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Isolation, identification and characterization of plant growth promoting bacteria and its effect on growth of different vegetables.

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ABSTRACT

Excessive chemical fertilizer usage in agriculture negatively impacts the ecosystem. Opting for plant growth-promoting microorganisms as a bioinoculants is a sustainable, eco-friendly approach potentially improving crop output and quality. Plant health, production, and soil fertility is influenced by interaction of plant growth-promoting bacteria with plants through siderophore, ammonia and indole acetic acid production. The present study aimed for isolation, identification of plant growth promoting microorganisms from agricultural field soil and their screening for siderophore, ammonia and indole acetic acid production and to evaluate their impact on vegetable plant growth by preparation of bio-inoculum. Twenty bacterial isolates were isolated and screened for plant growth promoting characters. 2 bacterial isolates K7 and A4 possessing all plant growth promoting characters were selected for bio-inoculum preparation. According to biochemical characterization based on Bergey's manual, two bacterial isolates K7 and A4 identified as the *Alcaligenes* sp. and *Acinetobacter* sp., respectively. By addition of consortium of A4 and K7 bacterial isolates as a bioinoculant, the root and shoot length of all vegetable plants viz. okra, brinjal, bottle gourd, and tomato plants was found to be increased as compared to the control. Thus, this bio-inoculum could be utilized as a bioinoculant for the growth of different vegetables.

Keywords: Plant growth-promoting bacteria, Ammonia production, Siderophore production, Indole acetic acid production, Bio-inoculum, consortium.

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INTRODUCTION

The increasing global population necessitates increased food production, but chemical fertilizers are often used, causing environmental damage and economic hardships. To boost sustainable agriculture, fewer fertilizers and increased plant resistance to abiotic stresses are needed. Use of plant growth-promoting bacteria (PGPB) can improve crop output, food security, and quality [1]. PGPB-based biofertilizers are viewed as an eco-friendly, cost-effective, and sustainable alternative to hazardous chemical fertilizers in soil applications [2].

Studies on plant growth promoting bacteria as a biofertilizer have shown that it can improve plant development, increase the availability of macronutrients and micronutrients, and reduce the requirement for artificial fertilization. For plants, nutrients like iron, phosphorus, and nitrogen are vital [1]. Plants and bacteria interact in symbiotic, endophytic, or associative ways [3]. Plant growth-promoting bacteria increase nutrient concentration and availability by locking in their supply, thereby promoting plant growth and productivity [4]. Microorganisms such as *Azospirillum, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Pseudomonas, Rhizobium, Frankia, Klebsiella, Clostridium, Trichoderma, Beauveria, Serratia,* and *Streptomyces* are employed to increase agricultural yield [5].

Iron is one of the most essential microelements for all living cells and is typically found in large amounts in the environment, especially in soils [6]. Accessibility of iron is restricted because ferric iron (Fe^{3+}) is not soluble in soil and is not available to plants as a micronutrient [7]. Bacteria produce Siderophore, low molecular weight metal-chelating compounds, including iron, which form soluble Fe^{3+} complexes and chelate iron from mineral phases, making it available to plants or bacterial cells [6]. Siderophores primarily scavenge iron, but they can also form complexes with other essential elements like Mo, Mn, Co, and Ni, making them accessible to microbial cells [8].

Plant growth and development are primarily influenced by the production of ammonia (NH_3) by Plant Growth Promoting Rhizobacteria (PGPR) [9,10]. PGPR promotes plant growth by producing ammonia, which increases plant biomass and root and shoot elongation, as it serves as a nitrogen source for host plants [11]. Several studies reported that PGPB influences plant growth by biological nitrogen fixation, siderophores production, and regulation of hormones and hormone like molecules like auxins, cytokinin, gibberellins, abscisic acid and ethylene[12,13,14]. Inoculation with a consortium of multiple bacterial strains could be a more effective method than individual strains due to their unique mechanisms. In recent years, plant growth-promoting bacteria (PGPB) have attracted increasing attention because they promote the growth of crops such as maize, rice, sugarcane, onion [15].

The present study aimed to isolate, identify and characterize plant growth-promoting bacteria from agricultural soil sample, and to evaluate the effect of a bio-inoculum prepared on growth of different vegetables.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from agricultural fields of Wai, Satara, Maharashtra, India and stored in the laboratory until use.

Isolation of bacteria from agricultural field soil

For the isolation of plant growth-promoting bacteria from the agriculture field soil sample, serial dilutions of the soil sample were prepared and were spread on sterile Nutrient Agar plates. The plates were incubated for 24 hours at 37^{o}C. After the incubation period, well-isolated colonies with different morphology were picked and sub-cultured through the streak plate method. For preservation, all bacterial isolates were streaked on sterile Nutrient agars slants. All (20) bacterial isolates were screened for different plant growth promoting activities.

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Screening of bacterial isolates for different plant growth-promoting characters

Siderophore production

For screening of Siderophore production, freshly grown 20 bacterial isolates were spot inoculated on sterile Chrome Azurol Sulphonate (CAS) agar plates. The plates were incubated at 37°C for 48 hours. After the incubation, plates were observed for Orange/yellow and light purple halo zone around the growth of bacteria which indicates positive results for siderophore production [16].

Catecholate type- Light purple/blue color zones
Hydroxamate type- Orange/yellow color zones

Ammonia production

Freshly grown bacterial cultures were inoculated in 5 ml of Sterile peptone water in each tube. The tubes were incubated for 48 hours at 37°C. After incubation, 0.5 ml of Nessler's reagent was added to each tube for detection of ammonia. After adding Nessler's reagent, if the brown to yellow color was formed it is indicative of positive results for ammonia production [17].

Indoleacetic acid (IAA) production

A loop full of overnight-grown bacterial cultures was inoculated in Sterile Nutrient broth enriched with tryptophan (1%). The tubes were incubated for 48-72 hours at 37° C. After observing the growth, the cultures were centrifuged at 5000 rpm for 20 min. After centrifugation, 2ml of supernatant was mixed with 4 ml of Salkowski reagent. After adding Salkowski reagent, if a pink color was observed it is indicative of positive results for IAA production [18].

Identification of selected plant growth promoting bacteria based on morphological and biochemical characterization

Based on plant growth-promoting activities, out of 20 bacterial cultures 2 cultures namely - K7 and A4 were selected for identification and further studies. They were morphologically and biochemically characterized as described in Bergey's Manual of Determinative Bacteriology [19]. Gram staining, Oxidase test, Catalase test, Sugar Utilization test, Nitrate reduction test, and Citrate utilization test were used for characterization of isolates.

Effect of plant growth-promoting bacteria on the growth performance of different vegetables:

Preparation of consortium of selected bacteria

Bacterial isolates K7 and A4 were selected for consortium preparation due to their positive growth-promoting activities. Both bacterial isolates were inoculated in nutrient broth, and incubated at 37°C for 24 hours. The number of bacteria in culture suspension was standardized using McFarland turbidity standards 0.5, which is equivalent to 1.5×10^8 ml.

Effect of Consortium on seed germination and pot assay

For a pot assay, Okra, Brinjal, Tomato, and Bottle gourd vegetable seeds were used. To ensure surface sterilization, the seeds were washed several times with distilled water. Two pots each for each plant were prepared, one as a control and the other for a test for each plant. Each pot was filled with garden soil. 10 seeds were sowed in each pot. Then 10 ml of bacterial consortium was inoculated to each pot containing garden soil. The pots were placed in sunlight and watered every 24 hours for 14 days. After 14 days, the length of the roots and shoots of all plants was measured.

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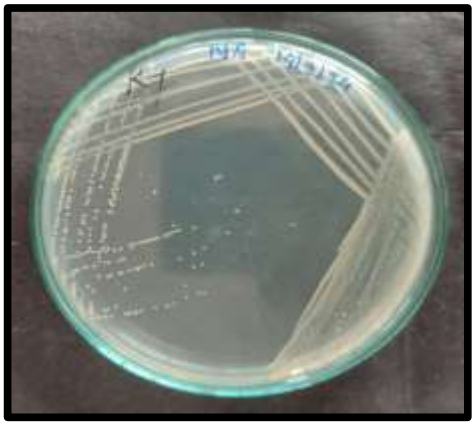
RESULTS

Isolation of bacteria from agricultural field soil sample

Serial dilutions of agriculture field soil were spread on sterile nutrient agar plates and incubated at 37°C for 24 hours. After 24 hours, isolated colonies were observed on nutrient agar plates. The streak plate method was used to purify and subculture well-isolated colonies with unique traits. Twenty bacterial isolates, viz. K2, K5, K6, K7, K8, K9, K10, K11, K12, K13, P1, P2, P3, P4, A1, A2, A3, A4, A5, and A6, were isolated from soil samples of agricultural fields. Figure 1 illustrates pure cultures of representative bacterial isolates K7 and A4. All bacterial isolates obtained from soil samples were streaked on sterile nutrient agar slants for storage.



Bacterial isolate A4 **Bacterial isolate K7**

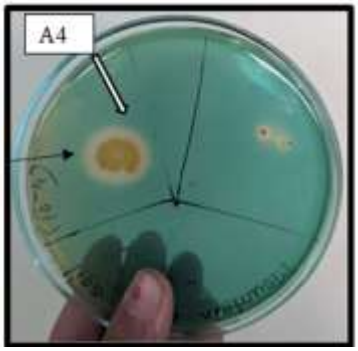
Figure 1: Pure cultures of bacterial isolates K7 and A4 obtained from agriculture field soil.

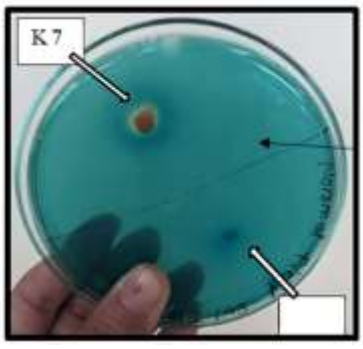
Screening of isolates for different plant growth-promoting characters

Twenty soil samples were analyzed for various plant growth-promoting activities, including Siderophore production, Ammonia production and Indole acetic acid production. Table 1 describes the plant growth-promoting activities of 20 bacterial isolates isolated from agricultural field soil.

Siderophore production

After spot inoculation, among 20 bacterial isolates, 12 isolates viz. K6, K7, K8, K10, K11, K13, P1, P4, A1, A3, A4 and A6 showed orange/yellow or light purple/ blue color zones around spots which indicate positive results for siderophore production. Figure 2 illustrates siderophore production activity of representative bacterial isolates K7 and A4.





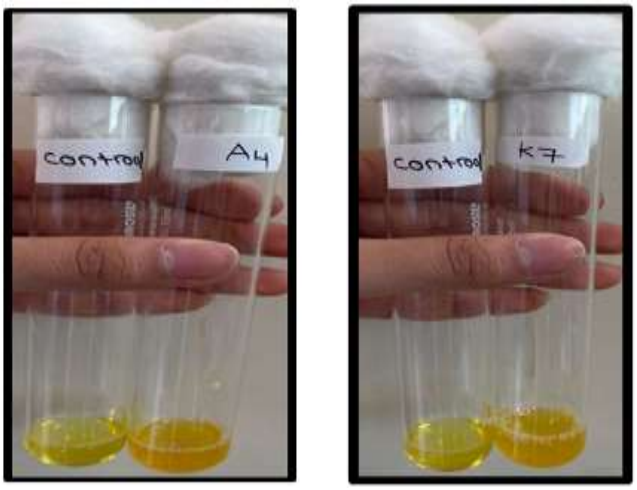
Bacterial isolate K7 **Bacterial isolate A4**

Figure 2: The appearance of orange and light purple/blue color zones indicating Siderophore

production by bacterial isolates K7 and A4

Ammonia Production

20 bacterial isolates were tested for ammonia production in peptone water. After observing turbidity in peptone water, 0.5 ml of Nessler's reagent was added for detection of ammonia. After adding Nessler's reagent brown to yellow colour was developed which indicated ammonia production. Out of 20, 12 bacterial isolates viz. K2, K6, K7, K8, K10, K11, K13, P1, P2, A2, A4, and A6 showed positive results. Figure 3 illustrates ammonia production by representative bacterial isolate A4 and K7.



Bacterial Isolate A4 **Bacterial Isolate K7**

Figure 3: Ammonia production by bacterial isolates A4 and K7

Indoleacetic acid (IAA) production

20 bacterial isolates were tested for Indole acetic acid production using Salkowski reagent. Positive results were observed for isolates K7, K13, P4, and A4, indicating pink color formation as shown in Figure 4.

a. Isolate K7 **b. Isolate K13** **c. Isolate P4** **d. Isolate A4**

Figure 4: IAA production by bacterial isolates K7, K13, P4 and A4.

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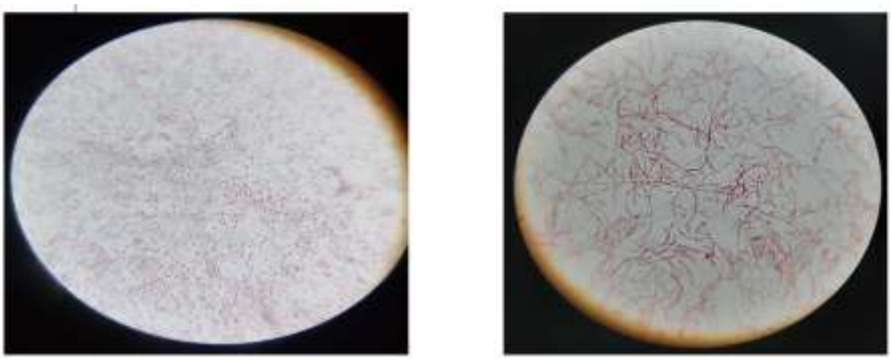
Table1: Plant growth-promoting activities of the 20 bacterial isolates.

Sr. No.	Isolates	Siderophore production	Ammonia production	Indole acetic acid (IAA) production
1	K2	-	+	-
2	K5	-	-	-
3	K6	+	+	-
4	K7	+	+	+
5	K8	+	+	-
6	K9	-	-	-
7	K10	+	+	-
8	K11	+	-	-
9	K12	-	-	-
10	K13	+	-	+
11	P1	+	+	-
12	P2	-	+	-
13	P3	-	-	-
14	P4	+	-	+
15	A1	+	-	-
16	A2	-	+	-
17	A3	+	-	-
18	A4	+	+	+
19	A5	-	-	-
20	A6	+	+	-

[(+): Positive result] [(-): Negative result]

Morphological and biochemical characterization and identification of selected plant growth promoting bacteria

Based on Plant growth-promoting activities, out of 20 bacterial cultures 2 cultures viz. K7 and A4 were selected for identification and further studies. Figure 5 illustrates Gram staining of representative bacterial isolate K7 and A4. Bacterial isolate K7 was found to be Gram negative short rods while A4 isolate was found to be Gram negative long, thin rods. Both cultures were found to be motile.



K7-Gram negative short rods **A4-Gram negative long, thin rods**

Figure 5: Gram staining of bacteria isolates K7 and A4

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Table 2 shows biochemical characterization of bacterial isolates K7 and A4.

Table 2: Biochemical characterization of bacterial isolates K7 and A4

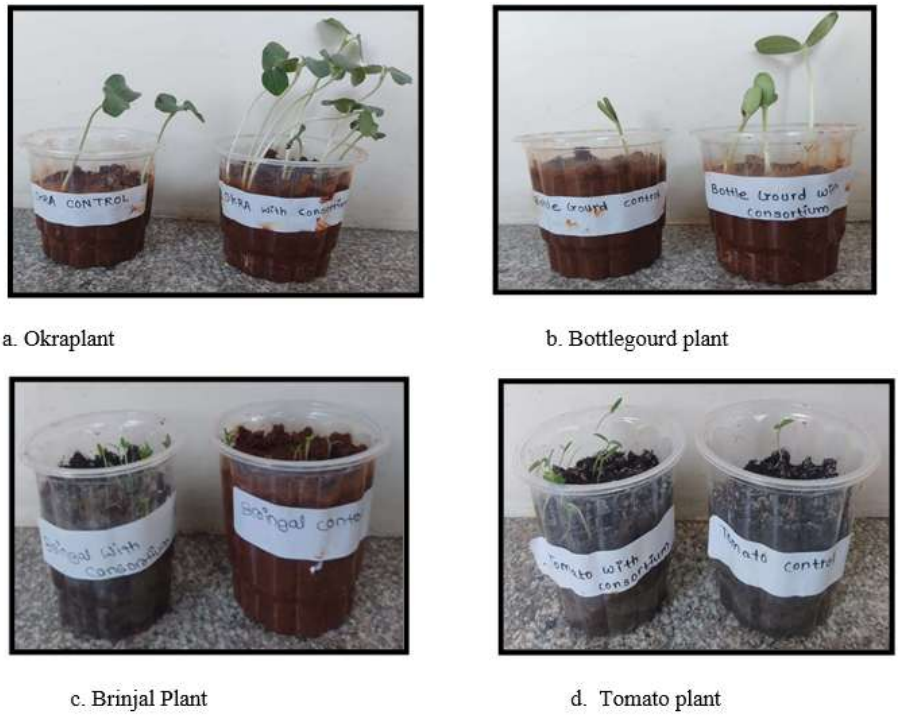
Sr. No.	Isolate	Oxidase Test	Catalase Test	Sugar Utilization Test			Nitrate Reduction Test	Indole Test	Citrate Utilization test
				D-Glucose	D-Xylose	D-Arabinose			
1.	K7	+	+	+	+	+	+	-	+
2.	A4	+	-	+	+	-	+	-	+

[(+): Positive Result] [(-): Negative Result]

For identification of isolates, Bergey's Manual of Determinative Bacteriology was referred. Based on morphological and biochemical characterization, the bacterial isolate K7 may belong to the *Alcaligenes* sp. and bacterial isolate A4 may belong to *Acinetobacter* sp.

Effect of plant growth promoting bacteria on the growth performance of different vegetables by pot assay

A pot assay was used to observe the effect of plant growth-promoting bacteria on growth performance of different vegetables. The results were shown in Figure 6,7,8,9 and 10.



a. Okraplant b. Bottlegourd plant

c. Brinjal Plant d. Tomato plant

Figure 6: Pot assay for different vegetables namely Okra, Bottle gourd, Brinjal and Tomato

According to Fig.7 and Table 3, when the consortium was inoculated in garden soil, the shoot and root length of the Okra plant was found to be increased as compared to the control after 10 days of incubation.

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Figure 7 - Root and shoot length of Okra plant with and without consortium

Table 3: Root and shoot length of Okra plant after 10days.

	Shoot length (cm)	Root length (cm)
Okra with consortium	15.8	7.3
Control	13.5	5.3

According to Fig.8 and Table 4, when the consortium was inoculated in garden soil the shoot and root length of the Bottle gourd plant was found to be increased as compared to the control after 10 days of incubation.

Figure 8: Root and shoot length of Bottle gourd plant with and without Consortium

Table 4: Root and shoot length of Bottle gourd plant after 10 days.

	Shoot length(cm)	Root length(cm)
Bottle gourd with consortium	16.8	11.5
Control	11.2	9.4

According to Fig.9 and Table 5, when the consortium was inoculated in garden soil the shoot and root length of the Brinjal plant was found to be increased as compared to the control after10 days of incubation.

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Figure 9: Root and shoot length of Brinjal plant with and without consortium.

Table 5: Root and shoot length of the Brinjal plant after 10days.

	Shoot length(cm)	Root length(cm)
Brinjal with consortium	7.3	3.7
Control	4	1.7

According to Fig.10 and Table 5, when the consortium was inoculated in garden soil, the shoot and root length of the Tomato plant was found to be increased as compared to the control after 10 days of incubation.

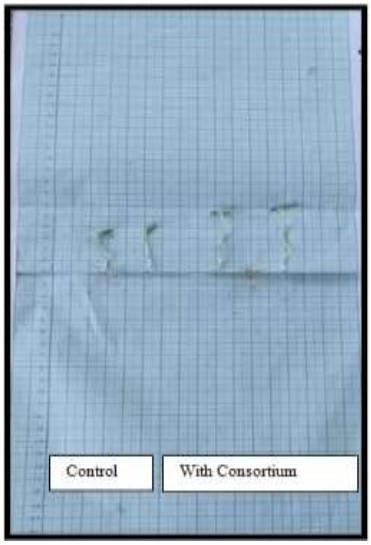

Figure 10: Root and shoot length of Tomato plant with and without consortium.

Table 6: Root and shoot length of Tomato plant after 10 days

	Shoot length(cm)	Root length(cm)
Tomato with consortium	5.6	3.5
Control	4.3	1.8

Thus, consortium of K7 and A4 bacterial isolates possessing plant growth promoting characteristics viz. siderophore, ammonia, and indole acetic acid production when added to the garden soil, the root and shoot length of all vegetables significantly increased. These findings suggest that consortium of bacteria K7 and A4 has promising potential as bioinoculant which can used for productive vegetable cultivation and promote sustainable agricultural practices.

DISCUSSION

The assessment of bacteria that promote plant growth is a fascinating field of study with significant implications for sustainable agriculture. In the present study, 20 bacteria were isolated from agricultural field soil, examining their cultural, morphological, biochemical, and metabolic characteristics. This study helped to identify bacterial species and understand their physiological and biochemical traits, crucial for sustainable agriculture and further research. PGPB, through various mechanisms like IAA synthesis, siderophore and ammonia production promotes plant growth. The growth of different vegetable seeds was positively impacted by the application of bioinoculant of the chosen isolates A4 and K7 possessing all plant growth promoting activities. Other PGPR strains have shown similar results. PGPR can indirectly increase seed germination and vigor index by decreasing the incidence of detrimental seed mycoflora [20].

Siderophores, produced by rhizospheric bacteria, aid in iron mineralization and plant uptake, with the CAS agar plate method being utilized to detect their production [21]. The CAS reagent's color change on CAS agar plates was used to screen siderophore-positive isolates, revealing twelve out of twenty bacterial isolates to produce siderophores. Iron bioavailability is limited by insoluble ferric complexes, preventing soil microorganisms and plants from accessing most of the iron due to its physiologically significant pH values [22]. Thus, soil bacteria secrete siderophores with Fe-scavenging properties, converting ferric Fe into ferrous (Fe^{2+}) form before transferring it into the cell.

This study found that four isolates were able to produce IAA, a common phytohormone in PGPR, indicating its widespread prevalence in various studies. IAA promotes plant growth by encouraging root development and nutrient uptake, with phytohormones regulating nitrogen availability and application [23]. Our study revealed that by inoculating bacterial strains K7 and A4 positively impacted plant growth without causing disease symptoms, and the inoculants also promoted lateral root growth. Another study reported that inoculating multiple bacteria with a bio inoculum has greater potential. Thus, our results are in agreement with the reports of Oliveria et al. [24].

Bacteria aid plant growth by producing ammonia, siderophores, and indole acetic acid. Pot assays and seed germination tests indicated plant growth capacity of the bioinoculant. The information obtained from the current research is valuable and may prove beneficial for sustainable agriculture.

CONCLUSION

This study investigated the isolation, identification, and characterization of plant growth promoting bacteria (PGPB) from agricultural field soil and their positive effect on the growth performance of various vegetables. 20 bacteria were isolated from agricultural field soil. Out of 20, two bacterial isolates viz. A4 and K7 were found to possess all plant growth promoting characters viz. ammonia, siderophore and IAA production. The identification of these two bacterial isolates by biochemical characterization and on the basis of Bergey's manual revealed that K7 and A4 isolates may belong to *Alcaligenes* sp. and *Acinetobacter* sp., respectively. The bio-inoculum of two bacterial strains viz. A4 and K7 was prepared and inoculated in pots containing seeds of different vegetables viz. Okra, Brinjal, Tomato, and Bottle gourd. In the pot assay, as compared to the control group, inoculation with these PGPB strains improved the growth parameters of all vegetables. These results imply that consortium of bacterial isolates A4 and K7 has encouraging potential as bioinoculant to support productive vegetable farming and sustainable agricultural methods. Thus, this study shows that PGPB has the potential to be an effective instrument for improving vegetable production. By utilizing these bacteria's advantageous qualities, we can have transition to a more environmentally responsible and sustainable method of farming.

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