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Research Article

Isolation and Characterization of Plant Growth-Promoting Rhizobacteria from Coffee Plantation Soils and Its Influence on Maize Growth

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Plant growth-promoting rhizobacteria (PGPR) are an important bacterial resource as biofertilizers, which can promote plant growth and increase crop yields. In this study, isolation, characterization, and its effect on plant promotion were assessed by isolating PGPR using soil samples collected from Jimma, Ethiopia. Out of 10 isolates, three of them (JEC3, JEC4, and JEC7) exhibited PSB traits in Pikovskaya media by solubilizing phosphate and producing IAA. Of the three isolates, JEC4 recorded the maximum phosphate solubilization index (4.98), soluble phosphate (283 μg/ml), and IAA production (10.21 μg/ml). The findings of the isolates' morphological and biochemical tests showed that JEC3, JEC4, and JEC7 as Bacillus sp., Pseudomonas sp., and Enterobacter sp., respectively. The optimization of phosphate solubilization was done using different incubation times and sources of carbon and nitrogen. Furthermore, the isolate (JEC4) was tested for plant growth-promoting (PGP) activities and indicated the production of ammonia, hydrogen cyanide, and siderophore. JEC4 isolate showed a significant increase in sideways (%) seed germination, root and shoot length, fresh and dry weight of root, and shoot of maize in field experiments than the untreated ones. The results indicate that the JEC4 isolate can be developed as a biological fertilizer for promoting crop productivity and ecosystem dynamics.

1. Introduction

Soil is the reservoir for a variety of microorganisms that have formed a complex interactive network [1]. Plant nutrient acquisition can be improved by soil microorganisms. They are capable of solubilizing and altering insoluble soil phosphorus to soluble forms for improving the growth of plants [2]. Among others, the rhizosphere exists around or on the root surface of the plants that supports the growth of plants by increasing plant biomass, nutrient efficiency, and crop yields [1, 3]. The bacteria that support the plant growth are known as plant growth-promoting rhizobacteria (PGPR), and these also act as phosphate-solubilizing bacteria (PSB) [4].

Phosphorus (P) is the second-most plant-essential macronutrient present in the soil after nitrogen and is found in organic and inorganic forms, which might be both insoluble or very poorly soluble inorganic forms [5]. Most of the P occurs in insoluble form as iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils. Some of them appear after the application of chemical fertilizers. Due to the formation of insoluble iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils, the deficiency of P occurs in soil [6]. To handle the P deficiency in different crops, chemical phosphate fertilizers are regularly added in various amount to the soil. However, this applied P is precipitated into an insoluble and stable form soon after the application and is available to

plants with limited only 5% or less of the total amount of P in the soil [7]. Besides, excessive application of chemical phosphate fertilizers can cause both environmental and economic problems [8, 9]. PSB plays a significant role in the release and mobilization of insoluble and fixed forms of P available to plants in a sustainable and eco-friendly approach to environmental protection. Numerous bacterial species such as *Alcaligenes* sp., *Aerobacter aerogenes*, *Achromobacter* sp., *Actinomadura oligospora*, *Burkholderia* sp., *Pseudomonas* sp., *Bacillus* sp., and *Rhizobium* sp. are capable of solubilizing phosphate in soils [7]. Several studies have investigated the effect of applying PSB [5–7, 10–16], but there is very limited information about isolation of *Pseudomonas* sp. from soils of coffee plants and examining its effect on plant growth.

Therefore, the present study has been carried out to isolate PSB from the soils of coffee plants and characterize isolated bacteria using morphological and biochemical characteristics and phosphate solubilization and indole acetic acid (IAA) production ability of the isolate. Furthermore, the study aims to identify the effect of isolated bacteria as inoculants on plant growth parameters.

2. Materials and Methods

2.1. Isolation and Characterization of PSB. The rhizospheric soil used for bacterial isolation was excavated from the 15–20 cm depth of roots of the coffee plant located in Jimma zone, Oromia regional state, Ethiopia. The town is located at around 7°41′ N latitude and 36° 50′ E longitude, with annual maximum and lowest temperatures of 30°C and 14°C, respectively [17]. The soil samples were collected from three sites (Sigimo, Kersa, and Setema). Bulk soil was removed from plants by shaking vigorously by hand for 10 min before collecting the rhizospheric soil from roots of coffee plants. Ten gram of rhizospheric soil samples were collected in sterilized plastic bags. The samples were stored in an icebox at 4°C and transported to the laboratory. Isolation of PSB was done through the serial dilution technique [3] and incubated at 30°C for 5 days. The selected bacterial isolates were coded according to their collection site. Potential isolates were screened and selected based on the halo zone produced in Pikovskaya's (PVK) agar for phosphate solubilization. Morphological (shape, color, edge, motility, and endospore formation and Gram stain) and biochemical tests (indole test, urease test, catalase test, Voges–Proskauer (VP) test, methyl red (MR) test, and citrate test) of the isolate were conducted according to methods in [15, 16], respectively.

2.2. P Solubilization and IAA Production. Qualitative estimation of phosphate solubilization was conducted using PVK agar plates that cultivated on the shaker incubator at 30°C for 5 days. Phosphate solubilization showed the formation of a clear zone around a growing colony, and this was determined by the calculation of phosphate solubilization index (PSI) through the following formula: [7]

$$\text{PSI} = \frac{\text{total diameter (colony + holo zone)}}{\text{colony diameter}}. \quad (1)$$

Additionally, a quantitative approximation of phosphorus was done in PVK broth corrected with 5.0 g/L tricalcium phosphate (TCP) [17], and the production of IAA was determined using the colorimetric method [18].

2.3. Characterization of Plant Growth-Promoting Activities of the Bacterium Strain JEC4

2.3.1. Hydrogen Cyanide Production. Production of hydrogen cyanide (HCN) was detected with the method adopted by Holt [19]. Isolate (JEC4) was tested for production of HCN by dispersing it on King's medium amended with 0.44% of glycine. A Whatman filter paper no. 1 was soaked in 2% sodium carbonate and 0.5% picric acid solution and placed at the top of the plate. Discoloration of the filter paper from orange to brown and yellow to light brown after incubation of 4-5 days indicated the production of hydrogen cyanide.

2.3.2. Siderophore Production. The bacterial inoculum was spotted on chrome azurol sulphonate (CAS) agar plates and incubated at 28 ± 2°C for 5 days for screening siderophore production [20]. Development of a yellowish, pinkish, or whitish color halo around the colonies in the dark blue medium indicated a positive test for siderophore production.

2.3.3. Ammonia Production. Freshly grown rhizobacteria cultures were inoculated in 10 ml peptone water and incubated at 28 ± 2°C for about 48–72 h followed by the addition of 0.5 ml of Nessler's reagent. The transformation from brown to yellow color indicated a positive test for ammonia production [20].

2.4. Optimization of P Solubilization. Isolate JEC4 was tested for optimization of P solubilization in PVK broth media. The effects of carbon source, nitrogen source, and incubation period on P solubilization were studied by inoculating 500 μl of inoculum (1.94×10^3 cfu/ml) into 100 ml PVK broth media in a 250 ml conical flask. The effect of different carbon sources on tricalcium phosphate (TCP) solubilization was assessed by replacing the glucose with fructose, starch, lactose, galactose, and mannitol, at a concentration of 10 g/l. The effect of the nitrogen source was also examined by replacing ammonium sulfate with ammonium nitrate, sodium nitrate, tryptone, urea, and peptone (0.5 g/l). The broth cultures were incubated at 30°C in a shaking incubator. P solubilization was estimated for 2, 5, 8, 11, and 14 days, and the range was selected based on Lebrazi et al. [21]. These sugars were sterilized separately and were added aseptically to the medium before inoculation. The standard PVK medium containing glucose and ammonium sulfate was used as a control.

2.5. Field Experiment. The field experiment was performed at Jimma Institute of Technology (JIT), Jimma, Ethiopia. The town is generally characterized by a warm climate with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C [17]. The maize field trial was conducted following the procedure used by

[7, 22–24]. The field experiment was conducted from November 2021 to January 2022. The experimental work consisted of PSB and control (uninoculated with PSB) was conducted on a plot size of 4 m × 4 m of soil in the agricultural field. The same type of soil was used for PSB and control experiments. The used soil had a moderately alkaline solution with a pH of 7.5, electrical conductivity (EC) of 1.87 $dS{\cdot}m^{-1}$, the organic carbon content of 4.6%, available N_2 content of 7.7 mg/kg, available P content of 4.6 mg/kg, and available potassium content of 32 mg/kg. Clay soil was used for testing the efficiency of isolated PSB, its effect was observed on the growth of maize plants, and maize seeds (the seeds were purchased from the local market, and the variety was identified by Jimma University College of Agriculture and Veterinary Medicine as flour maize) were surface-sterilized with sodium hypochlorite (2%) and ethyl alcohol (70%) for 1 min. Then, the seeds were washed with autoclaved distilled H_2O trace of chemicals. The broth culture of the isolate was made in the PVK medium. JEC4 strain was inoculated into a 250 mL Erlenmeyer flask containing 100 mL broth agar medium and incubated for 48 hours at 28°C with shaking (180 rpm) before soaking the seeds. The maize seeds were soaked with a cell density of 2×10^3 CFU/mL obtained through serial dilution. Then, the corn seeds were soaked in a broth culture for 30 min, and the uninoculated seeds without PSB were kept as control. Plants and row spacing were kept at 16 cm and 60 cm, respectively. The numbers of plants in each plot were ten, and the plots were fertilized by applying urea and watered slowly every one or two days. The available P, pH, EC, and the population of PSB (colony-forming unit (CFU)) in the soil of each experimental group (with and without PSB) were determined 25 days after seeding, and the experiment was performed triplicates.

2.6. Analysis of Soil Physicochemical Properties. The physical and chemical properties of the soil used for the field test were analyzed. The soil pH and EC were measured in 1 : 2.5 and 1 : 5 solutions of dried soil/water, respectively, with a portable pH-conductivity meter [25]. The organic matter content was measured using the potassium dichromate colorimetric method [26], the available potassium content was determined using the flame photometer method [27], and soil available nitrogen (N) and phosphorus (P) were measured using the alkali diffusion method [28] and molybdenum blue method [29], respectively.

2.7. Plant Growth Parameters. The growth parameter of the plant, number of leaves, shoot and root length, and dry weight were evaluated from randomly chosen plants 25 days after seeding. Ten plants were harvested, and the shoot was separated from the root. And then, the number of leaves, leaves' shoot, and root height were determined. Roots were rinsed with tap water to remove soil particles. After drying in an oven at 60°C for 48 hours, the dry weights of roots and shoots were measured and compared with control plants [7]. Percent (%) of seed germination was calculated by the following formula:

$$\%\ \text{seed germination} = \frac{\text{number of seeds germinated}}{\text{Number of seeds planted}} \times 100. \quad (2)$$

2.8. Experimental Design and Statistical Analysis. Experiments were performed using randomized complete block design (RCBD) with triplicates for each experiment. Plant growth parameters were performed by randomly selecting grown maize plants. One-way analysis of variance (ANOVA) of data was performed using Microsoft Excel version 2016, and the significance ($P < 0.05$) of PSB along control (uninoculated with PSB) were analyzed. The least significant difference (LSD) was computed to compare the differences between means in each treatment. $P \leq 0.05$ was considered significant.

3. Results and Discussion

3.1. Isolation and Characterization of PGPR Isolates. Among ten isolates from PVK agar medium, three of them were selected (JEC3, JEC4, and JEC7) based on phenotypic (morphological and biochemical tests) properties and P solubilization (Table 1). The phosphate-solubilization ability of the isolate was determined by observing the large clear zones around the bacterial colonies using PVK agar and calculated as PSI. PSI for the three isolates was between 3.97 and 4.98. From the three isolates, the maximum PSI was exhibited by PSB4. The reason for the formation of the halo zone around the bacterial colonies might be due to the production of organic acids, HCN and siderophore production, and other causes [3, 7, 30–32]. The morphology of three isolates with a wider clear halo zone surrounding their colony was examined under a microscope, and all three isolates were spherical elevated colonies and rod-shaped rods, motile, and most of them with a shiny surface (Table 2).

A variety of biochemical procedures were used to further describe the isolates (Table 2). All three microorganisms have proven negative for the urease and MR check. Bacterial isolates JEC3 and JEC4 have been poor for the citrate and indole check. However, microorganism isolate JEC7 is positive for VP, citrate, and indole check. The morphological and biochemical check effects of JEC3 are regular with the numerous phenotypic traits of the genus *Bacillus* sp. [32–34], while JEC4 and JEC7 are similar to *Pseudomonas* [33, 35] and *Enterobacter* sp. [27, 33], respectively. Therefore, it became concluded that the isolated JEC3, JEC4, and JEC7 have been recognized as *Bacillus* sp., *Pseudomonas* sp., and *Enterobacter* sp., respectively.

3.2. P Solubilization and IAA Production by PGPR Isolates. Quantitative evaluation of the isolate for P solubilization was performed by P solubilization and IAA production. Various bacterial isolates have different phosphate solubilization

Table 1: Phosphate-solubilizing index (PSI) of the isolates.

Bacterial isolates	Colony diameter (mm)	Halo zone diameter (mm)	PSI
JEC3	2.75	9.6525	4.51 ± 0.48
JEC4	1.62	6.4476	4.98 ± 0.62
JEC7	1.54	4.5738	3.97 ± 0.16

Table 2: Morphological and biochemical characterization of three plant growth-promoting rhizobacterial (PGPR) isolates.

Tests		PGPR isolates		
		JEC3	JEC4	JEC7
Morphological	Color	Reddish	Cream	White
	Shape	Rod-shaped	Rod-shaped	Rod-shaped
	Gram staining	+	−	−
	Motility	Motile	Motile	Motile
	Endospore formation	+	−	+
	Indole test	−	−	+
	Catalase test	+	−	+
Biochemical	Urease test	−	−	−
	Voges–Proskauer (VP) test	−	−	+
	Methyl red (MR) test	−	−	−
	Citrate utilization	−	−	+

Table 3: Phosphate (P) solubilization and indole acetic acid (IAA) production by plant growth-promoting rhizobacteria (PGPR) in liquid media.

Bacterial isolates	P solubilization (μg/ml)	IAA production (μg/ml)
JEC3	275 ± 0.17^{a}	9.1 ± 0.42^{a}
JEC4	283 ± 0.26^{b}	10.21 ± 0.26^{c}
JEC7	270 ± 0.15^{c}	8.76 ± 0.48^{b}

*Different letters in each column represent significant difference.

capacities. From the three identified isolates, JEC4 had shown high P-solubilization (283 μg/ml) and IAA production (10.21 μg/ml) after three days (Table 3). The result observed in this study is higher than that in [10], who isolated *Aneurinibacillus aneurinilyticus* CKMV1 from rhizosphere of *Valeriana jatamansi* and reported P solubilization of 260 μg/ml and IAA production of 8.1 μg/ml, while lower than that in other studies with IAA production 19.2–22 μg/ml [3] and phosphate solubilization 334 ± 0.8 μg/ml [36].

Phosphate solubilization by *Pseudomonas* genera has been stated by other scholars [3, 10, 16, 33]. Based on P solubilization in agar and broth media and IAA production isolate, JEC4 (*Pseudomonas*) was selected for determining its effects on growth-promoting parameters.

3.3. Characterization of PGP Activities of JEC4. After the incubation of samples on respective medium for 7 days, an analysis was carried out to observe the various plant growth-promoting activity of the isolate *Pseudomonas* sp. (JEC4) by formations of clear halos around the bacterial spots inoculated on the plates. The results indicated that tests were positive for the production of HCN, siderophore, and ammonia. This result was in agreement with Holt [37], who studies on enhancement of growth and yield parameters of wheat variety AAI-W6 by an organic farm isolate of PGP *Erwinia* species (KP226572).

3.4. P-Dissolving Capacity and Other Parameters in Experimental Soil. The available P content in the soil inoculated with JEC4 shows a significant difference ($P < 0.05$) compared to the uninoculated one (control) (Table 4), which indicated that JEC4 exhibited solubilizing ability in the experimental soil. There were also significant differences in the pH and EC of the soil treated with bacterial strains.

A higher population of PSB was found in soil treated with JEC4 than that in the untreated one. The solubilizing phosphate bacterium plays a significant role in the liberation of soluble P from insoluble phosphate [29]. This result is in agreement with studies on the characterization of PSB isolated from calcareous soils [33]. The available P, populations of the PSB, pH, and EC in the experimental soil was reported by many researchers [27, 37–40].

3.5. Effects of Incubation Time and Carbon and Nitrogen Sources on P Solubilization. The performance of P solubilization with the aid of using JEC4 strain was considered and located to be within the range of 92 μg/ml–284.4 μg/ml (from Figures 1(a)–1(c)), that is similar to the PSB isolated from the rhizosphere of *Allium hookeri* thwaites (124.8–266.4 μg/mL) [38] and better than PSB of *Bacillus* sp. (30–100.6 μg/ml) in PVK media [41].

The effect of incubation time on P solubilization by JEC4 strain is shown in Figure 1(a). 284.4 mg/l of soluble phosphate was obtained after 2 days of incubation. The soluble

Table 4: Effects of phosphate-solubilizing bacteria (JEC4) inoculation on the soil characteristics before and after the field experiment.

	Before	After	
		JEC4 inoculated	Control
Available phosphorus (mg/kg)	4.6	13.65 ± 0.40^{a}	8.07 ± 0.30^{b}
pH	7.5	6.5 ± 0.80^{b}	7 ± 0.84^{a}
Electrical conductivity ($dS \cdot m^{-1}$)	1.87	1.45 ± 0.06^{c}	$1.81.3 \pm 0.05^{d}$
Cell density (CFU/g of soil)	2×10^{3}	$2.51 \times 10^{3} \pm 0.20^{d}$	$2.031 \times 10^{3} \pm 0.32^{c}$

*Different letters in each row represent significant difference.

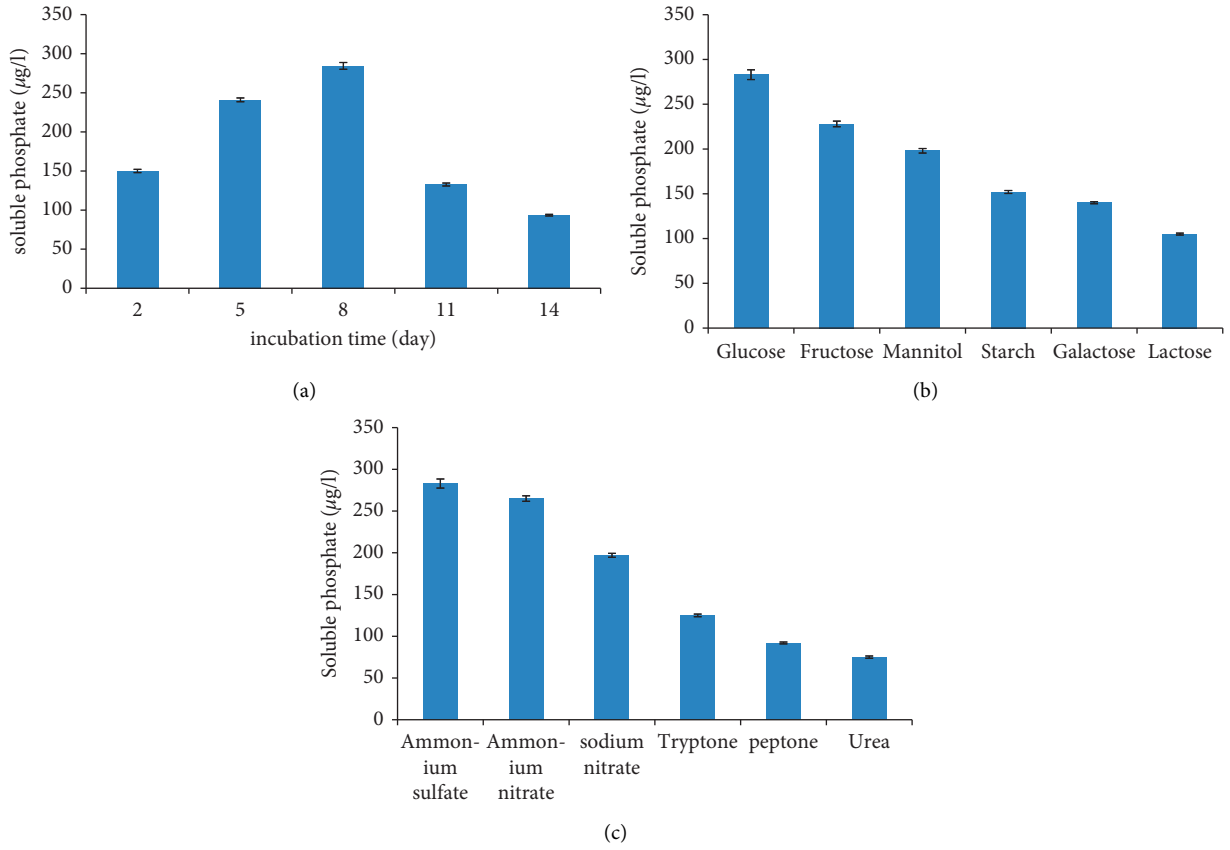

Figure 1: Effects of incubation time (a) carbon sources (b) and nitrogen sources (c) on phosphate solubilization by JEC4 strain with error value.

phosphate increased with the increasing incubation period, and maximum soluble phosphate (282.4 mg/l) was observed at 8 days of incubation time. These results were in correspondence with [7], and they observed that an incubation period of 8 days was ideal for achieving the highest level of phosphate solubilization. The authors of [30] studied the characterization of PSB isolated from calcareous soils P solubilization at different incubation times and attained the maximum concentration of water-soluble P (523.69 mg/l) at 7 days of incubation time. Additionally, the authors of [40] observed maximum soluble phosphate at 7 days of growth. The reason for the increased solubility of phosphate is due to the production of acids with the increase in the period of incubation [41]. But, the additional increase in incubation time has led to a decrease in phosphate solubilization activity; subsequently, the efficiency of the microorganism population to affect phosphate solubilization from tricalcium phosphate would be discounted if this variable were to be excessively high.

Different carbon sources were used to evaluate their effects on phosphorus solubilization, and the result is tabulated in Figure 1(b). Based on the results of the experiment conducted, glucose encouraged the greatest TCP solubilization (283 μg/ml) followed by fructose, mannitol, starch, galactose, and lactose. Statistical analysis showed that the effect of different carbon sources on TCP solubilization by the tested strains was significant ($P < 0.05$). These results are in agreement with earlier studies which stated that glucose was the best carbon source for phosphate solubilization [27, 42–44]. However, the authors of [45] observed higher phosphate solubilization by *Bacillus* sp. NPSBS 3.2.2 in the

Table 5: Effects of plant growth-promoting rhizobacteria (JEC4) on field-grown maize.

	% seed germination	Shoot			Root			No. of leaves/ plant	Leaves' length (cm)
		Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)		
JEC4	100 ± 0.35	5.474 ± 1.05	1.335 ± 1.49	0.45 ± 0.26	9.897 ± 1.09	0.558 ± 1.23	0.604 ± 0.22	3 ± 0.05	4.37 ± 1.10
Control	100 ± 0.35	2.656 ± 0.03	0.619 ± 0.14	0.191 ± 0.15	6.832 ± 0.06	0.26 ± 0.64	0.225 ± 0.2	3 ± 0.03	11.86 ± 0.64

appearance of sucrose, whereas the authors of [43] found maltose as the best carbon source using *Aspergillus awamori* S19 isolated from the rhizospheric soil. The possible reason for the release of P is the production of organic acids [46, 47].

The effect of different nitrogen sources showed that all the tested nitrogen sources have a significant effect in increasing the amount of soluble P. The results showed that the peak phosphate solubilization was noted in the presence of ammonium sulfate (283 μg/ml) followed by ammonium nitrate > sodium nitrate > tryptone > peptone > urease as shown in Figure 1(c). Similar results shown by [43, 45] also observed maximum solubilization via ammonium chloride followed by ammonium sulfate.

3.6. PSB Inoculation and Determination of Its Effect on Plant Growth Parameters. The plant growth parameters for JEC4 and control in field experiments were determined in maize plants. There is no significant difference in percentage germination of JEC4 treated and untreated seeds (Table 5). After 25 days of cultivation, maize plants treated with JEC4 had a significantly higher growth than those of untreated plants (Table 5). The bacterial strain JEC4 enhanced plant height, shoots fresh weight, shoots dry weight, roots length, roots fresh weight, roots dry weight, and leaf length than control. The results reveal that the differences between the treated seed and the control were significant ($P \leq 0.05$), which may be due to the production of IAA by the bacterial isolate [3]. AA-generating microorganisms stimulate the proliferation of existing roots and adventitious roots and increase the surface area and volume of roots [48]. Our results in this experiment are in line with many studies [13, 27, 46, 49, 50] which reported the effect of PSB in different crops to result in improving the growth parameters. Furthermore, similar works were reported previously in bacterial inoculation in melon [51], mung [20], tomato [5], *Acacia cyanophylla* ([18], oats (*Avenia sativa*), alfalfa (*Medicago sat*iva), and cucumber [26] and maize [7, 14].

4. Conclusions

In general, several helpful plant growth-promoting rhizobacteria (PGPR) strains can improve plant growth and yields. From isolated bacteria, JEC4 shows maximum phosphate solubilization efficiency in agar (PSI = 4.98) and broth media (283 μg/ml) and IAA production (10.21 μg/ml). Furthermore, P solubilization of JEC4 in PVK liquid medium at different parameters (incubation time, carbon source, and nitrogen source) was assessed for 8 days of the incubation period; glucose and ammonium sulfate shows high phosphate solubilization. Besides the isolate, JEC4, may well promote plant growth parameters (shoots height, shoots fresh weight, shoots dry weight, roots height, roots fresh weight, and roots dry weight and leaves height) more than the control. The finding point that the development of potentially active PGPR strain-based formulations can increase the availability of nutrient concentration in the rhizosphere by fixing nutrients, thus preventing them from leaching out. PGPR inoculation also increased soil phosphorus acquisition efficiency of maize cultivars. The current study demonstrated the potential of PGPR to improve plant growth-parameter effects on maize; however, further work on strain's molecular characteristics and application methods for inoculation of PGPR may assist in exploiting these microorganisms.

Abbreviations

IAA: Indole acetic acids
JEC: Jimma Ethiopia coffee
MR test: Methyl red test
VP test: Voges–Proskauer test
PSI: Phosphate solubilization index
PVK: Pikovskaya's
TCP: Tricalcium phosphate
PGPR: Plant growth-promoting rhizobacteria.

Data Availability

Data are included in the article/supplementary material/references in the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All authors contributed to the conception and experimental design study. Prof. Venkata conceived the research and edited the manuscript. The work was conceptualized by Yasin and Ermias. Material preparation, data collection, and analyses were performed by Mohammed and Yasin. The manuscript was written by Yasin, Ermias, and Mohammed, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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