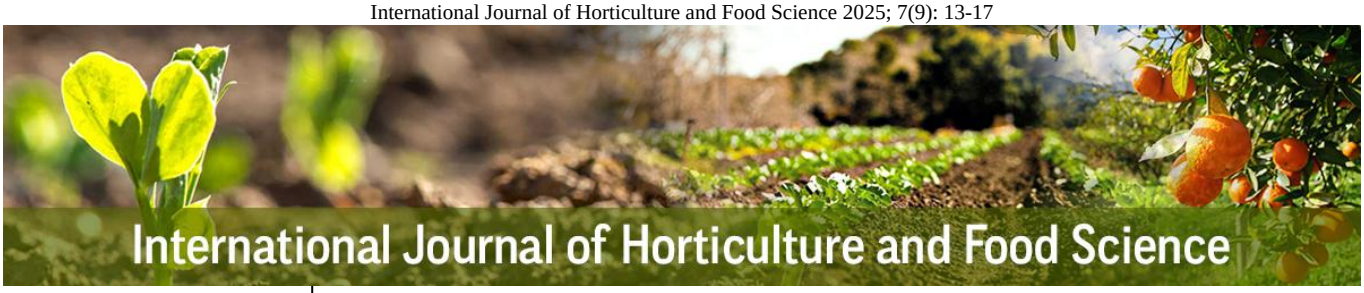

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Studies on plant growth promoting rhizobacteria in bottle gourd (*Lagenaria siceraria* L.)

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Abstract
The present investigation was conducted during kharif 2024-25 at the Experimental farm of Department of Plant Pathology and Microbiology, MPKV, Rahuri, to isolate, characterize and evaluate efficient plant growth promoting rhizobacteria for bottle gourd (*Lagenaria siceraria*). Nine bacterial isolates were obtained from the rhizosphere and identified as belonging to *Azotobacter*, *Pseudomonas* and *Bacillus* species based on morphological, physiological and biochemical traits. Laboratory assays revealed their potential for nitrogen fixation, phosphate solubilization and potassium mobilization. A field experiment laid out in randomized block design with seven treatments and three replications assessed the effect of PGPR with graded NPK levels. Results indicated that the combined application of PGPR with 100% NPK significantly improved vine length, number of primary branches, fruit yield compared to control and sole NPK application. The study highlights the potential of integrating PGPR with reduced fertilizer doses for sustainable cultivation of bottle gourd.

Keywords: PGPR, *Azotobacter*, *Pseudomonas*, *Bacillus*, biofertilizer, bottle gourd

1. Introduction
Bottle gourd (*Lagenaria siceraria* Mol. Standl.) is one of the most widely cultivated cucurbitaceous vegetables in India and other tropical and subtropical regions. It is valued not only for its tender green fruits consumed as a staple vegetable but also for its diverse medicinal and industrial applications. The crop is rich in water (96%), carbohydrates, minerals and vitamins, and is recognized for its therapeutic properties such as diuretic, cardiotonic and cooling effects. In India, it occupies more than 1.55 lakh hectares with a production of over 2.5 million tonnes, predominantly in states like Rajasthan, Uttar Pradesh, Maharashtra and Bihar. Despite its nutritional and economic importance, bottle gourd productivity remains constrained by imbalanced fertilizer use and declining soil fertility.
Plant Growth Promoting Rhizobacteria (PGPR) have emerged as an effective and sustainable tool to improve crop performance. PGPR are free-living soil bacteria that colonize the rhizosphere and promote plant growth through direct mechanisms such as nitrogen fixation, phosphate solubilization and potassium mobilization. Among the diverse PGPR genera, *Azotobacter*, *Pseudomonas* and *Bacillus* are widely studied for their efficiency in nutrient mobilization and biocontrol potential. Therefore, the present study was undertaken to (i) isolate, identify and characterize efficient PGPR strains from the rhizosphere of bottle gourd, (ii) evaluate their nutrient solubilizing abilities under laboratory conditions, and (iii) assess their impact on growth, nutrient uptake and yield of bottle gourd under graded levels of nitrogen, phosphorus and potassium fertilizers.

2. Materials and Methods
2.1 Experimental site
A field experiment was conducted during kharif 2024-25 at the Experimental Farm, Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra, India. Geographically, the site lies in the scarcity zone of Western Maharashtra, characterized by semi-arid climate with mean annual rainfall of 520 mm, received in two peaks (July and September).

The experimental soil was medium black with neutral reaction (pH 7.72), low in available nitrogen (203.7 kg ha^{-1}), low in available phosphorus (10.3 kg ha^{-1}), and medium in available potassium (330.45 kg ha^{-1}). Organic carbon content was 0.55%.

2.2 Plant material and fertilizers
Seeds of bottle gourd cultivar *Phule Samrat* were procured from the AICRP on Vegetable Crops, Department of Horticulture, MPKV, Rahuri. The recommended fertilizer dose (RDF) for bottle gourd was 50:50:50 $N:P_2O_5:K_2O$ kg ha^{-1} applied in the form of urea, single super phosphate, and muriate of potash, respectively.

2.3 Isolation and characterization of PGPR
Rhizospheric soil samples of bottle gourd were collected from AICRP vegetable Improvement project, MPKV Rahuri. One gram of soil was serially diluted up to 10^{-6} using sterile distilled water, and 0.1 mL aliquots were plated on selective media: Jensen's medium for *Azotobacter*, Pikovskaya's medium for phosphate solubilizing bacteria, and Aleksandrow's medium for potassium mobilizers. Plates were incubated at 28-30 °C for 7 days, and distinct colonies were purified by streak plate method.

2.4 Morphological and physiological characterization
Isolates were characterized based on colony morphology, pigmentation, Gram reaction and cell shape. Temperature tolerance was assessed at 30, 40 and 50 °C on nutrient agar.

2.5 Biochemical characterization
Standard assays were performed for catalase, urease, starch hydrolysis, methyl red, Voges-Proskauer, and gelatin hydrolysis following Cappuccino and Sherman (1987) [3].

2.6 Functional potential
Nitrogen fixation was estimated using Microkjeldahl method (Reis *et al*., 1994) [12] in cultures grown in Jensen's medium. Phosphate solubilization was assessed by halo zone formation on Pikovskaya's medium.
Potassium mobilization was determined qualitatively on Aleksandrow's medium containing muscovite mica.

2.7 Experimental design and treatments
The field trial was laid out in a randomized block design (RBD) with seven treatments replicated three times. Each gross plot measured 4.5 × 5.0 m with a net plot of 3.0 × 4.0 m, maintaining 1.5 m × 1.0 m plant spacing.

The treatments were as follows:

Treatment No.	Treatment details
T_1	Absolute control
T_2	Reference strain
T_3	25% NPK + PGPR
T_4	50% NPK + PGPR
T_5	75% NPK + PGPR
T_6	100% NPK + PGPR
T_7	100% NPK (alone) without PGPR

2.8 Crop management
Ten seeds were sown per plot in July 2024. Standard agronomic practices were followed. A light irrigation was given immediately after sowing, followed by irrigation at 10-15 days intervals. Hand weeding and intercultural operations were carried out as required. Fully matured fruits were harvested manually at physiological maturity.
Observations recorded from three randomly selected plants per plot: Germination percentage (7 DAS), Vine length (cm), Number of primary branches per vine, Days to 50% flowering, Fresh weight and dry weight of plant (g), Root length (cm), Number of fruits per vine, Average fruit weight (g), Fruit length and diameter (cm), Yield (q ha^{-1}).

2.9 Microbial population count
Soil samples (0-15 cm depth) were collected at sowing and harvesting to assess microbial populations using serial dilution and plate count technique (Alexander, 1977) [2].

2.10 Statistical analysis
Experimental data were analyzed using analysis of variance (ANOVA) for randomized block design as per Panse and Sukhatme (1967) [10]. Treatment means were compared at 1% and 5% levels of significance using critical difference (CD).

3. Results and Discussions
3.1 Isolation and characterization of PGPR
Nine bacterial isolates were obtained from rhizospheric soil of bottle gourd and designated as *Azotobacter* (Isolates 1, 4, 7), *Pseudomonas* (Isolates 2, 5, 8) and *Bacillus* (Isolates 3, 6, 9). Morphologically, *Azotobacter* colonies were yellow pigmented and rod-shaped, *Pseudomonas* isolates were light green with smooth margins, while *Bacillus* formed whitish colonies with rough circular margins. Gram staining confirmed *Azotobacter* and *Pseudomonas* as Gram-negative, and *Bacillus* as Gram-positive.
Physiological assays revealed that all isolates grew well at 30 °C, moderately at 40 °C, while only *Pseudomonas* isolates (2, 5, 8) survived at 50 °C, confirming their mesophilic nature. Biochemical characterization showed that *Pseudomonas* and *Azotobacter* were positive for catalase, starch hydrolysis and gelatin hydrolysis, while *Bacillus* isolates showed strong positive Voges-Proskauer reaction, reflecting their metabolic diversity. These findings are consistent with Soesanto and Mugiastuti (2011) [19], who reported similar biochemical traits in PGPR isolated from vegetable rhizospheres.
Functional analysis confirmed that *Azotobacter* isolates fixed nitrogen efficiently, *Pseudomonas* isolates produced clear phosphate solubilization halos on Pikovskaya's medium, and *Bacillus* isolates mobilized potassium from muscovite mica. These traits validate their potential as biofertilizers for nutrient mobilization. Similar reports were made by Richardson *et al*. (2009) [14] for phosphate solubilization and Kumar *et al*. (2020) [6] for potassium mobilization in *Bacillus* strains.

Table 1: Collection of isolates from soil sample

Location	Name of bacteria	Isolates	Medium
AICRP Vegetable project	Nitrogen-fixing bacteria	1 4 7	Jensen's
	Phosphate-solubilizing bacteria	2 5 8	Pikovskaya
	Potash-mobilizing bacteria	3 6 9	Aleksandrow

Table 2: Characterization of isolates based on morphology

Isolates	Gram Reaction	Colony colour	Shape	Size	Pigmentation
Isolate 1	Gram-ve	Yellow	Rod (Circular)	1-2 mm	Dark brown
Isolate 2	Gram-ve	Light green	Straight rod	1-2 mm	Yellow green
Isolate 3	Gram +ve	White	Rod (Roughly circular)	1-2 mm	Yellow white
Isolate 4	Gram-ve	Yellow	Rod (Circular)	1-2 mm	Dark brown
Isolate 5	Gram-ve	Light green	Straight rod	1-2 mm	Yellow green
Isolate 6	Gram +ve	White	Rod (Roughly circular)	1-2 mm	Yellow white
Isolate 7	Gram-ve	Yellow	Rod (Circular)	1-2 mm	Dark brown
Isolate 8	Gram-ve	Light green	Straight rod	1-2 mm	Yellow green
Isolate 9	Gram +ve	White	Rod (Roughly circular)	1-2 mm	Yellow white

Table 3: Biochemical characterization of isolates

Isolate no and Biochemical test	1	2	3	4	5	6	7	8	9
Catalase test	+	+	+	+	+	+	+	+	+
Urease test	+	-	-	+	-	-	+	-	-
Strach Hydrolysis test	+	+	-	+	+	-	+	+	-
MR test	-	-	-	-	-	-	-	-	-
VP test	-	-	+	-	-	+	-	-	+
Gelatin hydrolysis test	+	+	-	+	+	-	+	+	-

Table 4: Ability of isolates of *Azotobacter spp*. to fix Nitrogen by Microkjeldhals method

Sr. No	Isolates No.	Nitrogenase activity mg/g of sucrose consumed
1.	Isolate 1	13.40
2.	Isolate 4	12.52
3.	Isolate 7	10.05

Table 5: Phosphate solubilization activity (Zone of solubilization)

Sr. No.	Name of the isolate	Culture diameter	Solubilization zone	Solubilization efficiency (%)	Solubilization Index
1.	Isolate 2	10	20	200.0	3.0
2.	Isolate 5	9	13	144.4	2.4
3.	Isolate 8	11	26	236.3	3.3

Table 6: Potash solubilization activity (zone of solubilization) by different isolates

Sr. No.	Name of the isolate	Culture diameter	Solubilization zone	Solubilization efficiency (%)	Solubilization Index
1.	Isolate 3	10	25	250.0	3.5
2.	Isolate 6	11	19	172.7	2.7
3.	Isolate 9	12	21	175.0	2.7

3.2 Effect of PGPR on germination and growth attributes

Among the different inoculation treatments, T_6 (100% NPK + PGPR) showed the highest germination rate of 86.53%. This was followed by T_5 (75% NPK + PGPR) with a germination percentage of 85.94%. The lowest germination was observed in T_1, the absolute control, with 78.19%. Overall, the results indicate that the application of PGPR along with fertilizers enhanced the germination percentage compared to the use of fertilizers alone in bottle gourd. Similar enhancement of seed germination by PGPR was reported in *Crataegus pseudoheterophylla* (Fatemeh *et al*., 2014) [4].

3.3 Vine length

Observations on vine length were recorded at the time of final harvest. The shortest vine length was observed in T_1 (absolute control), measuring 71.55 cm at the flowering stage and 361.37 cm at harvest. In contrast, the longest vine length was recorded in T_6, with 93.01 cm at the flowering stage and 476.70 cm at harvest. Overall, these results indicate that the inoculation of *Azotobacter*, *Pseudomonas*, and *Bacillus*, particularly in combination with fertilizers, improved vine length in bottle gourd compared to the untreated control.

The improved vegetative growth with integrated nutrient management treatments was also recorded by Rekha and Gopalkrishnan (2001) [13] in bitter gourd, Londhe *et al*. (2002) [9] in cabbage, Singh and Krishna (2007) [20] in pointed gourd, Tavhare (2007) [20] in brinjal *cv*. *Krishna*.

3.4 Fresh weight and dry weight

The highest total fresh weight of shoot and root was recorded in treatment T_6 as 950.04 g, where PGPR inoculation was applied. In contrast, the lowest total fresh weight was observed in treatment T_1, which served as the absolute control as 825.23 g without any PGPR inoculation. The highest total dry weight of shoot and root was recorded in treatment T_6 as 226.34 g, where PGPR inoculation was applied. In contrast, the lowest total dry weight was observed in treatment T_1, which served as the absolute control as 154.50 g without any PGPR inoculation. A similar observation was reported by Samayoa *et al*. (2020) [15], who demonstrated that screening and evaluating potential plant growth-promoting rhizobacteria associated with *Allium cepa Linn*. Positively influenced growth parameters in onion.

3.5 Root length

The treatment T_6 which involved the application of 100% NPK combined with PGPR, recorded the highest root length of 45.00 cm. This was followed by treatment T_5, 75% NPK + PGPR which recorded 43.57 cm however both the treatments were at par with each other. In contrast, the lowest root length of 20.00 cm was observed in treatment T_1, i.e. absolute control without any PGPR application.

Table 7: Observations recorded

Tr. No.	Treatment	Germination percentage	Vine Length at harvesting	No of branches per vine	Days required for 50% flowering	Fresh weight (g)	Dry weight (g)	Root length (cm)
T_1	Absolute control	78.19	361.37	8.37	52.50	825.23	154.50	20.00
T_2	Reference strain	80.37	402.50	8.63	53.43	841.58	161.77	25.00
T_3	25% NPK + PGPR	81.28	382.70	8.90	55.50	850.44	180.44	28.00
T_4	50% NPK + PGPR	82.44	407.30	9.13	56.43	866.76	195.09	29.00
T_5	75% NPK + PGPR	85.94	454.37	9.59	57.30	948.18	221.10	43.57
T_6	100% NPK + PGPR	86.53	476.70	9.66	59.60	950.04	226.34	45.00
T_7	100% NPK (alone) without PGPR	84.57	350.93	9.46	56.80	919.33	204.06	37.24
	SE(m)±	0.27	0.30	0.03	0.16	0.69	1.81	0.63
	CD at 5%	0.85	0.90	0.09	0.52	2.15	5.60	1.96

3.6 Yield attributes and fruit yield

3.6.1 Number of fruits per vine.

The highest number of fruits per vine was recorded in treatment T_6 (7.93 fruits per vine), which was statistically at par with treatment T_5. The lowest number of fruits per vine was observed in the absolute control (T_1), with 6.67 fruits per vine.

3.6.2 Fruit weight at marketable stage (g).

The highest average fruit weight was recorded in treatment T_6 at 815.36 g, while the lowest was noted in the absolute control, T_1, at 575.10 g.

3.6.3 Length of fruit (cm)

The highest fruit length was recorded in treatment T_6, measuring 30 cm, while the shortest fruit length was observed in the absolute control treatment T_1, at 21.67 cm.

3.6.4 Diameter of fruits (cm).

The highest fruit diameter was recorded in treatment T_6, measuring 9.76 cm, while the smallest was observed in T_1, with 8.33 cm. Similar variations in fruit diameter for different varieties of ridge gourd were earlier reported by Rathore *et al*. (2017) [11], who recorded a diameter of 4.75 cm at the center. Comparable findings were also reported by Krishnamoorthy and Ananthan (2017) [20].

3.6.5 Yield

The highest yield of 418.67 q/ha was achieved with 100% NPK + PGPR (T_6). This was followed by 75% NPK + PGPR (T_5) of 417.57 q/ha and 100% NPK (alone) without PGPR (T_7) of 411.53 q/ha which was at par with treatment T_6. The lowest yield of 360.97 q/ha was from the control group (T_1), which received no biofertilizers. Control plots (T_1) recorded the lowest yield, while T_6 was superior to PGPR integrated treatments, highlighting the synergistic role of bio-inoculants in nutrient assimilation and crop productivity. These findings corroborate those of Kumari *et al*. (2018) [7], who reported enhanced yield of mungbean with PGPR inoculation, and Singh *et al*. (2017) [17], who demonstrated increased garlic yield with *Azotobacter* and phosphate solubilizing bacteria.

3.6.6 Microbial population

Soil analysis at harvest revealed higher available nitrogen, phosphorus and potassium in PGPR-treated plots compared to control and sole RDF plots. PGPR inoculation not only improved nutrient uptake by plants but also enhanced microbial population in the rhizosphere, suggesting positive microbial dynamics. These results are supported by Kurrey *et al*. (2018) [8], who reported that *Azotobacter* application improved soil organic carbon and nutrient availability in onion fields.

Table 8: Observations of fruit and yield

Tr. No.	Treatment	Number of fruits per plant	Marketable Fruit weight (g)	Length of fruit (cm)	Diameter of fruit (cm)	Yield (q/ha)
T_1	Absolute control	6.67	575.10	21.67	8.33	360.97
T_2	Reference strain	6.83	655.23	22.00	8.59	396.57
T_3	25% NPK + PGPR	7.10	658.70	24.67	9.05	399.40
T_4	50% NPK + PGPR	7.40	718.73	26.00	9.20	409.50
T_5	75% NPK + PGPR	7.87	740.53	29.26	9.55	417.57
T_6	100% NPK + PGPR	7.93	815.36	30.00	9.76	418.67
T_7	100% NPK (alone) without PGPR	7.73	735.80	28.90	9.43	411.53
	SE(m)±	0.04	38.10	0.38	0.15	2.40
	CD at 5%	0.12	117.40	1.15	0.48	7.42

4. Conclusion

The present investigation demonstrated that rhizospheric isolates of *Azotobacter*, *Pseudomonas* and *Bacillus* possess multiple plant growth promoting traits such as nitrogen fixation, phosphate solubilization and potassium mobilization, along with enzymatic activities that contribute to soil nutrient cycling. Field evaluation revealed that the integration of these PGPR with graded fertilizer levels significantly improved germination, vegetative growth, flowering, yield attributes of bottle gourd.

Among the different treatment combinations, the application of PGPR with 100% of the recommended NPK dose (T_6) was found to be the most efficient, producing yield equivalent to or better than the full recommended dose of fertilizers. This indicates that PGPR inoculation can effectively reduce the requirement of chemical fertilizers by at least 25% without compromising crop productivity. Moreover, PGPR treatments improved soil microbial population and nutrient status at harvest, suggesting their long-term role in enhancing soil fertility and sustainability.

The study thus provides strong evidence that PGPR-based biofertilizers, when combined with rational use of chemical fertilizers, can serve as an eco-friendly and cost-effective strategy for sustainable production of bottle gourd. Wider adoption of such microbial inoculants in integrated nutrient management programs will not only improve vegetable productivity but also mitigate the adverse effects of indiscriminate fertilizer use on soil health and environment.

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