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RESEARCH ARTICLE

Endophytic bacteria from *Lantana camara* and their effects on cowpea (*Vigna unguiculata*) germination and growth

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Abstract

Invasive weeds represent a promising but underexplored reservoir of beneficial endophytes. In this study, root-associated bacterial endophytes were isolated from *Lantana camara*, an aggressive invasive weed collected from a farm in Surat, South Gujarat, India. A total of 12 isolates were obtained and evaluated for key plant growth-promoting (PGP) traits, including phosphate and potassium solubilization, growth on nitrogen-free medium, siderophore and indole-3-acetic acid (IAA) production and hydrogen cyanide (HCN) production. Based on *in vitro* screening, selected isolates were assessed for their bioinoculant potential in *Vigna unguiculata* (L.) Walp. cv. Pant Grain Cowpea-14 (PGCP-14) under controlled conditions. Treatments included endophyte-inoculated seeds, uninoculated controls and seeds treated with either chemical fertilizer (CF) or farmyard manure (FYM). Among the isolates, strains LCR5 and LCR7 showed significantly enhanced germination (100 %) and superior performance across multiple growth parameters, including seedling vigor index, root and shoot length, plant height and biomass accumulation. Molecular identification using 16S rRNA gene sequencing confirmed LCR5 as *Paenibacillus graminis* (GenBank accession no. QR554129) and LCR7 as *Bacillus safensis* (GenBank accession no. OQ619180). The findings highlight the biofertilizer potential of these endophytic strains in promoting the growth of cowpea, a key leguminous crop cultivated in the Surat region.

Keywords: *Bacillus safensis*; bacterial endophytes; *Lantana camara*; *Paenibacillus graminis*; plant growth promotion

Introduction

Bacterial endophytes reside within the internal tissues of almost all plants around the globe. It has been reported that they benefit the host plant in diverse ways. They stimulate the growth of their host by enhancing nutrient uptake, producing a variety of compounds that help in the development and protection from biotic and abiotic stresses. Nonetheless, a holistic understanding of the mechanisms deployed by these plant growth-promoting bacteria (PGPB) has remained rather elusive, but it is understood that the bacterial endophytes employ different direct and indirect mechanisms to stimulate plant development (1, 2).

Although there have been numerous important studies conducted on endophytic bacteria, there is a lack of information about endophytes that inhabit weeds. Weeds existing in various ecosystems, grow and multiply aggressively withstanding harsh adverse environmental conditions. It might be possible that the endophytic population living within the weed host are reservoirs of beneficial bacteria with inimitable potentialities enabling the weed to survive and adapt in adverse conditions. (3). It is becoming more interesting for researchers to harness the potential of these interesting endophytic bacteria in crop production (4, 5). *Lantana camara* (*L. camara*) belonging to the family *Verbenaceae* is considered one of the most obnoxious weeds in the world that grows even in extremely harsh climatic conditions and has naturalized worldwide as an ornamental plant (6, 7). This invasive weed stands out because of its unique features of rapid and opportunistic spread and a high degree of resistance to any control strategies (8). The plant has a deep and penetrating root system with tremendous potential to survive unfavourable conditions (9). The current study was conducted to explore and identify the most promising PGPB endophytes residing within the roots of *L. camara*.

Materials and Methods

Collection of plant sample

Plants of *L. camara* growing as a weed were collected at the flowering stage during the summer season (March 2023) in Surat, Gujarat (Fig. 1). Its identity was authenticated by eminent taxonomist Dr. M H Parabia (Ex HOD, Dept. of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat). The plant materials were brought to the laboratory and washed carefully under running tap water to remove any adhering dirt and debris (10).

Fig. 1. *Lantana camara* at the collection site.

Isolation of endophytes

The roots were separated, cut into small pieces of about 2-3 cm in length, disinfected in 70 % ethanol for about 30 sec and then surface sterilized with 0.1 % $HgCl_2$ for 3 min. The tissue was then repeatedly rinsed about 10-12 times with sterile distilled water. To check the efficacy of the process of surface sterilization method, sterility test was performed. After the final wash, aliquots of water (0.1 mL) diluted with nutrient broth were transferred to a nutrient agar (NA) plate incubated at 28 ± 2 °C for 48 hr. No microbial growth after 48 hr of incubation ensures the effectiveness of the surface sterilization process and subsequent isolation procedures were carried out. The root tissues were aseptically macerated with homogenizers, serially diluted and plated in triplicate on NA plates (10). Isolates were selected based on variation in morphology, purified and maintained on NA slants and preserved at 4 °C for further investigations (11).

Morphological and biochemical characterization

The isolates were characterized morphologically and biochemically. Morphological characterization includes Gram staining, appearance of colour, elevation, size, shape, surface, margin, pigmentation and opacity. Biochemical characterization was conducted by the biochemical tests like utilizations of carbohydrates and organic acids using Methyl-Red (M-R) test, Voges-Proskauer (V-P) test, Citrate utilization test, urea hydrolysis test, nitrate reduction test, catalase test, triple sugar iron agar test was carried out (12).

In vitro screening of the isolates for their plant growth-promoting (PGP) traits

The obtained isolates were assessed for multiple PGP activities namely growth on nitrogen free media, phosphate and potassium solubilization, indole-3-acetic acid (IAA), siderophore and hydrogen cyanide (HCN) production.

Growth on nitrogen-free media

The isolates' ability to grow on nitrogen-free media was tested on semi-solid Rennie media. After autoclaving, filter-sterilized biotin and para-amino benzoic acid were added to final concentrations of 5 and 10 µg per litre. Plates were incubated at ambient temperature for 48 hr and observed for growth (12).

Phosphate solubilization

The screening for phosphate solubilization was carried out using spot inoculation technique on agar plate containing insoluble tricalcium phosphate (TCP) and incubated at a temperature of 28 ± 2 °C for 4-5 days and the zone of solubilization was observed (11).

The phosphate solubilization index (PSI) was calculated using the following formula:

PSI = Colony diameter + Zone of clearance/Colony diameter

Quantitative phosphate solubilization was measured using the method described by Lynn et al. with slight modifications (13).

Potassium solubilization

Qualitative evaluation of potassium solubilization was performed using Aleksandrow liquid medium. The composition of medium (g/L) was as follows: 5.0 glucose, 0.5 magnesium sulphate, 0.005 ferric chloride, 0.1 calcium carbonate, 2 calcium phosphate and 2 potassium alumino silicates minerals. The pH of the medium was adjusted to 7.2 by adding 1 N NaOH. The petri-plates were incubated at 28 ± 2 °C for 4-5 days and the zone of solubilization was observed (14).

The potassium solubilization index (KSI) was calculated using the following formula:

KSI = Colony diameter + Zone of clearance/Colony diameter

A spectrophotometric method was used for estimating potassium solubilization following method described by Rajawat et al. (15)

Siderophore production

Glass wares were immersed in 6 M hydrochloric acid overnight to eliminate any residual Fe contamination and then rinsed with double distilled water two to three times. The CAS (Chrome Azurol S) plate assay was employed to study siderophore production. After the solidification of the plates, bacterial isolates were spot inoculated on the CAS-blue agar and were incubated at 28 ± 2 °C for 4-5 days. The plates were then observed for the development of yellow-orange halo zone around the spot which indicates siderophore production (16).

Siderophore production by CAS liquid assay

To measure siderophores, the CAS (Chrome Azurol S) liquid assay method was employed. The CAS assay solution was mixed with 0.5 mL of 72 hr old cell free supernatant and 10 µL shuttle-solutions (sulfo-salicylic acid) were then added. After 15 min, the colour intensity of the solution was measured with UV-VIS spectrophotometer at 630 nm against reference. A decrease in blue colour as expressed in percent siderophore units (%SU) was seen because of siderophore synthesis (16).

Siderophore unit (SU) = Ar – As /Ar × 100 %

Where, Ar - the absorbance of the reference at 630 nm.

As - the absorbance of the sample (bacterial cultures) at 630 nm.

IAA production

Estimation of IAA production was carried out by inoculating 200 µL of bacterial suspension into 10 mL of Luria Bertani (LB) broth supplemented with L-tryptophan (100 µg/mL). The culture was incubated at 28 ± 2 °C for 48 hr. The IAA content in the culture supernatant was determined following a standard protocol, with experiments conducted on three separate dates to ensure reproducibility (17).

HCN production

For the detection of HCN production, nutrient broth was supplemented with 4.4 g/L of glycine and the isolated bacterial strain was streaked onto modified agar plates. Whatman filter paper No. 1, pre-soaked in a solution of 0.5 % picric acid and 2 % sodium carbonate (Na_2CO_3), was placed on the inner surface of the Petri dish lid. The Petri dish was then sealed with paraffin film to prevent gas escape. After incubating at 28 ± 2 °C for four days, the development of yellow to brown coloration on the filter paper indicated HCN production by the strain (10).

Seed germination and seedling growth

The *Vigna unguiculata* (*V. unguiculata*) (L.) Walp. cv. Pant Grain Cowpea-14 (PGCP-14) seeds were used to evaluate the PGP potential of the bacterial isolates. The bacterial strains were cultured in 250 mL conical flasks containing 200 mL of nutrient broth, incubated on an orbital shaker at 120 rpm for 72 hr at 28 ± 2°C. The cultures were then centrifuged at 15000 rpm for 1 min at 4 °C. The resulting pellets were washed twice with sterile distilled water, resuspended in 1 mL of sterile distilled water, vortexed and used for seed treatment. The cow pea seeds were surface sterilized in 5 % NaOCl for 1 min, followed by three thorough washes with sterile distilled water. The sterilized seeds were then dried on sterile blotting paper. The dried seeds were immersed in bacterial suspension (10^8 cfu/mL) for 1 hr before use (18, 19).

A total of 15 treatments (T1 to T15) were applied in the study, with each treatment conducted in four replicates. T1: Seeds soaked in distilled water (control), T2: Seeds treated with the recommended dose of chemical fertilizer (CF), T3: Seeds treated with the recommended dose of farmyard manure (FYM), T4 to T15: Seeds treated with bioinoculants from strains LCR1 to LCR12, respectively.

Following the treatments, seeds were incubated for 72 hr. Germinated seeds were counted and vital parameters including germination percentage, seedling vigor index I (SVI-I) and seedling vigor index II (SVI-II) were evaluated and calculated to assess plant growth performance (18, 19).

Germination percentage = Seeds germinated/Total seeds × 100

SVI-I = Germination percentage (%) × Seedling length (cm) [Seedling length = Shoot length + Root length].

SVI-II = Germination percentage (%) × Seedling dry weight (gm) [Seedling dry weight = Shoot dry weight + Root dry weight].

The germinated seeds were transplanted into a germination tray (5 × 5 cm diameter) filled with sterile soil. The trays were placed in a temperature-controlled growth chamber maintained at 28 ± 2 °C. Fifteen days after sowing (DAS), the plantlets were harvested and various growth parameters were measured, including shoot length, root length, total height, shoot and root fresh weight, shoot and root dry weight and the number of lateral roots (18, 20). Observations from all treatments were compared with one another and with the control.

Molecular characterization

Molecular characterization of the most efficient PGP endophytic bacterial isolates was performed based on 16S rRNA gene sequencing. The gene sequences were amplified using standard PCR techniques and the results were verified by running the amplified fragments on a 1 % agarose gel electrophoresis. The PCR amplicons were then purified using column purification to eliminate contaminants. The DNA sequencing of the purified PCR products was carried out using the primers 27F and 1391R with the BDT v3.1 Cycle Sequencing Kit on an ABI 3500xl Genetic Analyzer. The resulting sequences were identified by comparing them with the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic relationships were inferred using the Neighbour-Joining method (21). A bootstrap consensus tree was generated from 1000 replicates to represent the evolutionary history of the analysed taxa. Branches with less than 50 % bootstrap support were collapsed and bootstrap values (from 1000 replicates) were displayed next to the branches. Evolutionary distances were calculated using the Jukes-Cantor method, expressed as the number of base substitutions per site. This analysis included 11 nucleotide sequences and all ambiguous positions were removed using the pairwise deletion option. The final dataset comprised 1276 positions. Phylogenetic analysis of the sequences was conducted using Molecular Evolutionary Genetics Analysis Version 11 (MEGA-11) software (22). The sequences were submitted to the NCBI GenBank database and accession numbers were assigned.

Statistical analysis

The effects of potential endophytes used as bioinoculants were statistically analyzed using ASTATSA software. A one-way Analysis of Variance (ANOVA) was conducted, followed by multiple comparison tests, including Tukey HSD, Scheffé, Bonferroni and Holm methods, with a significance level of $p < 0.05$. The growth parameters of *V. unguiculata* treated with bioinoculants were statistically compared to the control to evaluate their effectiveness.

Results

Isolation, morphological and biochemical characterization of endophytic bacteria

A total of twelve endophytic bacterial isolates were successfully obtained from the roots of healthy *L. camara* plants. The isolates were morphologically characterized based on colony features and cell shape, while standard biochemical tests were performed to aid in preliminary identification. The morphological and biochemical profiles are summarized in Table 1 and 2, respectively.

In vitro screening of the isolates for their PGP activities

Growth on nitrogen-free medium: Five isolates-LCR5, LCR7, LCR9, LCR10 and LCR11-could grow on nitrogen-free Jensen's medium, indicating their potential to fix atmospheric nitrogen and contribute to plant nitrogen nutrition (Fig. 2).

Phosphate solubilization

Qualitative screening: Four isolates-LCR2, LCR5, LCR7 and LCR10-exhibited clear halo zones on Pikovskaya agar, suggesting their ability to solubilize inorganic phosphate (Table 3, Fig. 2).

Quantitative estimation: Phosphate solubilization by these isolates was further quantified spectrophotometrically at 830 nm using KH_2PO_4 as the standard. The solubilization potential varied among the isolates, with LCR5 and LCR7 showing comparatively higher phosphate solubilization efficiencies (Table 4).

Potassium solubilization

Qualitative screening: Seven isolates-LCR4, LCR5, LCR7, LCR9, LCR10, LCR11 and LCR12-formed halo zones on Aleksandrow agar, indicating the solubilization of insoluble potassium compounds (Table 3, Fig. 2).

Quantitative estimation: The potassium solubilizing activity of these isolates was measured spectrophotometrically at 620 nm using KCl as the standard. Isolates LCR5, LCR7 and LCR10 recorded higher potassium solubilization values (Table 4).

Siderophore production

Qualitative screening: All twelve isolates tested positive for siderophore production, as indicated by orange halo formation on Chrome Azurol S (CAS) agar plates (Table 3, Fig. 2).

Quantitative estimation: The type and amount of siderophores produced were further characterized and quantified. LCR5 and LCR7 were among the strongest siderophore producers (Table 4).

IAA production

IAA production was detected in five isolate-CR1, LCR5, LCR7, LCR10 and LCR12-through the development of a pink coloration upon reaction with Salkowski's reagent (Fig. 2). Optical density was measured at 530 nm and the concentration of IAA was calculated using a standard curve. LCR5 and LCR7 again showed the highest levels of IAA production (Table 4).

HCN production

HCN production was observed in four isolates-LCR1, LCR2, LCR5 and LCR7-through a color change in the filter paper saturated with picric acid solution. This trait may contribute to biocontrol potential against phytopathogens (Table 3, Fig. 2).

Seed germination and seedling growth of Cowpea (*V. unguiculata*)

The effect of endophytic bacterial inoculation on seed germination and seedling growth of *V. unguiculata* (L.) Walp. cv. PGCP-14 was evaluated under controlled conditions. Among all treatments, seeds inoculated with strains LCR5 (Treatment 8) and LCR7 (Treatment 10) achieved the highest germination rate (100 %), compared to 60 % in the uninoculated control (Treatment 1). These treatments also

Table 1. Morphological characteristics of the bacterial endophytic isolates

Isolate	Gram stain	Cell shape	Elevation	Surface	Odor	Consistency	Optical character
LCR1	+ve	Rod	Flat	Smooth	Moldy	Viscous	White
LCR2	+ve	Rod	Flat	Wrinkled	Earthy	Moist	White
LCR3	+ve	Rod	Raised	Smooth	Moldy	Moist	White
LCR4	–ve	Cocci	Flat	Smooth	Moldy	Viscous	White
LCR5	+ve	Rod	Raised	Smooth	Earthy	Moist	White
LCR6	+ve	Rod	Capitate	Wrinkled	Moldy	Viscous	White
LCR7	+ve	Rod	Raised	Wrinkled	Earthy	Viscous	White
LCR8	+ve	Rod	Flat	Wrinkled	Earthy	Moist	White
LCR9	+ve	Rod	Capitate	Smooth	Earthy	Moist	White
LCR10	–ve	Cocci	Flat	Wrinkled	Moldy	Viscous	White
LCR11	+ve	Rod	Capitate	Smooth	Earthy	Viscous	White
LCR12	+ve	Rod	Flat	Smooth	Moldy	Moist	White

"+": Positive; "-": Negative.

Table 2. Heat map analysis for biochemical characterization of the bacterial endophytic isolates

Isolate	MR test	VP test	Citrate utilization test	Urea hydrolysis test	TSI test	H_2S production	Catalase production	Nitrate reduction test	Ammonia production test	Indole production test
LCR1										
LCR2										
LCR3										
LCR4										
LCR5										
LCR6										
LCR7										
LCR8										
LCR9										
LCR10										
LCR11										
LCR12										

MR test: Methyl-Red; VP test: Voges-Proskauer test; TSI test: triple sugar iron agar test; H_2S production: Hydrogen sulfide production.

Positive: ; Negative: .

Table 3. Heat map analysis for in vitro screening of the isolates for their selected PGP activities

Isolate	Growth on nitrogen free media	Phosphate solubilization	Potassium solubilization	HCN production	Siderophore production	IAA production
LCR1						
LCR2						
LCR3						
LCR4						
LCR5						
LCR6						
LCR7						
LCR8						
LCR9						
LCR10						
LCR11						
LCR12						

HCN production: hydrogen cyanide production; IAA production: Indole acetic acid production.

Positive: ;Negative:

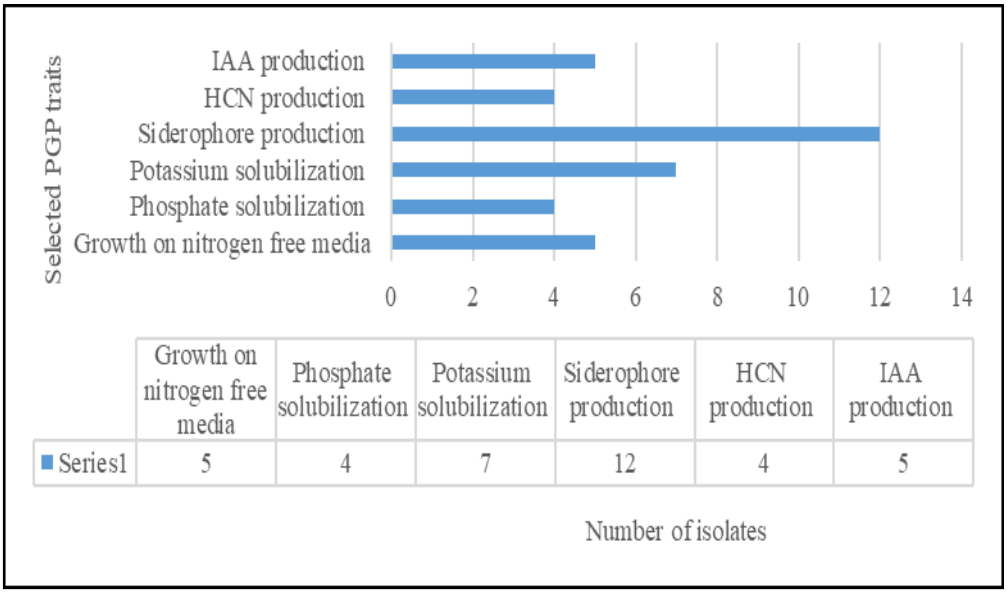

Fig. 2. Number of isolates showing positive results for the PGP traits.

Table 4. Quantitative estimation of the isolated endophytes for selected PGP traits

Isolate	Phosphate solubilization			Potassium solubilization			Siderophore production		IAA production
	Zone (cm)	PSI	Concentration (mg/mL)	KSI	Zone (cm)	Concentration (mg/mL)	% SU	Type	(mg/mL)
LCR1	NIL	NIL	NIL	NIL	NIL	NIL	63.403 ± 2.692	Catecholate	0.091 ± 0.003
LCR2	2.2	1.72	0.045 ± 0.034	NIL	NIL	NIL	49.413 ± 0.693	Hydroxamate	NIL
LCR3	NIL	NIL	NIL	NIL	NIL	0.028 ± 0.031	57.936 ± 1.556	Hydroxamate	NIL
LCR4	NIL	NIL	NIL	2.41	1.8	0.049 ± 0.024	64.866 ± 1.130	Catecholate	NIL
LCR5	3.2	2.33	0.156 ± 0.072	2.37	3.5	0.128 ± 0.022	41.853 ± 1.079	Catecholate	0.117 ± 0.002
LCR6	NIL	NIL	NIL	NIL	NIL	NIL	62.7 ± 2.421	Catecholate	NIL
LCR7	3.7	2.55	0.189 ± 0.062	2.89	3.7	0.184 ± 0.031	68.836 ± 0.577	Hydroxamate	0.151 ± 0.003
LCR8	NIL	NIL	NIL	NIL	NIL	NIL	37.196 ± 1.985	Hydroxamate	NIL
LCR9	NIL	NIL	NIL	1.93	1.7	0.023 ± 0.043	52.12 ± 1.281	Catecholate	NIL
LCR10	2.3	1.98	0.032 ± 0.013	2.56	2.5	0.173 ± 0.032	59.663 ± 1.550	Catecholate	0.109 ± 0.004
LCR11	NIL	NIL	NIL	2.46	3.3	0.169 ± 0.034	66.336 ± 1.075	Catecholate	NIL
LCR12	NIL	NIL	NIL	2.34	1.5	0.058 ± 0.052	47.736 ± 2.373	Hydroxamate	0.105 ± 0.003

significantly enhanced seedling growth parameters, including root length, shoot length, total plant height, fresh weight and dry weight (Fig. 3A and B). Notably, Seedling Vigor Index I (SVI I) increased by 158.1 % in LCR5 and 175.1 % in LCR7 treatments, while Seedling Vigor Index II (SVI II) rose by 127.7 % and 129.3 %, respectively, compared to the control (Fig. 4A and B). These results indicate that LCR5 and LCR7 are highly effective PGPB isolates, capable of significantly boosting early -stage growth in cowpea through improved germination and biomass accumulation.

Molecular identification based on BLAST analysis of 16S rRNA gene sequences confirmed the identity of the two most effective strains. Isolate LCR5 was identified as *Paenibacillus graminis* (*P. graminis*) strain NBRC100820 and LCR7 as *Bacillus safensis* (*B. safensis*) strain FO-36b. These sequences were submitted to NCBI and assigned GenBank accession numbers QR554129 (LCR5) and OQ619180 (LCR7), respectively.

The statistical analysis demonstrated that both bioinoculants had a significant impact on plant growth parameters compared to the control. However, no significant differences were detected between the individual treatments when compared to each other (Fig. 5).

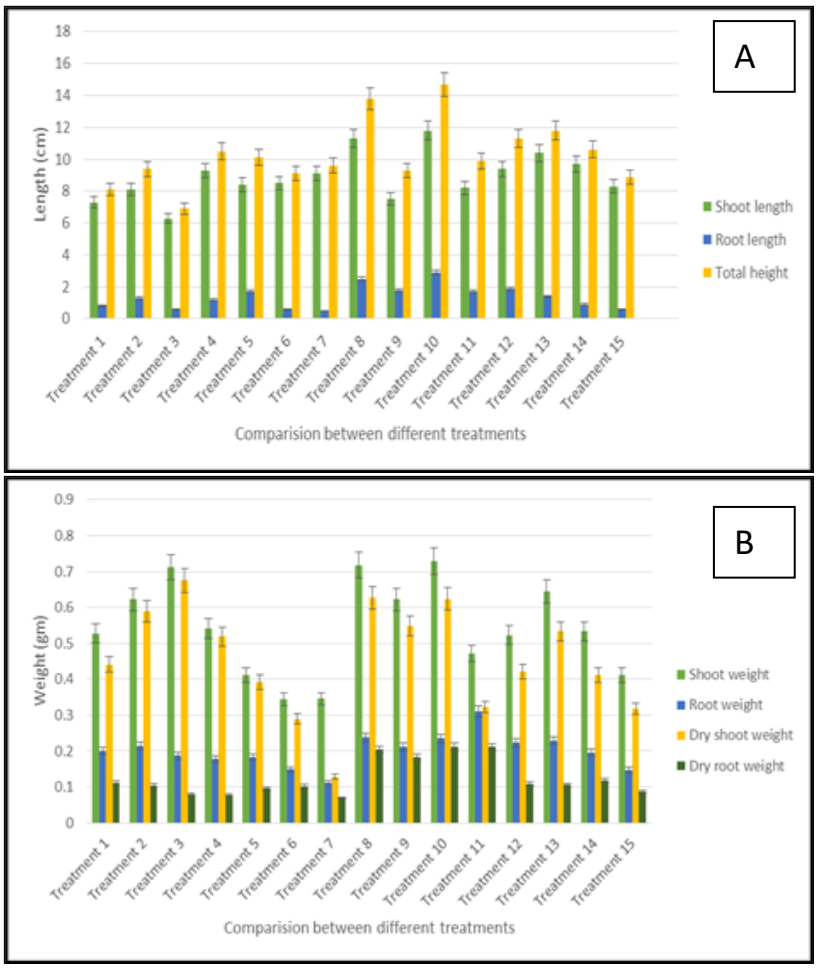

Fig. 3. (A) Effects of various treatments on the growth of root, shoot and total height of *V. unguiculata*. (B) Effects of various treatments on change in weight of *V. unguiculata*. Error bar indicated standard error (p value < 0.05).

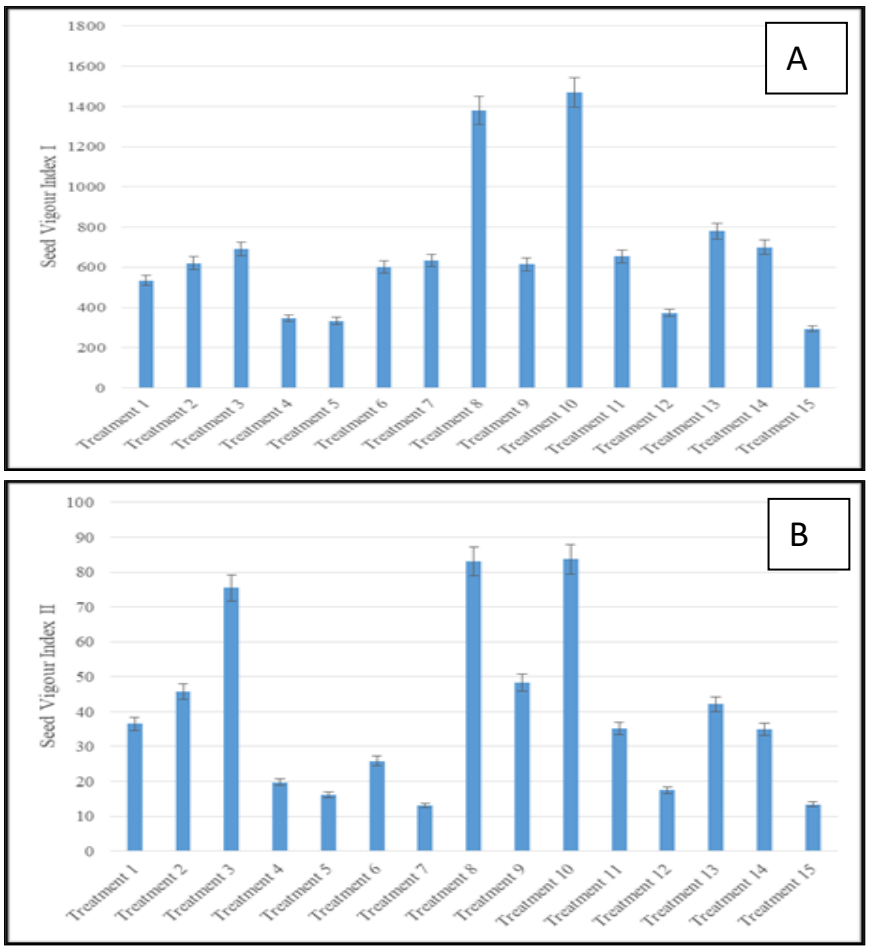

Fig. 4. (A) Seedling vigour index I; (B) Seedling vigour index II.

Tukey HSD results

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	3.6448	0.0444139	* p<0.05
A vs C	3.8241	0.0340390	* p<0.05
B vs C	0.1793	0.8999947	insignificant

Bonferroni and Holm results: only pairs relative to A simultaneously compared

treatments pair	Bonferroni and Holm *T*-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
A vs B	2.5773	0.0351348	insignificant	0.0175674	* p<0.05
A vs C	2.7040	0.0265809	* p<0.05	0.0265809	* p<0.05

Fig. 5. Effects of individual endophytes (LCR5 & LCR7) inoculation with control treatment comparison on *V. unguiculata* seeds. Parameter includes shoot length, root length, total height, shoot weight, root weight, dry shoot weight and dry root weight. Here A is control treatment, B is *P. graminis* LCR5 and C is *B. safensis* LCR7. Here A vs. B as well as A vs. C are showing significant where B vs. C are not significant.

Discussion

The present study was undertaken to isolate and characterize efficient PGPB endophytes from the roots of the invasive weed *L. camara*. Plant-associated bacteria, particularly endophytes, are well-documented for their ability to colonize internal plant tissues, including roots and promote plant growth through both direct mechanisms (e.g., nitrogen fixation, phytohormone production and nutrient solubilization) and indirect mechanisms (e.g., siderophore and HCN production) (23). While earlier studies have reported the presence of diverse endophytic bacteria in the stems, leaves and fruits of *L. camara*, there has been a notable lack of information regarding the endophytic communities associated with its root system (24). The current work fills this knowledge gap by successfully isolating twelve root-associated endophytic bacterial strains and evaluating their PGP potential through both qualitative and quantitative assays.

In the present study, twelve endophytic bacterial isolates were obtained from the roots of *L. camara*. *In vitro* screening for PGP attributes revealed that five isolates-LCR5, LCR7, LCR9, LCR10 and LCR11-could grow on Jensen's nitrogen-free medium, which selectively supports nitrogen-fixing microorganisms. The ability of these isolates to grow under nitrogen-limited conditions strongly indicates their potential for biological nitrogen fixation (25, 26). The capacity of root-associated nitrogen-fixing bacteria to inhabit non-leguminous hosts, particularly invasive species, has garnered increasing scientific interest. For instance, 131 nitrogen-fixing strains were isolated from the roots of the invasive plant *Ageratina adenophora*, highlighting the abundance and diversity of such bacteria in non-leguminous hosts. Likewise, endophytic bacteria from the roots of *Sorghum halepense*, another invasive species commonly found in nitrogen-deficient soils, have been shown to fix atmospheric nitrogen and promote host plant growth (27, 28). These findings, together with the current results, underscore the ecological significance and biotechnological potential of nitrogen-fixing endophytes in invasive weeds.

Phosphate solubilization is a vital trait of bacterial endophytes that contributes to improved plant growth by enhancing phosphorus availability in the rhizosphere. Although phosphorus is an essential macronutrient for plants, its bioavailability in soils is often limited due to its presence in insoluble mineral forms. Phosphate-solubilizing bacteria (PSBs) address this constraint by converting inorganic phosphate into soluble forms that can be readily absorbed by plants (29). In the present study, isolates LCR5 and LCR7 demonstrated the highest phosphate-solubilizing efficiency. Their ability to solubilize phosphate was initially indicated by the formation of clear halo zones around colonies in Pikovskaya's agar medium. Quantitative analysis in Pikovskaya's broth revealed that soluble phosphate concentration increased progressively during incubation, reaching a peak on the sixth day (30). Isolate LCR7 recorded the highest solubilization level, with a soluble phosphorus concentration of 0.189 ± 0.062 mg/mL, followed by LCR5 at 0.156 ± 0.072 mg/mL. These findings are consistent with earlier studies reporting high-efficiency PSBs from maize root endophytes, particularly in the PEEHM-5 hybrid variety, where soluble phosphorus concentrations continued to rise up to the seventh day of incubation (31, 32). Further supporting evidence from advanced analytical approaches, such as X-ray imaging, spectroscopy and proteomic analysis, has directly demonstrated the role of endophytic PSBs in enhancing phosphorus uptake in wild poplar (*Populus* spp.) (33). Collectively, these results highlight the multifaceted role of phosphate-solubilizing endophytes in promoting plant nutrient acquisition, root development and overall growth and productivity (34).

Interestingly, the present study revealed that several phosphate-solubilizing endophytic isolates also possessed the ability to solubilize potassium. A total of seven isolates formed distinct halo zones on Aleksandrow agar medium, indicating their capacity to mobilize insoluble potassium compounds and confirming their identity as potassium-solubilizing bacteria (KSBs) (15). Quantitative assessment of potassium solubilization demonstrated a steady increase in soluble potassium concentrations from the first to the sixth day of incubation, with peak activity recorded on day six. Among the isolates, LCR7 showed the highest solubilization efficiency, releasing 0.184 ± 0.031 mg/mL of soluble potassium, followed by LCR10 (0.173 ± 0.032 mg/mL) and LCR5 (0.128 ± 0.022 mg/mL). While previous studies have generally reported that bacterial endophytes exhibit comparatively lower potassium-

solubilizing activity than phosphate solubilization, the present findings challenge this notion, with a greater number of isolates demonstrating potassium-solubilizing potential than phosphate-solubilizing ability (32). These results suggest that endophytic bacteria from *L. camara* roots may harbour a unique and underexplored capacity for potassium mobilization, offering further promise for their application as multifunctional biofertilizers.

Bacterial endophytes enhance plant growth through the synthesis of various bioactive compounds, including phytohormones such as IAA and siderophores (35). IAA, a principal auxin, plays a pivotal role in regulating plant development by promoting cell division, elongation and differentiation (11). Numerous endophytic bacterial species have been reported to produce IAA in substantial quantities. Notable examples include *Burkholderia kururiensis* isolated from peanut roots, *Enterobacter hormaechei* and *Bacillus aryabhattai* from *Chrysopogon zizanioides* and *Pseudomonas stutzeri* and *Bacillus* sp. TA_AJ2 from saline desert environments (36-38). These IAA-producing bacteria complement the endogenous auxin pool of host plants, thereby functioning as effective bioinoculants that promote root and shoot development (39, 40). In the present study, five endophytic isolates were found to produce IAA, with concentrations ranging from 0.091 ± 0.003 mg/mL to 0.151 ± 0.003 mg/mL. Among these, isolate LCR7 exhibited the highest IAA production, followed by LCR5, which synthesized 0.117 ± 0.002 mg/mL. These results indicate the potential of these strains to stimulate plant growth through enhanced auxin biosynthesis, reinforcing their candidacy as promising biofertilizer agents.

Siderophores are another crucial class of PGP metabolites produced by bacterial endophytes. These low-molecular-weight, iron-chelating compounds facilitate iron acquisition under iron-limiting conditions, thereby enhancing plant growth and metabolic functions (29). Although siderophore production is recognized as beneficial, it has often been reported as a relatively rare trait among plant-associated bacterial endophytes, with earlier studies noting limited occurrences of siderophore-producing strains (41, 42).

Contrary to these observations, the present study revealed that all twelve endophytic isolates from *L. camara* roots exhibited siderophore production, marking a noteworthy and exceptional finding in the field of endophyte research. Quantitative estimation of siderophore activity, expressed as siderophore units (S.U.), ranged from 37.196 ± 1.985 % in isolate LCR8 to a maximum of 68.836 ± 0.577 % in isolate LCR7. Further characterization of siderophore types showed that seven isolates produced catecholate-type siderophores, while the remaining five synthesized hydroxamate-type siderophores. These results are consistent with previous reports of diverse siderophore classes being produced by endophytic bacteria (42). The widespread occurrence and diversity of siderophore production among the isolates underscore their potential utility in biofertilizer development, particularly for enhancing plant nutrition under micronutrient-deficient conditions.

Beyond their role in nutrient acquisition, siderophores also contribute to plant health by functioning as biocontrol agents. By sequestering iron from the rhizosphere, these compounds create iron-limiting conditions that inhibit the growth of iron-dependent phytopathogenic fungi and bacteria. This competitive exclusion benefits the host plant and contributes to disease suppression. The dual functionality of siderophore-producing endophytes-enhancing nutrient uptake and inhibiting pathogens-underscores their value in sustainable agriculture and integrated plant health management strategies (43-45). Similarly, HCN, a volatile secondary metabolite synthesized by certain endophytic bacteria, exhibits potent antimicrobial properties. HCN disrupts key metabolic processes in phytopathogens and nematodes, effectively suppressing their proliferation (29). Thus, the ability of endophytes to produce both siderophores and HCN highlights their multifunctional potential not only as biofertilizers but also as eco-friendly biocontrol agents. In the current study, four endophytic bacterial isolates demonstrated the ability to produce HCN, as indicated by a color change of the filter paper from yellow to brown. This qualitative observation confirms the potential of these isolates to synthesize HCN, a known secondary metabolite with antimicrobial activity. Both siderophore and HCN-producing bacterial endophytes are considered promising candidates for biocontrol applications, as they inhibit the growth of phytopathogens and pests through iron competition and metabolic disruption. Their dual functionality offers a sustainable and environmentally friendly approach to managing plant diseases and improving crop resilience (45).

The potential of the endophytic isolates as PGPB was evaluated using cowpea (*V. unguiculata*) seeds, a highly nutritious legume cultivated globally. Previous studies have underscored the beneficial effects of applying microbial inoculants to enhance the growth and productivity of cowpea, both under optimal and stress conditions (46). Among the isolates tested, LCR5 and LCR7 exhibited the most significant PGP effects. The enhanced SVI I and SVI II values indicate the potential of these isolates to significantly improve the overall health and growth of *V. unguiculata* seedlings, contributing to better plant establishment and growth under field conditions. These results corroborate earlier findings that endophytic bacteria not only promote seed germination but also improve overall seedling growth and development by enhancing nutrient availability, promoting hormonal balance and suppressing pathogens. Such enhancements are particularly valuable for crops like cowpea, where early-stage growth is essential for maximizing yield potential. These findings are in line with previous studies that have reported similar growth-promoting effects of endophytic bacteria in legume crops (46). The enhanced growth observed in this study suggests that these bacterial isolates have the potential to serve as effective bioinoculants, contributing to sustainable agricultural practices by reducing the reliance on CF and improving crop resilience under various environmental conditions.Molecular phylogenetic analysis, based on 16S ribosomal RNA sequencing, identified LCR5 as *P. graminis* strain NBRC100820 and LCR7 as *B. safensis* FO-36b. Notably, earlier studies have reported that the majority of bacterial endophytes isolated from *L. camara* belonged to the genus *Bacillus* (24). However, *Paenibacillus*, previously classified under *Bacillus*, has since been reclassified into a distinct genus based on 16S rRNA

sequence analysis (47). Several species from both *Paenibacillus* and *Bacillus* are well-established for their PGP properties and are widely recognized as prominent endophytic bacteria (48-50). For example, *B. safensis* R173 and *P. graminis* R200, isolated from the wheat rhizosphere, were shown to enhance the growth of soybean and wheat, which aligns with the findings of the current study involving *V. unguiculata* (cowpea). The results of this study are consistent with previous findings, demonstrating that bio-inoculation of *V. unguiculata* (cowpea) seeds with *P. graminis* LCR5 and *B. safensis* LCR7 significantly improved seed germination and enhanced seedling growth compared to the control and other treatments.

Conclusion

The present study demonstrates that *P. graminis* LCR5 and *B. safensis* LCR7, isolated from the roots of the invasive weed *L. camara*, possess significant PGP traits. These include nitrogen fixation, phosphate and potassium solubilization, production of IAA, siderophores and HCN, underscoring their multifaceted role in enhancing plant health and growth. When evaluated on *V. unguiculata* (cowpea), both isolates significantly improved seed germination and seedling development. Bio-inoculation with these strains resulted in marked increases in seedling vigor. Specifically, Treatment 8 and Treatment 10 recorded 157.99 % and 174.97 % increases in Seedling Vigor Index I (SVI I) and 127.7 % and 129.3 % increases in Seedling Vigor Index II (SVI II), respectively, compared to the control. These results strongly suggest that *P. graminis* LCR5 and *B. safensis* LCR7 can serve as promising bioinoculants for sustainable agriculture. Overall, these endophytic strains exhibit immense functional potential and merit further exploration for their application as eco-friendly biofertilizers and biostimulants in crop improvement strategies.

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Author's contributions

SZ collected samples, conducted experiments and compiled the data. SDG supervised the research, drafted the manuscript and performed the review.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Data availability

The generated sequences LCR5 and LCR7 in this study are available in the Gene Bank Database under the accession number QR554129 and OQ619180 respectively.

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