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Characterization of plant growth-promoting rhizobacteria associated with *Sesbania cannabin*a (Retz.) Pers.in long An Province, Vietnam

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Abstract

This study investigated characteristics of plant growth-promotion (PGP) of rhizobacteria associated with *Sesbania cannabina* (Retz.) Pers. in the Tan Hung district of Long An province, Vietnam. The rhizobacteria were studied colony, cell morphology and assessed capabilities of nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) production and siderophore synthesis. Colorimetric assays were employed to quantify nitrogen fixation, phosphate solubilization, and IAA synthesis. The Chrome Azurol Sulfonate (CAS) assay was used to detect bacterial siderophore production. A total of twenty-four rhizobacteria were isolated, exhibiting predominantly opaque white, flat colonies with diverse shapes, margins, and diameters ranging from 1 to 4 mm, a balanced distribution of Gram-positive and Gram-negative bacteria with rod and coccus shapes, demonstrated significant plant growth-promoting capabilities. Notably, isolates DDS24 and DDS21 displayed the highest nitrogen fixation, DDS23 exhibited the highest phosphate solubilization and DDS07, DDS05, and DDS06 exhibited superior IAA production. Fifty percent isolates demonstrated siderophore production abilities. The identified *Sesbania cannabina* (Retz.) Pers. rhizobacteria show significant promise as biofertilizers, and require further studies on their application to sustainable agricultural practices.

Keywords: IAA production; Nitrogen fixation; Plant growth promotion; Phosphate solubilization; Rhizobacteria; *Sesbania cannabina* (Retz.) Pers

1. Introduction

The genus *Sesbania*, a diverse group of roughly seventy species, holds significant ecological and agricultural importance, particularly within the warm embrace of tropical and subtropical climates [1]. It is applied to revitalize and enrich soil fertility, specially, in sustainable agriculture practices [2]. Notably, several Sesbania species have been deployed as effective green manure, a traditional technique that leverages plant biomass to improve soil structure and nutrient content. Among these, *Sesbania cannabina* was historically utilized green manure on oceanic islands as well as in Fiji. Moreover, in India, it was applied as green manure to ameliorate soil conditions, fostering the growth of vital crops like coconut, rice, and sugarcane [2]. Similarly, *Sesbania grandiflora* was commonly integrated into rice paddy ecosystems in Southeast Asia, acting as a natural fertilizer. In South India, *Sesbania speciosa* fulfilled this critical role. Research comparing three *Sesbania* species (*S. bispinosa*, *S. rostrata*, and *S. speciosa*) for their potential as rice biofertilizers revealed *S. rostrata* as superior in nitrogen contribution. This species, uniquely possessing nodules on both roots and stems, held double the nitrogen levels of the other two while *S. rostrata* also exhibited the highest concentrations of manganese, zinc, and copper, *S. bispinosa* showed the highest iron content. Overall, *S. rostrata's* substantial nitrogen and micronutrient profile suggested it's a particularly effective biofertilizer [2].

The elevated nitrogen content observed in *Sesbania* is primarily attributed to its intricate symbiotic relationships with a diverse array of diazotrophic bacteria. Approximately forty species within the *Sesbania* genus engage in nodule

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formation, a specialized process that facilitated nitrogen fixation through these bacterial associations [3]. The bacterial partners involved in these symbioses span a wide taxonomic range, encompassing genera such as *Rhizobium*, *Mesorhizobium, Sinorhizobium, Azorhizobium, Neorhizobium, Bradyrhizobium*, *Ensifer*, and *Agrobacterium* [3, 4]. Numerous specific symbiotic pairings have been documented, highlighting the specificity and diversity of these interactions. For instance, *Neorhizobium huautlense* forms nodules with *Sesbania herbacea* [5], while *Azorhizobium caulinodans* and *Bradyrhizobium sp*. establishe symbioses with *S. rostrata* [6, 7, 8]. *Agrobacterium doebereinerae* was known to associate with *S. virgate* [9, 10]. Furthermore, *Ensifer teranga* and *E. saheli* have been identified in association with both *S. rostrata* and *S. cannabina* [6]. *Mesorhizobium plurifarium* and *Neorhizobium huautlense* have been isolated from a variety of *Sesbania* species [11, 12]. *Rhizobium gallicum* has been observed in symbiotic relationships with *S. sericea* and *S. sesban*, and *Agrobacterium salinitolerans* have been isolated from *S. cannabina* [13]. Notably, *Sesbania sesban* demonstrates a remarkable ability to interact with a broad spectrum of nodule-inducing rhizobia species [14, 15]. Within Vietnam, eight *Sesbania* species are used for soil enhancement, with *Sesbania rostrata* distinguished by its unique ability to fix atmospheric nitrogen in both its stems and roots, making it a prevalent green manure crop in rice cultivation [16, 17]. Studies have consistently revealed that incorporating *Sesbania sesban* significantly boosts soil nitrogen availability, and that cultivating *Sesbania* enhances nutrient content in saline rice soils, leading to improved rice yields [10, 18].

While extensive research has focused on the nodule-associated, nitrogen-fixing bacteria that interact with *Sesbania*, the investigation of other plant growth-promoting bacteria, particularly within the context of Vietnamese agriculture, remain relatively unexplored. This study seeks to bridge this knowledge gap by comprehensively examining the broader spectrum of plant growth-promoting bacteria associated with *Sesbania cannabina* (Retz.) Pers.. Specifically, the objectives of this research are to isolate bacteria from the rhizosphere soil of Sesbania cannabina (Retz.) Pers. in the Tan Hung district of Long An province, Vietnam and to evaluate their plant growth promotion capabilities based on the levels of nitrogen fixation, phosphate solubilization, IAA production, and siderophore synthesis.

2. Material and methods

2.1. Sample collection and preparation

Sesbania cannabina (Retz.) Pers. plants with rhizosphere soil growing in Tan Hung district, Long An province, Vietnam were collected, placed in sterile plastic bags, transported to the laboratory in an ice box, and stored at 4 - 5 ℃ for later use. The loose soil detached from the roots and root sheath was removed by vigorously shaking the root system. The remaining soil (rhizosphere soil) adhered to the root system was collected using sterile tools and rubber gloves for. The rhizosphere soil was stored at 4-5℃ for the following experiments [19].

2.2. Isolation of rhizobacteria

Processed rhizosphere soil was ground. A 10g sample was mixed with 90mL sterile water, shaken for 3 hours, and filtered. The filtrate was then serially diluted 10-fold (up to 10-6 dilution). Samples of 500 µL were added to test tubes with 3.0 mL of semi-solid nutrient agar medium and incubated at 28 ± 2 ℃ for 2-4 days. Bacteria that grew and formed a white or yellow film 1-4 mm below the surface, were then transferred to solid nutrient agar plates. Individual colonies were subsequently isolated for purification [20].

2.3. Morphological characterization of bacterial isolates

First, bacterial colonies grown on nutrient agar medium for 48 hours were characterized by their form, elevation, margin, surface, and size. Next, light microscopy was used to examine the size, shape, and movement of individual bacterial cells. Finally, the Gram staining of the isolates was determined using the method described by Nguyen et al. (2003) [21].

2.4. Preparation of standard bacterial suspensions

Rhizobacterial strains were grown in Burk's nitrogen-free liquid medium for quantifying nitrogen fixation, IAA production, and liquid NBRIP medium and phosphate solubilization for assessing phosphate solubilization, respectively. Cultures were incubated at 30°C and 120 rpm for 1-2 days. Following incubation, the cell density of each strain was determined spectrophotometrically at 600 nm and adjusted to a 0.5 McFarland standard (1.5 x 10^8 CFU/mL), creating standardized bacterial suspensions [22, 23].

2.5. Evaluating plant growth promotion capability of rhizobacteria

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

The concentrations of NH_4^+, P_2O_5, and IAA produced by the bacterial isolates were determined using colorimetric assays. Standardized bacterial suspensions (500 µL) were cultured in 20 mL of the appropriate liquid media: Burk's nitrogen-free medium for the quantitative analysis of NH_4^+ and IAA, and NBRIP medium for the quantitative analysis of P_2O_5. Cultures were incubated on a shaker at 30°C and 100 rpm. Culture fluids were collected at specific time points (2, 4, 6, and 8 days for NH_4^+ and IAA; 5, 10, 15, and 20 days for P_2O_5), and supernatants were collected after centrifugation at 12,000 rpm for 5 minutes. Each supernatant was then mixed with phenol nitroprusside (5:1), ammonium molybdate (5:1), or Salkowski reagent (2:1), respectively, and incubated for 30 minutes for color development. The optical densities (OD) of the resulting-colored solutions were measured at 640 nm (NH_4^+), 880 nm (P_2O_5), and 530 nm (IAA). Concentrations (mg/L) were determined by comparing the OD values to standard curves for NH_4^+, IAA, and P_2O_5 [24].

2.5.2. Screening siderophore production

Standardized bacterial suspensions were used for the experiment. The Chrome Azurol Sulfonate (CAS) assay, developed by Schwyn and Neilands (1987), was used to detect siderophore production of rhizobacteria. A color shifted from blue to orange, purple, or green within the CAS medium signified the presence of these iron-chelating compounds. Growth medium for bacteria was solid or liquid Luria Bertani medium (LB). The CAS medium was prepared following the protocol detailed by Chaitanya et al. (2014), while the CAS-Growth medium plates, used in the paper-disc agar diffusion method for siderophore detection, were prepared according to Srivastava et al. (2013) [25, 26]

2.6. Experiment design and data analysis

Experiments were set up in a completely randomized design, with three replicates. Statistical analysis was performed using IBM SPSS Statistics 20.0, employing one-factor ANOVA and Duncan's test ($\alpha = 0.05$) to determine significant differences.

3. Results and discussion

3.1. Morphological characteristics of rhizobacteria

Table 1 Colony morphology of bacterial isolates on nutrient agar medium

Colony Morphology	Number	Percentage (%)
Color		
Opaque white	14	58.3
Creamy white	6.0	25.0
White	4.0	16.7
Shape		
Irregular	13	54.2
Round	11	45.8
Margin Form		
Entire	11	45.18
Serrated	10	41.7
Filamentous	3.0	12.5
Elevation		
Flat	17	70.8
Raised	7.0	29.2

Twenty-four rhizobacteria were isolated from the rhizosphere of *Sesbania cannabina* (Retz.) Pers. collected in Tan Hung district, Long An province, Vietnam and designated as DDS1 to DDS24. Table 1 and Figure 1 describe the colony

characteristics of the 24 bacterial strains when cultured on a Nutrient agar medium. The most frequent color was opaque white, observed in 14 isolates (58.3%) of the strains. In terms of colony shape, 13 isolates (54.2%) and 11 isolates (45.8%) were round and irregular, respectively. For colony margin, 11 strains (45.8%) had entire margins, and 10 strains (41.7%) had serrated margins. Most of colony elevation was flat, (17 strains, 70.8%). The diameters of the colonies ranged from 1.0 mm to 4.0 mm. The most common diameters were 3.0 mm, observed in 4 strains.

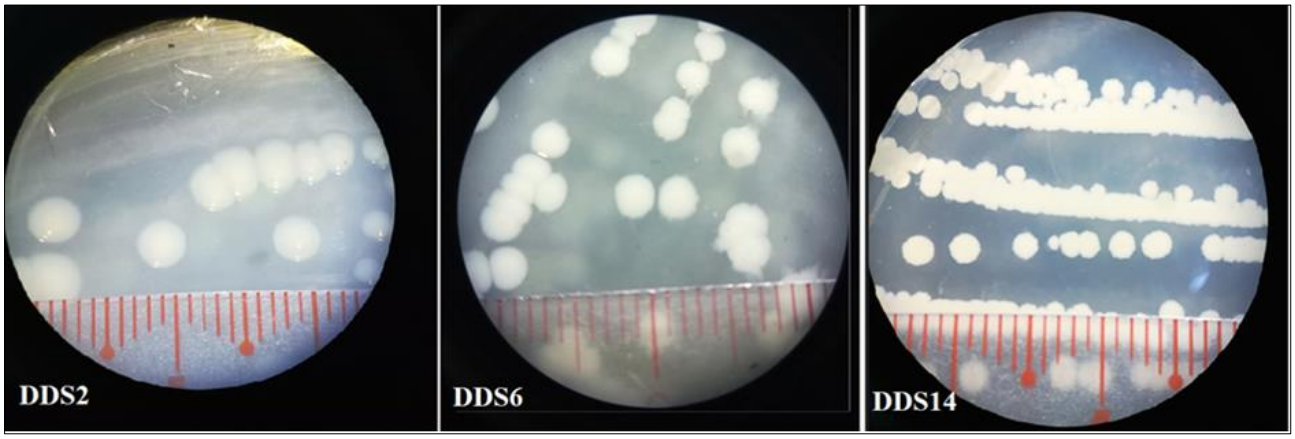
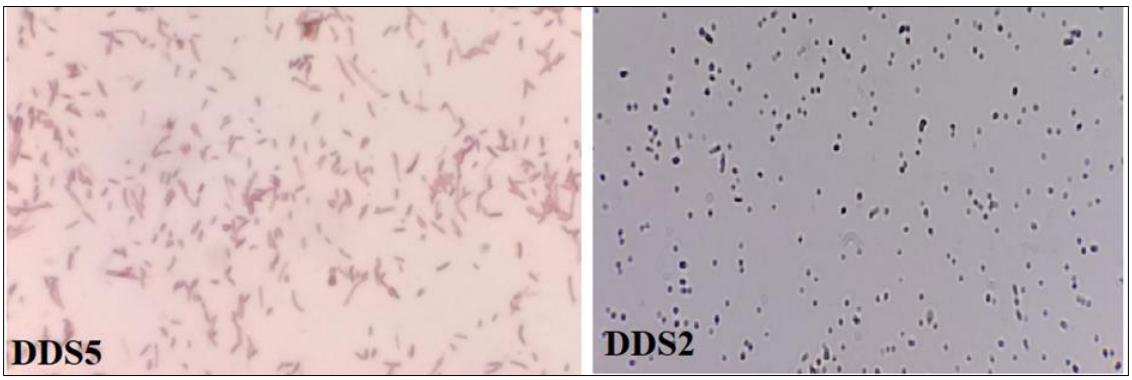


Figure 1 Morphology of some bacterial colonies

Regarding cell shape, the strains were classified into two categories: rods and cocci (Table 2). Among the 24 strains, 14 (58.3%) were short rods, while 10 (41.7%) were cocci. In terms of Gram staining, 12 (50%) strains were Gram-negative, and 12 (50%) isolates were Gram-positive (Figure 2). This shows an equal distribution between Gram-negative and Gram-positive bacteria within the sample.

Table 2 Cell characteristics of bacterial isolates on nutrient agar medium

Cell feature	Number	Percentage (%)
Cell Shape		
Short rod	14.0	58.3
Coccus	10.0	41.7
Gram		
Positive	12.0	50.0
Negative	12.0	50.0



DDS5 Gram (-), DDS2 Gram (+)

Figure 2 Gram of some bacteria

3.2. Evaluating plant growth promotion capabilities of rhizobacteria

3.2.1. Nitrogen fixation capability of rhizobacteria

Table 3 Nitrogen fixation activity of 24 rhizobacterial trains

Isolate	Concentration of NH_4^+ (mg/L)				
	Day 2th	Day 4th	Day 6th	Day 8th	Average
Control (-)	0.00 ± 0.00^{c}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00
DDS01	2.15 ± 0.42^{ab}	1.81 ± 0.23^{b-e}	0.98 ± 0.08^{de}	2.11 ± 0.44^{ab}	1.76
DDS02	2.16 ± 0.19^{ab}	2.42 ± 0.87^{b}	1.25 ± 0.26^{cde}	1.27 ± 0.26^{c}	1.77
DDS03	2.32 ± 0.34^{ab}	2.38 ± 1.04^{bc}	1.10 ± 0.08^{cde}	2.10 ± 0.03^{ab}	1.98
DDS04	2.28 ± 0.50^{ab}	2.04 ± 0.11^{b-e}	1.08 ± 0.22^{cde}	2.19 ± 0.35^{ab}	1.90
DDS05	1.94 ± 0.04^{ab}	1.74 ± 0.17^{b-e}	0.87 ± 0.08^{e}	2.09 ± 0.12^{ab}	1.66
DDS06	2.21 ± 0.24^{ab}	1.68 ± 0.22^{b-e}	1.04 ± 0.13^{de}	2.39 ± 0.32^{ab}	1.83
DDS07	1.74 ± 0.65^{ab}	1.55 ± 0.20^{e}	0.97 ± 0.04^{de}	2.16 ± 0.29^{ab}	1.60
DDS08	2.07 ± 0.06^{ab}	1.57 ± 0.04^{de}	0.83 ± 0.07^{e}	2.05 ± 0.21^{ab}	1.63
DDS09	2.53 ± 0.62^{ab}	2.45 ± 0.73^{bc}	0.81 ± 0.02^{e}	2.12 ± 0.10^{ab}	1.98
DDS10	1.98 ± 0.18^{ab}	1.54 ± 0.15^{b-e}	2.32 ± 1.12^{a}	2.12 ± 0.18^{ab}	1.99
DDS11	1.62 ± 0.14^{b}	1.64 ± 0.09^{de}	1.67 ± 0.59^{abc}	2.02 ± 0.40^{ab}	1.74
DDS12	2.14 ± 0.40^{ab}	1.83 ± 0.30^{b-e}	1.18 ± 0.24^{de}	2.00 ± 0.20^{ab}	1.79
DDS13	2.27 ± 0.39^{ab}	1.61 ± 0.11^{cde}	1.01 ± 0.05^{cde}	2.22 ± 0.30^{ab}	1.78
DDS14	2.10 ± 0.35^{ab}	1.74 ± 0.12^{b-e}	0.98 ± 0.10^{e}	2.09 ± 0.28^{ab}	1.73
DDS15	2.12 ± 0.18^{ab}	1.79 ± 0.08^{b-e}	1.04 ± 0.05^{de}	2.36 ± 0.48^{a}	1.83
DDS16	2.06 ± 0.10^{ab}	2.01 ± 0.21^{b-e}	1.02 ± 0.12^{de}	2.04 ± 0.28^{b}	1.78
DDS17	2.51 ± 0.72^{ab}	1.86 ± 0.11^{b-e}	1.13 ± 0.11^{cde}	2.17 ± 0.11^{ab}	1.92
DDS18	2.04 ± 0.18^{ab}	1.85 ± 0.16^{b-e}	0.93 ± 0.12^{e}	2.16 ± 0.24^{ab}	1.75
DDS19	1.95 ± 0.35^{ab}	1.98 ± 0.18^{b-e}	1.05 ± 0.07^{de}	2.44 ± 043^{ab}	1.88
DDS20	2.07 ± 0.35^{ab}	2.23 ± 0.42^{b-e}	1.34 ± 0.12^{de}	2.12 ± 0.53^{a}	1.94
DDS21	1.92 ± 0.20^{b}	1.80 ± 0.20^{b-e}	2.24 ± 0.91^{ab}	2.15 ± 0.18^{ab}	2.03
DDS22	2.46 ± 0.90^{a}	2.29 ± 0.34^{bcd}	1.20 ± 0.42^{b-e}	1.95 ± 0.30^{ab}	1.97
DDS23	1.93 ± 0.04^{b}	2.38 ± 0.36^{b-e}	1.75 ± 0.22^{cde}	1.82 ± 0.18^{ab}	1.97
DDS24	2.55 ± 0.68^{ab}	3.98 ± 0.94^{a}	1.75 ± 0.22^{a-d}	1.19 ± 0.14^{c}	2.23

Values in the same vertical column followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

Table 3 presents the quantitative analysis results of ammonium (NH_4^+) concentration for 24 rhizobacterial strains over an eight-day culture period, with measurements taken on days 2, 4, 6, and 8. The results indicate that all strains exhibited nitrogen fixation capability with varying efficiency. Average ammonium concentrations of testing rhizobacteria ranged from 1.60 mg/L to 2.23 mg/L. Strains DDS24 and DDS21 showed the highest average concentrations (2.23 mg/L and 2.03 mg/L, respectively), followed by DDS10 (1.99 mg/L), DDS09 (1.98 mg/L), and DS03 (1.98 mg/L), while DDS07 had the lowest average concentration (1.60 mg/L). Analyzing the fluctuations in ammonium (NH_4^+) concentrations throughout the experimental period revealed diverse nitrogen fixation trends among the rhizobacterial isolates. From day 2 to day 4, a majority of the isolates (DDS01-19, DDS21, and DDS22) showed a decrease in ammonium concentration, whereas a smaller subset (DDS02, DDS03, DDS11, DDS20, DDS23, and DDS24) exhibited

an ammonium concentration increase. Subsequently, between day 4 and day 6, the trend reversed for some strains, with DDS10, DDS11, and DDS21 showing an ammonium concentration increase, and the remaining isolates showing an ammonium concentration decrease. From day 6 to day 8, most isolates showed an increase in ammonium concentration, except for DDS10 and DDS24, which decreased ammonium concentration. Overall, all isolates demonstrated fluctuations in ammonium concentrations throughout the experiment. Considering the overall ammonium production, strains DDS24, DDS21, DDS10, DDS09, and DS03 demonstrated higher average ammonium concentrations, suggesting they are more effective nitrogen-releasing isolates

3.3. Phosphate solubilization of rhizobacteria

Table 4 presents the phosphorus solubilization activity converting insoluble phosphate into soluble phosphate (P_2O_5) of 24 rhizobacterial isolates over a twenty-day culture period. Measurements were taken on days 5, 10, 15, and 20. The isolates displayed significant differences in their solubilization performance. The average P_2O_5 concentration of all rhizobacteria ranged from 116.55 mg/L to 145.87 mg/L. Specifically, isolates DDS23 (145.87 mg/L), DDS24 (142.41 mg/L), DDS19 141.62 mg/L), and DDS11 (134.93 mg/L) expressed the highest overall solubilization while DDS01 showed the lowest ability with a P_2O_5 concentration of 116.55 mg/L. P_2O_5 concentrations among isolates generally increased from day 5 to day 20, although individual patterns exhibited variability. Specifically, the majority of isolates showed an increase from day 5 to day 10. Between day 10 and day 15, some isolates continued to show an increase, while others (DDS05, DDS06, DDS07, DDS08, and DDS12) experienced a decrease in their P_2O_5 concentration. From day 15 to 20, most increased, though some (DDS14, DDS17, DDS18, DDS19, DDS20, DDS21, DDS22, DDS23, and DDS24) decreased their P_2O_5 concentration. Overall, isolates showed fluctuating P_2O_5 concentrations despite the general upward trend. In general, DDS23 performed most consistently, while DDS10 showed strong potential by the end of the study. Other isolates were less effective. These findings could help in choosing the best isolates for agricultural applications.

Table 4 Phosphate solubilization activity of 24 rhizobacterial trains

Isolate	Concentration of P_2O_5 (mg/L)				
	Day 5th	Day 10th	Day 15th	Day 20th	Average
Control (-)	0.00 ± 0.00^{n}	0.00 ± 0.00^{h}	0.00 ± 0.00^{n}	0.00 ± 0.00^{j}	0.00
DDS01	97.47 ± 3.62^{def}	97.45 ± 1.53^{g}	117.32 ± 1.97^{lm}	153.97 ± 6.36^{c-h}	116.55
DDS02	75.77 ± 2.15^{m}	124.17 ± 4.21^{def}	127.16 ± 2.43^{i-m}	152.84 ± 4.41^{c-h}	119.99
DDS03	81.60 ± 2.25^{klm}	119.06 ± 3.28^{ef}	128.32 ± 2.45^{i-l}	157.13 ± 7.42^{b-f}	121.53
DDS04	83.05 ± 3.50^{klm}	124.81 ± 11.14^{cde}	125.38 ± 8.36^{i-l}	145.42 ± 6.59^{f-i}	119.67
DDS05	83.52 ± 3.54^{jkl}	122.60 ± 3.37^{ef}	115.28 ± 5.53^{lm}	170.72 ± 9.70^{ab}	123.03
DDS06	87.90 ± 1.86^{h-k}	122.63 ± 7.48^{def}	117.05 ± 2.21^{lm}	165.00 ± 2.03^{a-d}	123.15
DDS07	87.98 ± 1.26^{ghi}	129.92 ± 3.58^{cde}	120.32 ± 3.85^{j-m}	161.80 ± 6.49^{a-e}	125.01
DDS08	90.59 ± 3.21^{g-j}	128.02 ± 5.70^{cde}	111.46 ± 0.72^{m}	162.58 ± 3.53^{a-e}	123.16
DDS09	85.00 ± 6.83^{i-l}	120.08 ± 6.33^{def}	123.08 ± 2.70^{lm}	163.37 ± 2.30^{a-e}	122.88
DDS10	88.07 ± 3.86^{fgh}	120.08 ± 1.92^{def}	120.15 ± 17.43^{klm}	174.16 ± 15.20^{a}	125.61
DDS11	93.87 ± 5.85^{fgh}	124.85 ± 1.82^{f}	153.97 ± 18.78^{ef}	167.01 ± 4.47^{abc}	134.93
DDS12	94.38 ± 1.59^{e-h}	145.11 ± 3.73^{a}	137.21 ± 3.52^{g-j}	158.67 ± 7.03^{a-f}	133.84
DDS13	95.15 ± 1.24^{def}	137.35 ± 6.17^{bcd}	146.58 ± 9.08^{f-i}	167.22 ± 8.12^{abc}	136.57
DDS14	89.82 ± 0.73^{fgh}	136.77 ± 2.45^{abc}	149.37 ± 2.52^{fg}	148.28 ± 9.61^{e-i}	131.06
DDS15	82.65 ± 1.71^{i-l}	145.96 ± 6.37^{ab}	137.65 ± 5.00^{h-k}	155.43 ± 1.01^{b-g}	130.43
DDS16	85.38 ± 1.74^{lm}	143.27 ± 3.67^{ab}	144.36 ± 1.91^{f-i}	153.05 ± 5.74^{c-h}	131.52
DDS17	96.67 ± 3.16^{def}	133.50 ± 1.98^{abc}	153.42 ± 3.02^{def}	148.11 ± 4.76^{e-i}	132.92
DDS18	95.18 ± 1.00^{efg}	143.78 ± 17.46^{abc}	153.05 ± 12.08^{fgh}	143.51 ± 3.13^{f-i}	133.88

DDS19	98.44 ± 1.85^{cde}	146.34 ± 10.57^{ab}	171.44 ± 5.31^{cd}	150.26 ± 10.88^{d-i}	141.62
DDS20	97.47 ± 2.57^{def}	125.44 ± 6.82^{ab}	178.62 ± 4.41^{cde}	139.56 ± 3.64^{ghi}	135.27
DDS21	105.98 ± 4.43^{cd}	98.77 ± 0.87^{g}	178.08 ± 20.59^{bc}	138.23 ± 1.72^{hi}	130.27
DDS22	108.84 ± 7.35^{c}	128.60 ± 11.39^{def}	192.55 ± 3.47^{ab}	135.54 ± 26.16^{i}	141.39
DDS23	114.60 ± 5.93^{a}	131.76 ± 3.27^{cde}	200.39 ± 6.34^{a}	136.73 ± 2.10^{i}	145.87
DDS24	113.01 ± 2.73^{b}	127.34 ± 4.46^{cde}	195.06 ± 8.53^{a}	134.21 ± 10.03^{i}	142.41

Values in the same vertical column followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

3.4. IAA synthesis activity of rhizobacteria

Table 5 presents the quantitative analysis results of IAA concentration for 24 rhizobacterial strains over an eight-day culture, with measurements taken on days 2, 4, 6, and 8. The results indicate that all strains exhibited IAA synthesizing capability with various efficiency. Over an eight-day culture period, the average IAA concentrations of the bacterial isolates ranged from 3.75 mg/ml to 5.56 mg/ml. Isolate DDS07 produced the highest average IAA concentration at 5.56 mg/ml, closely followed by DDS05 (5.03 mg/ml) and DDS06 (4.85 mg/ml). Several other isolates, including DDS15, DDS14, DDS13, DDS04, and DDS02, also showed high average IAA production, with concentrations between 4.36 mg/ml and 4.60 µg/ml. A second group of isolates, including DDS09, DDS17, DDS19, DDS20, DDS21, DDS22, DDS23, DDS24, DDS12, DDS10, and DDS11, exhibited moderate IAA production. In contrast, DDS08 produced the lowest average IAA concentration among all the tested isolates. The study tracked IAA production in bacterial isolates over eight days, revealing various patterns. Initially, all isolates showed increased IAA from day 2 to 4. After this, between days 4 and 6, isolates DDS01, DDS02, DDS03, DDS10, and DDS11 continued to produce more IAA, while a larger group, including DDS05-09 and DDS12-24, decreased, with DDS04 and DDS08 fluctuating. From day 6 to 8, DDS03, DDS08, DDS09, DDS10, and DDS12 increased IAA concentration, but most others decreased, and DDS13-18 fluctuated. Overall, while DDS03, DDS09, and DDS10 showed a general increase over the eight days, the majority of isolates displayed fluctuating IAA concentrations throughout the experiment. All 24 rhizobacterial strains demonstrated IAA synthesis, though with varying levels. While DDS07, DDS05, and DDS06 exhibited high average IAA production, and DDS03, DDS09, and DDS10 showed consistent increases in IAA concentration, most strains displayed fluctuating IAA levels over the eight-day culture period.

Table 5 IAA synthesis activity of 24 rhizobacterial trains

Isolate	**Concentration of IAA (mg/L)**				
	Day 2th	**Day 4th**	**Day 6th**	**Day 8th**	**Average**
Control (-)	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{f}	0.00
DDS01	3.64 ± 0.38^{abc}	4.24 ± 1.10^{f}	5.04 ± 0.72^{a}	4.11 ± 0.64^{bcd}	4.26
DDS02	3.44 ± 0.33^{de}	4.63 ± 0.62^{def}	4.72 ± 0.40^{abc}	4.66 ± 0.64^{abc}	4.36
DDS03	3.30 ± 0.08^{de}	4.26 ± 0.31^{f}	4.36 ± 0.12^{a-e}	5.02 ± 0.44^{ab}	4.23
DDS04	3.19 ± 0.08^{e}	5.48 ± 0.52^{cde}	4.24 ± 0.34^{a-f}	5.05 ± 0.65^{ab}	4.49
DDS05	3.32 ± 0.11^{de}	7.08 ± 0.64^{b}	4.27 ± 0.52^{a-f}	5.44 ± 0.90^{ab}	5.03
DDS06	3.57 ± 0.43^{a-d}	7.22 ± 0.17^{b}	4.49 ± 0.79^{a-d}	4.11 ± 0.28^{bcd}	4.85
DDS07	3.67 ± 0.17^{ab}	8.26 ± 1.78^{a}	4.95 ± 0.85^{ab}	5.37 ± 1.04^{ab}	5.56
DDS08	3.37 ± 0.11^{b-e}	3.67 ± 0.39^{f}	2.94 ± 0.23^{g}	5.00 ± 0.93^{ab}	3.75
DDS09	3.30 ± 0.03^{de}	4.15 ± 1.07^{f}	3.58 ± 0.30^{d-g}	5.71 ± 0.80^{a}	4.19
DDS10	3.18 ± 0.16^{e}	3.65 ± 0.27^{f}	3.74 ± 0.65^{c-g}	5.02 ± 0.51^{ab}	3.90
DDS11	3.64 ± 0.16^{abc}	3.83± 0.41^{f}	3.74 ± 0.64^{c-g}	4.06 ± 0.90^{bcd}	3.82
DDS12	3.33 ± 0.08^{cde}	4.50 ± 0.11^{ef}	3.28 ± 0.41^{fg}	4.68 ± 0.73^{abc}	3.95
DDS13	3.30 ± 0.03^{de}	5.71 ± 0.92^{cd}	4.26 ± 0.41^{a-f}	4.88 ± 0.88^{a-d}	4.54

DDS14	3.44 ± 0.11^{a-e}	7.00 ± 0.59^{b}	3.65 ± 0.90^{c-g}	4.22 ± 0.21^{e-i}	4.58
DDS15	3.18 ± 0.16^{e}	6.95 ± 0.26^{b}	3.57 ± 0.26^{d-g}	4.70 ± 0.61^{abc}	4.60
DDS16	3.21 ± 0.14^{e}	6.42 ± 0.36^{bc}	3.39 ± 0.31^{efg}	4.36 ± 0.43^{a-d}	4.35
DDS17	3.30 ± 0.08^{de}	5.53 ± 0.17^{cde}	3.09 ± 0.34^{g}	4.79 ± 0.45^{abc}	4.18
DDS18	3.25 ± 0.08^{de}	5.62 ± 0.03^{cd}	3.99 ± 0.41^{b-g}	4.04 ± 2.41^{bcd}	4.23
DDS19	3.23 ± 0.11^{e}	5.62 ± 0.21^{cd}	3.81 ± 0.92^{c-g}	3.39 ± 0.39^{cde}	4.01
DDS20	3.74 ± 0.09^{a}	5.78 ± 0.13^{c}	3.28 ± 0.77^{fg}	3.42 ± 0.79^{cde}	4.06
DDS21	3.23 ± 0.11^{e}	5.87 ± 0.18^{c}	3.92 ± 0.27^{b-g}	3.00 ± 0.46^{de}	4.00
DDS22	3.46 ± 0.29^{a-e}	5.76 ± 0.24^{c}	3.42 ± 0.69^{d-g}	3.02 ± 0.52^{de}	3.92
DDS23	3.30 ± 0.08^{de}	5.80 ± 0.27^{c}	3.90 ± 0.38^{c-g}	2.24 ± 0.03^{e}	3.81
DDS24	3.33 ± 0.08^{cde}	5.87 ± 0.14^{c}	3.46 ± 0.45^{d-g}	3.14 ± 0.67^{de}	3.95

Values in the same vertical column followed by one or more of the same letters were not significantly different at the 0.01 significance level according to Duncan's test.

3.5. Siderophore synthesizing activity of rhizobacteria

Siderophore produced from the tested rhizobacteria was indicated by the discoloration of the blue dye in the CAS reagent and formed halo zones on the CAS agar medium. Table 6 and Figure 3 exhibit results of assessing the siderophore producing ability of the twenty-four rhizobacteria isolates. Of the 24 distinct isolates tested, 09 isolates, constituting roughly 37.5 % of the tested strains, displayed positive siderophore production. 15 isolates, representing approximately 62.5 % of the sample, exhibited a negative result.

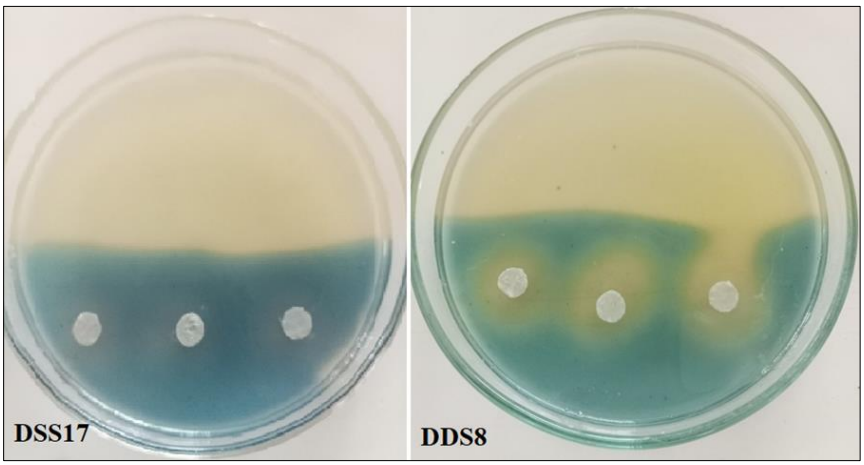


Figure 3 Siderophore synthesis of some rhizobacteria

Table 6 Siderophore synthesis capability of rhizobacteria

Isolate	**Siderophore production**	**Isolate**	**Siderophore production**
Control (-)	-	DDS13	+
DDS01	-	DDS14	+
DDS02	-	DDS15	+
DDS03	-	DDS16	-
DDS04	+	DDS17	+
DDS05	-	DDS18	-

DDS06	-	DDS19	-
DDS07	+	DDS20	-
DDS08	+	DDS21	-
DDS09	-	DDS22	+
DDS10	+	DDS23	-
DDS11	-	DDS24	-
ĐĐS12	-		

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria found in rhizosphere in association with roots and root surfaces or free living in soil and promote plant growth directly or indirectly [27, 28]. A significant portion of endophytic bacteria are believed to originate from the rhizosphere [29]. Root exudates including organic acids, amino acids, and proteins may be involved in recruiting bacterial endophytes from the rhizosphere and becoming rhizobia [30]. Several investigations isolating plant associated bacteria from *Sesbania* genus and assessing their plant growth promotion activity have been carried out. Most of surveys have focused on rhizobia while studies on rhizosphere bacteria have been limited. Studies on bacteria from *Sesbania* species revealed that 20 isolated from *Sesbania bispinosa* root nodules demonstrated growth in nitrogen-free media and positive ammonia production [31]. Several endophytic bacteria displayed phosphate solubilization and IAA synthesis capabilities. From *Sesbania grandiflora* nodules, twenty rhizobia were isolated, with 80% exhibiting phosphate solubilization. The phosphate solubilization index (P-SI) for these isolates ranged from 1.96 to 4.85, with five isolates showing excellent phosphate solubilization potential for biofertilizer applications [32]. A *Rhizobium* strain, *Rhizobium sp*. U9709-SC, isolated from S. cannabina, also showed phosphate solubilization [33]. Further research on *Sesbania bispinosa* showed that twenty nodule isolates exhibited formation of halo zones on NBRIP medium and high IAA production [31]. A study on *Sesbania sesban* (L.) Merr. resulted in the isolation of 26 *Rhizobium* strains from root nodules in Andhra Pradesh, all capable of IAA synthesis. Among these, 6 strains produced maximum IAA with 2.5 mg/mL L-tryptophan supplementation [33]. Similarly, when studied PGPR inhabiting the rhizosphere of *Sesbania bispinosa*, Dasgupta et al., (2015) found seven out of 12 rhizobacteria with phosphate solubilization and IAA synthesis capabilities [27]. Another study on Plant growth promotion characterization of endophytic bacteria from *Sesbania sesban* (L.) Merr. collected in Tan Hung district, Long An province, Vietnam isolated eighteen endophytic strains showing nitrogen fixation with NH_4^+ concentration of 0.183 to 0.357 mg/L, phosphate solubilization with P_2O_5 concentration of 56.052 to 69.66 mg/L and IAA biosynthesis with IAA concentration of 0.796 mg/L to 1.543 mg/L [34]. With regard to siderophore synthesis, siderophore production was not a common trait among rhizobia but was limited to a few specific strains [35]. A study on siderophore producing rhizobia from *Sesbania sesban* using different types of Indian soils showed that six out of 14 rhizobia isolated from *Sesbania sesban* were able to produce siderophore. Jenifer et al. (2013) reported that four out of the 11 bacteria isolated from rhizosphere soil were found to produce siderophore [36]. Kumar et al., (2024) selected 16 out of 70 rhizobia from *Sesbania sesban* (L) Merr. for investigating their phosphate solubilization and siderophore production. The result showed that only six out of 16 strains exhibited siderophore production [37].

4. Conclusion

This study successfully isolated and characterized 24 rhizobacteria from the rhizosphere of *Sesbania cannabina*, revealing a diverse population with varying morphological and physiological traits. The isolates, exhibiting predominantly opaque white, flat colonies with diverse shapes, margins, and diameters ranging from 1 to 4 mm, showed a balanced distribution of Gram-positive and Gram-negative bacteria with rod and cocci shapes, and demonstrated significant plant growth-promoting activities. Notably, isolates DDS24 and DDS21 displayed the highest nitrogen fixation, DDS23 exhibited the highest phosphate solubilization and DDS07, DDS05, and DDS06 exhibited superior IAA production. Furthermore, nearly half of the isolates demonstrated siderophore production capabilities. These findings highlight the potential of these *Sesbania cannabina* (Retz.) Pers. rhizobacteria as effective biofertilizers, and further investigation on their application and optimization for sustainable agriculture is needed.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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