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# Investigating Bacteria inhabiting Root Nodule of Velvet bean [*Mucuna pruriens* (L.) DC.] for their Plant Growth Promotional Traits

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**Abstract**

This study investigates the plant growth-promoting (PGP) potential of bacterial strains isolated from root nodules of velvet bean (*Mucuna pruriens*), a medicinal legume valued for its therapeutic and agricultural applications.While rhizobia within root nodules are extensively studied, other microbial communities associated with these nodules remain largely unexplored. A total of 43 bacterial isolates, including rhizobial and non-rhizobial strains, were obtained through standard microbiological techniques. Morphological and biochemical characterizations revealed significant diversity among the isolates. Screening for PGP activities demonstrated capabilities such as phosphorus and potassium solubilization, siderophore and ammonia production and nitrogen fixation. Notably, 17 isolates exhibited all five PGP traits, indicating their potential for enhancing nutrient availability and supporting sustainable agriculture. Among these, seven isolates (NN8, NN10, NJ1, NJ2, NJ6, NJ8 and NP2) were identified as particularly promising based on their high indices of solubilization and nitrogen fixation. This study highlights the importance of root nodule-associated microbes of velvet bean in promoting plant growth. Further, *In vivo* evaluation is required to validate their agricultural utility. The findings highlight the role of microbial diversity in root nodules and its potential for biofertilizer development.

*Keywords* : Biochemical characterization, PGP traits, Root nodule associated microbes, Velvet bean

Medicinal plants with wide variety of applications have been used in *Ayurveda* since the ancient times in India and around the world for treating various ailments or diseases and also have cosmetic value. These medicinal plants are recognized as rich sources of secondary metabolites with therapeutic properties, making them prominent for their use in both traditional and modern pharmacology. Velvet bean is one of the prominent medicinal plants with promising pharmaceutical and cosmeceutical bioactive compounds. The plant is well known for its therapeutic properties and it is being used in treating various diseases including its potential in managing neurological disorders, improving male fertility, anti-inflammatory, anti-diabetic, anti-oxidant, anti - microbial, anti - epileptic properties (Sathiya narayanan *et al*., 2007). The seeds are especially known for their high content of L-3, 4-dihydroxy phenylalanine (L-DOPA) (4-7%), velvet bean is a commercial source of this compound, a precursor of dopamine, which makes it a valuable bioactive compound in treating Parkinson's disease (Lucia *et al.,* 2012).

The velvet bean, belonging to the family Fabaceae and subfamily Papilionaceae, comprises approximately 150 species of annual and perennial legumes. Among the various underutilized wild

legumes, the velvet bean is widely distributed across tropical and subtropical regions worldwide. In addition to its diverse therapeutic applications, it is recognized as a valuable source of dietary protein (23-35%) (Janardhanan *et al*., 2003 and Pugalenthi *et al*., 2005). Its protein content is comparable to that of other pulses such as soybean, rice bean and lima bean (Gurumoorthi *et al*., 2003).

However, being the under-utilized wild crop having diverse health and agriculture applications, the associated microbial communities and their plant growth promoting (PGP) potentials have been paid least attention. Specifically, those microbes which reside inside the plant particularly in root nodules have been least studied. Most of the previous studies have focused only on the nodule inhabiting rhizobia but latest literatures of metagenomic studies have confirmed the presence of other microbial communities along with rhizobia and they have shown plant growth promoting potential (Jinfeng *et al.,* 2021 and Cao *et al.,* 2024). In this context, the investigation on microbes associated with the root nodules of velvet bean is first of its kind and could provide valuable insights into novel bacterial strains with PGP activities for sustainable agriculture, biotechnology and commercial applications. Hence the present study aims to isolate and test the bacterial communities associated with the root nodules of velvet bean for their PGP activities.

## Material and Methods

### Sample Collection

Root nodules were collected from velvet bean plant grew wildat Krishi Vignana Kendra (KVK), Hadonahalli, Doddaballapura, Bengaluru, India (13.37°N, 77.55°E). Root nodules were immediately transferred into an ice box and brought to the laboratory for isolation of bacteria associated with root nodules (Plate 1).

### Isolation of Bacteria

For isolation of native bacteria including Rhizobia from root nodules of velvet bean was carried out following procedure given by Zhen *et al*. (2011). First the collected root nodules were washed thoroughly to remove adhering soil particles and dirt. Then, nodules were surface sterilized with 70 per cent ethanol for 1 min and then with 3 per cent sodium hypochlorite for 1 min followed by serial washing with sterile distilled water for 7 consecutive washes. Sterilized root nodules were crushed in small quantity (10 ml) of sterile distilled water and aliquot of 1 ml was serially diluted in 9 ml sterile distilled water up to $10^{-4}$. An aliquot of 0.1 ml from $10^{-3}$ was spread on petri plates having Yeast Extract Mannitol Agarwith Congo Red (CRYEMA) for isolation of rhizobium while an aliquot of 0.1 ml from $10^{-4}$ was spread on petri plates having Pikovskaya'sagar (PA), Aleksandrov's agar (AA),

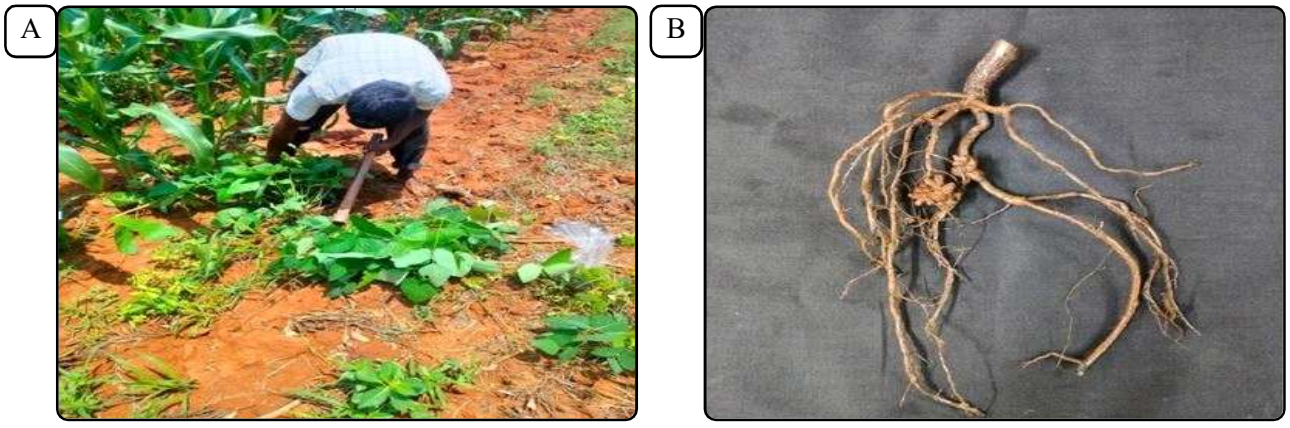


Plate 1 : Collection of root nodule sample for isolation of bacteria.
A. Wildly grown Velvet bean plant (KVK,Hadonahalli). B. Root nodules of Velvet bean

Jensen's agar (JA) and Nutrient agar (NA) media for enumeration and isolation of phosphate solubilizing, potassium solubilizing, nitrogen fixing and other non fastidious bacteria respectively. Plates were incubated at 30°C for 24 - 96 h. The microbial count was expressed in CFU/ml.

**Morphological Characterization**

Morphologically distinct bacterial colonies were selected based on the distinct characters such as colony colour, shape, elevation and margin.

**Confirmatory Tests for Rhizobial Isolates**

The following tests were carried out to differentiate rhizobial isolates from *Agrobacterium.*

**Hofer's Alkaline Test**

Hofer's alkaline medium (YEMA with pH adjusted to pH 10.8 - 11.2) was used to differentiate rhizobial isolates from *Agrobacterium*. No growth of culture in the medium confirms the isolates as *Rhizobium,* as Hofer's alkaline medium is selective medium is for *Agrobacterium* isolation because of its ability to grow at higher pH while inhibiting *Rhizobium* spp. at such alkaline pH (Hofer, 1935).

**Growth on YEMA with BTB**

Production of acid/alkali by the root nodulating rhizobia was studied as per the procedure outlined by Vincent (1970). One day old rhizobial cultures were streaked on plates containing YEMA with 0.5 per cent alcoholic BTB having a pH of 7.0 The change in colour of medium from green to yellow confirms the rhizobial isolates as fast growing acid producers while, change in the colour of BTB dye from green to blue confirms the rhizobial isolates as fast or slow growing rhizobia.

**Growth on YEMA with Congo Red**

YEMA with 2.5 ml of 1 per cent Congo red (CR) per litre was prepared. Fresh cultures of test isolates were streaked on the plates containing YEMA+CR and incubated at 28±2°C for 2-3 days. Little or no absorption of Congo red on CRYEMA is characteristic feature of rhizobia (Vincent, 1970).

**Screening for Plant Growth Promoting (PGP) Activities**

**Phosphate Solubilization**

Screening for phosphate solubilizationability of bacterial isolates was done by a plate assay method using Pikovskaya's agar medium (Pikovskaya, 1948) supplemented with tricalcium phosphate. The plates were spot inoculated with 1.5 µL of three days old bacterial cultures ($OD_{600}$ = 0.8) and incubated for upto 7 days at 30°C. The formation of clear zone around bacterial colonies indicates positive for solubilizing ability of the isolatesand the results were expressed as phosphorus solubilization index (PSI) (Tariq *et al.,* 2022).

**Potassium Solubilization**

Bacteria isolates were screened for potassium solubilization following a plate assay method using Aleksandrov's agar medium supplemented with insoluble micaas a potassium bearing mineral. The plates were spot inoculated with 1.5 µL of three days old bacterial cultures ($OD_{600}$ = 0.8) and incubated for upto 7 days at 30°C. Formation of clear zone around bacterial colonies indicates positive for solubilizing ability of the isolates and the results were expressed as Potassium Solubilization Index (KSI) (Hu *et al.,* 2006).

**Siderophore Production**

Bacterial isolates were screened of forsiderophore production which was carried outby following method given by Schwyn and Neilands (1987) in Chrome Azurol S (CAS) agar medium. The plates containing bluish green CAS agar were spot inoculated with 1.5 µL of bacterial cultures ($OD_{600}$ = 0.8) and incubated at 30°C for 3-5 days. Change in bluish green colour of the CAS agar medium around the bacterial colony to yellow, blue, orange or red indicated as positive for production of siderophore. Siderophore Producing Index (SPI) was calculated as the ratio of (colored zone diameter + colony diameter)/ colony diameters (Desai *et al.,* 2012).

**Nitrogen Fixing Ability**

Screening for nitrogen fixing ability of the bacterial isolates was carried out by streakingon nitrogen free Jensen's medium (Norris and Chapman, 1968). Growth of bacterial isolates was considered as positive for atmospheric nitrogen fixing ability.

**Ammonia Production**

Ammonia production ability of the bacterial isolates was carried out by following the method given by Cappuccino and Sherman (1992). 1.5µl of bacterial cultures ($OD_{600}$= 0.8) were inoculated in 10 ml peptone broth and incubated at 30 °C for 48-72 h in anorbital shaking incubator. After incubation, 0.5 ml of Nessler's reagent was added in each test tube. Development of a yellow to dark brown colour indicated the production of ammonia. For quantification, 2 ml of the broth was taken in an eppendorf tube and centrifuged at 10,000 rpm for 5 min. Then, 0.5 ml of Nessler's reagent was added to each tube containing the supernatant of bacterial isolates and the absorbance was read at 450 nm. The concentration of the $NH_4^+$ was calculated using the standard curve of ammonium sulphate solution and expressed as µg/mL (Mukherjee *et al*., 2017)

**Biochemical Characterization**

The isolates were biochemically characterized as per the procedures described by Cappuccino and Sherman (1992). The tests conducted are detailed below.

**Catalase Test**

Bacterial colonies, grown for 24 hours, were placed on glass slides and a drop of 30 per cent hydrogen peroxide ($H_2O_2$) was added. The formation of gas bubbles signified the presence of the catalase enzyme.

**Oxidase Test**

A strip of filter paper was dipped in Kovac's reagent and air-dried. The bacterial isolates were considered positive if the colour of filter paper turns to lavender colour, which then turns dark purple to black color within 5 min after inoculation with bacterial cultures.

**Urease Test**

The bacterial isolates were inoculated to the test tube containing 5 ml of sterilized urea broth with phenol red as pH indicator and incubated at 30± 2°C for 24 - 48 hours. The development of dark pink color was recorded as positive for urease activity.

**Citrate Utilization Test**

The citrate utilization test was performed by inoculating the one day cultures separately onto Simmon's citrate agar with bromothymol blue as an indicator, where sodium citrate was the only carbon and energy source. After 48 h of incubation at 30°C, the cultures were observed for the growth and change in the colour of the medium from green to blue indicating positive for citrate utilization.

**Starch Hydrolysis**

The bacterial isolates were streaked on starch agar medium and incubated the plates at room temperature (25-37 °C) for 24 hrs. Hydrolysis of starch was tested by flooding the plates with iodine solution. Formation of clear zones around the bacterial colonies was considered as positive for starch hydrolysis.

**Hydrogen Sulfide Production**

Sulfide indole motility (SIM) agar stabs were inoculated with the bacterial isolates and incubated at 30 °C for 48 hrs. Black coloration along the line of stab inoculation indicated $H_2S$ production.

**Statistical Analysis**

The data was analyzed through a one-way ANOVA test and Duncan's Multiple Range Test (DMRT) was applied to separate the means at a significance level of $P<0.05$, using OPSTAT (Duncan, 1995).

## Results and Discussion

The root nodules sample of velvet bean were collect from KVK, Hadonahalli, Doddaballapura, Bengaluru

(13.37°N, 77.55°E). Standard serial dilution and plate count (SDPC) method was followed for isolation and enumeration of bacterial isolates from root nodules of velvet bean. The Rhizobial isolates with other bacterial isolates were isolated on five different medianamely CRYEMA, PA, AA, JA and NA. Total population of bacterial isolateson mentioned media were enumerated and expressed in CFU/ml as represented in Table 1. A total of 45.23, 40.33, 52.67 and 12.33 on PA, AA, JA and AA were recorded at dilution $10^4$ and 25.13 rhizobial colonies were recorded on CRYEMA medium at dilution $10^3$.

A total of forty-three distinct bacterial isolates including eleven rhizobial isolates associated with root nodules of velvet bean were obtained on five different media based on distinct morphological characters (Table 1). These forty-three bacterial isolates includes twelve bacteria (NN1 to NN12) from NA, four bacteria (NA1 to NA4) from AA, nine bacteria (NJ1 to NJ9) from JA, seven bacteria (NP1 to NP7) from PA and elevenrhizobial isolates (NR1 to NR8 and NRL1 to NRL3) from CRYEMA. Out of eleven rhizobial isolates, eight (NR1-NR8) were observed to be fast growers as they showed growth on CRYEMA medium within 24 h of incubation whereas NRL1, NRL2 and NRL3 were seen on CRYEMA after 72 h of incubation. They were purified on culture media and stored at 4°C for further studies.

**Table 1**

**Isolation and enumeration of bacterial isolates from root nodules of Velvet bean on different media**

| Root Nodule | Population of bacteria | Isolates obtained |
|---|---|---|
| Media | CFU/ml | |
| NA | $45.23 \times 10^4$ | 12 |
| PA | $40.33 \times 10^4$ | 7 |
| JA | $52.67 \times 10^4$ | 9 |
| AA | $12.33 \times 10^4$ | 4 |
| YEMA | $25.13 \times 10^3$ | 11 |

*Note* : Numerical values are mean of three replicates. Nutrient agar (NA), Pikovskaya's agar (PA), Jensen's medium (JA), Aleksandrov's agar (AA) and Yeast Extract Mannitol Agar with Congo red (CRYEMA)

Morphological characteristics of bacterial isolates obtained are presented in Table 2. Isolates exhibited six different colony colours ranging from creamish white, yellow, pale yellow, white, light orange to translucent. Out of forty-three isolates, thirty-three isolates were observed to have round shape and entire margin; nine isolates

**Table 2**

**Morphological Characteristics of bacteria isolated from root nodules of Velvet bean**

| Isolate code | Colony colour | Shape | Elevation | Margin | Gram's reaction | Shape |
|---|---|---|---|---|---|---|
| NN1 | Translucent | Round | Flat | Entire | + | Rod |
| NN2 | Yellowish | Round | Raised | Entire | + | Rod |
| NN3 | Creamish White | Round | Raised | Entire | + | Rod |
| NN4 | Creamish White | Irregular | Flat | Undulated | + | Rod |
| NN5 | White | Round | Convex | Entire | + | Cocci |
| NN6 | Creamish White | Round | Raised | Entire | + | Cocci |
| NN7 | Creamish White | Round | Raised | Entire | + | Rod |
| NN8 | Pale Yellow | Irregular | Convex | Undulated | + | Rod |
| NN9 | Pale Yellow | Filamentous | Flat | Lobate | + | Cocci |
| NN10 | Pale Yellow | Round | Raised | Entire | - | Rod |

Continued....

**Table 2 Continued....**

| Isolate code | Colony colour | Shape | Elevation | Margin | Gram's reaction | Shape |
|---|---|---|---|---|---|---|
| NN11 | Light orange | Round | Raised | Entire | + | Rod |
| NN12 | Creamish White | Round | Flat | Entire | + | Rod |
| NA1 | Creamish White | Round | Convex | Entire | + | Cocci |
| NA2 | Creamish White | Round | Raised | Entire | - | Rod |
| NA3 | Creamish White | Irregular | Raised | Undulated | + | Rod |
| NA4 | Creamish White | Irregular | Raised | Undulated | + | Cocci |
| NJ1 | Creamish White | Round | Raised | Entire | + | Rod |
| NJ2 | Creamish White | Round | Raised | Entire | + | Rod |
| NJ3 | Translucent | Irregular | Raised | Undulated | + | Rod |
| NJ4 | Creamish White | Round | Raised | Entire | + | Rod |
| NJ5 | Creamish White | Irregular | Convex | Undulated | + | Rod |
| NJ6 | Creamish White | Round | Convex | Entire | + | Cocci |
| NJ7 | Creamish White | Round | Convex | Entire | - | Rod |
| NJ8 | Creamish White | Round | Raised | Entire | + | Rod |
| NJ9 | Creamish White | Round | Raised | Entire | + | Rod |
| NP1 | Creamish White | Irregular | Convex | Undulated | + | Cocci |
| NP2 | Creamish White | Round | Convex | Entire | + | Rod |
| NP3 | Creamish White | Round | Raised | Entire | + | Rod |
| NP4 | Creamish White | Irregular | Raised | Undulated | + | Rod |
| NP5 | Creamish White | Round | Flat | Entire | - | Rod |
| NP6 | Creamish White | Round | Raised | Entire | - | Rod |
| NP7 | Creamish White | Irregular | Raised | Undulated | + | Cocci |
| NR1 | Translucent | Round | Convex | Entire | - | Rod |
| NR2 | Translucent | Round | Convex | Entire | - | Rod |
| NR3 | Translucent | Round | Convex | Entire | - | Rod |
| NR4 | Translucent | Round | Convex | Entire | - | Rod |
| NR5 | Translucent | Round | Convex | Entire | - | Rod |
| NR6 | Translucent | Round | Convex | Entire | - | Rod |
| NR7 | Translucent | Round | Convex | Entire | - | Rod |
| NR8 | Translucent | Round | Convex | Entire | - | Rod |
| NRL1 | Translucent | Round | Convex | Entire | - | Rod |
| NRL2 | Translucent | Round | Convex | Entire | - | Rod |
| NRL3 | Translucent | Round | Convex | Entire | - | Rod |

*Note* : '+'-positive; '-'- negative.

have irregular shape with undulated margin and one isolate had filamentous shape with lobes. Majority of the isolates were showed convex to raised elevation and very few had flat elevation. Most of the isolates were observed as Gram-positive, rod shaped.

**Confirmatory Tests for the Rhizobial Isolates.**

The rhizobial isolates were observed for the growth on YEMA+Bromothymol Blue, YEMA + Congo red (CR) and Hofer's alkaline broth to confirm isolates as rhizobium and differentiate them from *Agrobacterium* (Table 3). The rhizobial isolates namely NR1, NR2, NR4 and NR8 were acid producing fast growers as they turned green colour of the YEMA+BTB medium into yellow. The isolates namely NR3, NR5, NR6, NR7, NRL1, NRL2 and NRL3 were alkaline producing slow growers as they turned green colour of the YEMA + BTB medium into blue. The obtained rhizobial isolates showed no growth in Hofer's alkaline broth and were observed that the isolates did not absorb the Congo red dye amended in YEMA medium. This confirms the isolates as rhizobia. These results are in similar to the confirmatory tests of rhizobia isolated from cowpea (Erana *et al.,* 2022).

**TABLE 3**

**Confirmatory tests for the rhizobial isolates obtained from root nodules of velvet bean**

| Isolate code | YEMA with BTB | Growth on CRYEMA | Hoffer's alkaline test |
|---|---|---|---|
| NR1 | Yellow | - | - |
| NR2 | Yellow | - | - |
| NR3 | Blue | - | - |
| NR4 | Yellow | - | - |
| NR5 | Blue | - | - |
| NR6 | Blue | - | - |
| NR7 | Blue | - | - |
| NR8 | Yellow | - | - |
| NRL1 | Blue | - | - |
| NRL2 | Blue | - | - |
| NRL3 | Blue | - | - |

*Note* : Growth on CRYEMA: absorbed Congo red dye (+) and no absorbance of dye (-).

Growth in Hoffer's alkaline broth: "+" indicates growth and "-" indicates no growth in alkaline broth

**Screening of Bacterial Isolates for Plant Growth Promoting Traits**

The bacterial isolates were screened for different PGP activities (Table 4). Among forty-three bacterial isolates, forty isolates showed positive for phosphorus solubilization with phosphorus solubilization indices (PSI) ranging from 2.30 to 6.66. The isolate NN3 showed the highest PSI (6.66) followed by NJ6 with 6.02 whereas, isolate NRL2 showed the least PSI of 2.30. Among the rhizobial isolates, NR5 showed the highest PSI of 5.51. Similarly, twenty-seven isolates showed positive for potassium solubilization with KSI ranging from 2.27 to 4.60, where the highest KSI was recorded by isolate NR7 (6.61) and the lowest by NJ5 (2.27).

Majority of the isolates were observed to produce siderophore. Out of forty-three isolates, thirty-One isolates were positive for siderophore production by forming yellow or blue coloured zones around the bacterial colonies with SPI ranging from 2.30 (NJ6) to 7.50 (NN5). Among the rhizobial isolates, five isolates namely, NR1, NR2, NR3, NR4 and NR8 showed siderophore production were in highest SPI was recorded by NR4 (2.76) and least by NR8 (2.35). Two isolates (NN4 and NN6) showed negative for nitrogen fixation as well as ammonia production whereas, the isolate NR6 showed the highest ammonia production with 57.03 mmol/L followed by NR2 with 56.42mmol/L and the least was recorded by NJ9with 10.33mmol/L. All rhizobial isolates showed the ability to produce ammonia which ranged from 0.38 (NR1) to 0.92 mg/ml (NR6).

Out of forty-three isolates, seventeen isolates showed all the five PGP activities and remaining isolates failed to show one or more PGP activities. This shows the potential of isolates obtained from root nodule of velvet bean for *In vitro* plant growth promoting activities such as,phosphorus and potassium solubilization, nitrogen fixation helps in providing available form of nutrients such as $N_2$, P and K for uptake by the plants. Siderophore production by isolates aids in chelating iron in soil

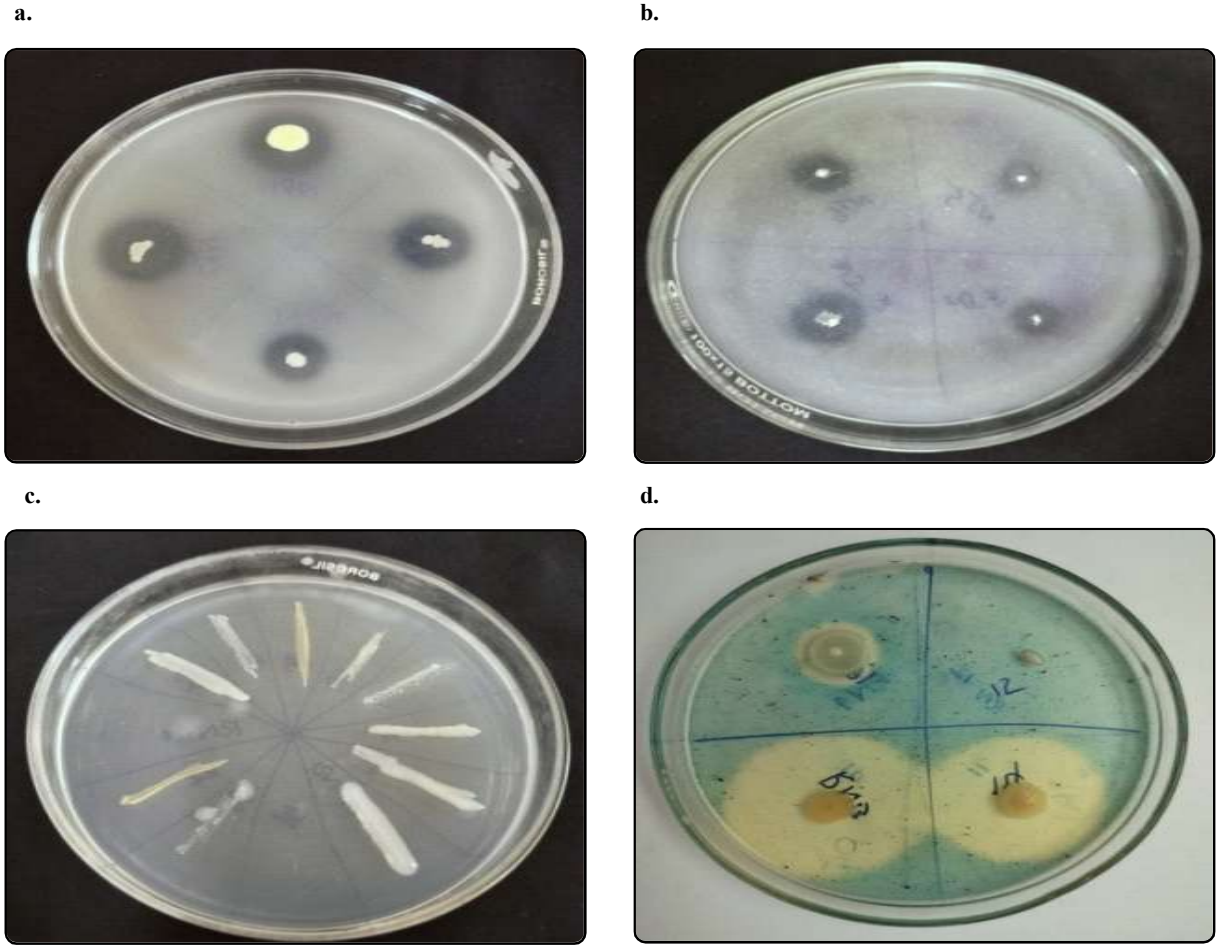


Plate 2 : Plant growth promoting traits of bacterial isolates.
a. Phosphorus solubilization, b. Potassium solubilization, c. Growth on N- free medium and d. Siderophore production

**TABLE 4**

**Screening of bacterial isolates for PGP activities**

| Isolate code | P-solubilization | | K-solubilization | | Nitrogen fixation | | Siderophore production | |
|---|---|---|---|---|---|---|---|---|
| | Qualitative assay | Solubilization Index | Qualitative assay | Solubilization Index | Growth on $N_2$-free medium | $NH_3$ Production (mmol/L) | Qualitative assay | Siderophore production Index |
| NN1 | | 0.00 $^{x}$ | | 0.00 $^{q}$ | + | 39.13 $^{m}$ | | 0.00 $^{p}$ |
| NN2 | + | 4.09 $^{hi}$ | | 0.00 $^{q}$ | + | 36.07 $^{t}$ | | 0.00 $^{p}$ |
| NN3 | + | 6.66 $^{a}$ | + | 3.86 $^{l}$ | + | 32.88 $^{w}$ | | 0.00 $^{p}$ |
| NN4 | + | 4.24 $^{g}$ | + | 4.20 $^{k}$ | | 0.00 $^{L}$ | + | 5.50 $^{gh}$ |
| NN5 | | 0.00 $^{x}$ | + | 2.66 $^{o}$ | + | 38.67 $^{n}$ | + | 7.50 $^{a}$ |
| NN6 | | 0.00 $^{x}$ | + | 0.00 $^{q}$ | - | 0.00 $^{L}$ | | 0.00 $^{p}$ |
| NN7 | + | 3.92 $^{jk}$ | + | 5.34 $^{e}$ | + | 39.47 $^{l}$ | | 0.00 $^{p}$ |
| NN8 | + | 4.81 $^{e}$ | + | 4.29 $^{j}$ | + | 47.07 $^{h}$ | + | 6.33 $^{d}$ |

Continued....

**TABLE 4 Continued....**

| Isolate code | P-solubilization | | K-solubilization | | Nitrogen fixation | | Siderophore production | |
|---|---|---|---|---|---|---|---|---|
| | Qualitative assay | Solubilization Index | Qualitative assay | Solubilization Index | Growth on $N_2$-free medium | $NH_3$ Production (mmol/L) | Qualitative assay | Siderophore production Index |
| NN9 | + | 3.21 $^{o}$ | | 0.00 $^{q}$ | + | 36.22 $^{s}$ | + | 2.45 $^{no}$ |
| NN10 | + | 5.01 $^{d}$ | + | 4.28 $^{j}$ | + | 39.38 $^{l}$ | + | 6.40 $^{d}$ |
| NN11 | + | 2.33 $^{vw}$ | | 0.00 $^{q}$ | + | 34.96 $^{v}$ | + | 2.39 $^{no}$ |
| NN12 | + | 3.74 $^{l}$ | + | 4.81 $^{g}$ | + | 35.78 $^{u}$ | + | 6.00 $^{f}$ |
| NA1 | + | 4.01 $^{ij}$ | + | 5.01 $^{f}$ | + | 38.45 $^{o}$ | + | 5.67 $^{g}$ |
| NA2 | + | 3.30 $^{o}$ | + | 5.02 $^{f}$ | + | 25.98 $^{C}$ | + | 4.56 $^{j}$ |
| NA3 | + | 3.45 $^{n}$ | + | 3.64 $^{m}$ | + | 25.93 $^{C}$ | + | 6.36 $^{d}$ |
| NA4 | + | 2.36 $^{uvw}$ | + | 3.41 $^{n}$ | + | 23.43 $^{G}$ | + | 2.76 $^{l}$ |
| NJ1 | + | 4.32 $^{fg}$ | + | 5.81 $^{c}$ | + | 18.94 $^{I}$ | + | 2.53 $^{mn}$ |
| NJ2 | + | 3.50 $^{n}$ | + | 5.51 $^{d}$ | + | 26.63 $^{B}$ | + | 3.72 $^{k}$ |
| NJ3 | + | 2.90 $^{r}$ | | 0.00 $^{q}$ | + | 48.58 $^{f}$ | + | 5.54 $^{gh}$ |
| NJ4 | + | 4.34 $^{f}$ | + | 4.14 $^{k}$ | + | 44.43 $^{i}$ | + | 0.00 $^{p}$ |
| NJ5 | + | 4.01 $^{ij}$ | + | 2.27 $^{p}$ | + | 37.93 $^{q}$ | + | 5.63 $^{gh}$ |
| NJ6 | + | 6.02 $^{b}$ | + | 6.02 $^{b}$ | + | 24.02 $^{E}$ | + | 2.30 $^{o}$ |
| NJ7 | + | 4.13 $^{h}$ | + | 4.38 $^{i}$ | + | 23.81 $^{F}$ | + | 6.70 $^{c}$ |
| NJ8 | + | 3.71 $^{lm}$ | + | 6.01 $^{b}$ | + | 24.52 $^{D}$ | + | 6.10 $^{ef}$ |
| NJ9 | + | 3.41 $^{n}$ | + | 4.61 $^{h}$ | + | 10.33 $^{K}$ | + | 5.44 $^{h}$ |
| NP1 | + | 3.94 $^{jk}$ | | 0.00 $^{q}$ | + | 27.46 $^{A}$ | + | 5.11 $^{i}$ |
| NP2 | + | 4.88 $^{e}$ | + | 5.41 $^{e}$ | + | 27.37 $^{A}$ | + | 2.79 $^{l}$ |
| NP3 | + | 3.63 $^{m}$ | + | 4.68 $^{h}$ | + | 15.13 $^{J}$ | + | 6.11 $^{ef}$ |
| NP4 | + | 3.27 $^{o}$ | | 0.00 $^{q}$ | + | 20.83 $^{H}$ | + | 6.22 $^{de}$ |
| NP5 | + | 4.33 $^{fg}$ | | 0.00 $^{q}$ | + | 31.33 $^{y}$ | | 0.00 $^{p}$ |
| NP6 | + | 3.88 $^{k}$ | | 0.00 $^{q}$ | + | 30.08 $^{z}$ | + | 7.25 $^{b}$ |
| NP7 | + | 3.00 $^{q}$ | | 0.00 $^{q}$ | + | 32.11 $^{x}$ | + | 7.22 $^{b}$ |
| NR1 | + | 2.70 $^{s}$ | | 0.00 $^{q}$ | + | 48.46 $^{g}$ | + | 2.52 $^{mn}$ |
| NR2 | + | 2.38 $^{uvw}$ | | 0.00 $^{q}$ | + | 56.42 $^{b}$ | + | 2.50 $^{mn}$ |
| NR3 | + | 2.33 $^{vw}$ | | 0.00 $^{q}$ | + | 49.85 $^{e}$ | + | 2.67 $^{lm}$ |
| NR4 | + | 2.35 $^{vw}$ | | 0.00 $^{q}$ | + | 54.18 $^{c}$ | + | 2.76 $^{l}$ |
| NR5 | + | 5.51 $^{c}$ | + | 0.00 $^{q}$ | + | 38.33 $^{p}$ | | 0.00 $^{p}$ |
| NR6 | + | 3.64 $^{m}$ | + | 3.57 $^{m}$ | + | 57.03 $^{a}$ | | 0.00 $^{p}$ |
| NR7 | + | 2.50 $^{t}$ | + | 6.61 $^{a}$ | + | 47.18 $^{h}$ | | 0.00 $^{p}$ |
| NR8 | + | 3.10 $^{p}$ | | 0.00 $^{q}$ | + | 51.22 $^{d}$ | + | 2.35 $^{no}$ |
| NRL1 | + | 2.42 $^{tuv}$ | | 0.00 $^{q}$ | + | 42.63 $^{j}$ | | 0.00 $^{p}$ |
| NRL2 | + | 2.30 $^{w}$ | + | 5.01 $^{f}$ | + | 40.28 $^{k}$ | | 0.00 $^{p}$ |
| NRL3 | + | 2.45 $^{tu}$ | + | 5.34 $^{e}$ | + | 36.95 $^{r}$ | | 0.00 $^{p}$ |

*Note* : '+'-positive; '-'- negative. Numerical values are mean of three replicates. Treatments with the different superscripts represent a significant difference as determined by DMRT ($p \leq 0.05$)

and making it available for plants and thereby enhances the plant growth as well as yield as reported by Cao *et al.* (2024), where they isolated bacterial endophytes from root nodules of *Abrusmollis* which exhibited PGP traits and enhanced the seedling growth upon inoculation.

Among these nineteen isolates, potential isolates showing phosphorus and potassium solubilization indices more than 4.50 and 5.00 respectively, which also exhibiting nitrogen fixation were further screened for biochemical characterization. Out of seventeen isolates NN8, NN10, NJ6 and NP2 isolates recorded PSI of 4.81, 5.01, 6.02 and 4.88 respectively, whereas isolates NJ1, NJ2, NJ6, NJ8 and NP2 recorded KSI of 5.81, 5.51, 6.02, 6.01 and 5.41 respectively. These isolates also exhibited nitrogen fixation. Hence, these isolates were further biochemically characterized.

**Biochemical Characterization**

The selected potential isolates were biochemically characterized for various enzymatic and metabolic traits, such as oxidase, catalase, citrate utilization, urea hydrolysis, starch hydrolysis, hydrogen sulfide ($H_2S$) production and results are presented in Table 5. All the seven isolates showed positive for catalase, oxidase, urease enzyme, citrate utilization and starch hydrolysis activity except isolates NJ2 showed negative for citrate utilization, NJ2 and NN8 showed negative for starch hydrolysis. All seven isolates were negative for $H_2S$ production. The results of biochemical tests indicated that the isolates are aerobic utilization of citrate and starch as a source carbon, presence of urease enzyme which is responsible for ammonia production (Bindushree and Shivaprakash, 2022). These traits of the isolates combined with the PGP traits such as solubilization of phosphorus and potassium, ammonia and siderophore production make them potential candidates for plant growth promotion.

**Phytohormone Production by Efficient Bacterial Isolates**

Efficient bacterial isolates were further tested for their ability to produce phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA), which play avital role in plant growth promotion. All the bacterial isolates were able to produce phytohormones as represented in Table 6. The IAA production by the bacterial isolates ranged from 27.51-61.05 µg/ml and GA production ranged from 1.57-11.66 µg/ml. The highest IAA production was recorded by the isolate NP2 with 61.05 µg/ml and the lowest of 27.51 µg/ml by the isolate NJ2. Similarly, isolate NJ1 has produced the highest levels of (11.66 µg/ml) gibberellic acid (GA) and the lowest by isolate NJ8 with production of 1.57 µg/ml. The phytoharmones secretion (IAA and GA) is essentially involved in plant growth and development mainly

**TABLE 5**

**Biochemical characteristics of bacterial isolates**

| Isolate code | Oxidase | Catalase | Citrate utilization | Urea hydrolysis | Starch hydrolysis | $H_2S$ production |
|---|---|---|---|---|---|---|
| NN8 | + | + | + | + | - | - |
| NN10 | + | + | + | + | + | - |
| NJ1 | + | + | + | + | + | - |
| NJ2 | + | + | - | + | + | - |
| NJ6 | + | + | + | + | - | - |
| NJ8 | + | + | + | + | + | - |
| NP2 | + | + | + | + | + | - |

*Note* : '+'-positive; '-'- negative

**TABLE 6**

**Quantitative estimation of phytoharmones secreted by efficient bacterial isolates**

| Isolates | Indole acetic acid (µg/ml) | Gibberellic acid (µg/ml) |
|---|---|---|
| NN8 | 35.29 $^{d}$ | 8.56 $^{c}$ |
| NN10 | 39.75 $^{c}$ | 5.70 $^{e}$ |
| NJ1 | 50.88 $^{b}$ | 11.66 $^{a}$ |
| NJ2 | 27.51 $^{f}$ | 6.68 $^{d}$ |
| NJ6 | 31.38 $^{e}$ | 5.36 $^{e}$ |
| NJ8 | 61.05 $^{a}$ | 1.57 $^{f}$ |
| NP2 | 49.82 $^{b}$ | 9.54 $^{b}$ |

*Note* : Numerical values are mean of three replicates. Treatments with the differen super scripts represent a significant difference as determined by DMRT ($p \leq 0.05$)

root elongation and shoot growth and also influence the interaction between microorganisms and plants. These results were in accordance with Nalini *et al.* (2023). This suggests that IAA producing bacteria have profound effects on plant growth promotion.

The present study provides the isolation of non-rhizobial and rhizobial isolates from root nodules of velvet bean, presents their morphological characterization and PGP traits. The obtained isolates exhibited PGP traits such as phosphorus, potassium solubilization, nitrogen fixing and siderophore production abilities. These results reveals the potential PGP ability of the isolates obtained from root nodules of velvet bean and their role in enhancing plant growth by providing available nutrients in soil there by increases yield and supports sustainability of agriculture. Further, suggested to test their effect on the growth of the plants *In vivo* for the plant growth promotion upon their inoculation will confirm their efficiency in plant growth promotion.

## REFERENCES

BINDUSHREE, C. AND SHIVAPRAKASH, M. K., 2022, Exploring the plant growth promoting traits of the Actinobacteria isolated from compost. *Mysore J. Agric. Sci.,* **56** (1) : 32 - 38.

CAO, K., CHEN, J., LI, Q., GU, P., LI, L. AND HUANG, R., 2024, Bacteria from nodules of *Abrusmollis* Hance: genetic diversity and screening of highly efficient growth promoting strains. *Front. Microbiol.* **15** : 1345000.

CAPPUCCINO, J. G. AND SHERMAN, N., 1992, Microbiology: A laboratory manual.

DESAI, S., KUMA, G. P. SULTANA, U., PINISETTY, S., AHMED, S. M. H., AMALRAJ ELD., 2012, Potential microbial candidate strains for management of nutrient requirements of crops. *Afr. J. Microbiol. Res.,* **6** : 3924 - 3931.

DUNCAN, D. B., 1995, New multiple range and multiple F-test. *Biometrics*., **11** : 1 - 42.

ERANA, K., AMSALU, B., ARGAW, A. AND TAMIRU, S., 2022, Nodulation potential and phenotypic diversity of rhizobia nodulating cowpea isolated from major growing areas of Ethiopia. *Agrosys. Geosci. Environ.,* **5** : e20308.

GURUMOORTHI, P., PUGALENTHI, M. AND JANARDHANAN, K., 2003, Nutritional potential of five accessions of a south Indian tribal pulse *Mucunapruriens* var. *utilis* ; II. Investigation on total free phenolics, tannins, trypsin and chymotripsin inhibitors, phytohaemagglutinins and in vitro protein digestibility. *J. Trop. Subtrop. Agroecosys*, **1** : 153 - 158.

HOFER, A. W., 1935, Methods for distinguishing between legume bacteria and their most common contaminant. *J. Am. Society of Agron*., **27** (3) : 228 - 230.

HU, X. F., CHEN, J. AND GUO, J. F., 2006, Two phosphate and potassium solubilizing bacteria isolated from Tiannu mountain, Zhejiang, China. *World J. Microbiol. Biotech.,* **22** : 983 - 990.

JANARDHANAN, K., GURUMOORTHI, P. AND PUGALENTHI, M., 2003, Nutritional potential of five accessions of a South Indian tribal pulse, Mucunapruriens var. utilis. Part I. The effect of processing methods on the contents of L-DOPA, phytic acid and oligosaccharides. *J. Trop. Subtrop. Agroecosys*., **1** : 141 - 152.

JINFENG, P., MARIKE, P., HENRY, J. S., CALE, O. S., LING, Z., BRIAN, P. H. AND FANJIANG, ZENG., 2021, Diversity of root nodule-associated bacteria of diverse legumes along an elevation gradient. In the Kunlun Mountains, China. *Front. Microbiol*., **12** : 633141.

LUCIA, R. L., CORTELAZZO, A. GUERRANTI, R., STICOZZI, C. AND VALACCHI, G., 2012, The Magic Velvet Bean of *Mucunapruriens. J. Tradit. Complement Med.,* **2** (4) : 331 - 339.

MUKHERJEE, P., ROYCHOWDHURY, R. AND ROY, M., 2017, Phytoremediation potential of rhizobacterial isolates from Kans grass (*Saccharum spontaneum*) of fly ash ponds. *Clean Technol. Environ. Policy*, **19** : 1373 - 1385.

NALINI, B. S. AND MUTHURAJU, R., 2023, Plant growth promoting traits of *rhizospheric actinobacteria*. *Mysore J. Agric. Sci.,* **57** (1) : 176 - 185.

NORRIS, J. R. AND CHAPMAN, H. M., 1968, Classification of Azotobacter. In: Identification methods for Microbiologists (Gibbs, B. M. and Shapton, D. A., Eds.). Academic Press, London, New York, pp. : 19 - 27.

PIKOVSKAYA, R. I., 1948, Mobilization of phosphates in soil in connection with the vital activities of some microbial species. *Microbiol.,* **17** : 362 - 370.

PUGALENTHI, M., VADIVEL, V. AND SIDDHURAJU, P., 2005, Alternative food/feed perspectives of an underutilized legume *Mucunapruriens* var. *utilis* - A review. Plant foods for human nutrition, **60** (4) : 201 - 218.

SATHIYANARANAN, L. AND ARULMOZHI, S., 2007, *Mucuna pruriens.* A comprehensive review. *Pharmacogn. Rev.,* **1** : 157 - 162.

SCHWYN, B. AND NEILANDS, J. B., 1987, Universal chemical assay for the detection and determination of siderophores. *Ann. Biochem.,* **160** : 47 - 56.

TARIQ, M. R., SHAHEEN, F., MUSTAFA, F., ALI, S., FATIMA, A., SHAFIQ, M., SAFRDAR, W., SHEAS, M. N., HAMEED, A. AND NASIR, M. A., 2022, Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. *Peer J.* **12** : 1 - 19.

VINCENT, J. M., 1970, A manual for the practical study of the root nodule bacteria. Blackwell Scientific Publications. Oxford and Edinburgh. pp. : 1 - 3.

ZHEN, S. D., ZHAO, L. F., KONG, Y. Z., YANG, W. Q., LINDSTROM, K.,WANG, E. T. AND WEI, H. G., 2011, Diversity of endophytic bacteria within nodules of the *Sphaerophysasalsula*in different regions of Loess Plateau in China. *FEMS Microbiol. Ecolo.,* **76** : 463 - 475.

VINCENT, J. M., 1970, A manual for the practical study of the root nodule bacteria. Blackwell Scientific Publications. Oxford and Edinburgh. pp. : 1 - 3.

ZHEN, S. D., ZHAO, L. F., KONG, Y. Z., YANG, W. Q., LINDSTROM, K.,WANG, E. T. AND WEI, H. G., 2011, Diversity of endophytic bacteria within nodules of the *Sphaerophysasalsula* in different regions of Loess Plateau in China. *FEMS Microbiol.Ecolo.,* **76** : 463 - 475.