hence proving the sterility check.

Morphological characterization

Grams staining: To study the morphological features of bacterial isolates, a smear from its suspension was prepared on glass slide; air dried and stained by Hucker's modified Gram's staining method. The air dried stained slides were examined under compound microscope under high power and oil immersion objectives.

Identification by culture characteristics: The bacterial colonies were examined by naked eyes as well as under stereo-binocular microscope for the external features such as size, shape, margins, elevation, optical characteristics and pigmentation. The characteristics of the bacterial isolates were noted by standard protocol.

Biochemical and physiological characterization

On the basis of morphological and cultural characteristics of colonies, the isolates were differentiated and subjected to other biochemical tests:

Protease test

The isolates were initially screened by conducting a proteolytic activity experiment on Milk Agar plates. The composition of the agar plates included 3g/L yeast extract, 5g/L peptone, 12g/L agar, and 100ml/L sterile UHT non-fat milk. The plates were injected with bacteria and then incubated for a duration of 24 hours. A clear zone of skim hydrolysis gave an indication of protease producing organisms.

Gelatinase assay

The pre-selected isolates were introduced into 10 mL of Nutrient Gelatin (NG) solution, which consisted of 13g/L of dehydrated Nutrient Broth and 120g/L of gelatin, using a stab inoculation method. A negative control was prepared with uninoculated NG. The tubes were placed in an environment with a constant temperature of around 20^0C for a duration of 48 hours. After incubation, the tubes were placed in the freezer for a duration of 15 minutes to allow them to harden. Cultures that remained liquefied produced gelatinase and demonstrate rapid gelatin hydrolyses.

Catalase test

The enzyme catalase test was performed by inoculating and incubating the isolate in LB broths for 24 to 48 hours at 37^0C. a loop of bacterial culture was spreaded on slide and few drops of of 3% H_2O_2 was added. The presence of effervescence indicated positive result.

H_2S production

The ability of organism to produce H_2S from sulfur containing amino acid or inorganic sulfur compounds is done by using lead acetate paper strips from HiMedia. Bacteria were inoculated in peptone water A lead acetate paper strip was inserted between the plug and inner wall of culture tube and incubated at 30°C for 18-24 hours. Change in the color of the tip or the entire strip to black indicates the production of H_2S.

Indole production

The development of indole was identified by applying Kovac's reagent and examining the forming of a red circle. New isolates were inoculated in tryptone broth with 0.2 ml Kovac's reagent (conc. HCl, 25 ml; amyl alcohol, 75 ml; p-dimethyl amino benzaldehyde, 5g) and thoroughly combined.

Amylase

"Starch hydrolysis test was done to study the activity of amylase. The endophytic isolates were streaked on nutrient agar plates containing 2 per cent insoluble starch and incubated at room temperature. Hydrolysis of starch was tested by flooding with iodine solution and the plates were observed for the presence of clear zones surrounding the colonies and considered for positive reaction".

Urease activity

Inoculated cultures were in urea broth with pH indicator phenol red. The organisms producing ammonia raises the pH of the broth. Within 4-5 days of developing, extreme change in color from yellow to deep pink of the broth was observed as positive result.

Indole Production test

This test determines the ability of microbes to degrade the amino acid tryptophan with the help of an enzyme tryptophanase. In this SIM agar, containing substrate tryptophan is used. After inoculation and incubation Kovacs reagent is added and agitated. Production of cherry red colored layer indicates the production of indole

Methyl Red and Voges Proskauer Test

"The Methyl red and Voges-Proskauer (MR VP) broth prepared in two sets was inoculated with the endophytic isolates and incubated for 48 hours at 30°C. To the first set of tubes, few drops of an alcoholic solution of methyl red were added. The development of distinct red color was indicative of positive reaction for MR test. α-naphthol solution (5 per cent solution in 70 per cent ethyl alcohol) was added to the second set of tubes and shaken gently for 15 min". The positive reaction of acetyl methyl carbinol production was indicated by development of red color. This indicates positive result for the VP test**.**

Citrate utilization

Simmon's Citrate Agar was used to verify citrate use. Plates were incubated and observed for the change in colour of the medium from greenish to yellow.

Phosphate solubilization

Phosphate is one of the most vital nutrients next to nitrogen for microbes. Several species of bacteria and fungi degrade and solubilize the insoluble phosphates into soluble forms through the mechanism of secretion of some organic acids Pikovskaya's agar medium, was used to determine the ability of bacteria to solubilize phosphate. Spot inoculation was done on Pikovskaya's agar medium. After incubation of 48-72 hours formation of clear zone around the colonies were considered as positive.

Siderophore production

Siderophore are the iron chelator that complexes with iron and make them readily available to the plant root surfaces. In soil a competition exists among the microorganism for iron uptake. Siderophore production increases with the amount of organic substrates added to the soil. Production of siderophore was determined by using Chrome Azurol S agar medium given by Schwyn and Neilands, 1987. Bacteria culture was spotted on CAS plate. After incubation at 28°C for 3 days, siderophore production was assessed on the basis of development of orange halo against a dark blue background.

Results and discussion

The morphological and biochemical characteristics of the endophytic bacterial isolates from the rhizospheric soil of

Tecomella undulata from Jodhpur are summarized in Table 1. All isolates were identified as Gram-negative rods, indicating a consistent morphological trait among the tested strains. This finding aligns with previous studies suggesting that rod-shaped bacteria are prevalent in soil ecosystems.

Biochemical Test Results

The biochemical tests revealed diverse metabolic capabilities among the isolates. The catalase test showed that all strains, except JRs4 and JRs11, produced catalase, indicating their ability to detoxify hydrogen peroxide. This trait is crucial for survival in oxidative environments.

Amylase production was observed in JRs1, JRs3, JRs4, JRs5, and JRs8, suggesting their potential in starch hydrolysis, which can be beneficial for plant growth by increasing nutrient availability. Conversely, JRs2, JRs4, and JRs9 did not exhibit amylase activity, indicating varying degrees of starch degradation capabilities. Amylase production by endophytic bacteria is helpful for the plants since amylase enzyme aids in breakdown of starch into simpler sugars such as maltose and glucose, therefore presence of amylase-producing bacteria aids the plant in hydrolyzing the starch, thereby making it more accessible for plants. The simpler sugars released during this process serve as source of energy for plant growth and is particularly beneficial in nutrient-poor soils wherein plant roots struggle to access available carbohydrates. Apart from this, amylase production can directly alter the soil nutrient dynamics, thereby enabling indirect plant growth. Presence of amylase producing bacteria is particularly beneficial for unlocking the locked soil nutrition, facilitating its uptake by the plant (Hu *et al.*, 2022 [11]; Dat *et al.*, 2021) [7].

The methyl red (MR) test indicated that JRs1, JRs3, JRs5, and JRs10 are capable of mixed acid fermentation, which can be advantageous in nutrient-poor conditions. The Voges-Proskauer (VP) test showed positive results for JRs1, JRs3, JRs5, and JRs8, indicating the ability to produce acetoin, another metabolic product of significance. The methyl red test is indicative of the bacterial ability to perform mixed acid fermentation, leading to production of acidic metabolic products (including lactic acid, acetic acid, and formic acid), all of which are particularly beneficial in lowering the pH of the surrounding environment. Apart from this, mixed acid fermentation aids in nutrient mobilization, enabling acidification of the rhizosphere to release essential nutrients from the soil and facilitates their uptake by the plant roots. Mixed acid fermentation also supports increased plant nutrient uptake by lowering the ph of the root hair zone, enabling the plants to better absorb nutrients from the soil. It is particularly favourable for absorption of nutrients that are less soluble under neutral or alkaline conditions. Therefore, endophytic bacteria, being able to show positive methyl red test shows the ability of bacteria to augment nutrient availability to the plants, aiding in increased plant growth (Anjum *et al.*, 2015 [2]; Bhagya *et al.*, 2019) [3].

Lipase production was noted in JRs1, JRs3, JRs4, JRs5, JRs7, JRs8, and JRs10, highlighting their potential in lipid degradation, which can enhance nutrient cycling in the rhizosphere. Lipases aid in breakdown of lipids to fatty acids and glycerol. The soil harbouring plants is rich in lipid containing organic matter, however, the availability of lipids to plants is restricted owing to presence of lipase enzyme. In this context, lipase producing bacteria aid in degradation of lipids to fatty acids and glycerol, making these lipids available to plants in the form of fatty acids. Therefore, In this context, lipase producing bacteria are essential for maintaining optimum plant health. Lipase producing bacteria aid in promoting plant growth in a number of ways, including:

- **Nutrient cycling:** Lipase-producing bacteria induce breakdown of lipids to produce fatty acids, thereby improving nutrient cycling in the soil. Fatty acids and glycerol thus produced by bacteria can be used by plants or other soil organisms, augmenting the soil nutrient content.

- **Improved soil structure:** The presence of lipase producing bacteria aids in decomposition of organic matter present in the soil, thereby contributing to improvement of soil texture as well as improved water retention in the soil. Therefore, presence of lipase producing bacteria aids in enhancement of root growth by providing a better medium for root penetration as well as root expansion.

- **Protection against pathogens:** Production of antimicrobial compounds by lipase-producing bacteria aids in providing plant protecting by restricting the growth of malicious plant pathogens (Lestari *et al.*, 2018 [15]; Cardoso *et al.*, 2020 [4]; Khan *et al.*, 2023; Prajitha *et al.*, 2024) [23].

Gelatinase activity was demonstrated by several isolates, while urease activity was consistently positive among most strains, suggesting their role in nitrogen cycling. Gelatinase activity was demonstrated by 4 endophytic bacterial strains, including, JRs4, JRs5, JRs7 and JRs8. Gelatinase activity by endophytic bacteria aids in contributing to plant growth in a number of ways, including, enhancement of soil nutrient availability, improvement of soil structure, leading to promotion of overall plant health.

Degradation of Organic Matter: Endophytic bacteria produce gelatinase enzyme, which causes hydrolysis of gelatin to smaller peptides and amino acids, increasing their bioavailability to the plants. Therefore, gelatinase producing bacteria decompose complex organic matter in the soil, facilitating release of nitrogenous compounds to plants. Nitrogen being a critical macronutrient to plant growth, contributes to increased plant growth by augmenting the biosynthesis of amino acids and proteins, leading to increased plant growth.

Breakdown of gelatin by gelatinase producing bacteria ensures nutrient rich rhizosphere, ensuring beneficial microbial interaction with plant roots. Increased microbial activity therefore ensures increased nutrient uptake, promoting higher plant growth and augmented ability of plants to fight biotic and abiotic stresses (Van Phoung N et al., 2021; Mai et al., 2022) [19].

Urease activity was consistently positive among most strains, suggesting their role in nitrogen cycling. Out of the 12 reported rhizospheric soil bacterial strains, 6 strains showcased potent urease activity, including, JRs2, JRs3, JRs4, JRs5, JRs7 and JRs8. Urease activity by endophytic bacteria plays a crucial role in promoting plant growth in a number of ways, which include- Augmentation of nitrogen availability, Improvement of soil fertility, Promotion of Plant Growth and Development, Soil pH Regulation, Reduction of Soil Stress, Enhancement of Plant-Microbe Symbiosis (Yang *et al.*, 2024 [30]; Haque *et al.*, 2023 [10]; Das *et al.*, 2022) [6].

Citrate utilization was positive in JRs1, JRs5, JRs7, and JRs8, indicating their potential to utilize citrate as a sole carbon source, which is beneficial in nutrient-deficient soils. Citrate-utilizing bacteria aid in nutrient cycling in the soil, particularly in the context of nutrient-deficient or acidic soils. Such bacteria can be beneficial for the plants, owing to several reasons, that include- Increased nutrient availability, Soil pH regulation, Utilizing of citrate by the bacteria aids in balancing soil pH, particularly in alkaline or nutrient-poor soils, ensuring favourable environment for plant growth (Mahmood *et al.*, 2012) [18].

Out of the 12 isolated rhizospheric soil bacterial endophytes, 10 were found to be motile. Motility aids in bacterial colonization as well as migration of the bacteria in rhizosphere. Motile bacteria are therefore better equipped to reach plant roots and establish colonization there. Furthermore, high motility of the bacteria enables them to move towards root exudates, which is important for exchange of nutrients and promotion of plant growth. Motile bacteria are therefore more likely to set up beneficial interactions with roots of plants and showcase improved nutrient uptake coupled with increased disease resistance and increased plant growth via biofilm formation (Knights *et al.*, 2021 [14]; Compant *et al.*, 2010 [5]; Turnbull *et al.*, 2001) [27].

10 of the isolated rhizospheric soil bacterial endophytes showed protease production. Protease production by endophytic bacteria aids in degradation of rhizospheric proteins to release amino acids as well as other nitrogen rich compounds in the soil. This ensures increased bioavailability of the nitrogen for plants, contributing to increased protein synthesis, chlorophyll production, and improved overall plant metabolism. Furthermore, protease producing bacteria can aid in killing of plant phytopathogens, thereby aiding in plant production (Rana *et al.*, 2020 [25]; Rana *et al.*, 2022) [24].

Only 3 of the isolated rhizospheric soil bacterial endophytes showed hydrogen sulfide production. Hydrogen sulphide production aids in efficient sulfur cycle in the soil, since these bacteria can reduce sulphur compounds, converting them into easily assimilable forms for plants. Also, production of hydrogen sulfide in moderate concentrations aids in plant defense and strengthens its stress tolerance capacity, especially in soils containing heavy metal toxicity (Eida *et al.*, 2022; Soumyamol *et al.*, 2023) [26].

Plant Growth-Promoting Properties

Indole production by endophytic bacteria is a crucial trait for promotion of plant growth. The ability to produce indole by the bacteria JRs9 is indicative of its ability to contribute to auxin like activity, which aids the plant in cell division and root elongation, thereby contributing to overall plant growth. Also indole production by bacteria enables the plant in adjusting to the environmental stressful conditions by modulation of plant growth patterns and augmentation of nutrient uptake. This trait enables the bacteria JRs9 in promotion of plant health in nutrient deficient conditions (Etesami *et al.*, 2015 [9]; Khan *et al.*, 2016 [12]; Panigrahi *et al.*, 2020 [20]; ALKahtani *et al.*, 2020) [1].

The isolates demonstrated various plant growth-promoting properties, such as phosphate solubilization and siderophore production. Phosphate solubilization was observed in multiple strains, indicating their potential to enhance phosphorus availability for plants, a critical nutrient for plant growth.

This was followed by analysis of phosphate solubilization traits of the isolated endophytic bacteria from rhizosperic soil of *Tecomella undulata* in Jodhpur region. The results identified that nearly 10 (83.3%) of the isolated endophytic bacteria were found to be efficient in phosphate solubilization. Phosphate solubilization enables the growth and well being of host plants in a number of ways, which include- Phosphate solubilizing bacteria (PSB) enable increased bioavailability of phosphorus to plant by converting the insoluble phosphorus from soil to its soluble form via action of phosphatases; Increased bioavailability of soil phosphorus causes increased root development, better flowering and stronger fruit formation; Better phosphorus absorption enables better adaptability of plants towards abiotic stresses like drought, salinity, and temperature extremes; Increased availability of phosphorus indirectly augments the uptake of other essential nutrients, such as nitrogen and potassium; PSB act as natural biocontrol agents and prevent the growth of harmful pathogens that infect plants or compete for space and resources (Walia *et al.*, 2017 [29]; Oteino *et al.*, 2015 [21]; Mei *et al.*, 2021) [20].

Apart from phosphate solubilization, siderophore production was observed in nearly 11 isolates, reflecting their ability to chelate iron from the environment, which is essential for plant health and growth. Iron is present in the soil in limited quantity, and is not easily available to the plants due to poor solubility. Endophytic bacteria produce siderophores which chelate iron, making it easily available to the plants. Siderophores easily facilitate iron uptake by plants by augmenting photosynthesis, respiration, and nitrogen fixation. Furthermore, siderophores also aid in plant protection from pathogens, by restricting the growth of iron-dependent microbes. Siderophore producing bacteria augment resistance of plants to abiotic stresses such as salinity, nutrient deficiency and drought, improving overall resilience (Maheshwari *et al.*, 2019 [18]; Li *et al.*, 2023).

Table 1: Morphological, Biochemical and plant growth promoting test of isolates isolated from rhizospheric soil of Jodhpur district.

Strain code	Gram staining	shape	catalase	amyalse	MR	lipase	Indole	VP	gelatinase	Urease	Citrate	Motility	protease	H2S	Phosphate solublization	Siderophore test
JRs1	-	Rod	+	+	+	+	-	+	-	-	+	+	+	+	+	+
JRs2	-	Rod	+	-	-	-	-	+	-	+	+	+	+	+	+	+
JRs3	-	Rod	+	+	-	+	-	+	-	+	+	+	+	+	+	+
JRs4	-	Rod	-	+	-	+	-	+	+	+	+	+	+	-	-	+
JRs5	-	Rod	+	+	-	+	-	+	+	+	+	+	+	-	+	+
JRs6	-	Rod	+	+	-	+	-	+	+	+	+	+	+	-	+	+
JRs7	-	Rod	+	-	-	+	-	+	+	+	+	-	+	-	+	+
JRs8	-	Rod	+	+	-	+	-	+	+	+	+	+	+	-	+	+

JRs9	-	Rod	+	-	-	-	+	+	-	-	-	+	+	-	+	+
JRs10	-	Rod	+	+	+	-	-	-	-	-	-	+	+	-	+	+
JRs11	-	Rod	+	-	-	-	-	-	-	-	-	+	+	-	+	+
JRs12	-	Rod	+	-	-	-	-	-	-	-	-	+	+	-	+	+

Conclusion

The isolation and identification of endophytic bacteria from the rhizospheric soil of *Tecomella undulata* in Jodhpur, Rajasthan, reveal a diverse array of bacterial strains with significant plant growth-promoting properties. The characterized isolates exhibited various biochemical and physiological traits, including protease and amylase production, catalase activity, and phosphate solubilization, all of which contribute to enhanced nutrient availability and improved plant health.

The findings underscore the crucial role of endophytic bacteria in promoting plant growth, particularly in nutrient-poor and arid environments. These bacteria not only facilitate the breakdown of organic matter and the mobilization of essential nutrients but also enhance plant resilience against biotic and abiotic stresses. Their ability to produce siderophores further emphasizes their potential to improve iron availability, thereby supporting plant vitality and growth.

In conclusion, the endophytic bacteria associated with *Tecomella undulata* present a valuable resource for developing sustainable agricultural practices and enhancing soil fertility. Future research should focus on exploring the mechanisms underlying these beneficial interactions and their application in improving the growth and health of economically and ecologically significant plant species in arid regions. The insights gained from this study could contribute to more sustainable forestry practices and the conservation of biodiversity in desert ecosystems.

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