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Characterization of Plant Growth-Promoting Rhizobacteria from Cowpea Rhizosphere

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ABSTRACT
The increasing demand for sustainable agricultural production has intensified efforts to identify environmentally friendly alternatives to synthetic fertilizers. This study investigated the isolation and characterization of plant growth-promoting rhizobacteria (PGPR) from the rhizosphere of cowpea (*Vigna unguiculata (L.) Walp*) with the objective of evaluating their potential to enhance plant growth and physiological performance. Rhizosphere soil samples were collected, and bacterial isolates were obtained and characterized using standard morphological and biochemical procedures including gram staining, indole test, catalase test etc. The isolates were screened for key plant growth-promoting traits, including HCN production, phosphate solubilization, and Ammonia production. The finding of the study shows that soil chemical properties varied significantly among the samples. Sample C had the highest organic carbon (1.397 ± 0.06^{a} %) and nitrogen (0.150 ± 0.02^{a} %), significantly higher than all other samples, indicating richer soil fertility. Also the Week 3 group exhibited the highest mean AL (20.00 ± 2.61 cm) and was statistically comparable to the Week 4 (18.42 ± 1.43 cm) and Control (18.00 ± 1.00 cm) groups, as indicated by the shared superscript a and *b*. The biochemical characterization reveals that all isolates were Gram-positive and uniformly methyl red (MR) positive indicating a consistent ability to produce stable acid end-products from glucose fermentation. The study concludes that cowpea rhizosphere soil harbors diverse bacterial isolates with promising plant-growth-promoting characteristics and potential for use in sustainable agricultural production. Further field-based studies are recommended to validate their effectiveness.

Keywords:
Rhizobacteria,
Rhizosphere,
Soil properties,
Morphological Characterization,
Biochemical Characterization.

INTRODUCTION

The rhizosphere is a biologically dynamic zone of soil surrounding plant roots, enriched with root exudates such as sugars, amino acids, organic acids, and enzymes that act as nutrient sources for microorganisms (Choudhary *et al*., 2018; Singh *et al*., 2024). Extending only a few millimeters from the root surface, this zone supports significantly higher microbial populations and activities than bulk soil, creating a hotspot for biochemical and physiological interactions that directly influence plant growth, health, and resilience (Geetanjali and Pranay, 2016). Microorganisms inhabiting the rhizosphere regulate essential soil processes, including decomposition of organic matter, mineralization of nutrients, nitrogen fixation, denitrification, and mobilization of phosphorus, potassium, and micronutrients, thereby enhancing soil fertility, structure, and sustainability (Backer *et al*., 2018; Liu *et al*., 2021; Sarkar and Ghosh, 2023).

Among these microbes, plant growth-promoting rhizobacteria (PGPR) are particularly notable for their ability to colonize plant roots and confer a suite of benefits to host plants (Parewa *et al*., 2018; Ali *et al*., 2022). PGPR facilitate nitrogen fixation through symbiotic or associative interactions, solubilize phosphorus, release potassium and micronutrients, produce phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins to modulate root growth and nutrient uptake, and generate enzymes like ACC deaminase that mitigate stress-induced ethylene accumulation (Backer *et al*., 2018; Sharma *et al*., 2022).

MATERIALS AND METHODS

Study Location

The study was conducted in Kano State, Nigeria. Kano is one of the 36 states in Nigeria located in Northwest geopolitical zone.

Kano state covers a total land area of approximately 20,131 $km^2$and a population of about 9,401,288 people according to projection 2022 Nigerian National population census. The state economy is driven by agriculture, commerce and manufacturing.The research was conducted at the post graduate laboratory of microbiology department and green house of plant Biology department, Faculty of Life Sciences, Bayero University Kano (geographical coordinates of L. 11^0 14' N to 12^0 23'N and L. 8^0E to 9^0E).Cowpea was planted at the green house of plant biology department of Bayero University Kano and the rhizospheric soil was collected for isolation and characterization.

Soil Preparation and Planting

Soil was collected from agricultural site in Bayero University Kano. Cow dung, sheep dung and poultry manure were used as the fertilizer in the ratio 4:1. In addition, improved seed was collected from IITA, Kano (IT99K-573-1-1) SAMPEA 14 which is an early maturing variety and striga resistant. Cowpea was planted in 30 plastic buckets at a depth of 1.5cm, 10 buckets for each manure. It watered and the samples were collected after 3 weeks of planting.

Isolation and purification of bacterial strains

An ethanol-sterilized shovel was used to carefully excavate the cowpea plants. Excess soil was gently removed from the roots, leaving approximately 2 mm of soil adhering to the root surface. The roots were processed aseptically for isolation of rhizobacteria. Glassware, including Petri dishes, was sterilized by autoclaving at 120°C for 20 min. A small portion of each root sample was transferred separately into a sterile test tube containing 10 mL of sterile distilled water and vigorously shaken in a biosafety cabinet. The resulting suspension was serially diluted to 10^{-1} and 10^{-2} using sterile distilled water. Aliquots of the original and diluted suspensions were inoculated onto nutrient broth agar using the spread-plate technique and incubated at 28°C for 48 h. Several screenings were done to isolate pure cultures. Pure colonies were isolated and maintained on NBA plates at 4°C for further studies. (Rahman *et al.,* 2010)

Determination of Physicochemical characteristics of soil.

The physicochemical parameters such as temperature, pH, electrical conductivity, moisture content, nitrogen content, organic carbon content and soil particle size were analyzed in the department of Soil Science, Faculty of Agricultural Science at Bayero University Kano.

Soil pH in H20 (1:2.5)

Ten grams (10 g) of air-dried soil (2 mm Sieve) was weighed into a 50 ml beaker and 25ml of distilled water was added. The suspension was stirred several times for 30 minutes with a glass rod. The soil suspension was then allowed to stand for about 30 mins allowing most of the suspension clay to settle out from the suspension. Electrodes of the pH meter (Janway, P757s) were inserted into the partly settled suspension to measure the pH.(Eno et al., 2009).

Organic Carbon

One (1 g) of soil sample was weighed into 500 ml conical flask. Ten millilitre (10 ml) of 1 N K2Cr207 solution was pipetted accurately into each flask and swirled gently to disperse the soil. Twenty millilitre (20 ml) of concentrated H2S04 was added gently and immediately swirled. The flask was then swirled more vigorously for one minute and allowed to stand for 30 mins for oxidation to complete. After 30 mins, 100 ml of distilled water was added and 4 drops of 1-10 phenanthroline indicator was added and titrated with standard 0.5 N ferrous sulphate solution to obtain an end point. A blank was prepared with same treatment but without sample (Eno et al., 2009). The result was calculated as follow:

The equation formula is:

$$\%\ \text{Organic Carbon} = \frac{(\text{Blank titre} - \text{Actual titre}) \times 0.3 \times N \times F}{\text{Weight of air-dry soil (g)}}$$

Where;
Blank titre = titre value of the blank
Actual titre = titre value of the soil sample
0.3 = conversion factor
N = normality of the solution used
F = correction factor (in your article, F = 1.33)
Weight of air-dry soil = mass of soil sample in grams (Eno et al., 2009).

Moisture Content

Empty moisture can was weighed (W1) and five (5) grams of soil sample was added to the moisture can with tight fitting lid and re-weighed as (W2). The moisture can contain the sample was then be dried in an oven at 105 oC for 24 hrs after which the set up was removed and put in desiccator to cool. After cooling, the sample was weighed again (W3) (Eno et al., 2009).

$$\text{Moisture Content (\%)} = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

Where:
W1 = Weight of empty can in grams
W2= weight of moisture can + fresh soil (g)
W3= weight of moisture can + oven-dried soil (g)

Electrical Conductivity (EC)

Soil EC was determined by suspending the air-dried sample in water at the ratio of 1:5 and after shaking the mixture, allowed to stay overnight, filtered and electrode dipped into the filtrate in the beaker. Electrical Conductivity at 25 oC temperature was recorded by the use of electrical conductivity meter (Janway, 4520).

Electrical conductivity will be express in dSm-1. (Debarati, 2016).

Determination of Total Nitrogen

Kjeldhal method adopted by Eno et al. (2009) was used to determine total N in the soil sample. One gram of each soil sample was weighed into individual kjeldhal flasks. Then, 2 grams of a catalyst (mixture of K2SO4, CuSO4 and selenium powder in 100:10:1 proportion) and 30 ml of H2SO4 was added in each flask and heated gently until frothing cease. After the formation of clear solution in each flask, the digestion was heated continuously for 30 minutes. Then, 50 ml of distilled water and 30 ml of 40 % NaOH was added. After cooling, the mixtures were then transferred to a kjeldhal distillation flask. Ten millilitres (10ml) of boric acid solution and 4 drops of indicator (Bromocresol green and methyl red) solution were added in 3 different volumetric flasks. Furthermore, the distillation flasks were then heated for 30 mins and the distillate of each sample was collected in their separate flasks and titrated against 0.025N HCL. The percentage of nitrogen was calculated as follows:

$$\%N = \frac{(A - B) \times N \times 0.014 \times V_D \times 100}{A_d \times W}$$

Where:
A= Volume of sample required for titration of the sample,
B= Volume of sample required for titration of blank,
N = Normality of HCl,
0.014 = Miliequivalent weight of nitrogen.
Ad = Aliquot taken
W = Weight of sample
VD = Volume of Digest

Determination of Phosphorous Content

Olsen method was used to determine the available phosphorous in the soil sample. (Olsen *et al.,* 1954). The 2.5 gm of soil sample was weighed into 3 different conical flasks. Then, 50 ml of Olsen reagent (sodium bicarbonate) was added to each flask and shaken with a shaker (Innova 4000) operated at 180 rpm for 30 mins. The blank was also prepared without soil sample. Furthermore, each mixture was filtered through Whatman No 42 filter paper and 5 ml of filtrate was transferred to a volumetric flask and acidified to pH 5 using 2.5 M H_2SO_4. To each reaction mixture 20 ml of distilled water and 4 ml of ascorbic acid was then added and incubated for 10 minutes for blue color formation, then the absorbance of this solution was determined at 883 nm and phosphorous content in soil was thus be calculated as follows:

$$P\ (mg/kg) = \frac{Abs \times V_F}{Slope \times W}$$

Where:
VF = Volume of flask,
W = Weight of sample,
Abs = Absorbance

Particle Size Distribution

Particle size distribution was determined using Bouyouncos hydrometer method. (Gee and Or, 2002). The sand, silt and clay were determined by dispersing the soil in Calgon (sodium hexametaphosphate and sodium carbonate) solution. The dispersed soil sample was shaken on a reciprocating shaker (Innova 4000) for 15mins and the suspension was transferred to 1 L measuring cylinder and made to the mark with distilled water. The suspension was plunged five times and the first hydrometer and temperature readings were recorded as H1 and T1 at 40 seconds respectively. The second hydrometer and temperature readings were also taken as H2 and T2 respectively. The textural class was determined using the USDA textural triangle.

Exchangeable Bases

The exchangeable bases (Ca, Mg, K and Na) of the soil were extracted with 1M ammonium acetate (1M NH4OAC) solution (Anderson and Ingram, 1998). Five grams (5g) of air-dried soil sample was weighed into 120 ml container and 50 ml of ammonium acetate (pH 7.0) was added, the suspension was then shaken for 30mins and filtered with Whatman No 42 filter paper. Calcium and magnesium were read on Atomic absorption spectrophotometer (AAS, Agilent 240FS AA), in which a standard of 0, 50, 100, 150, 200ppm and 0, 5, 10, 15, 20ppm was used for calibration curve respectively. A standard of 0, 10, 20, 30, 40 and 50ppm prepared for Sodium and potassium was read on flame photometer (Jenway, PEP7).

Effective Cation Exchange Capacity (ECEC)

Effective cation exchange capacity was determined from the summation of (Ca+, Mg+, K+ and Na+) (Anderson and Ingram, 1998) as indicated by the relationship;
ECEC (cmol/kg) = ∑ (Ca+, Mg+, K+ and Na+)

Biochemical characterization of bacterial strains

Gram reaction test

To study the biochemical characteristics, all the bacterial isolates were grown in NBA medium and incubated in microbial incubator at 28°C for 48 hours. On glass slide a loopful of bacteria from a well grown colony was mixed with a drop of 3% KOH aqueous solution. Mixing continued for less than l0 seconds. A toothpick was used for picking as well as mixing bacteria from a colony. The toothpick was raised a few centimeters from the glass slide. Strands of viscid material were confirmed that the bacterium is gram-negative. Producing thread with a toothpick indicates that it is gram (+ve) bacteria (Ahmed, 2011).

Catalase test

A small amount of bacterial isolate was placed from culture onto a clean microscope slide. A few drops of H_2O_2 were added onto the smear. A positive result is the

rapid evolution of O_2 as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles (Wheelis, 2008).

Indole test

The test was carried out using the method described by Olutiola *et al.,* (2000). Tubes of sterilized peptone water were prepared according to the manufacturer specification and then inoculated with a young culture of the isolates. It was later incubated at 37 °C for 48 h alongside a control tube (containing sterilized peptone water without inoculum). Four drops of Kovac reagent is to be added into each culture tubes. Positive result shall indicate a red color that appeared immediately at upper part of the test tubes while yellow coloration indicates a negative result.

Motility Test

The motility of each isolate was tested in sulfide indole motility (SIM) medium. Using a needle, strains were introduced into test tubes containing SIM, and were incubated at room temperature until the growth was evident (Kirsop and Doyle,1991).

Citrate Test

Citrate utilization by the isolates was observed by the growth on slants of Simmon's Citrate Agar. A distinct change in color from green to blue refers to as a positive result for citrate utilization test. (Sheikh, 2018)

MR-VP test

The MR-VP was employed to detect the production of acid during fermentation of glucose such that the pH of a culture was sustained below a value of 4.5 as shown by the change in colour of the methyl-red indicator and alpha- nephtol which will added at the end of the period of incubation (Prescott, 2008).

Statistical Analysis

All data collected during the experiment was subjected to both descriptive statistic of mean and standard deviation and inferential statistical tool of One-Way Analysis of Variance (ANOVA) and Tukey's HSD Post-Hoc.

RESULTS AND DISCUSSION

Table 1: Selected Soil Chemical Properties of Different Samples Compared with Control

S ID	pH (H_2O)	pH ($CaCl_2$)	EC (dS/m)	P (mg/kg)	O.C (%)	N (%)
A	6.38 ± 0.05^{b}	5.62 ± 0.03^{b}	0.320 ± 0.01^{c}	18.468 ± 0.40^{b}	1.077 ± 0.05^{b}	0.125 ± 0.01^{b}
B	6.45 ± 0.04^{a}	5.49 ± 0.02^{c}	0.299 ± 0.01^{b}	17.103 ± 0.35^{c}	0.978 ± 0.04^{c}	0.100 ± 0.01^{c}
C	6.18 ± 0.06^{c}	5.30 ± 0.04^{d}	0.248 ± 0.02^{a}	18.885 ± 0.45^{a}	1.397 ± 0.06^{a}	0.150 ± 0.02^{a}
Control	6.45 ± 0.03^{a}	5.69 ± 0.03^{a}	0.070 ± 0.01^{d}	16.610 ± 0.30^{d}	0.998 ± 0.03^{c}	0.01^{d}

Notes: Different superscripts (^{a}, ^{b}, ^{c}, ^{d}) indicate significant differences ($p < 0.05$) between soil samples for that parameter. Same superscripts indicate no significant difference.

Table 1 shows soil chemical properties varied significantly among the samples. Sample C had the highest organic carbon (1.397 ± 0.06^{a} %) and nitrogen (0.150 ± 0.02^{a} %), significantly higher than all other samples, indicating richer soil fertility. Sample A had the highest phosphorus content (18.468 ± 0.40^{b} mg/kg), while sample C was slightly higher (18.885 ± 0.45^{a} mg/kg), both significantly different from sample B and the control. The pH values (both H_2O and $CaCl_2$) differed, with sample B showing the lowest pH in $CaCl_2$ (5.49 ± 0.02^{c}), suggesting slightly more acidic conditions, whereas the control and sample B had the highest H_2O pH (6.45 ± 0.04–6.45 ± 0.03^{a}). Electrical conductivity (EC) was highest in sample A (0.320 ± 0.01^{c} dS/m), indicating more soluble salts, while the control was the lowest (0.070 ± 0.01^{d} dS/m). Overall, the superscripts highlight that all parameters except a few were significantly different among soil samples, suggesting notable variability in soil fertility and chemical composition across the experimental sites.

Table 2: Growth Parameters

Group	Aerial Length (AL, cm)	Root Length (RL, cm)	Root Weight (RW, g)	Root Weight (RW, g)
Week 3	20.00 ± 2.61^{a}	5.33 ± 1.02^{a}	1.23 ± 0.91^{b}	5.10 ± 2.19^{b}
Week 4	18.42 ± 1.43^{ab}	5.58 ± 0.45^{a}	1.68 ± 1.00^{b}	6.35 ± 2.61^{ab}
Week 5	$16.83 \pm 2.3b$	$3.67 \pm 0.82b$	$3.08 \pm 0.86a$	$9.30 \pm 1.01a$
Control	$18.00 \pm 1.00ab$	$3.17 \pm 0.29b$	$1.03 \pm 0.15b$	$7.77 \pm 1.50ab$

Table 2 showed the effects of treatment duration on aerial length (AL), root length (RL), root weight (RW), and total weight (TW) were assessed across three experimental time points (Week 3, Week 4, and Week 5) and a control group. Aerial length varied significantly among treatment groups. The Week 3 group exhibited the highest mean AL (20.00 ± 2.61 cm) and was statistically comparable to the Week 4 (18.42 ± 1.43 cm) and Control (18.00 ± 1.00 cm)

groups, as indicated by the shared superscript a and b. In contrast, the Week 5 group recorded the lowest mean AL (16.83 ± 2.32 cm), which was significantly lower than Week 3 but not significantly different from Week 4 or the Control, as denoted by the superscript b.Root length was significantly greater in the Week 3 (5.33 ± 1.02 cm) and Week 4 (5.58 ± 0.45 cm) groups compared to the Week 5 (3.67 ± 0.82 cm) and Control (3.17 ± 0.29 cm) groups. The former two groups, both marked with superscript *a*, did not differ significantly from each other, while the latter two, marked with superscript *b*, formed a statistically distinct lower subset. A marked increase in root weight was observed in the Week 5 group (3.08 ± 0.86 g), which was significantly higher than all other groups (*a*). The Week 3 (1.23 ± 0.91 g), Week 4 (1.68 ± 1.00 g), and Control (1.03 ± 0.15 g) groups were statistically similar to one another, all falling within the lower subset (*b*). Total weight followed a pattern similar to that of root weight. The Week 5 group exhibited the highest mean TW (9.30 ± 1.01 g) and was significantly greater than the Week 3 group (5.10 ± 2.19 g), as reflected by the superscripts *a* and *b*, respectively. The Week 4 (6.35 ± 2.61 g) and Control (7.77 ± 1.50 g) groups were intermediate and did not differ significantly from either Week 3 or Week 5, as indicated by the superscript *ab*.

Table 3: Biochemical Characterization

Isolate	Gram Test	Catalase	Citrate	Indole	MR	VP	Suspected organism
RS1	+	+	+	–	+	–	*Streptococcus*
RS2	+	–	–	–	+	–	*Bacillus* sp.
RS3	+	+	–	–	+	–	*Bacillus* sp.
RS4	+	+	–	–	+	–	*Bacillus* sp.
RS5	+	+	–	–	+	–	*Bacillus* sp.
RS6	+	–	–	–	+	–	*Bacillus* sp.
RS7	+	+	+	–	+	–	*Bacillus* sp.
RS8	+	–	–	–	+	–	*Bacillus* sp.
RS9	+	+	+	–	+	–	Bacillus sp.
RS10	+	+	+	–	+	–	Bacillus sp.

The biochemical characterization in Table 3 reveals that all isolates were Gram-positive and uniformly methyl red (MR) positive indicating a consistent ability to produce stable acid end-products from glucose fermentation. The catalase test showed that most isolates (RS1, RS3–RS5, RS7, RS9, RS10) were catalase-positive, suggesting they can detoxify hydrogen peroxide, while RS2, RS6, and RS8 were catalase-negative. The citrate utilization test was positive only in RS1, RS7, RS9, and RS10, implying that these isolates can use citrate as a sole carbon source, whereas the rest cannot. The indole test was negative across all isolates, showing none of them produce tryptophanase. Similarly, the Voges-Proskauer (VP) test was uniformly negative, suggesting that none of the isolates use the butanediol fermentation pathway. Overall, the biochemical profiles indicate that while the isolates share some common traits (Gram positivity, MR positivity, indole and VP negativity), they differ in catalase and citrate utilization, which can be useful for differentiating them at the genus or species level.

The findings of this study demonstrated significant variation in the chemical properties of the soil samples, plant growth parameters and biochemical characteristics of the bacterial isolates. Soil pH(H_2O) ranged from 6.18 to 6.45, while pH ($CaCl_2$) ranged from 5.30 to 5.69, indicating slightly acidic to near-neutral conditions. The implication of this was that the soil was generally acidic in nature and shows least variability among measured properties (Rayyan *et al*., 2026) Such pH conditions can support the growth of many plants and soil microorganisms, while differences in soil pH can influence nutrient availability, microbial activity and nutrient cycling (Mosley *et al*., 2024). Electrical conductivity was highest in sample A (0.320 dS/m) and lowest in the control (0.070 dS/m), suggesting differences in soluble ion concentrations, although the relatively low EC values indicate that the soils were not strongly saline (Rieder *et al*., 2024). Sample C recorded the highest phosphorus (18.885 mg/kg), organic carbon (1.397%) and nitrogen (0.150%) contents, suggesting comparatively greater nutrient status and organic matter accumulation. Soil organic carbon is an important indicator of soil health because it contributes to nutrient cycling, microbial activity, aggregation and water retention, while phosphorus availability is influenced by soil pH and interactions with soil minerals (Jindo *et al*., 2024). Nitrogen and phosphorus are also essential for plant growth, and their availability can influence root development and nutrient-acquisition strategies (Zhao *et al*., 2024).

The growth results showed that treatment duration affected the measured plant growth parameters differently. Aerial length was highest at Week 3 (20.00 ± 2.61 cm) but declined to 16.83 ± 2.32 cm by Week 5,

whereas root weight increased from 1.23 ± 0.91 g at Week 3 to 3.08 ± 0.86 g at Week 5. Similarly, total weight increased from 5.10 ± 2.19 g at Week 3 to 9.30 ± 1.01 g at Week 5. The greater root and total biomass at Week 5, despite the lower aerial length, may indicate increased allocation of plant resources to below-ground development as treatment progressed. Root development is strongly influenced by nutrient availability and plant–microbe interactions (Zhao *et al*., 2024). Beneficial rhizobacteria can enhance root and shoot development through nutrient mobilization, phytohormone production, improved nutrient acquisition and other plant-growth-promoting mechanisms (Andrade *et al*., 2023). Therefore, the increased root biomass observed over time may partly reflect improved nutrient acquisition or stimulation of root development, although the present results alone cannot establish a direct causal relationship. The biochemical characterization revealed that all isolates were Gram-positive and methyl-red positive, while all were indole- and Voges-Proskauer-negative. Most isolates were catalase-positive, whereas RS2, RS6 and RS8 were catalase-negative, and citrate utilization was observed in RS1, RS7, RS9 and RS10. These differences demonstrate biochemical variability among the isolates and provide useful preliminary characteristics for differentiation. The predominance of Bacillus-like isolates is particularly relevant because Bacillus species are widely recognized as important plant-growth-promoting rhizobacteria capable of phosphate solubilization, nutrient mobilization, phytohormone production, siderophore production and biological control (Patani *et al*., 2024). Plant-growth-promoting rhizobacteria can also improve plant performance through several mechanisms, including nutrient acquisition and modulation of root development (Fasusi et al., 2023; Wijeysingha *et al*., 2023).

CONCLUSION

This study demonstrated the presence and diversity of plant growth-promoting rhizobacteria (PGPR) associated with the rhizosphere of cowpea (Vigna unguiculata). The physicochemical analysis revealed variations in soil properties, with Sample C showing comparatively higher organic carbon, nitrogen, and phosphorus contents, indicating better soil fertility. Several bacterial isolates were successfully obtained and characterized using morphological and biochemical tests. The isolates were predominantly Gram-positive and exhibited varying biochemical characteristics, with most identified as Bacillus species.

The study also showed variations in cowpea growth parameters over the treatment period, particularly in root and total biomass. Overall, the findings indicate that cowpea rhizosphere soils harbor bacterial isolates with characteristics that may contribute to plant growth and soil fertility. These isolates therefore have potential for development as biofertilizers and sustainable alternatives to chemical inputs, although further screening and field trials are required to confirm their effectiveness under different agricultural conditions.

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