GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Isolation and Characterization of Plant Growth-Promoting Rhizobacteria Associated with *Sesbania sesban* (L.) Merr. in Tan Hung commune, Tay Ninh Province, Vietnam

Minh Tam Hoang * and Thi Ngoc Thanh Dang

Faculty of Natural Science Pedagogy, Saigon University, 273 An Duong Vuong Street, Cho Quan Ward, Ho Chi Minh City, Vietnam.

GSC Biological and Pharmaceutical Sciences, 2026, 35(02), 089-099

Publication history: Received on 25 March 2026; revised on 04 May 2026; accepted on 07 May 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.35.2.0173

Abstract

This study aimed to isolate and characterizes indigenous plant growth-promoting rhizobacteria (PGPR) associated with *Sesbania sesban* (L.) Merr. in Tan Hung commune, Tay Ninh province, Vietnam. Twenty bacterial isolates were obtained from rhizosphere soil and quantitatively screened for key functional traits, including biological nitrogen fixation, phosphate and potassium solubilization, and indole-3-acetic acid (IAA) production. Morphological analysis revealed a diverse bacterial community, dominated by rod-shaped (70%) and Gram-positive (55%) isolates. All isolates demonstrated nitrogen-fixing and phosphate-solubilizing capabilities, although their efficiencies varied significantly. Strains DD04 and DD11 exhibited the highest nitrogen fixation capacity (1.66 mg/L NH_4^+). For phosphate solubilization, DD19 showed the highest average value (119.01 mg/L P_2O_5), while DD09 reached the maximum concentration (172.66 mg/L) after 20 days. Additionally, DD10 and DD20 were the most efficient IAA producers, with peak concentrations of 3.95 mg/L and 4.02 mg/L, respectively. Potassium solubilization was observed in 30% of the isolates, with DD13 showing the highest efficiency (1.41). These findings highlight the potential of S. sesban rhizosphere as a reservoir of beneficial bacteria, with several elite strains representing promising candidates for sustainable biofertilizer development in Vietnam.

Keywords: *Sesbania sesban (*L.) Merr.; PGPR; Nitrogen fixation; Phosphate solubilization; Indole-3-acetic acid; Tay Ninh province.

1. Introduction

Leguminous plants play an essential role in sustainable agriculture due to their capacity to establish symbiotic relationships with nitrogen-fixing microorganisms, which convert atmospheric nitrogen into forms available for plant uptake. This biological nitrogen fixation significantly reduces dependence on chemical nitrogen fertilizers while improving soil fertility and ecosystem sustainability [1, 2]. In addition to nitrogen fixation, legumes often interact with diverse plant growth-promoting bacteria (PGPB) inhabiting the rhizosphere, root nodules, and internal plant tissues. These microorganisms enhance plant growth through multiple mechanisms such as nutrient solubilization, phytohormone production, and improved nutrient acquisition [3, 4]. Consequently, plant-associated beneficial bacteria have received increasing attention as a sustainable strategy for developing biofertilizers.

Among leguminous plants, species of the genus *Sesbania* (Fabaceae) are widely recognized for their ecological and agricultural importance. The genus *Sesbania* comprises more than 60 species distributed mainly in tropical and subtropical regions of Africa, Asia, and the Americas [5]. Although its evolutionary origin remains uncertain, Africa is considered the primary center of diversity, from which *Sesbania* species spread to other tropical regions. These plants are characterized by rapid growth, adaptability to diverse environments, and tolerance of marginal soils [6]. One of the

* Corresponding author: Minh Tam Hoang.

most widely cultivated species is *Sesbania sesban* (L.) Merr., a fast-growing shrub or small tree that has been widely used in agroforestry systems and sustainable farming practices.

S. sesban is particularly valued for its ability to improve soil fertility and productivity. The species has been widely used as a green manure crop, cover crop, and soil improvement plant in tropical agricultural systems. When incorporated into the soil, *Sesbania* biomass contributes significant amounts of organic matter and nutrients, thereby enhancing soil structure, microbial activity, and nutrient availability [7]. In many rice-based agricultural systems across Asia, *Sesbania* species are commonly cultivated as green manure to increase soil nitrogen content and improve crop yields [6]. In addition to its role in soil fertility management, *S. sesban* also provides other benefits, such as livestock fodder, fuelwood, erosion control, and shade in agroforestry systems [5]. These multifunctional characteristics make *S. sesban* particularly valuable in smallholder farming systems.

A key factor contributing to the soil-fertility benefits of *Sesbania* species is their ability to form symbiotic relationships with nitrogen-fixing bacteria collectively known as rhizobia. Several rhizobial genera have been reported to associate with *Sesbania* plants. One of the most notable examples is *Azorhizobium caulinodans*, a nitrogen-fixing bacterium capable of forming nodules not only on roots but also on stems of certain *Sesbania* species [8]. This unique nodulation ability allows *Sesbania* species to fix nitrogen efficiently even under flooded conditions. Additional species within the genus *Azorhizobium*, such as *Azorhizobium doebereinerae*, have also been described as symbiotic nitrogen-fixing bacteria associated with *Sesbania* plants [9]. These findings highlight the taxonomic diversity and functional complexity of rhizobial symbiosis in *Sesbania* species.

Besides *Azorhizobium*, other rhizobial genera have been reported to nodulate *Sesbania* plants. Members of the genus *Mesorhizobium* have been isolated from nodules of various leguminous plants and are known to exhibit considerable genetic diversity and adaptability [10]. Furthermore, recent taxonomic studies have revealed that bacteria belonging to related genera such as *Agrobacterium* may also form symbiotic relationships with certain *Sesbania* species. For example, *Agrobacterium salinitolerans* was isolated from root nodules of *Sesbania cannabina* growing in saline-alkaline soils, suggesting that diverse bacterial taxa can participate in symbiotic or associative relationships with these plants [11]. These findings demonstrate that the microbial communities associated with *Sesbania* are highly diverse and may include both classical rhizobia and other plant-associated bacteria.

In addition to symbiotic nitrogen-fixing bacteria, the rhizosphere of leguminous plants hosts a wide range of PGPR. These bacteria enhance plant growth through several direct and indirect mechanisms. One of the most important mechanisms is the solubilization of mineral nutrients in soil. Many soil microorganisms are capable of converting insoluble forms of phosphorus into soluble forms that plants can absorb, thereby improving phosphorus availability in nutrient-limited soils [12]. Similarly, certain bacteria can solubilize mineral-bound potassium from mineral sources, making this essential nutrient more accessible to plants. Potassium-solubilizing bacteria isolated from rhizosphere soils have been shown to enhance plant growth and nutrient uptake in various crops [13].

Another important mechanism by which rhizosphere bacteria promote plant growth is the production of phytohormones, particularly indole-3-acetic acid (IAA). IAA is a key plant hormone involved in root development and cell elongation. Bacterial production of IAA can stimulate root growth, increase root surface area, and enhance the ability of plants to absorb water and nutrients from soil [3]. Through these combined mechanisms including nitrogen fixation, nutrient solubilization, and phytohormone production, plant growth-promoting bacteria play a crucial role in improving plant productivity and soil fertility.

The rhizosphere represents a highly dynamic environment where intense interactions occur among plant roots, soil, and microorganisms. Root exudates released by plants contain sugars, organic acids, amino acids, and other compounds that serve as nutrients and signaling molecules for soil microorganisms [14]. These compounds influence microbial community structure and activity in the rhizosphere, leading to the establishment of beneficial plant–microbe interactions. Investigating the diversity and functional potential of rhizosphere microorganisms associated with important agricultural plants such as *Sesbania* provides valuable insights into microbial resources that may be exploited for sustainable agriculture.

In Vietnam, *Sesbania* species are widely used in traditional agricultural systems, particularly in rice cultivation and agroforestry practices. Farmers often incorporate *Sesbania* biomass into soil as green manure to improve soil fertility and support crop productivity. Several studies in Vietnam have documented the presence of beneficial bacteria associated with *Sesbania* plants, including rhizosphere and endophytic bacteria capable of promoting plant growth through various mechanisms [15, 16]. These studies indicate that *Sesbania* plants harbor diverse microbial communities with significant potential for biofertilizer development. However, despite the agricultural importance of *Sesbania*

species in Vietnam, information on the diversity and functional characteristics of plant growth-promoting bacteria associated with *S. sesban* remains limited.

Understanding the microbial communities inhabiting the rhizosphere of *Sesbania* is particularly important because these microorganisms play a key role in nutrient cycling and plant productivity. Isolation and characterization of beneficial bacteria from the rhizosphere can help identify strains capable of enhancing plant growth through multiple mechanisms. Such bacteria may serve as potential candidates for the development of microbial inoculants or biofertilizers that support sustainable agricultural practices while reducing the reliance on chemical fertilizers.

This study aims to isolate and characterize indigenous PGPR from the rhizosphere of *Sesbania sesban* (L.) Merr. in Tan Hung commune, Tay Ninh Province, Vietnam. Isolates were screened for essential functional traits, including biological nitrogen fixation, phosphate and potassium solubilization, and IAA production. By evaluating these biochemical properties, this research contributes to the understanding of *S. sesban*-associated microbial diversity and highlights the potential of these bacterial isolates for biofertilizer development to support sustainable agriculture.

2. Materials and Methods

2.1. Sample collection and preparation

Rhizosphere soil samples were collected from healthy *Sesbania sesban* (L.) Merr. plants growing in agricultural fields in Tan Hung commune, Tay Ninh province, Vietnam. The plants, along with their adhering rhizosphere soil, were placed in sterile plastic bags, transported to the laboratory and stored at 4 – 5 °C. To separate the soil fractions, root systems were shaken to remove loosely attached soil particles. The soil remaining tightly adhered to the root surfaces (rhizosphere soil) was then carefully collected using sterile tools and transferred into sterile polyethylene bags. Finally, these soil samples were thoroughly homogenized before being used for bacterial isolation [17-19].

2.2. Isolation of rhizobacteria

Rhizobacteria were isolated using the serial dilution and streaking plate method as described by Cao [4]. Briefly, 10 g of rhizosphere soil was suspended in 90 mL sterile distilled water and vigorously shaken to obtain a homogeneous suspension. Serial dilutions were prepared up to 10^{-6}. Aliquots (500 µL sample) were inoculated into test tubes containing 3.0 mL semi-solid nutrient medium. These tubes were then incubated at 30 ± 2 °C for a duration of 2 – 4 days. Bacteria that had developed into a white or yellow film 1 - 4 mm beneath the surface were streaked onto solid nutrient agar plates (NA) and incubated at 30 ± 2 °C for 24 – 48 h. Colonies with different morphological characteristics were selected and purified by repeated streaking on fresh NA plates. Pure isolates were maintained on nutrient agar slants at 4 °C for further characterization [20].

2.3. Morphological characterization of bacterial isolates

After an incubation period of 24 – 48 h on nutrient agar plates, the morphological characteristics of the bacterial colonies were examined using a magnifying glass. Colony characteristics, including color, shape, margin, and elevation, were systematically recorded. To determine cell morphology, Gram staining was performed, and cell shapes were observed under a light microscope [20, 21].

2.4. Preparation of standardized bacterial inocula

Pure bacterial isolates were cultured in specific liquid media to obtain actively growing cells. Burk's nitrogen-free medium was used for nitrogen fixation and IAA production assays, while NBRIP medium was used for phosphate solubilization assessments. All cultures were incubated at 30 ± 2 °C for 24 h with continuous shaking at 120 rpm. Following incubation, bacterial density was measured spectrophotometrically at 600 nm and adjusted to approximately 10^8 CFU/mL, corresponding to the 0.5 McFarland turbidity standard, to ensure standardized inocula for all subsequent experiments [22, 23].

2.4. Evaluating plant growth promotion capability of rhizobacteria

The plant growth-promoting potential of rhizobacterial isolates was evaluated based on their ability to fix nitrogen, solubilize phosphate, solubilize potassium, and produce IAA, which are key traits of PGPR.

2.4.1. Quantification of nitrogen fixation, phosphate solubilization and IAA synthesis

Quantification of nitrogen fixation, phosphate solubilization, and IAA synthesis was performed using standardized bacterial suspensions. For nitrogen fixation, 5 mL of bacterial suspension was inoculated into 20 mL of nitrogen-free broth medium (Burk's) and incubated at 30 ± 2 ℃ for 8 days. Ammonium production was determined every 2 days using the indophenol blue colorimetric method at 640 nm, and the results were expressed as mg/L NH_4^+ [24, 25].

Phosphate solubilization was determined by inoculating 5 mL bacterial suspension into 20 mL of NBRIP broth medium and incubating at 30 ± 2 ℃ for 20 days. Soluble phosphorus concentration was measured every 5 days using the molybdenum blue colorimetric method at 880 nm and expressed as mg P_2O_5/L [24,26].

IAA production was determined by culturing bacterial isolates in Burk's nitrogen-free medium, at a ratio of 5 mL of bacterial suspension to 20 mL of medium, at 30 ± 2 ℃ for 8 days. Culture samples were collected every 2 days and reacted with Salkowski reagent; the absorbance was then measured at 530 nm. The IAA concentration was calculated using a standard calibration curve and expressed in mg/L [24, 27].

2.4.2. Screening for potassium solubilization capacity

The potassium-solubilizing ability of rhizobacterial isolates was screened using the agar well diffusion method on Alexandrov agar medium containing insoluble potassium compounds as the sole potassium source. Standard bacterial suspensions (approximately 10^8 CFU/mL**)** prepared as described in Section 2.4 were used as inocula. Wells of approximately 6 mm diameter were aseptically prepared in agar plates, and 50 µL of the standardized bacterial suspension was added to each well. The plates were incubated at 30 ± 2 ℃ for 5–7 days**.** After incubation, the agar surface was flooded with Congo red solution to enhance the visualization of the potassium solubilization halo surrounding the wells. The presence of a clear halo zone indicated potassium solubilization. Potassium solubilization efficiency (KE) was calculated by the following method: KE = D/d, where D is the total diameter (halo zone + colony) and d is the colony diameter. A higher KE value indicated a greater potassium-solubilizing capacity of the bacterial isolates [28,29]**.**

2.5. Statistical analysis

All experiments were performed in triplicate, and results were presented as mean ± standard deviation. Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for mean comparisons (α = 0.05). Data were analyzed using IBM SPSS Statistics (version 20.0).

3. Results and Discussion

3.1. Isolation and morphological characteristics of rhizosphere bacteria

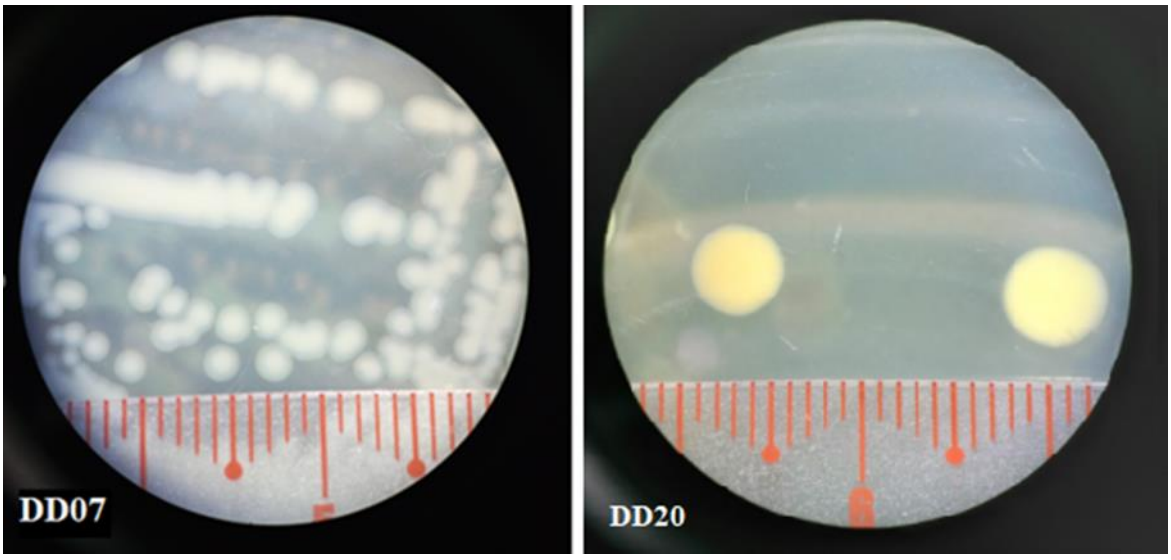

Figure 1 Colony morphology: DD07 presents as opaque white, while DD20 is yellow; both exhibit circular shapes, entire margins, and flat elevations

A total of 20 bacterial isolates were successfully isolated from the rhizosphere soil of *Sesbania sesban* (L.) Merr. using nutrient agar medium. The isolates exhibited diverse colony morphologies, indicating the presence of a heterogeneous bacterial community associated with the rhizosphere of this leguminous plant (Fig. 1).

Colony analysis showed that opaque white colonies were the most common (45%), followed by white colonies (25%), creamy white colonies (20%), and yellow colonies (10%). Colony shape analysis indicated that 14 isolates (70%) were circular, while 6 isolates (30%) displayed irregular forms. Regarding colony elevation, 18 isolates (90%) presented flat colonies, whereas 2 isolates (10%) exhibited raised elevation. Colony margins were mainly entire (60%), while 40% had serrated or irregular margins.

Microscopic observation further revealed that 14 isolates (70%) were rod-shaped, whereas 6 isolates (30%) were cocci. Gram staining results showed that 11 isolates (55%) were Gram-positive and 9 isolates (45%) were Gram-negative bacteria (Fig 2). The presence of both Gram-positive and Gram-negative bacteria indicates a complex rhizosphere microbial community.

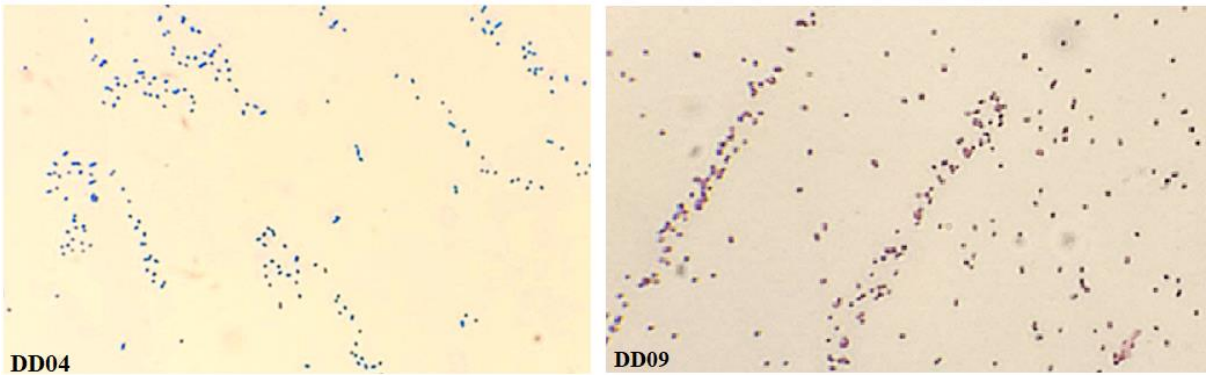

Figure 2 Gram staining of isolates (DD04 and AA09)

The observed morphological diversity reflects the dynamic microbial ecosystem in the rhizosphere, which is strongly influenced by root exudates. Root exudates supply carbon sources, amino acids, and organic acids that support microbial growth and stimulate the proliferation of diverse bacterial taxa [30]. Similar findings have been reported in rhizobacteria associated with *Sesbania cannabina*, where isolates showed varied colony shapes, colors, and Gram reactions, indicating a diverse microbial population [16]. This diversity is particularly important for plant growth because different bacterial groups contribute to different plant growth-promoting mechanisms such as nitrogen fixation, phosphate solubilization, phytohormone production, and pathogen suppression [31]. Therefore, the presence of diverse bacterial populations in the rhizosphere of *S. sesban* (L.) Merr. suggests a potentially rich reservoir of beneficial microorganisms.

3.2. Nitrogen fixation capability of rhizobacteria

The nitrogen fixation activity of the 20 rhizobacterial isolates (DD01–DD20) was evaluated by measuring ammonium (NH_4^+) accumulation in nitrogen-free medium during an eight-day incubation period. The results showed that all isolates exhibited measurable nitrogen fixation activity, with mean NH_4^+ concentrations ranging from 1.38 mg/L to 1.66 mg/L, while the control treatment showed no detectable ammonium production. Among the isolates, strains DD04 and DD11 were identified as the most efficient diazotrophs, both achieving the highest average concentration observed in the study of 1.66 mg/L. This was closely followed by isolate DD01 at 1.65 mg/L and DD08 at 1.64 mg/L. Other high-performing isolates, including DD02, DD03, and DD06, also maintained average values exceeding 1.50 mg/L.

Temporal analysis revealed a non-linear, fluctuating progression of ammonium accumulation during the incubation period. Following an initial increase from Day 2 to Day 4, where isolates such as DD01 peaked at 2.10 mg/L, a decrease was observed on Day 6. This was followed by a recovery phase on Day 8, during which DD03 reached the experiment's maximum concentration of 2.34 mg/L. Despite these temporal variations, DD04 and DD11 consistently maintained the highest mean nitrogen-fixing activity throughout the study.

The ability of these isolates to fix nitrogen is consistent with the well-known association between *Sesbania* species and diazotrophic bacteria. Many *Sesbania* species form symbiotic relationships with nitrogen-fixing bacteria belonging to

genera such as Rhizobium, Azorhizobium, Bradyrhizobium, and Mesorhizobium [16]. These bacteria convert atmospheric nitrogen (N_2) into ammonium, which can be utilized by plants and contributes to soil fertility.

Previous studies have demonstrated that *Sesbania* species possess particularly efficient nitrogen-fixing symbioses. For example, all 20 strains of bacteria isolated from the roots and stems of *Sesbania rostrata* demonstrated the ability to grow using nitrogen gas under microaerobic conditions. When evaluated via an Acetylene Reduction Assay, these strains exhibited significant nitrogenase activity. Specifically, the activity level was measured at approximately 30 nmol of acetylene produced per milligram of protein per minute [8]. In another study, researchers analyzed 20 isolates previously collected from the root nodules of *Sesbania bispinosa*. All isolates demonstrated the ability to thrive on nitrogen-free Jensen's medium, confirming their nitrogen-fixing potential. Additionally, every isolate was found to produce ammonia during the growth process [32]. Similarly, rhizobacteria associated with *Sesbania cannabina* in Vietnam were reported to produce measurable ammonium concentrations in nitrogen-free media, confirming their diazotrophic capacity [16]. The tested rhizobacteria produced average ammonium concentrations between 1.60 mg/L and 2.23 mg/L. Rather than increasing steadily, the bacterial isolates showed periodic fluctuations in their ammonium output throughout the study. Most isolates experienced a decrease in ammonium between days 2 and 4, though a smaller group showed an increase during this time. These declining levels continued from day 4 to day 6. By day 8, the majority of the isolates reached their peak ammonium production.

The results confirm that these isolates demonstrate robust nitrogen fixation, aligning with the established symbiotic efficiency of *Sesbania* species. Despite temporal fluctuations in ammonium production, the sustained performance of strains like DD04 and DD11 confirms their diazotrophic potential, highlighting their value as effective agents for atmospheric nitrogen conversion and soil enrichment.

3.3. Phosphate solubilization ability of rhizobacteria

The phosphate solubilization activity of the isolates (DD01–DD20) was determined by measuring soluble phosphorus (P_2O_5) concentrations in liquid medium during a 20-day incubation period. All isolates exhibited phosphate solubilization capability, though the level of activity varied significantly among strains. Mean soluble phosphorus concentrations ranged from 99.36 mg/L to 119.01 mg/L, whereas the negative control treatment showed no detectable concentration of P_2O_5. Among the isolates, DD19 demonstrated the highest phosphate solubilization capacity at 119.01 mg/L, followed by DD18 at 114.67 mg/L. Other strains showing relatively strong phosphate solubilization capacity included DD10 at 107.78 mg/L, DD03 at 107.74 mg/L, and DD17 at 107.48 mg/L.

The phosphate solubilization activity across all twenty isolates followed a consistent, progressive upward trend throughout the twenty-day incubation period. During the initial phase between day five and day ten, a significant increase in soluble phosphorus was observed as most isolates transitioned from an early average of approximately 60 mg/L to over 95 mg/L. For example, strains such as DD18 and DD13 showed rapid early mobilization, both exceeding 100 mg/L by the ten-day mark, which established a strong baseline for sustained microbial activity.

This growth pattern continued steadily into the intermediate phase. By day fifteen, the majority of the strains had surpassed the 110 mg/L threshold, with isolate DD19 recording a particularly sharp rise to 138.15 mg/L. Unlike the fluctuations often observed in nitrogen fixation processes, the solubilization of phosphate in this study appeared more cumulative and stable. The concentrations measured during this middle period suggest that the bacteria maintained a high metabolic state, continuously secreting the organic acids necessary for the dissolution of mineral phosphate.

The final measurement on day twenty revealed the most significant surge in activity, particularly for a select group of high-performing isolates. While almost all strains reached their maximum concentrations at this final stage, isolate DD09 exhibited the most dramatic late-stage acceleration, increasing from 114.11 mg/L on day fifteen to a study-high 172.66 mg/L on day twenty. Similarly, DD19 and DD10 showed robust final increases, reaching 168.03 mg/L and 161.62 mg/L, respectively. This widespread late-stage peaking indicates that the solubilizing capacity of these rhizobacteria remains highly effective over extended periods without reaching an early plateau.

Phosphorus is a critical macronutrient involved in energy transfer, nucleic acid synthesis, and membrane formation in plants. However, a large proportion of phosphorus in soil occurs in insoluble forms such as calcium phosphate, iron phosphate, or aluminum phosphate, which cannot be directly utilized by plants [3]. Phosphate-solubilizing bacteria (PSB) can release organic acids such as gluconic acid, citric acid, and oxalic acid that dissolve insoluble phosphate compounds and convert them into plant-available forms.

Similar phosphate solubilization capacities have been reported in rhizobacteria associated with *Sesbania* species. Rhizosphere bacteria isolated from *Sesbania bispinosa* have been reported to solubilize large amounts of phosphate and significantly enhance plant phosphorus uptake [33]. Singh et al., (2018) reported that 80% of rhizobial isolates from *Sesbania grandiflora* nodules possessed phosphate solubilization with Phosphate Solubilization Indices (P-SI) ranging from 1.96 to 4.85, demonstrating their potential as biofertilizers [34]. This high frequency of phosphate-solubilizing traits was further supported by Nguyen et al., (2023), who reported that 100% of the eighteen endophytic bacteria isolated from *Sesbania sesban* were capable of solubilizing phosphate, yielding P_2O_5 concentrations between 56.05 mg/L and 69.66 mg/L, with isolates DD18 and DD16 demonstrating the highest efficacy at 69.66 mg/L and 68.24 mg/L, respectively [15]. In another study of twenty-four rhizobacteria isolated from *Sesbania sesban (L.) Merr.* on solubilization efficiency, Dang et al., (2025) observed that average P_2O_5 concentrations ranged from 116.55 mg/L to 145.87 mg/L, with elite isolates such as DDS23 (145.87 mg/L), DDS24 (142.41 mg/L), and DDS19 (141.62 mg/L) achieving the highest overall solubilization [16]. Quantitative analysis of P_2O_5 concentration trends reveals a dynamic relationship between incubation duration and solubilization efficiency. Both studies observed that average P_2O_5 concentrations generally increased from day 5 to day 20 [15,16]. This upward trend during the 20-day period indicates sustained metabolic activity and acid production by the bacteria, which is essential for providing a steady supply of available phosphorus during critical growth stages.

The results demonstrate that the tested rhizobacterial isolates possess a significant and sustained capacity for phosphate solubilization. The consistent, cumulative increase in soluble phosphorus concentrations over the 20-day incubation period—peaking at 172.66 mg/L for isolate DD09—highlights the robust metabolic endurance and acid-producing efficiency of these strains. By effectively converting insoluble phosphate into plant-available forms, elite isolates such as DD09, DD19, and DD10 show great promise as potent biofertilizers. These findings suggest that these bacteria could play a crucial role in enhancing phosphorus availability in agricultural soils, providing a stable nutrient supply essential for optimal plant growth and sustainable crop production.

3.4. Indole-3-acetic acid (IAA) production

IAA production by the twenty rhizobacterial isolates (DD01–DD20) was quantitatively evaluated over an eight-day incubation period. All tested strains successfully synthesized the phytohormone, whereas the negative control consistently showed no detectable IAA production (0.0 mg/L). The collective average across all strains ranged from 2.40 mg/L to 3.03 mg/L.

Among the twenty isolates, DD10 and DD20 emerged as the most potent candidates for IAA producers. DD10 achieved the highest overall average concentration of 3.03 mg/L, peaking on the sixth day at 3.95 mg/L. Close behind was DD20, which maintained an average of 3.02 mg/L and recorded the single highest concentration observed in the study: 4.02 mg/L on day four. Other notable performers included DD12 (average 2.88 mg/L) and DD17 and DD18 (both averaging 2.81 mg/L). These isolates are particularly significant because they maintain higher-than-average concentrations throughout the middle stages of growth.

The trend in IAA concentration over time followed a distinct pattern: a sharp increase in the early stages, followed by stabilization in the later stages. Significant increases were observed from day 2 to day 4 for most isolates, with many reaching maximum yield during this period. For example, DD20 and DD12 showed strong early-stage growth, nearly doubling their initial concentrations by day four. While some isolates, such as DD10 and DD16, continued their upward trend until day 6, a general decline was observed on day 8. Despite this eventual decline, the consistent presence of IAA throughout the study suggests the potential of these isolates as efficient IAA-producing bacteria to enhance overall plant growth and development.

Previous studies have reported similar results in rhizobacteria associated with *Sesbania* species. For example, seven bacteria isolated from the rhizosphere of *Sesbania bispinosa* were found to produce significant amounts of IAA and stimulate plant growth [33]. In addition, in a study of IAA production ability of rhizobacteria isolated from *Sesbania bispinosa,* out of 21 bacterial samples collected from the nodules and surrounding soil of *Sesbania bispinosa,* eight isolates including species of *Bacillus, Enterobacter, Klebsiella, Chryseobacterium,* and *Herbaspirillum* were identified as IAA producers using the Salkowski colorimetric assay [35]. These findings align with studies of other *Sesbania* species, where Rhizobium strains harvested from the root nodules of *S. procumbens* and stem nodules of both *S. rostrata* and *S. procumbens* also demonstrated the ability to synthesize IAA in media containing L-tryptophan. In those instances, peak IAA accumulation occurred at the 72-hour mark, coinciding with the transition of the bacterial cultures into the stationary growth phase [36].

Moreover, researche on *Sesbania* species from Vietnam revealed substantial variations in the hormonal output of their associated bacteria. For instance, all eighteen endophytic strains isolated from *Sesbania sesban* (L.) Merr. demonstrated the ability to biosynthesize IAA, with relatively modest yields ranging from 0.796 mg/L to 1.543 mg/L, led by isolate DD16 [15]. In contrast, 24 rhizobacterial strains from *Sesbania cannabina* (Retz.) Pers. exhibited dramatically higher production levels, with average concentrations between 3.75 and 5.56 mg/L. While isolate DDS07 from *S. cannabina* emerged as the most efficient producer at 5.56 mg/L, temporal monitoring over eight days showed that most strains experienced fluctuating or declining yields after an initial increase by day four. Notably, only isolates DDS03, DDS09, and DDS10 maintained a consistent upward trend, underscoring the diverse and time-dependent metabolic efficiency of *Sesbania*-associated rhizobacteria in hormonal biosynthesis [16].

The consistent synthesis of IAA across diverse *Sesbania*-associated isolates confirms their significant potential as plant growth promoters. While production levels vary between species and strains, the observed peak concentrations during middle growth stages align with established metabolic patterns. These findings underscore the diverse, time-dependent efficiency of these rhizobacteria, positioning potent candidates like DD10 and DD20 as effective biological tools for enhancing plant development through sustainable IAA biosynthesis

3.5. Potassium solubilization ability

The assessment of the potassium (K) solubilization efficiency of 20 bacterial isolates (DD01–DD20) compared against a negative control revealed a clear distinction between active and inactive strains, with roughly 70% of the tested population showing no capacity for K-solubilization. This large group of inactive isolates, which includes the control and 14 specific strains including isolates DD01–DD04, DD07, DD09–DD11, DD14, DD15, and DD17–DD20, serves as a baseline, confirming that the ability to solubilize potassium-bearing minerals is not a universal trait among all rhizospheric bacteria.

Conversely, six isolates DD05, DD06, DD08, DD12, DD13, and DD16 (approximately 30%) exhibited potassium-solubilizing activity. Among these active strains, isolate DD13 emerged as the most efficient performer, achieving a peak potassium solubilization efficiency of 1.41 ± 0.12. A secondary group comprising isolates DD06, DD12, and DD16 also showed high efficiency, with values ranging from 1.16 to 1.19. Finally, isolates DD05 and DD08 exhibited the lowest positive activity among the active group, with efficiency scores of 1.04 and 1.05, respectively. These findings indicate that while many rhizosphere isolates may be inactive, specific strains like DD13 hold significant potential for K-nutrient mobilization.

Potassium exists in four soil states: mineral, non-exchangeable, exchangeable, and water-soluble. Although plants only take up the small available fraction of soluble and exchangeable K, these reserves are often depleted by erosion, leaching, and runoff. To combat this deficiency, potassium solubilizing bacteria (KSB) serve as a potent, sustainable solution. These beneficial microbes release available K from insoluble minerals like mica and feldspar by excreting organic acids. Naturally occurring in the rhizospheres of various sources including cereals (maize, wheat), root crops (potato), and fruit trees (apple, walnut), these bacteria effectively bridge the gap between fixed soil minerals and plant requirements [37, 38].

The variability in KSB efficiency observed in this study is consistent with previous research in diverse agricultural environments. Khanghahi et al. (2017) isolated potassium-solubilizing bacteria (KSB) from paddy rhizosphere soil using a modified Aleksandrov medium. Among the isolates, three strains exhibited the highest potassium-solubilizing capacity. After 21 days of incubation, these strains released 35.36, 76.04, and 56.58 $\mu g\ mL^{-1}$ of potassium from mica, respectively [39]. Similarly, studies on cereal crop rhizospheres have identified bacteria capable of mobilizing mineral-bound potassium. Fatharan et al. (2018) isolated seven bacterial strains from the rice rhizosphere that were able to solubilize potassium from feldspar [40]. In another study, 137 bacterial isolates were obtained from wheat rhizosphere soil, of which 20 strains, approximately 15% demonstrated the ability to extract potassium from mica. Using atomic absorption spectrometry, these strains were shown to release potassium in the range of 15 to 48 mg L^{-1} into the culture medium [41].

More recently, Zhao et al. (2024) isolated 77 bacterial strains representing diverse colony morphologies from cotton rhizosphere soil in Xinjiang. Among these, 49 isolates (approximately 67%) exhibited potassium-solubilizing ability on Alexandrov agar supplemented with feldspar, with solubilization indices (D/d) ranging from 1.16 to 3.71 [28]. Furthermore, research on sugarcane rhizosphere bacteria demonstrated that 100% of the 31 isolated strains could solubilize insoluble K into available K_2O, with concentrations increasing over a 21-day period to reach 35.23 mg/L [38]. These comparative studies validate the results of the current screening, suggesting that isolates like DD13 are part of a specialized group of microbes capable of significantly improving potassium availability in depleted soils.

4. Conclusion

This study evaluated the plant growth-promoting potential of 20 indigenous rhizobacterial strains isolated from the rhizosphere of *Sesbania sesban* (L.) Merr. in Tan Hung commune, Tay Ninh Province. Several elite isolates exhibited strong functional traits, including nitrogen fixation, phosphate and potassium solubilization, and indole-3-acetic acid (IAA) production. Strains DD04 and DD11 demonstrated the highest nitrogen-fixing capacity, while DD19 and DD09 showed superior phosphate solubilization. Notably, DD13 exhibited the strongest potassium-solubilizing ability. These findings highlight the potential of indigenous rhizobacteria to enhance nutrient availability and promote plant growth through multiple mechanisms. Although this study was limited to *in vitro* conditions, the observed multifunctional traits suggest strong potential for agricultural application. Future studies should focus on field validation to assess the effectiveness and stability of these strains under natural conditions. Overall, these rhizobacteria represent promising candidates for sustainable biofertilizer development in Vietnam.

Compliance with ethical standards

Acknowledgments

This study was conducted as part of the research project coded CSB.2025.091, funded by Saigon University.

Disclosure of conflict of interest

There is no conflict of interest.

References

[1] Peoples MB, Herridge DF, Ladha JK. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant Soil. 1995;174:3–28.

[2] Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571–586.

[3] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World J Microbiol Biotechnol. 2012;28:1327–1350.

[4] Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63:541–556.

[5] Bunma S, Balslev H. A review of the economic botany of Sesbania (Leguminosae). Bot Rev. 2019;85:185–251.

[6] Gutteridge RC. The perennial Sesbania species. In: Gutteridge RC, Shelton HM, editors. Forage tree legumes in tropical agriculture. Wallingford: CAB International; 1994. p. 49–64.

[7] Young JPW. Phylogeny and taxonomy of rhizobia. Plant Soil. 1997;186:45–52.

[8] Dreyfus B, Garcia JL, Gillis M. Characterization of Azorhizobium caulinodans gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from Sesbania rostrata. Int J Syst Bacteriol. 1988;38:89–98.

[9] Moreira FMS, Cruz LM, De Faria SM, Marsh TL, Martínez-Romero E, Pedrosa FO, et al. Azorhizobium doebereinerae sp. nov., microsymbiont of Sesbania virgata. Syst Appl Microbiol. 2006;29:197–206.

[10] Wang ET, Kan FL, Tan ZY, Toledo I, Chen WX, Martínez-Romero E. Diverse Mesorhizobium plurifarium populations native to soils. Arch Microbiol. 2003;180:444–454.

[11] Yan J, Li Y, Yan H, Chen WF, Zhang X, Wang ET, et al. Agrobacterium salinitolerans sp. nov., a saline–alkaline-tolerant bacterium isolated from root nodules of Sesbania cannabina. Int J Syst Evol Microbiol. 2017;67:1906–1911.

[12] Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009;321:305–339.

[13] Zhang C, Kong F. Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl Soil Ecol. 2014;82:18–25.

[14] Dakora FD, Phillips DA. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil. 2002;245:35–47.

[15] Nguyen TPV, Dang TNT, Pham VN, Hoang MT. Plant growth promotion characterization of endophytic bacteria from Sesbania sesban (L.) Merr. collected in Long An Province, Vietnam. GSC Biol Pharm Sci. 2023;25:84–92.

[16] Dang TTN, Pham LTT, Hoang TM. Characterization of plant growth-promoting rhizobacteria associated with Sesbania species in Vietnam. GSC Biol Pharm Sci. 2025;31:104–115.

[17] Soils and Fertilizers Institute, 1998. Handbook of Soil, Water, Fertilizer, and Crop Analysis. Vietnam: Agricultural Publishing House; 1998.

[18] Li M, Wang J, Yao T, Wang Z, Zhang H, Li C. Isolation and characterization of cold-adapted PGPB and their effect on plant growth promotion. J Microbiol Biotechnol. 2021 Sep 28;31(9):1218-1230. doi: 10.4014/jmb.2105.05012.

[19] Barillot CDC, Sarde C-O, Bert V, Tarnaud E, Cochet N. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. Ann Microbiol. 2013;63(2):471–476. doi:10.1007/s13213-012-0491-y

[20] Cao ND. Endophytic bacteria of plants. Vietnam: Can Tho University Publishing House. 2010.

[21] Nguyen DL, Phan TH, Nguyen AT. Practice of biotechnology (Vol.2). Vietnam: Vietnam National University Ho Chi Minh City Press; 2003.

[22] Mahesh PP, Kolape J, Sultana H, Neelakanta G. McFarland Standards-Based Spectrophotometry Method for Calculating Approximate Multiplicity of Infection for an Obligate Intracellular Bacterium Anaplasma phagocytophilum. Microorganisms. 2025;13(3):662. doi: 10.3390/microorganisms13030662.

[23] Nix ID, Idelevich EA, Storck LM, Sparbier K, Drews O, Kostrzewa M, et al. Detection of methicillin resistance in Staphylococcus aureus from agar cultures and directly from positive blood cultures using MALDI-TOF mass spectrometry-based direct-on-target microdroplet growth assay. Front Microbiol. 2020;11:232. doi: 10.3389/fmicb.2020.00232.

[24] Hoang MT, Cao ND. Characteristics of rhizosphere bacteria of sugarcane (Saccharum spp.) grown on acrisol and ferrasol soils of Dong Nai province, the Southeast of Vietnam. In: Rad MRN. Emerging Issues in Agricultural Sciences. 2023; 7: 116–135. https://doi.org/10.9734/bpi/eias/v7/5934B

[25] Santoni S, Bonifacio E, Zanini E. Indophenol blue colorimetric method for measuring cation exchange capacity in sandy soils. Commun Soil Sci Plant Anal. 2001;32(15):2519-30.

[26] Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31-36. doi: 10.1016/S0003-2670(00)88444-5.

[27] Pramanik RS, Chandwani S, Amaresan N. Screening of Endophytes for Plant Growth-Promoting Metabolites. In: Sankaranarayanan A, Amaresan N, Dwivedi MK, editors. Endophytic Microbes: Isolation, Identification, and Bioactive Potentials. New York (NY): Humana; 2023. p. 231-240. (Springer Protocols Handbooks). Available from: https://doi.org/10.1007/978-1-0716-2827-0_19

[28] Zhao Y, Liang H, Zhang J, Chen Y, Dhital YP, Zhao T, Wang Z. Isolation and characterization of potassium-solubilizing rhizobacteria (KSR) promoting cotton growth in saline-sodic regions. Microorganisms. 2024;12(7):1474. doi:10.3390/microorganisms12071474. PMID: 39065241; PMCID: PMC11279176.

[29] Vo XT, Dang NQ, Vu DC, Nguyen NN. Presence and potential for isolation of potassium solubilizing bacteria in mango, pitaya, and banana rhizospheric soils. AIP Conf Proc. 2021;2406:040007. doi:10.1063/5.0067228.

[30] Shafi Z, Shahid M. Root exudates as molecular architects shaping the rhizobacterial community: A review. Rhizosphere. 2025;36:101212. doi:10.1016/j.rhisph.2025.101212.

[31] Saeed Q, Xiukang W, Haider FU, Kučerik J, Mumtaz MZ, Holatko J, et al. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. Int J Mol Sci. 2021 Sep 29;22(19):10529. doi: 10.3390/ijms221910529.

[32] Chakrabarty M, Hossen F, Begum A, Akhter H. Plant Growth Promoting (PGP) Activities of Rhizobial Isolates from Sesbania bispinosa in Response to Pesticides. Original ArticleBangladesh J Microbiol. 2021; 38(2): 31-37.

[33] Dasgupta D, Sengupta C, Paul G. Screening and identification of phosphate solubilizing and IAA producing PGPR inhabiting the rhizosphere of Sesbania bispinosa. Int J Innov Res Sci Eng Technol. 2015;4(6):3968–3972.

[34] Singh K, Gera R. Assessing phosphate solubilization ability of Sesbania grandiflora rhizobia isolated from root nodules using diverse agroecological zones of Indian soils for biofertilizer production. Int J Chem Stud. 2018;6(4):398–402.

[35] Islam S, Ahmed T, Rahman A, Ahsan N. Assessing the IAA production ability of rhizobacteria isolated from Sesbania bispinosa and their impact on root growth promotion. Int J Agron. 2025: 2206310. https://doi.org/10.1155/ioa/2206310.

[36] Sridevi M, Mallaiah KV. Production of indole-3-acetic acid by Rhizobium isolates from Sesbania species. Afr J Microbiol Res. 2007 Dec;1(7):125-8. Available from: http://www.academicjournals.org/ajmr

[37] Torabian S, Farhangi-Abriz S, Qin R, Noulas C, Sathuvalli V, Charlton B, Loka DA. Potassium: A Vital Macronutrientin Potato Production—A Review. Agronomy. 2021; 11(3):543. https://doi.org/10.3390/agronomy1103054

[38] Hoang TM, Dang TTN. Investigation on starch and cellulose degradation, siderophore production and potassium solubilization of rhizosphere bacteria isolated from sugarcanes cultivated in Ben Cau district, Tay Ninh province, Vietnam. GSC Biol Pharm Sci. 2025;31(1):10–19. Available from: https://doi.org/10.30574/gscbps.2025.31.1.0137

[39] Khanghahi MY, Pirdashti H, Rahimian H, Nematzadeh G, Sepanlou MG. Potassium solubilising bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. Symbiosis. 2018;76(1):13-23. Available from: https://doi.org/10.1007/s13199-017-0533-0

[40] Fatharani R, Rahayu YR. Isolation and characterization of potassium-solubilizing bacteria from paddy rhizosphere (Oryza sativa L.). 2018; J. Phys.: Conf. Ser.. DOI 10.1088/1742-6596/1108/1/012105

[41] Parmar P, Sindhu SS. Potassium solubilization by rhizosphere bacteria: Influence of nutritional and environmental conditions. J Microbiol Res. 2013;3(1):25-31. doi:10.5923/j.microbiology.20130301.04