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Research paper

Isolation and Characterization of Plant Growth-Promoting Actinomycetes from the Rhizosphere of *Azadirachta indica*

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KEYWORDS

Azadirachta indica

actinomycetes

rhizosphere

plant growth-promoting microorganisms

phosphate solubilization

indole-3-acetic acid

ABSTRACT

Actinomycetes are an important group of rhizosphere microorganisms that contribute to nutrient cycling and plant growth through diverse metabolic activities. The present study aimed to isolate and characterize culturable actinomycetes from the rhizosphere of Azadirachta indica and evaluate their qualitative plant growth-promoting attributes. Rhizosphere soil samples were processed by the serial dilution spread plate technique on Starch Casein Agar, resulting in the isolation of eight morphologically distinct actinomycete isolates (ACN01–ACN08). The isolates were characterized on the basis of morphological, physiological and biochemical characteristics. Considerable phenotypic diversity was observed among the isolates with respect to colony pigmentation, mycelial structure and physiological characteristics. All isolates exhibited positive catalase, oxidase, starch hydrolysis, urease and citrate utilization reactions, whereas differences were observed in hydrogen sulfide production, methyl red reaction and salt tolerance. Qualitative screening of plant growth-promoting attributes revealed that six isolates exhibited phosphate-solubilizing activity, four showed zinc and potassium solubilization, seven produced indole-3-acetic acid (IAA), and six produced ammonia. Among the recovered isolates, ACN02, ACN05 and ACN07 expressed multiple plant growth-promoting attributes. Overall, the findings demonstrate that the rhizosphere of Azadirachta indica harbours phenotypically diverse actinomycetes with multiple plant growth-promoting characteristics and highlights the potential of this ecological niche as a source of beneficial microorganisms for future agricultural applications.

1. Introduction

The rhizosphere is a biologically active zone of soil surrounding plant roots where microorganisms interact closely with plants. These interactions influence nutrient cycling, organic matter decomposition and several processes that contribute to plant growth and soil fertility. Root exudates released into the rhizosphere provide carbon sources and other nutrients that support diverse microbial communities, resulting in distinct microbial populations associated with different plant species (Vishwakarma et al., 2020).

Actinomycetes constitute an important group of filamentous, Gram-positive bacteria commonly found in rhizosphere soils. They play a significant role in the decomposition of complex organic matter and the recycling

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of nutrients within soil ecosystems. Many actinomycetes also produce extracellular enzymes, antibiotics and other bioactive metabolites that enhance their ecological competitiveness. In addition, several species have been reported to promote plant growth through nutrient solubilization, phytohormone production and other beneficial activities, making them important microorganisms for sustainable agricultural applications (Elshafie & Camele, 2022).

Azadirachta indica A. Juss. (Neem) is a medicinal tree widely distributed in tropical and subtropical regions. The plant contains several biologically active compounds, including azadirachtin, nimbin, salannin and diverse phenolic constituents, which contribute to its medicinal and antimicrobial properties (Yadav, 2024). These metabolites, together with root exudates released into the surrounding soil, influence the composition and activity of rhizosphere microbial communities. Consequently, the neem rhizosphere represents a suitable ecological niche for isolating actinomycetes with diverse physiological and functional characteristics.

Plant growth-promoting actinomycetes enhance plant development through multiple mechanisms. These include the solubilization of insoluble forms of phosphorus, zinc and potassium, thereby improving nutrient availability in the rhizosphere. Many isolates also produce indole-3-acetic acid (IAA), which promotes root development, and ammonia, which contributes to nitrogen availability. The presence of multiple plant growth-promoting traits within a single isolate is considered advantageous because plant growth promotion generally results from the combined action of several biological processes rather than a single mechanism (Anwar et al., 2016).

The rhizosphere of *Azadirachta indica* has received comparatively less attention as a source of culturable actinomycetes. Consequently, information on their morphological, physiological, biochemical and plant growth-promoting characteristics remains comparatively scarce. Characterization of these properties provides baseline information for identifying promising actinomycete isolates for future taxonomic and functional investigations.

Accordingly, the present study was undertaken to isolate culturable actinomycetes from the rhizosphere of *Azadirachta indica* and characterize them using morphological, physiological and biochemical approaches. The isolates were further screened qualitatively for selected plant growth-promoting attributes, including phosphate, zinc and potassium solubilization, indole-3-acetic acid production and ammonia production.

2. Materials and Methods

2.1 Study Site and Sample Collection

Rhizosphere soil samples were collected from healthy *Azadirachta indica* A. Juss. (Neem) trees growing within the campus of Patanjali Bio Research Institute, located at Patanjali Food & Herbal Park, Laksar Road, Haridwar, Uttarakhand, India. Prior to sampling, surface litter and loose soil were carefully removed to minimize contamination. Rhizosphere soil adhering closely to the root system was collected from a depth of approximately 5–15 cm using sterile spatulas. Approximately 200–300 g of soil was aseptically transferred into sterile polyethylene bags and transported to the laboratory for microbiological analysis. The samples were processed within 24 h of collection and stored at 4°C until further use. All microbiological procedures were performed under aseptic conditions throughout the study.

2.2 Isolation of Actinomycetes

Actinomycetes were isolated from rhizosphere soil by the serial dilution spread plate technique (Muleta & Assefa, 2018). Briefly, 10 g of rhizosphere soil was suspended in 90 mL of sterile distilled water and mixed thoroughly to obtain the initial soil suspension. Serial ten-fold dilutions were prepared up to 10^{-6} using sterile distilled water. Aliquots (0.1 mL) from appropriate dilutions were spread uniformly onto Starch Casein Agar (SCA) plates and incubated at 28 ± 2°C for 7–10 days. Colonies exhibiting characteristic actinomycete morphology, including dry, chalky, leathery or powdery colonies with filamentous growth, were selected and purified for further characterization.

2.3 Purification and Maintenance of Actinomycete Isolates

Presumptive actinomycete colonies exhibiting distinct morphological characteristics were purified by repeated streaking on fresh Starch Casein Agar plates until pure cultures were obtained. Purified isolates were maintained on Starch Casein Agar slants at 4°C and periodically subcultured to preserve their viability throughout the study.

2.4 Phenotypic Characterization of Actinomycete Isolates

2.4.1 Morphological Characterization

Purified actinomycete isolates were examined for their morphological characteristics following growth on Starch Casein Agar. Colony characteristics including aerial mass colour, reverse-side pigmentation, melanoid pigment production and mycelial structure were recorded. The isolates were differentiated according to the phenotypic characteristics commonly employed for the characterization of actinomycetes (Li et al., 2016).

2.4.2 Physiological Characterization

The physiological characteristics of the actinomycete isolates were evaluated by assessing their ability to grow under different environmental conditions. Growth was evaluated at 20°C and 44°C, at pH 5 and pH 10, and in media supplemented with 10% and 20% NaCl. Growth under each condition was recorded qualitatively based on visible colony development (Mondal & Thomas, 2022).

2.4.3 Biochemical Characterization

Biochemical characterization of the actinomycete isolates included catalase activity, oxidase activity, starch hydrolysis, gelatin hydrolysis, urease activity, hydrogen sulfide production, methyl red (MR) test, Voges–Proskauer (VP) test and citrate utilization following standard microbiological protocols (Mondal & Thomas, 2022). Carbon source utilization was evaluated using media supplemented individually with L-arabinose, sucrose, xylose, inositol, mannitol, fructose, rhamnose, maltose, raffinose and cellulose. Growth was recorded qualitatively following incubation.

2.5 Screening of Plant Growth-Promoting Attributes

2.5.1 Phosphate Solubilization

Phosphate-solubilizing activity was evaluated on Pikovskaya's agar medium containing insoluble tricalcium phosphate as the sole phosphorus source (Hussain et al., 2016). Fresh cultures of the actinomycete isolates were spot inoculated onto the medium and incubated at 28 ± 2°C for 5–7 days. Formation of a clear halo zone surrounding the colonies was considered indicative of phosphate-solubilizing activity.

2.5.2 Zinc Solubilization

Zinc-solubilizing activity was determined using Zinc Solubilizing Medium (ZSM) (Jalaluddin et al., 2024). Fresh cultures were spot inoculated onto the medium and incubated at 28 ± 2°C for 5–7 days. Formation of a clear halo zone around the colonies was considered indicative of zinc solubilization.

2.5.3 Potassium Solubilization

Potassium-solubilizing activity was evaluated using Aleksandrov medium containing an insoluble potassium source (Patel & Patel, 2021). Fresh cultures were spot inoculated onto the medium and incubated at 28 ± 2°C for 5–7 days. Formation of a clear halo zone around the colonies was considered indicative of potassium solubilization.

2.5.4 Indole-3-Acetic Acid (IAA) Production

Indole-3-acetic acid (IAA) production was determined using the Salkowski colorimetric method (Gang et al., 2019). Fresh actinomycete cultures were inoculated into Nutrient Broth supplemented with L-tryptophan and incubated at 28 ± 2°C for 48 h. Following incubation, the cultures were centrifuged, and the cell-free supernatant was mixed with an equal volume of Salkowski reagent. Development of a pink colour after incubation in the dark was considered indicative of IAA production.

2.5.5 Ammonia Production

Ammonia production was determined by inoculating fresh actinomycete cultures into sterile peptone water followed by incubation at 28 ± 2°C for 48–72 h. After incubation, Nessler's reagent was added to each culture. Development of a yellow to brown colour was considered indicative of ammonia production (Borah & Thakur, 2020).

3. Results

3.1 Isolation of Actinomycetes from the Rhizosphere of Azadirachta indica

Serial dilution and culture-dependent isolation of rhizosphere soil collected from healthy *Azadirachta indica* trees resulted in the successful recovery of morphologically diverse actinomycetes. Following incubation on Starch Casein Agar, colonies exhibiting characteristic actinomycete morphology, including dry, chalky to powdery appearance with filamentous growth, were readily distinguished from other microbial colonies. Based on colony morphology, eight representative isolates were selected, purified by repeated streaking and designated ACN01–ACN08 for subsequent characterization.

The purified isolates exhibited distinct colony characteristics, indicating the presence of a heterogeneous culturable actinomycete population associated with the neem rhizosphere.

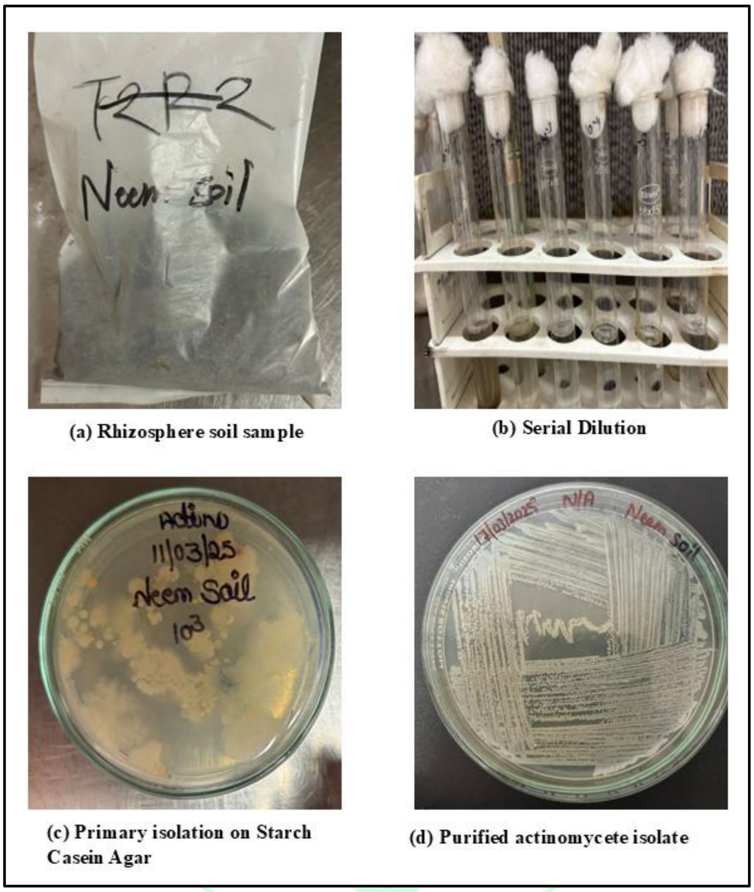

Fig. 1 Isolation of actinomycetes from the rhizosphere of *Azadirachta indica*. (a) Rhizosphere soil collected from the root zone of *A. indica*; (b) preparation of serial dilutions of the soil sample; (c) growth of actinomycete colonies on Starch Casein Agar; and (d) purification of selected actinomycete isolates by the streak plate method

3.2 Morphological Characterization of Actinomycete Isolates

The morphological characteristics of the eight actinomycete isolates are presented in Table 1. Considerable variation was observed among the isolates with respect to aerial mass colour, reverse-side pigmentation, melanoid pigment production and mycelial structure.

Six isolates (ACN01–ACN05 and ACN08) produced white aerial mycelia, whereas ACN06 exhibited light lavender aerial growth with blackish lavender pigmentation, and ACN07 developed brown aerial mycelia accompanied by dark brown reverse pigmentation. Reverse-side pigmentation was absent in six isolates but was distinctly observed in ACN06 and ACN07. Melanoid pigment production was detected only in ACN07, while the remaining isolates were non-melanoid producers.

The isolates also differed in their mycelial morphology. Flexible mycelia were observed in ACN01 and ACN05, whereas ACN03, ACN04 and ACN06 exhibited straight mycelia. ACN02 showed flexible, irregularly branched mycelia, ACN07 displayed irregularly branched and straight mycelia, while ACN08 was characterized by a zigzag mycelial arrangement. These observations demonstrate appreciable morphological diversity among the actinomycete isolates recovered from the rhizosphere of *Azadirachta indica*.

Table 1 Morphological characteristics of actinomycete isolates recovered from the rhizosphere of *Azadirachta indica*

S.N.	Isolates	Aerial Mass Colour	Reverse Side Pigmentation	Melanoid Production	Mycelial Structure
1	ACN01	White	–	–	Flexible
2	ACN02	White	–	–	Flexible, Irregularly branched
3	ACN03	White	–	–	Straight
4	ACN04	White	–	–	Straight
5	ACN05	White	–	–	Flexible
6	ACN06	Light Lavender	Blackish Lavender	–	Straight
7	ACN07	Brown	Dark Brown	Blackish brown	Irregularly branched, Straight
8	ACN08	White	–	–	Zigzag

3.3 Physiological and Biochemical Characterization of Actinomycete Isolates

The physiological and biochemical characteristics of the eight actinomycete isolates are presented in Table 2. All isolates were non-motile and exhibited positive reactions for catalase and oxidase activities, indicating their aerobic metabolic characteristics. Similarly, all isolates demonstrated positive reactions for starch hydrolysis, urease activity and citrate utilization, whereas none of the isolates exhibited gelatin hydrolysis or a positive Voges–Proskauer (VP) reaction.

Variation was observed in hydrogen sulfide production and methyl red (MR) reactions. Hydrogen sulfide production was detected in seven isolates (ACN02–ACN08), while ACN01 did not produce hydrogen sulfide. A positive MR reaction was observed only in ACN02, whereas the remaining isolates were negative.

Carbon source utilization studies revealed that all actinomycete isolates were capable of utilizing the tested carbon sources, including L-arabinose, sucrose, xylose, inositol, mannitol, fructose, rhamnose, maltose, raffinose and cellulose, demonstrating broad metabolic versatility.

The physiological responses of the isolates under different environmental conditions also exhibited distinct patterns. All isolates showed growth at 20°C and pH 5, whereas none of the isolates grew at 44°C, pH 10 or in the presence of 20% NaCl. Growth in medium containing 10% NaCl was observed only for ACN01 and ACN02, suggesting comparatively greater salt tolerance in these two isolates than in the remaining isolates.

Overall, the physiological and biochemical characterization demonstrated a high degree of similarity among the isolates with respect to their core metabolic traits, while differences in hydrogen sulfide production, methyl red reaction and salt tolerance provided useful characteristics for differentiating individual isolates.

Table 2 Physiological and biochemical characteristics of actinomycete isolates recovered from the rhizosphere of *Azadirachta indica*

Characteristics	ACN01	ACN02	ACN03	ACN04	ACN05	ACN06	ACN07	ACN08
Motility	Non-motile	Non-motile	Non-motile	Non-motile	Non-motile	Non-motile	Non-motile	Non-motile
Catalase activity	+	+	+	+	+	+	+	+
Oxidase activity	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+
Gelatin hydrolysis	–	–	–	–	–	–	–	–
Urease activity	+	+	+	+	+	+	+	+
Hydrogen sulfide production	–	+	+	+	+	+	+	+
Methyl red (MR) test	–	+	–	–	–	–	–	–
Voges–Proskauer (VP) test	–	–	–	–	–	–	–	–
Citrate utilization	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+
Cellulose	+	+	+	+	+	+	+	+
Growth at 20°C	+	+	+	+	+	+	+	+
Growth at 44°C	–	–	–	–	–	–	–	–

Growth at pH 5	+	+	+	+	+	+	+	+
Growth at pH 10	–	–	–	–	–	–	–	–
Growth in 10% NaCl	+	+	–	–	–	–	–	–
Growth in 20% NaCl	–	–	–	–	–	–	–	–

Note: (+) Positive reaction or growth; (–) Negative reaction or no detectable reaction/growth

3.4 Plant Growth-Promoting Attributes of Actinomycete Isolates

The plant growth-promoting (PGP) potential of the actinomycete isolates was evaluated qualitatively by assessing phosphate, zinc and potassium solubilization, indole-3-acetic acid (IAA) production, and ammonia production. Considerable variability was observed among the isolates with respect to the intensity and distribution of these traits, as summarized in Table 3 and illustrated in Fig. 2.

3.4.1 Phosphate Solubilization

Phosphate-solubilizing activity was detected in six of the eight isolates (75%). Among these, ACN02 and ACN05 exhibited strong phosphate-solubilizing activity (+++), while ACN01 and ACN07 showed moderate activity (++). ACN04 and ACN08 demonstrated weak phosphate solubilization (+), whereas ACN03 and ACN06 did not exhibit detectable phosphate-solubilizing activity under the experimental conditions.

3.4.2 Zinc Solubilization

Zinc-solubilizing activity was observed in four isolates (50%). Moderate zinc solubilization (++) was recorded for ACN05 and ACN08, whereas ACN02 and ACN06 exhibited weak activity (+). The remaining isolates did not produce detectable zinc-solubilizing activity.

3.4.3 Potassium Solubilization

Potassium-solubilizing ability was detected in four isolates (50%). ACN07 showed strong potassium-solubilizing activity (+++), while ACN03 exhibited moderate activity (++). Weak potassium solubilization (+) was observed in ACN01 and ACN05, whereas the remaining isolates did not produce detectable halo zones.

3.4.4 Indole-3-Acetic Acid Production

IAA production was one of the most frequently observed plant growth-promoting traits among the recovered actinomycetes. Positive IAA production was detected in seven isolates (87.5%), with ACN01 and ACN05 exhibiting strong activity (+++). Moderate IAA production (++) was observed in ACN02, ACN04 and ACN08, while ACN03 and ACN07 exhibited weak activity (+). Only ACN06 failed to produce detectable IAA under the conditions employed.

3.4.5 Ammonia Production

Ammonia production was observed in six isolates (75%). Strong ammonia production (+++) was detected in ACN02 and ACN06, while ACN01, ACN05 and ACN07 exhibited moderate activity (++). ACN04 showed weak ammonia production (+), whereas ACN03 and ACN08 were negative for this trait.

The qualitative screening demonstrated that the recovered actinomycete isolates differed considerably in their plant growth-promoting potential. Among the isolates, ACN05 exhibited the broadest spectrum of PGP attributes, showing strong phosphate solubilization and IAA production together with moderate zinc solubilization, potassium solubilization and ammonia production. Similarly, ACN02 displayed strong phosphate solubilization and ammonia production along with positive zinc solubilization and IAA production, whereas ACN07 demonstrated strong potassium-solubilizing activity in addition to phosphate solubilization and ammonia production. These observations indicate that several isolates possess multiple plant growth-promoting characteristics and may therefore be considered promising candidates for further evaluation under greenhouse and field conditions.

Table 3 Qualitative screening of plant growth-promoting attributes of actinomycete isolates recovered from the rhizosphere of *Azadirachta indica*

Isolate	Phosphate solubilization	Zinc solubilization	Potassium solubilization	IAA production	Ammonia production
ACN01	++	–	+	+++	++
ACN02	+++	+	–	++	+++
ACN03	–	–	++	+	–
ACN04	+	–	–	++	+
ACN05	+++	++	+	+++	++
ACN06	–	+	–	–	+++
ACN07	++	–	+++	+	++
ACN08	+	++	–	++	–

Note: (–) No detectable activity; (+) Weak activity; (++) Moderate activity; (+++) Strong activity

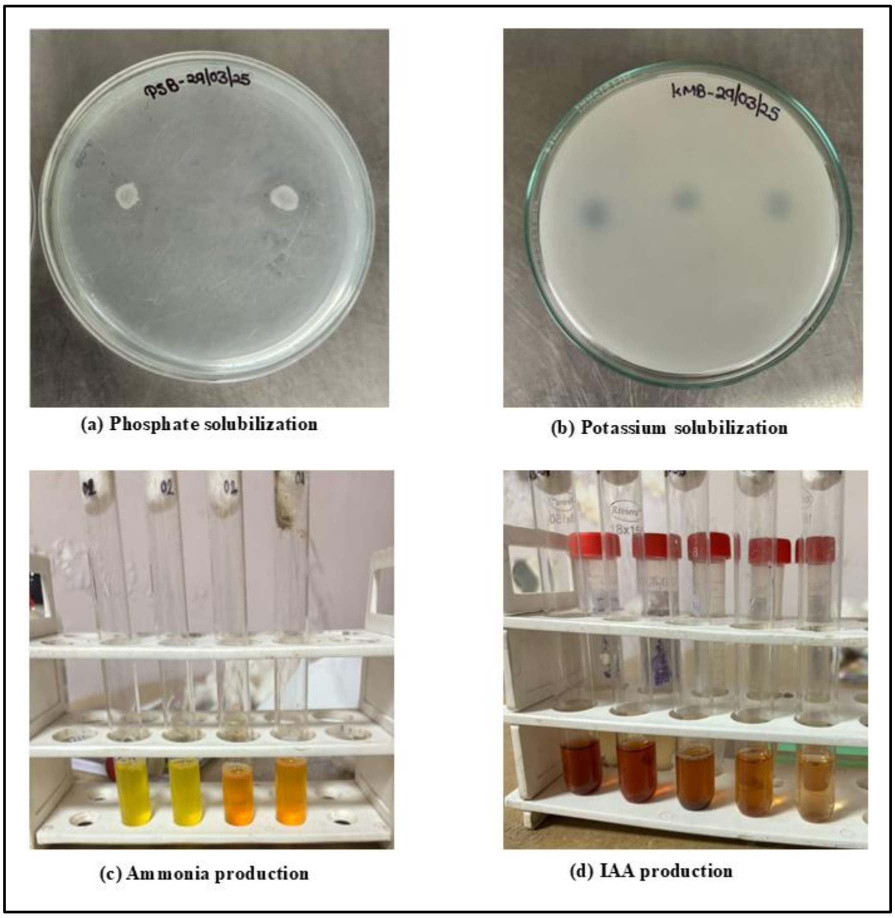

Fig. 2 Representative qualitative screening of plant growth-promoting attributes of actinomycete isolates recovered from the rhizosphere of *Azadirachta indica*: (A) phosphate solubilization on Pikovskaya's agar, (B) potassium solubilization on Aleksandrov medium, (C) indole-3-acetic acid production following reaction with Salkowski reagent, and (D) ammonia production following the addition of Nessler's reagent

4. Discussion

Actinomycetes constitute an important component of the rhizosphere microbiome and play a significant role in maintaining soil health through organic matter decomposition, nutrient cycling and the production of a wide range of bioactive metabolites (Bhatti et al., 2017). The rhizosphere of medicinal plants provides a chemically dynamic environment that supports diverse microbial communities, largely influenced by root exudates and secondary metabolites (Singh & Dubey, 2018). The successful recovery of eight morphologically distinct actinomycete isolates in the present investigation demonstrates that the rhizosphere of *Azadirachta indica* represents a favourable ecological niche for culturable actinomycetes exhibiting diverse phenotypic and functional characteristics.

The recovered isolates displayed considerable variation in aerial mycelial colour, reverse-side pigmentation, melanoid pigment production and mycelial structure. White aerial mycelia predominated among the isolates, whereas pigmentation was observed only in ACN06 and ACN07. Colony morphology remains one of the primary criteria used for the preliminary differentiation of actinomycetes prior to molecular identification (Madhurama et al., 2014). Similar variation in colony pigmentation and mycelial morphology has been reported among rhizosphere-associated actinomycetes isolated from medicinal and agricultural plants (Muleta & Assefa, 2018). Collectively, these observations suggest that the recovered isolates represent a phenotypically diverse actinomycete population adapted to the rhizosphere environment of *A. indica*.

Although morphological characteristics alone are insufficient for taxonomic identification, they provide valuable preliminary information for selecting isolates for further characterization. Reverse pigmentation and melanoid production observed in selected isolates may reflect differences in secondary metabolite biosynthesis and ecological adaptation. Pigment production has frequently been associated with protection against oxidative stress and enhanced competitiveness under diverse soil conditions (Chaturvedi et al., 2024; Lima et al., 2017). Consequently, the phenotypic diversity observed among the recovered isolates may represent adaptive responses to the chemically complex rhizosphere environment of neem.

Physiological and biochemical characterization further demonstrated the metabolic versatility of the recovered actinomycetes. All isolates exhibited catalase and oxidase activities together with positive starch hydrolysis, citrate utilization and urease activity. Catalase and oxidase activities are characteristic of aerobic microorganisms and contribute to protection against reactive oxygen species, whereas extracellular hydrolytic enzymes enable actinomycetes to utilize complex organic substrates present in soil (Behie et al., 2017; Gomes et al., 2018). The consistent occurrence of these characteristics among all isolates suggests that these metabolic functions are well conserved within the culturable actinomycete population associated with the neem rhizosphere.

Variation in hydrogen sulfide production, methyl red reaction and tolerance to saline conditions further distinguished the recovered isolates. Only ACN01 and ACN02 exhibited growth in the presence of 10% NaCl, whereas none of the isolates tolerated 20% NaCl. Such physiological variation indicates differences in environmental adaptability among individual isolates. Likewise, the ability of all isolates to utilize the tested carbon sources demonstrates broad metabolic flexibility, enabling them to exploit a wide range of organic compounds present within the rhizosphere (Mohammadipanah & Wink, 2016). These characteristics are likely to contribute to their ecological persistence under varying soil conditions.

Evaluation of plant growth-promoting attributes revealed that most of the recovered actinomycetes possessed one or more beneficial characteristics, although the intensity of individual activities differed among isolates. Approximately three-quarters of the isolates exhibited phosphate-solubilizing activity, whereas half of the isolates demonstrated zinc- and potassium-solubilizing abilities. Indole-3-acetic acid production was the most frequently detected plant growth-promoting attribute, being observed in seven of the eight isolates. The occurrence of multiple beneficial traits among the recovered isolates suggests that multifunctional plant growth-promoting characteristics are common among culturable actinomycetes associated with the rhizosphere of *A. indica*.

Phosphate solubilization was particularly evident in ACN02 and ACN05, both of which exhibited strong activity during qualitative screening. Microbial phosphate solubilization is recognized as an important mechanism for improving phosphorus availability through the production of organic acids and phosphatases (Alori et al., 2017). The predominance of phosphate-solubilizing isolates observed in the present investigation suggests that phosphorus mobilization may represent an important ecological function of actinomycetes inhabiting the neem rhizosphere. In addition, several isolates demonstrated the ability to solubilize zinc and potassium, indicating the presence of multiple nutrient-mobilizing mechanisms. Similar observations have been reported for rhizosphere actinomycetes capable of improving the availability of essential mineral nutrients required for plant growth (Rani et al., 2018).

IAA production represented the most prevalent plant growth-promoting characteristic among the recovered isolates. Microbial synthesis of IAA influences root elongation, lateral root formation and overall root system development, thereby improving the uptake of water and mineral nutrients by plants (Hakim et al., 2021). The high frequency of IAA-producing isolates recovered from the neem rhizosphere indicates that phytohormone production may be a common functional characteristic of these microorganisms. Ammonia production was detected in most isolates, suggesting an additional mechanism that may contribute to nitrogen availability within the rhizosphere.

Among all recovered isolates, ACN02, ACN05 and ACN07 expressed the widest range of plant growth-promoting attributes. ACN05 exhibited strong phosphate solubilization together with positive zinc solubilization, potassium solubilization, IAA production and ammonia production. ACN02 combined strong phosphate-solubilizing ability with high ammonia production, whereas ACN07 demonstrated the strongest potassium-solubilizing activity while also expressing several additional plant growth-promoting traits. Although these observations were obtained from qualitative assays, the coexistence of multiple beneficial characteristics within individual isolates identifies ACN02, ACN05 and ACN07 as suitable candidates for future physiological, molecular and greenhouse investigations. Similar multifunctional actinomycetes have been considered promising microbial resources because they may influence plant growth through several complementary mechanisms rather than a single biological activity (Boukhatem et al., 2022; Sáhó et al., 2024). The present investigation has certain limitations that should be considered while interpreting the findings. Identification of the recovered isolates was based on classical morphological, physiological and biochemical characterization, whereas molecular identification using 16S rRNA gene sequencing was beyond the scope of the study. Similarly, plant growth-promoting activities were assessed qualitatively, and quantitative estimation of nutrient-solubilizing efficiency and metabolite production was not performed. In addition, the investigation was restricted to a single sampling site and did not include greenhouse or field validation of plant growth-promoting activity. Nevertheless, these findings provide a useful foundation for identifying promising actinomycete isolates for detailed characterization and future evaluation.

Future investigations should focus on molecular identification of the recovered isolates, quantitative assessment of their plant growth-promoting activities and evaluation of their effectiveness under greenhouse and field conditions. Investigation of their antimicrobial properties, secondary metabolite profiles and interactions with economically important crops would further improve understanding of their ecological significance and agricultural potential. Collectively, the findings of the present investigation demonstrate that the rhizosphere of *Azadirachta indica* harbours a functionally diverse population of culturable actinomycetes possessing multiple plant growth-promoting attributes and represents a valuable source of microorganisms for future microbiological and agricultural research.

5. Conclusion

The present study demonstrated that the rhizosphere of *Azadirachta indica* is a valuable source of culturable actinomycetes exhibiting considerable morphological, physiological and biochemical diversity. Eight actinomycete isolates were successfully recovered and characterized, revealing variation in colony morphology, physiological responses and biochemical properties. Qualitative screening further demonstrated that several isolates possessed important plant growth-promoting attributes, including phosphate, zinc and potassium solubilization, together with indole-3-acetic acid and ammonia production. Among the recovered isolates, ACN02, ACN05 and ACN07 exhibited multiple plant growth-promoting traits, indicating their potential for further evaluation as beneficial rhizosphere microorganisms. Although the present investigation was limited to qualitative screening and classical characterization, the findings provide a valuable foundation for future studies involving molecular identification, quantitative evaluation of plant growth-promoting activities and validation under greenhouse and field conditions. Overall, the study highlights the diversity of neem rhizosphere-associated actinomycetes and supports their further investigation as promising microbial resources for the future development of sustainable bioinoculants for agricultural applications.

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