



Research article

Endophytic bacteria of *Ficus microcarpa* L.f. enhance rice root formation through phytohormone production

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Abstract

Importance of the work: Endophytic bacteria play a crucial role in promoting plant growth and resilience through the production of phytohormones, offering promising applications in sustainable agriculture.

Objectives: To isolate and characterize endophytic bacteria from the aerial roots of *Ficus microcarpa* L.f. and to evaluate their abilities to produce gibberellins (GAs) and indole-3-acetic acid (IAA), as well as their effects on the root development of rice seedlings.

Materials and Methods: In total, 28 bacterial strains were isolated from the aerial roots, screened for IAA and GA production and identified through molecular techniques. Three promising isolates (*Bacillus* sp. LV34, *Pantoea* sp. CM21 and *Rhizobium* sp. TN43) were selected for inoculation on rice seedlings, with root growth parameters assessed after 9 d.

Results: *Bacillus* sp. LV34 showed the highest IAA production ($427.17 \pm 16.59 \mu\text{g/mL}$), whereas *Rhizobium* sp. TN43 showed the highest GA production ($179.99 \pm 7.41 \mu\text{g/mL}$). Inoculation with *Bacillus* sp. LV34 significantly increased both the number of roots (7.5 roots per plant on average) and total root length (450 mm) in the rice seedlings, with *Rhizobium* sp. TN43 and *Pantoea* sp. CM21 also promoting root growth to varying degrees.

Main finding: The high phytohormone-producing endophytic bacteria isolated from *Ficus microcarpa* demonstrated notable potential to enhance plant growth, highlighting their applicability as biofertilizer candidates for sustainable crop management.

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Introduction

Ficus microcarpa L.f., commonly known as the Chinese banyan, is a tropical tree species renowned for its remarkable adaptability and unique aerial root system; this species can thrive in diverse environmental conditions, making it a vital component of many ecosystems (Chan et al., 2017). The aerial roots of *F. microcarpa* serve not only as structural supports but also as an interface for complex interactions with various microorganisms, including endophytic bacteria (Tuo et al., 2019). These bacteria inhabit the internal tissues of the plant and can have major effects on the host's health and growth. Among the various metabolites produced by these endophytic microorganisms, gibberellins (GAs) and indole-3-acetic acid (IAA) are of particular interest due to their essential roles in regulating plant growth and development (Egamberdieva, 2012).

GAs are a group of plant hormones that play crucial roles in growth processes such as seed germination, stem elongation, flowering and fruit development (Gray, 2004). They are involved in various physiological responses, allowing plants to adapt to changing environmental conditions (Colebrook et al., 2014). The discovery of GAs dates back to the early 20th century when they were identified as the causative agents of the "foolish seedling" disease in rice, highlighting their profound impact on cereal crops (Tai-ping, 2008). In more recent years, the potential of endophytic bacteria to synthesize GAs has garnered attention, as these bacteria can contribute to enhanced plant growth and stress resilience (Baliyan et al., 2022). On the other hand, IAA is the most prevalent auxin in plants and is critical for numerous growth processes, including cell elongation, root formation and apical dominance (Egamberdieva, 2012). The production of IAA by endophytic bacteria has important implications for plant health, as it can improve nutrient uptake, enhance root development and promote overall plant vigor (Duca et al., 2014; Kumar et al., 2020). Research has shown that certain bacterial strains can produce IAA from tryptophan, an amino acid that is abundant in root exudates (Naveed et al., 2015). This mechanism is especially relevant for crops like rice, where root architecture directly influences yield and stress tolerance (Belaouni et al., 2023).

The interactions between endophytic bacteria and their host plants are complex and multifaceted. For example, these bacteria can influence plant growth through direct mechanisms, such as hormone production and indirect mechanisms, including

the enhancement of nutrient availability and the suppression of pathogenic organisms (Trivedi et al., 2020). By producing phytohormones, such as GAs and IAA, endophytic bacteria can help plants better cope with biotic and abiotic stresses, thereby improving their overall resilience. To evaluate the agricultural potential of these bacteria, the current study focused on rice (*Oryza sativa* L.) as a model crop, given its global importance and sensitivity to phytohormone-mediated root development (Wang et al., 2018). The potential of these bacteria to serve as natural growth promoters has led to increased interest in their application in sustainable agriculture, where they may function as biofertilizers to enhance crop yields while reducing the need for chemical inputs (Prasad et al., 2020).

Understanding the role of endophytic bacteria in hormone production is more relevant than ever, given the increasing interest in sustainable agricultural practices. The ability of these microorganisms to enhance plant growth through natural processes can lead to innovative strategies for improving crop resilience and productivity (Afzal et al., 2019). Furthermore, the exploration of endophytic bacteria associated with *F. macrocarpa* could provide insights into the ecological dynamics of these communities and their potential applications in agricultural systems. The current study aimed to isolate and characterize endophytic bacterial strains from the aerial roots of *F. microcarpa*, evaluate their ability to produce GAs and IAA and assess their growth-promoting effects on rice seedlings. By identifying strains with strong hormone production capabilities, it was hoped to elucidate the potential of these bacteria as biofertilizers and their role in promoting plant health.

Materials and Methods

Sample collection

Aerial roots of *F. microcarpa* were collected from four distinct locations: Cho Moi (10°25'24.6"N;105°30'30.0"E) and Long Xuyên (10°22'10.1"N;105°25'27.4"E) in An Giang Province, Vietnam; Lap Vo (10°22'26.5"N;105°31'40.9"E) in Dong Thap Province, Vietnam; and Thot Not (10°16'19.8"N;105°31'07.0"E) in Can Tho City, Vietnam. At each sampling site, 10 root segments, each approximately 10 cm in length, were harvested from individual plants. The collected samples were placed immediately in sterile plastic bags and stored at 4°C until further processing.

Isolation of endophytic bacterial strains

The collected aerial root samples were washed thoroughly with sterile distilled water to eliminate surface contaminants. Subsequently, the roots underwent surface sterilization based on sequential immersion in 70% (volume per volume, v/v) ethanol for 30 s, followed by immersion in sodium hypochlorite (0.1% (v/v) available chlorine) for 1 min and a final immersion in 70% (v/v) ethanol for 30 s. After this process, the roots were rinsed three times in sterilized distilled water, with each wash lasting 1 min (Yu et al., 2016). The sterilized roots were ground in a sterile mortar with 5 mL of sterile distilled water and the resulting mixture was centrifuged at 10,000 revolutions per minute (rpm) at 4°C for 10 min. Then, the supernatant was diluted and plated on nutrient agar medium supplemented with 5% sucrose to isolate endophytic bacteria. The plates were incubated at 28°C for 48 hr. Catalase activity was determined by adding a drop of 3% (v/v) H₂O₂ onto fresh bacterial colonies and the immediate formation of bubbles was recorded as a positive reaction.

Screening for indole-3-acetic acid production

IAA production was measured according to Yu et al. (2016), with some modifications. Briefly, the isolated endophytic bacterial strains were cultured for 24 hr in 1 mL of nutrient broth (NB) medium. Following this, 100 µL of the bacterial inoculum was transferred into the same medium supplemented with 100 µg/mL of L-tryptophan (Sigma-Aldrich). The cultures were incubated in the dark at 30°C on a shaker set to 180 rpm for 10 d. After incubation, the bacterial cells were removed from the culture medium by centrifugation at 8,000 rpm for 10 min. Subsequently, 1 mL of culture supernatant was mixed with 2 mL of Salkowski reagent (composed of 49 mL of 35% HClO₄ and 1 mL of 0.5 M FeCl₃). The mixture was allowed to stand at room temperature for 35 min, after which the absorbance was measured at 530 nm. Uninoculated tryptophan-containing medium mixed with the Salkowski reagent served as a blank control. A standard curve was generated using serial dilutions of an IAA stock solution. The results are presented as mean ± standard error from three independent cultivations.

Screening for gibberellic acid production

GAs were assessed colorimetrically according to the standard method of Holbrook et al. (1961), with slight modifications (Baliyan et al., 2022). After 72 hr of incubation in NB, 15 mL of culture supernatant was collected and mixed

with 2 mL of zinc acetate reagent. Then, after 2 min, 2 mL of potassium ferrocyanide (10.6%) was added and the mixture was centrifuged at 2,000 rpm for 15 min. Next, the supernatant was treated with 5 mL of 30% HCl and incubated at 20°C for 75 min. Absorbance was measured at 254 nm and GA production was quantified using gibberellic acid as a standard.

Phylogenetic analysis of endophytic bacteria

The endophytic bacterial strains producing high levels of IAA and GAs were identified using 16S rRNA gene sequencing. The amplification of the 16S rRNA gene was performed using polymerase chain reaction (PCR) with the primers 27f (5'-AGAGTTTGTATCTGGCTCAG-3') and 1492r (5'-GGCTACCTTGTTACGACTT-3'). Subsequently, the PCR products were cloned and sequenced at Phu Sa Genomics Joint Stock Company (Vietnam). The sequencing results obtained were analyzed for genetic similarity using the EzBioCloud platform (Chalita et al., 2024) and the closest related type-strain sequences were used for phylogenetic analysis. A neighbor-joining phylogenetic tree was constructed using the MEGA7 software (Kumar et al., 2016), incorporating 1,000 bootstrap replicates to assess the robustness of the phylogenetic relationships (Yu et al., 2016).

Rice growth promoting experiment

Rice seeds (*Oryza sativa* L.) were surface-sterilized by soaking in 70% ethanol for 1 min, followed by treatment with 2% sodium hypochlorite for 10 min and rinsing five times with sterile distilled water. Then, the sterilized seeds were germinated on sterile moist filter paper in Petri dishes and incubated in a growth chamber at 28°C under a 16 hr light/8 hr darkness photoperiod. For each treatment, 10 uniformly germinated rice seedlings were used and the experiment was conducted with three independent replicates. Three endophytic bacterial isolates (LV34, CM21, TN43) were cultured in NB at 28°C for 24 hr. The bacterial suspensions were adjusted to an optical density (OD₆₀₀) of 0.5 before inoculation. Each replicate consisted of 10 seedlings grown in a sterile hydroponic container and germinated rice seedlings were transferred to sterile hydroponic containers and inoculated with 1 mL of bacterial suspension. The control group received sterile NB without bacteria. After 9 d of growth, seedlings were removed carefully from the hydroponic containers and their roots were rinsed with sterile distilled water. The number of roots per seedling and the total root length (measured in millimeters)

were measured using a ruler. Root length was recorded as the sum of all primary and lateral roots per seedling (Yu et al., 2016).

Statistical analysis

Data obtained from the IAA and GA quantification were analyzed using one-way analysis of variance (ANOVA). The results were expressed as mean $\pm$ SE values. Differences among bacterial strains in hormone production were considered significant at $p < 0.05$. *Post hoc* comparisons were performed using Duncan's multiple range test. All statistical analyses were conducted using the Centurion XVIII software (Statgraphics).

Results

Isolation of endophytic bacteria

In total, 28 distinct bacterial strains were isolated successfully from the aerial roots of *F. microcarpa*. Most of the isolated strains had positive catalase activity, while strain TN13 was catalase-negative, as summarized in Table 1. Each strain was identified based on colony morphology and subsequent molecular analysis. The diversity of the isolated strains reflected the rich endophytic microbial community residing within the aerial root system. As shown in Table 1, Gram-negative bacteria accounted for 64.29% (18 out of 28 strains) of the total isolates, while Gram-positive bacteria comprised 35.71% (10 out of 28 strains). Upon examining the cell morphology of the bacteria, it was observed that rod-shaped bacteria constituted 78.57% (22 out of 28 strains), whereas cocci represented 21.43% (6 out of 28 strains). Based on the results of the catalase test, detailed in Table 1, most of the isolated strains had positive results for catalase activity, evidencing the presence of the catalase enzyme in 96.43% of the isolates. However, one strain, TN13, tested negative for catalase, accounting for 3.57% of the total bacterial strains analyzed.

Indole-3-acetic acid and gibberellic acid production

The ability of the isolated strains to produce IAA and GAs was assessed qualitatively. As shown in Table 2, approximately 17.86% (5 out of 28 strains) produced IAA at concentrations exceeding 100 $\mu\text{g/mL}$. Strain LV34 had the highest IAA production at $427.17 \pm 16.59 \mu\text{g/mL}$ among all tested strains. Following LV34, strains CM21 and CM22 produced IAA concentrations of $323.86 \pm 6.18 \mu\text{g/mL}$ and $293.73 \pm 3.11 \mu\text{g/mL}$, respectively. Conversely, strain LX13 had the lowest IAA production at $4.15 \mu\text{g/mL}$ (Table 2).

Table 2 Production of indole-3-acetic acid (IAA) and gibberellins (GAs) by endophytic bacteria isolated from aerial roots of *F. microcarpa*

Bacterial isolate	Concentration ($\mu\text{g/mL}$)	
	IAA	GAs
LX11	$25.30 \pm 8.87^{\text{hi}}$	$25.83 \pm 9.67^{\text{h}}$
LX12	$27.80 \pm 1.02^{\text{h}}$	$38.07 \pm 10.73^{\text{gh}}$
LX13	$4.15 \pm 0.31^{\text{k}}$	$93.86 \pm 9.67^{\text{b-e}}$
LX14	$7.04 \pm 2.73^{\text{ik}}$	$80.61 \pm 10.79^{\text{c-f}}$
LX15	$6.42 \pm 1.40^{\text{ik}}$	$84.36 \pm 9.80^{\text{b-f}}$
LX16	$83.31 \pm 1.60^{\text{f}}$	$113.87 \pm 9.41^{\text{bc}}$
CM21	$323.86 \pm 6.18^{\text{b}}$	$75.65 \pm 8.17^{\text{d-f}}$
CM22	$293.73 \pm 3.11^{\text{c}}$	$87.72 \pm 9.73^{\text{b-f}}$
CM23	$4.98 \pm 3.35^{\text{k}}$	$41.77 \pm 34.86^{\text{gh}}$
CM24	$120.26 \pm 10.19^{\text{e}}$	$102.77 \pm 10.94^{\text{b-d}}$
CM25	$0.00 \pm 0.00^{\text{k}}$	$108.42 \pm 9.90^{\text{b-d}}$
LV31	$8.10 \pm 0.30^{\text{ik}}$	$26.97 \pm 8.42^{\text{h}}$
LV32	$225.91 \pm 22.22^{\text{d}}$	$102.38 \pm 11.77^{\text{b-d}}$
LV33	$0.00 \pm 0.00^{\text{k}}$	$96.43 \pm 9.54^{\text{b-e}}$
LV34	$427.17 \pm 16.59^{\text{a}}$	$114.83 \pm 9.06^{\text{b}}$
LV35	$94.44 \pm 2.04^{\text{f}}$	$85.14 \pm 17.33^{\text{b-f}}$
TN11	$4.17 \pm 0.36^{\text{k}}$	$65.83 \pm 13.11^{\text{c-g}}$
TN12	$9.47 \pm 0.33^{\text{ik}}$	$60.80 \pm 10.48^{\text{fg}}$
TN13	$0.00 \pm 0.00^{\text{k}}$	$97.36 \pm 11.64^{\text{b-e}}$
TN14	$10.94 \pm 0.32^{\text{ik}}$	$65.06 \pm 16.20^{\text{c-g}}$
TN15	$4.93 \pm 0.52^{\text{k}}$	$95.22 \pm 11.75^{\text{b-e}}$
TN21	$29.56 \pm 4.11^{\text{h}}$	$95.52 \pm 9.06^{\text{b-e}}$
TN31	$31.27 \pm 0.90^{\text{h}}$	$93.38 \pm 13.69^{\text{b-e}}$
TN32	$68.92 \pm 8.68^{\text{g}}$	$97.58 \pm 10.68^{\text{b-e}}$
TN41	$34.66 \pm 1.32^{\text{h}}$	$107.72 \pm 17.68^{\text{b-d}}$
TN42	$29.61 \pm 0.68^{\text{h}}$	$82.57 \pm 10.52^{\text{b-f}}$
TN43	$35.67 \pm 2.18^{\text{h}}$	$179.99 \pm 7.41^{\text{a}}$
TN44	$20.98 \pm 2.07^{\text{h-j}}$	$93.54 \pm 10.98^{\text{b-e}}$

^{a-k} = mean $\pm$ standard error values with different lowercase superscripts in each column are significantly different among bacterial isolates, based on Duncan's multiple range test at $p < 0.05$.

Table 1 Microbiological and biochemical characteristics of endophytic bacteria isolated from aerial roots of *F. microcarpa*

Microscopic observation		Catalase	Number of strains	ID ^a
Gram	Morphology			
Positive	Bacilli	+	7	LX12, LX13, LX15, LX16, CM22, LV34, TN31
Negative	Bacilli	+	15	LX11, LX14, CM21, CM24, CM25, LV31, LV33, TN11, TN12, TN14, TN15, TN21, TN32, TN42, TN43
Negative	Coccobacilli	+	3	LV32, TN41, TN44
Positive	Coccobacilli	+	2	CM23, LV35
Positive	Coccobacilli	-	1	TN13

^a ID = identification code of the bacterial isolates.

All 28 strains were capable of producing GAs, with concentrations varying significantly across strains. The GA production was in the range 25.83 ± 9.67 – 179.99 ± 7.41 $\mu\text{g/mL}$. Strain TN43 was identified as the highest GA producer, yielding 179.99 ± 7.41 $\mu\text{g/mL}$, which was significantly greater than all other strains. In total, 24 strains produced GAs at levels exceeding 50 $\mu\text{g/mL}$, accounting for 85.71% of the strains tested. Strain LX11 had the lowest GA production at 25.83 ± 9.67 $\mu\text{g/mL}$ (Table 2).

Molecular identification

Two strains with high IAA production (LV34 and CM21) and one strain with significant GA production (TN43) were identified through molecular techniques. Molecular identification of the most effective strains revealed that strain LV34 showed a 99.74% 16S rRNA gene sequence similarity to *Bacillus* species, strain TN43 showed a 100% similarity to *Rhizobium* species and strain CM21 showed a 99.65% similarity to *Pantoea* species, based on EzBioCloud identification (Chalita et al., 2024). Accordingly, the isolates were designated as *Bacillus* sp. LV34, *Rhizobium* sp. TN43 and *Pantoea* sp. CM21. Fig. 1 presents the phylogenetic tree constructed for *Bacillus* sp. LV34, *Rhizobium* sp. TN43 and *Pantoea* sp. CM21. The selected isolates had distinct phenotypic characteristics on nutrient agar after 48 hr at 28 °C. At 1,000 $\times$ magnification, *Pantoea* sp. CM21 formed creamy-white, smooth colonies with entire margins and consisted of short, Gram-negative rods. *Bacillus* sp. LV34 produced opaque, convex colonies with smooth surfaces and exhibited Gram-positive rod-shaped cells. *Rhizobium* sp. TN43 developed small, whitish colonies with smooth margins and contained short, Gram-negative rods. These features were consistent

with the molecular identification at the genus level. Overall, the molecular and phenotypic characterization confirmed the suitability of these isolates for further evaluation of their plant growth-promoting potential.

Effects of endophytic bacteria on rice growth

The effects of different endophytic bacterial isolates on rice growth were assessed by measuring the number of roots and total root length. The data, presented in Fig. 2, indicated that inoculation with endophytic bacteria significantly influenced root development compared to the control.

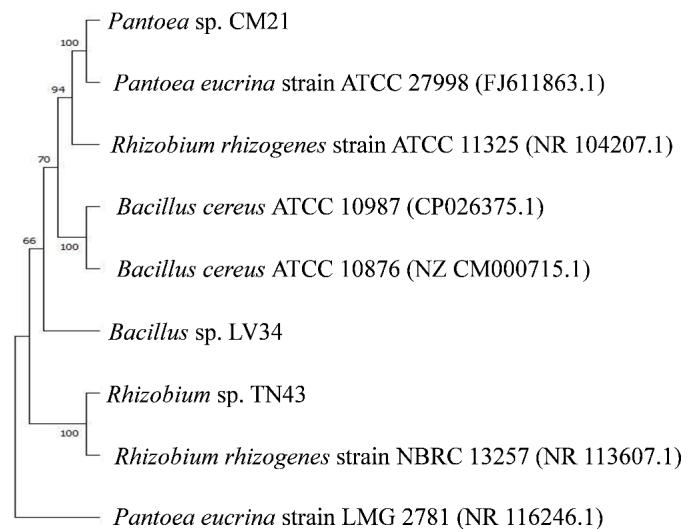


Fig. 1 Phylogenetic tree based on 16S rRNA gene sequences showing relationship of *Bacillus* sp. LV34, *Rhizobium* sp. TN43 and *Pantoea* sp. CM21 with related type strains retrieved from EzBioCloud (Chalita et al., 2024) and bootstrap values (1,000 replicates) shown only for nodes with support $\geq 50\%$

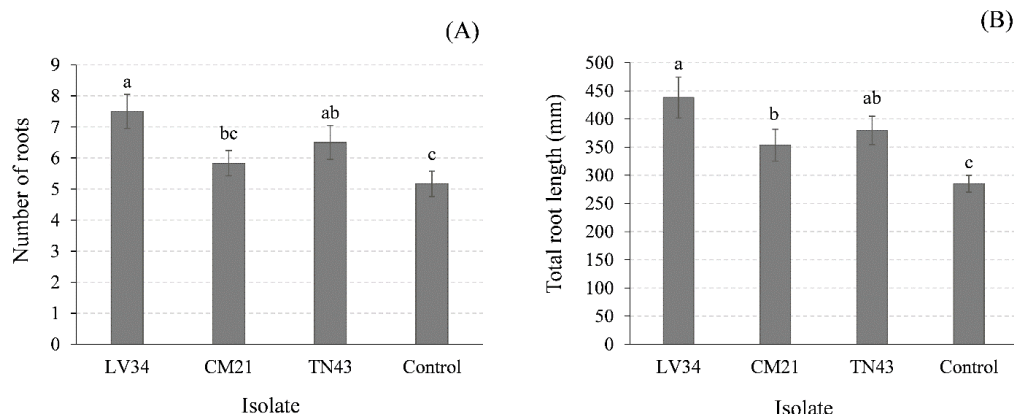


Fig. 2 Effects of endophytic bacterial isolates on root growth for rice seedlings inoculated with different bacterial isolates (*Bacillus* sp. LV34, *Rhizobium* sp. TN43, *Pantoea* sp. CM21) compared to the control: (A) number of roots per plant; (B) total root length (mm), where error bars = $\pm$ SD and different letters above the bars indicate statistically significant differences between treatments based on Duncan's multiple range test at $p < 0.05$

Fig. 2A shows that the *Bacillus* sp. LV34 isolate produced the highest number of roots, with an average of approximately 7.5 roots per plant, followed by slightly lower numbers of roots by *Rhizobium* sp. TN43 and *Pantoea* sp. CM21, respectively. The control group had the lowest number of roots (around 5.5), indicating that bacterial inoculation generally enhanced root proliferation. Fig. 2B illustrates the total root length measurements, revealing that plants treated with *Bacillus* sp. LV34 had the longest roots, reaching nearly 450 mm. In addition, plants inoculated with *Rhizobium* sp. TN43 had substantial root elongation, whereas *Pantoea* sp. CM21 resulted in moderately shorter roots. The control group had the shortest root length (approximately 300 mm). Representative root images are provided in Supplementary Fig. S1 and were consistent with the quantitative trends in root number and total root length.

Based on these results, endophytic bacteria positively influenced rice root growth by promoting both root number and root length. *Bacillus* sp. LV34 isolate had the most substantial effect, significantly increasing root proliferation and elongation. These effects may have been mediated through mechanisms such as phytohormone production, nitrogen fixation, or improved nutrient uptake. The current study did not evaluate additional plant growth potential traits (such as phosphate solubilization, siderophore production or N₂ fixation); therefore, the specific mechanisms remain to be determined. The variation among isolates indicated that different bacterial strains may have distinct modes of action. For example, *Rhizobium* sp. TN43 also significantly increased root growth, suggesting a strong growth-promoting ability, while *Pantoea* sp. CM21 exhibited a moderate effect. The control group's limited root growth highlighted the natural constraints on root development in the absence of beneficial microbial interactions.

Discussion

The diversity of endophytic bacteria isolated from the aerial roots of *F. microcarpa* reflected a rich microbial community that plays an important role in plant health and growth. In the current study, 28 distinct bacterial strains were identified, with a predominance of Gram-negative bacteria, which was consistent with findings from other studies on various plant species. For example, research on the endophytic communities of medicinal plants reported high diversity, often dominated by genera such as *Bacillus* and *Pantoea* (Khaskheli et al., 2020;

Castro et al., 2014). Comparatively, studies focusing on tree species, such as *Mangifera indica* and *Eucalyptus* spp., have documented varying levels of endophytic diversity, often influenced by environmental factors and the specific plant's ecological niche (Kumar et al., 2021; Wang et al., 2022). While the reported isolated strains from *F. microcarpa* include well-known plant growth-promoting bacteria, such as *B. cereus* (Kulkova et al., 2023), the presence of other genera, such as *Rhizobium* and *Pantoea*, highlights the potential for these endophytes to contribute to nutrient cycling and stress resilience. Further exploration of these microbial communities could lead to the discovery of novel strains with beneficial traits.

The ability of the endophytic bacteria isolated from the aerial roots of *F. microcarpa* to produce IAA highlights their potential as natural growth promoters. In the current study, *Bacillus* sp. LV34 was identified as a notable IAA producer (427.17 ± 16.59 µg/mL), which was higher than the findings from other studies. For example, endophytic bacteria from the root of *Chrysopogon zizanioides* (L.) had significant IAA production, with levels reaching 246 µg/mL (Khianngam et al., 2023). This consistency across diverse plant species suggests that endophytic bacteria possess inherent capabilities to synthesize IAA, likely due to the presence of tryptophan in root exudates. The high IAA production observed in the strains from *F. microcarpa* is particularly noteworthy as it may enhance root development and overall plant vigor, contributing to the plant's adaptability in varying environmental conditions. Furthermore, the correlation between IAA production and the presence of beneficial traits, such as increased nutrient uptake and stress tolerance, underscores the ecological importance of these bacteria (Duca et al., 2014).

The GA-production ability of endophytic bacteria is an important aspect of their potential role in enhancing plant growth. In the current study, *Rhizobium* sp. TN43 was able to synthesize GAs, which are crucial for various plant developmental processes, including seed germination, stem elongation and flowering (Roopa et al., 2023). In addition, many studies have reported on the ability to produce GAs from endophytic bacteria originating from different plants (Ambawade and Pathade, 2013; Shahzad et al., 2016). The production of GAs by these endophytic bacteria highlights their potential to have a positive influence on their host plant's physiological responses, particularly in adapting to environmental stresses (Halo et al., 2015). The presence of GA-producing bacteria in the aerial roots of *F. microcarpa* indicates their potential role in promoting the growth and resilience of this species in various conditions.

Endophytic bacteria have been known to enhance plant growth through multiple mechanisms, including the production of auxins (e.g., IAA), solubilization of phosphorus and improvement of water uptake efficiency (Yu et al., 2016). The significant increase in root number and total root length observed in this study further supports the hypothesis that these bacteria facilitate root system expansion, which is critical for enhancing plant resilience to abiotic stresses such as drought, salinity and nutrient deficiency. A more extensive root system not only improves the plant's capacity to absorb water and essential nutrients like nitrogen, phosphorus and potassium but also strengthens its anchorage in the soil, reducing susceptibility to lodging (Shah et al., 2019).

Moreover, the production of IAA and GAs by strains such as *Bacillus* sp. LV34 and *Rhizobium* sp. TN43 may contribute to modulating root architecture by promoting lateral root formation and root hair development, thereby increasing the rhizosphere's surface area for microbial interactions and nutrient exchange. In this study, the evaluation was limited to root number and total root length and root fresh and dry weights were not measured; therefore, the observed effects primarily reflect changes in root architecture rather than overall root biomass or root health. In addition, phytohormone production quantified *in vitro* may not reflect phytohormone production under plant-associated conditions (*in planta*). This hormonal regulation may also enhance the plant's ability to tolerate biotic stresses by stimulating systemic resistance against pathogens (Yang et al., 2013). For instance, a well-developed root system can physically hinder pathogen invasion while supporting beneficial microbial communities that outcompete harmful microbes (Mendes et al., 2013). Importantly, high IAA/GA production is not exclusively associated with beneficial bacteria because some pathogenic strains can also exploit phytohormones during disease development; therefore, additional functional characterization and plant-safety assessments are required. In addition, re-isolation of the inoculated bacterial strains from rice tissues was not performed in the present study; therefore, direct evidence of bacterial colonization and interaction with the host plant could not be confirmed.

The endophytic bacteria isolated from *F. microcarpa* present promising potential applications in sustainable agriculture. Their ability to produce phytohormones, such as IAA and GAs, suggests they can enhance plant growth, improve nutrient uptake and bolster stress resilience (Sharma et al., 2023). By harnessing these beneficial microorganisms, farmers could develop biofertilizers that reduce reliance on chemical inputs,

thereby promoting environmentally friendly practices (Ahmed et al., 2024). The potential of *B. cereus* and *Rhizobium* sp. as a biofertilizer has been well established elsewhere (Hapsoh et al., 2022, Mendoza-Arroyo et al., 2020). Additionally, the diverse strains identified in the current study may contribute to soil health by enhancing microbial diversity and nutrient cycling (Ding et al., 2024). Given the adaptability of *F. microcarpa* in various environments, the application of its associated endophytes could be effective, particularly in marginal or degraded soils. Nevertheless, the underlying biochemical and genetic mechanisms driving these plant-microbe interactions remain to be fully elucidated. Future studies should include re-isolation and re-identification of the inoculated strains from rice plants to verify successful colonization and their direct involvement in the observed growth-promoting effects, together with the evaluation of additional plant growth potential traits (such as phosphate solubilization, siderophore production and N₂ fixation) and the quantification of phytohormone production under plant-associated conditions.

Conclusion

The significant potential was demonstrated of endophytic bacteria isolated from the aerial roots of *F. microcarpa* to produce GAs and IAA, key phytohormones that enhance plant growth and resilience. The isolation of diverse bacterial strains, notably *Bacillus* sp. LV34 and *Rhizobium* sp. TN43, highlights the rich microbial community associated with this adaptable species. The inoculation of rice seedlings with selected bacterial isolates resulted in a significant enhancement of root growth. *Bacillus* sp. LV34 produced the highest impact, increasing both root number and total root length, while *Rhizobium* sp. TN43 and *Pantoea* sp. CM21 had moderate but significant effects compared to the control. However, the observed growth-promoting effects cannot be attributed solely to IAA/GA production, as other plant growth-promoting traits were not evaluated; furthermore, phytohormone production measured *in vitro* may not reflect plant-associated conditions. Therefore, these isolates should be considered as promising candidates with potential plant growth potential activity rather than confirmed biofertilizers. Further functional characterization, including additional plant growth potential traits and plant-safety assessments, is required before practical application.

Conflict of interest

The authors declare that there are no conflicts of interest.

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