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PLANT GROWTH PROMOTING RHIZOBACTERIA AND THEIR SCREENING, CHARACTERIZATION AND ENHANCING SEED GERMINATION IN MUNG BEAN (VIGNA RADIATA L.)

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ABSTRACT

Global food security is increasingly challenged by a growing population, shrinking agricultural land, and climate change. Utilizing Plant Growth-Promoting Rhizobacteria (PGPR) as bio-stimulants offers a sustainable strategy to enhance crop productivity. The present study aimed to isolate, screen, and characterize efficient PGPR from the rhizospheric soil to improve the seedling vigour and germination of mung bean (Vigna radiata). Rhizospheric soil samples were collected to isolate bacterial strains, which were subsequently evaluated for vital plant growth-promoting (PGP) attributes. Screening criteria included Indole Acetic Acid (IAA) synthesis. Furthermore, the isolates were tested for phosphate solubilization, siderophore production, ammonia production, and biocontrol traits such as Hydrogen Cyanide (HCN) production. The most promising isolates were then evaluated in vitro for their impact on mung bean seed germination and Seed Vigour Index (SVI) over a 7-day period. Out of the isolates screened, seven efficient strains demonstrated superior PGP potentialities. Notably, the most potent isolates, PBN13 and PGY11, exhibited high levels of phosphate solubilization and IAA production. In vitro assays revealed that bacterial inoculation significantly augmented seedling development; isolate PBN13 achieved a maximum germination rate of 97.33% and a Seed Vigour Index of 1499.85. This represents a 148.89% increase in Vigour Index over the uninoculated control (SVI = 602.61), a result that highlights the efficiency of these strains in accelerating early plant development. The rhizobacterial isolates PBN13 and PGY11 possess a robust suite of PGP attributes, making them promising candidates for the formulation of biofertilizers. These results suggest that such PGPR-based applications can effectively enhance crop improvement and protection, supporting the transition toward sustainable agricultural practices.

KEYWORDS: PGPR (Plant Growth-Promoting Rhizobacteria), Mung bean (*Vigna radiata L.*), Seed Vigour Index (SVI), Sustainable agriculture, Phosphate Solubilization, Indole-3-acetic Acid, *Pseudomonas sp.*, *Azotobacter sp.*, Biofertilizers

INTRODUCTION

Microorganisms that inhabit the soil are an essential reservoir for many different types of organisms that work together in complex and interconnected system of networks throughout the rhizosphere [1].

Plant-Growth Promoting Rhizobacteria (PGPR) have emerged as a safe, non-toxic alternative way to help address the systemic issues created by long-term use of chemical fertilizers on agricultural ecosystems. These beneficial bio-stimulant microorganisms or microbial inoculants represent a complete paradigm shift to a new 'green revolution' focused on sustainability. These microorganisms provide a low carbon way of improving the health and productivity of soils, which puts biological methods at the forefront of today's regenerative agricultural practices [2].

Plant-Growth Promoting Rhizobacteria (PGPR) are specialized soil bacteria found in the plant rhizosphere that enhance growth through increased availability of nutrients. These microorganisms will metabolically solubilize phosphate and potassium from the soil, converting them into forms that are plant-available, via processes such as acidolysis, ion exchange, & chelation (also referred to as the conversion of insoluble mineral fractions to plant available forms). Diazotrophic PGPR aid soil fertility and enhance soil productivity by fixing nitrogen biologically (BNF) and directly enhance nutrient pools available for plants by reducing N2 from the atmosphere into NH3 utilizing the nitrogenase enzyme complex [3].

The following plant growth-promoting rhizobacteria (PGPR) genera; (i.e. *Agrobacterium*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Beijerinckia*, *Erwinia*, *Enterobacter*, *Pseudomonas*, and *Rhizobium*) are capable of increasing crop production by multiple mechanisms, including the direct stimulation of plant growth through solubilizing mineral phosphates, biological nitrogen fixation, and the production of plant hormones; and indirectly through production of siderophores, hydrocyanic acid (HCN) and diverse antibiotics, by suppressing phytopathogens when used as seed inoculants or soil amendments [4].

In addition to their ecological contributions, the main benefit of PGPR is their diverse means of enhancing plant growth. Recent research shows that PGPR improve soil ecology by promoting the establishment of soil microbial balance, reducing soil acidity, and speeding biogeochemical recycling of plant nutrients, thus enhancing the availability of key nutrients and

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improving their uptake by plants. In addition, specific PGPR strains produce and secrete plant hormones, mainly indole-3-acetic acid (IAA), which is the primary driver for stimulating root and shoot development [5, 6, 7, 9].

In India, Mung beans (*Vigna radiata* L.) are one of the primary staple foods in the country. In addition to its fast-growing cycle, mung beans are very well-suited for different types of crop production systems and can be grown in the Kharif (monsoon) season as a catch crop. Currently, Mung beans are being grown across around 4.25 million ha, with an estimated total production of around 2.41 million tonnes. Even though Mung beans are being grown widely, the average national productivity of Mung beans is only 567 kg ha^{-1}, indicating a large yield gap and the need to investigate the use of biological interventions, such as PGPR, to improve production levels [8]

MATERIALS AND METHODS

Rhizosphere soil sample collection site

Rhizospheric soil samples were collected from the village of Pandva Gir in the Gir Somnath district of Gujarat, India (Latitude: 20.9671 Longitude:70.5057). The intact root system was excavated, and the rhizospheric soil that was attached to the root systems were collected with sterile tools, placed in sterile bags, labelled, and shipped to the Microbiology Department at Monark University in Vahelal, Ahmedabad, in a box packed with ice. All samples will be stored at -20°C until analysis.

Isolation of rhizobacteria

Rhizospheric Soil samples (approximately 1 gram) were diluted serially with 9ml sterile distilled water. For each series of dilutions were made, 0.1ml of the dilution was taken after mixing, and 0.1ml was plated to both Sterilised Nutrient Agar (NA) and Nitrogen-free Mannitol Agar plates (Ashbey's) to promote the growth of the microorganisms. The inoculated plates were incubated at 30 ± 2°C for 48 hours. The colonies which were observed on the agar plates were transferred onto new sterile NA plates using the streak-plate method to isolate the pure cultures, and preserved as stocks in 50% glycerol at -20°C for future analysis.

Qualitative screening of isolates for Indole acetic acid production

The qualitative evaluation of the isolates' production of Indole-3-acetic acid (IAA) was performed using the method of Bric et al. (1991). Bacterial isolates were inoculated into 10 ml of nutrient broth, with 0.1% L-tryptophan, and incubated for 48 hours at 30 ± 2 °C. Inoculations were made with a loopful of isolate, and uninoculated 10 ml broth containing 0.1% tryptophan served as the negative control. At the completion of the incubation period, 1 ml of each culture was harvested, and the cultures were centrifuged at 10,000 rpm for 10 minutes. Supernatants were combined with an equal amount of Salkowski reagent and incubated in the dark for 30 minutes. The development of a pink to red in the sample was considered to be a positive result for the production of IAA.

Morphological and biochemical characterization

A nutrient agar and Ashbey's Mannitol agar were used to perform morphological characteristics of the isolates (pure culture) following 48 hours at 30 ± 2 °C. After the incubation period, different colony attributes (shape, margin, size, elevation, pigment) were recorded for each isolate. Structural characteristics were determined by Gram and Endospore stain. Biochemical characteristics of each isolate were determined using the techniques outlined by Clarke and Cowan. The biochemical assays included: catalase, oxidase, indole, citrate, V-P (Voges Proskauer), hydrogen sulphide (H_2S), and hydrolysis of starch. All of the assay tests were also conducted in order to evaluate the carbohydrate fermentation profile of each isolate based on their metabolic ability to use different sugars (glucose, fructose, mannitol, sucrose, maltose, lactose).

In Vitro Assessment of Plant Growth-Promoting (PGP) Traits

Bacterial isolates were tested for the following characteristics associated with plant growth:

Qualitative Assessment of Siderophore Production

The procedure for detecting the presence of siderophores in bacterial isolates was described by Schwyn and Neilands (1987) using the Chrome Azurol S (CAS) assay method. To qualitatively assess siderophore production, each bacterial isolate was inoculated onto CAS agar plates and incubated for 48–72 hours at 30±2 °C. If the presence of an orange or yellow halo around a bacterial colony was observed, it was recorded as a positive response indicating iron chelation.

Qualitative Assessment of Hydrogen cyanide production

To quantify the amount of hydrogen cyanide (HCN) produced by bacterial isolates, we utilized the method described by Castric (1975). Isolates were streaked onto sterile Nutrient agar supplemented with glycine (4.4 g/L). A piece of Whatman filter paper was soaked in a solution of 2% sodium carbonate (Na_2CO_3) and 0.5% picric acid and attached to the underside of each Petri dish cover. The Petri dishes were tightly wrapped with Parafilm to prevent gas loss. The Petri dishes were then incubated at 30 ± 2 °C for 48-72 hours. After incubation, we examined the Whatman filter paper for the presence of yellow to reddish-brown reaction products, indicating that HCN had been produced.

Qualitative Assessment of Ammonia production

According to the Cappuccino and Sherman (2005) method, the ammonia production by means of bacterial isolates was evaluated. 10 ml of sterile peptone water was inoculated with an overnight grown culture of an isolate and placed in a shaker at 120 rpm and 30 ± 2 °C for 48 h. After the incubation period, 0.5 ml of Nessler's reagent was added to each tube. The production of ammonia was indicated by the production of a colour change from pale yellow to dark brown.

Qualitative Assessment of Phosphate solubilization

According to Verma et al. (2001), the phosphate solubilizing activity of bacteria was assessed by determining their ability to solubilize inorganic phosphate. The inorganic phosphate solubilizing ability of the bacterial isolates was assessed in using Pikovskaya's (PVK) agar medium supplemented with

0.5% tri calcium phosphate [$Ca_3(PO_4)_2$]. The bacterial isolates were spot inoculated onto the agar and incubated for 48 to 72 h at 30 ± 2 °C. Positive solubilization by the bacterial isolates was demonstrated by the presence of a distinguishable halo around the colonies.

Quantitative Assessment of IAA production

The quantitative assessment of Indole-3-acetic acid (IAA) was accomplished according to the colorimetric method as described by Chaiharn & Lumyong (2011). The addition of Salkowski reagent to the samples demonstrated the production of IAA due to the formation of a distinct pink colour. Spectrophotometric absorbance was obtained at 530 nm and the quantity of IAA produced by the bacterial isolates was estimated according to a standard curve established with pure IAA.

Seed Germination and Vigour Index Assay

In order to assess the in vivo plant growth promoting (PGP) characteristics of bacterial isolates, seed germination assays were performed under strictly controlled laboratory conditions. Mung bean (*Vigna radiata* L.) were used as the plant test species and high-quality, surface-sterilized seeds were produced by placing them in ethanol (70% v/v) for 1 minute followed by sodium hypochlorite (2% v/v) for 2 minutes. The final wash of seeds was conducted using sterile deionized water, which consisted of 6 wash cycles. The surface sterilization procedure was validated by taking 0.1 ml aliquots from the final wash and plating on to Nutrient Agar (NA) plates. The lack of bacterial growth on NA plates following incubation validated that surface sterilization was successful. Prepared Petri dishes contained sterile filter paper lining; each Petri dish was then inoculated with a 10 ml aliquot of the appropriate rhizobacterial suspension, diluted to a concentration of 10^{-7} CFU/ml. Ten sterile mung bean seeds were then equidistantly distributed on the appropriate rhizobacterial suspension that had been inoculated on the filter paper of each dish. The experiment was performed in triplicate for each treatment to ensure repeatability. Watery distilled sterile controls were used in place of bacterial inoculation on the control plates. The Petri dishes were all kept at 28 ± 2 °C for a total of 7 days in a controlled environment. Once the 7-day incubation period was over, the % of seedlings that germinated and the measurements of root length and shoot length were collected and used to assess the effects of the isolates on plant growth.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Vigour index=(Mean root length+mean soot length)×germination (%)

Statistical analysis

IBM SPSS Statistics, Version 30.0.0.0, was the tool used to carry out statistical analyses and computations (IBM, 2024). The statistical outcomes have been examined using Analysis of Variance (One Factor ANOVA) when appropriate, and are reported as mean values from three distinct replications ± standard error (SE). Duncan's multiple range test is then carried out at the $P < 0.05$ level of significance. The study's experiments were carried out in triplicate, with a completely randomized design used for each experiment's two repetitions.

RESULTS AND DISCUSSION

Isolation, morphological and biochemical characterization of rhizobacteria

A total of 16 pure rhizobacterial isolates were obtained from these samples. The isolates were further assessed by their capacity to produce Indole-3-Acetic Acid (IAA). From the 16 initial isolates, the seven demonstrating a positive IAA response were retained for further research. These positive isolates were PAN13, PBND1, PBN13, PCY13, PCND3, PFYD1, and PGY11. A detailed account of morphological, physiological, and biochemical characteristics provided the basis for identifying the isolates. After 48 hours of incubation on nutrient agar (NA) and Ashbey's Mannitol agar plates at 30 °C, colony characteristics such as size, shape, colour, and growth pattern were recorded. Cell morphology and size were recorded using a light microscope, and identification of the isolates was based on the observations and biochemical analysis reported in Tables 1 and 2. The techniques used for identification were based on those outlined in Bergey's Manual of Determinative Bacteriology (JF, M. 2000). As a result of this identification process, PAN13 was designated as *Pseudomonas sp*. and PGY11 was designated as *Azotobacter sp.*

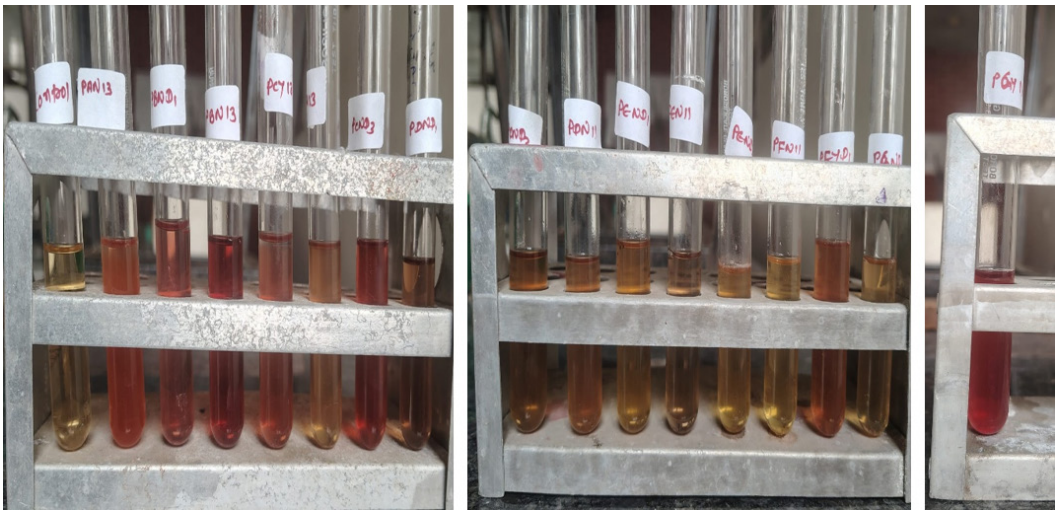


Figure 1: IAA production

Isolates	Parameters							
	Size	Shape	Margin	Elevation	Opacity	Texture	Pigment	Gram reaction
PAN13	M	R	E	C	O	S	Milky White	+Ve
PBND1	L	I	U	F	O	R	Cream	+Ve
PBN13	S	R	E	C	T	S	Light Green	-Ve
PCY13	S	R	E	C	O	S	Pale yellow	+Ve
PCND3	M	I	U	R	O	G	White	+Ve
PFYD1	L	I	L	F	O	R	Cream	+Ve
PGY11	M	R	E	C	T	M	Off white	-Ve

Table 1: Morphological characterization of the isolated rhizobacteria

Colony characteristics (S: Small colonies; M: Medium colonies; L: Large colonies; R: Round; I: Irregular; F: Flat; C: Convex; R: Raised; E: Entire; L: Lobate; U: Uneven; S: Smooth; R: Rough; M: Mucoid; G: Glossy; T: Translucent; O: Opaque; +Ve: Positive; -Ve: Negative)

Isolates	Parameters										
	Indole test	Methyl red test	Voges Prausker test	Citrate utilization	Oxidase test	Catalase test	Glucose	Lactose	Sucrose	H_2S Production	Starch utilization test
PAN13	-	+	+	-	-	+	+	-	-	-	-
PBND1	-	+	+	+	+	+	+	-	+	+	+
PBN13	-	-	+	+	+	+	+	-	-	+	+
PCY13	-	-	-	+	+	+	+	+	+	-	+
PCND3	+	-	+	-	+	+	+	-	-	-	+
PFYD1	-	-	-	+	+	+	+	+	+	-	+
PGY11	+	-	+	+	+	+	+	+	+	+	+

(+) Positive; (-) Negative

Table 2: Biochemical characterization of the isolated rhizobacteria

In vitro plant growth-promoting activities of the rhizobacterial isolates

Table 3 outlines the capacity to promote plant growth of the rhizobacteria that were analysed. There are several direct or indirect ways in which the rhizobacteria can promote plant growth. For example, rhizobacteria will solubilize inorganic phosphates, produce ammonia and synthesize phytohormones such as indole-3-acetic acid (IAA). Strains PBN13 and PGY11 of the screened isolates also had the highest capacity to biosynthesise IAA, producing 131.47±1.71 µg/mL and 128.81±1.77 µg/mL respectively, indicating that the strain has the potential to significantly modify root structure and therefore improve nutrient uptake by the plant host. In addition, rhizobacteria will control the growth of pathogenic microorganisms by producing hydrogen cyanide (HCN) and siderophores to sequester iron from the environment, thus depriving competing pathogenic microorganisms of iron.

Isolates	IAA production	Phosphate solubilization	Siderophore production	HCN production	Ammonia production
PAN13	+	+	+	-	-
PBND1	+	+	+	-	-
PBN13	+++	+++	++	++	++
PCY13	+	+	+	-	-
PCND3	+	+	+	+	+
PFYD1	+	+	-	-	-
PGY11	+++	++	++	++	+++

(+) presence of trait, (++) Moderately presence, (+++) highly presence, (−) absence of trait

Table 3: Selected rhizobacterial isolates with multiple plant growth-promoting traits

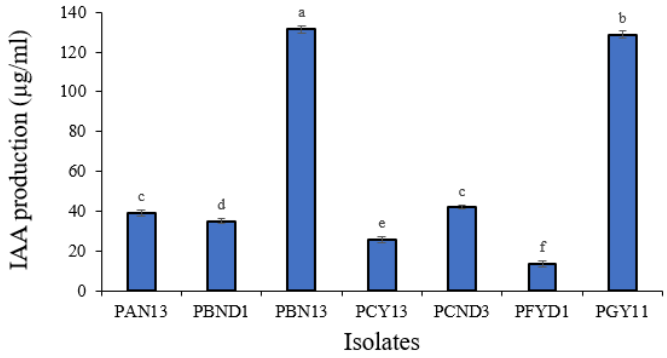


Figure 2: Quantitative evaluation of the selected rhizobacterial isolates' ability to produce indole acetic acid (IAA).

Note: According to Duncan's Multiple Range Test (DMRT), values in the same column which are followed by the same letter are not different significantly at $p<0.05$. The mean of three replicates (n=3) ± standard error (SE) is represented by the data. Ranking is presented by alphabetical superscripts, with 'a' representing as the most significant value and the subsequent letters, in descending order, representing significantly lower values.

Effect of PGPR inoculation on Seed Germination and Vigour Index

There was a significant increase in the germination rates and seedling vigour of plant growth-promoting rhizobacteria (PGPR) inoculated seeds as compared to untreated seeds. Mung Bean seed germination rates were increased from 78% to 97% and were assessed on 10 test isolates with PBN13 and PGY11 having the largest effect on seedling growth and development. The seeds treated with PBN13 and PGY11 had the highest mean seed vigour indices (SVI) of 1499.85 and 1429.95 respectively, which was an increase of 148% over the control treatment (Table 4, Fig.3).

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control	78.67	4.14±0.12c	3.54±0.09c	602.65±9.2d
PBND1	85.33	5.45±0.09c	4.25±0.03b	827.70±2.2b
PBN13	97.33	9.16±0.13a	6.25±0.04a	1499.85±9.8a
PCND3	82.67	4.85±0.08d	3.82±0.09c	716.75±10.6c
PGY11	95.33	8.92±0.06b	6.08±0.14a	1429.95±6.3ab

Table 4: Impact of rhizobacteria on V. radiata seedling germination, root and shoot lengths, and vigour index

Note: According to Duncan's Multiple Range Test (DMRT), values in the same column which are followed by the same letter are not different significantly at $p<0.05$. The mean of three replicates (n=3) ± standard error (SE) is represented by the data. Ranking is presented by alphabetical superscripts, with 'a' representing as the most significant value and the subsequent letters, in descending order, representing significantly lower values.



Figure 3: Effect of treatment on *V. radiata*. Under controlled condition.

CONCLUSION AND RECOMMENDATION

Worldwide, increasing agricultural output has been made possible through the widespread use of synthetic agricultural chemicals. Unfortunately, this practice has resulted in serious reductions in environmental integrity, as well as both human and animal health. This has led to an urgent need for the introduction of an alternative, sustainable means of increasing agricultural yields through the use of Plant Growth Promoting Rhizobacteria (PGPR). As a result, this study was conducted to isolate and biochemically characterize Rhizobacteria for the purpose of developing PGPR products that could be used as a biofertilizer or biopesticide. From initial screening, PBN13 and PGY11 were found to have an excellent array of PGP attributes including: nutrient mobilization (phosphate solubilization and Siderophore production, production of Indole-3-Acetic Acid (IAA) and ammonia); biocontrol and defence (hydrogen cyanide (HCN) production). The results of this study provide an excellent starting point for developing PGPR products based on the positive results of this study as PGPR formulations designed to improve the yield and quality of food crops. It is important to conduct further research regarding the application of these PGPR strains in field settings to evaluate their efficacy as biofertilizers in sustainable agricultural systems.

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