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Endophytic Luteibacter yeojuensis strains stimulate banana plant growth

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Abstract: This study isolated endophytic bacteria from tissue-cultured banana ultivars Grand Nain and Saba. The bacteria were further characterized and identified through morphological, cultural, and molecular analyses. The bacteria had morphological and colony characteristics resembling those of Luteibacter species. Colonies were white to yellow, round, and slightly raised, with the entire margin in nutrient agar medium. The bacterial isolates were Gram-negative based on the potassium hydroxide test (KOH) test. Phylogenetic analysis of the 16S ribosomal gene region grouped the three isolates in the Luteibacter yeojuensis clade. The three Luteibacter yeojuensis isolates were not pathogenic to banana 'Grand Nain,' 'Lakatan,' and 'Saba' in both wounded and unwounded assays conducted in controlled assays. No stunting, wilting, and corm tissue browning were observed 14 days post-inoculation when the bacteria were inoculated on tissue-cultured plants; two of the three isolates significantly increased plant height of cv. Lakatan (p<0.05) and one isolate, L. yeojuensis GN11-20, enhanced shoot proliferation in cv. Grand Nain. The study reports L. yeojuensis as an endophytic bacterium with growth-promoting activity in tissue-cultured banana plants. The endosymbiotic association of L. yeojuensis in bananas could enhance plant growth and resistance to banana diseases.

1. Introduction

Banana (*Musa* spp.) is a high-value fruit and cash crop widely grown as one of the staple foods in Asia (Rossman *et al.*, 2012). The Philippines remains the top producer of bananas in Asia and the second largest exporter globally, following Ecuador (FAO, 2023). There are three major varieties of banana produced in the country: Cavendish (51%), Saba (29%), and Lakatan (10%) (Anzures *et al.*, 2022). Data from the Philippine Statistics Authority (2023) show that the Davao region is the top producer of bananas, with 868.19 thousand metric tons (mt) or 37.4% of the total banana production in the last quarter of 2023. This was followed by Northern Mindanao with 431.86 thousand mt (19%) and Soccsksargen Region with 279.20 thousand mt (12.3%) shares in production, respectively (Philippine Statistics Authority, 2023).

Cultivated banana genotypes are triploids that are generally sterile and seedless (Uma, 2021). Thus, conventional production of banana planting materials is made through sword suckers. However, these vegetative materials may also harbour plant pathogens. They may subsequently result in the carry-over of diseases in succeeding planting materials; hence, plant tissue culture is a widely practiced method as an alternative for banana production. Aside from preventing the carry-over of diseases, the technology allows the plants to aseptic production with uniform genotypes in a relatively shorter period (Souza et al., 2000). The technology only requires a small portion (1-3 mm²) of the plant parts (meristems) for in-vitro mass production and germplasm conservation (Agbadje et al., 2021); this allowed bananas rapid propagation from a single corm with favourable pests and pathogen-free genotypes (Agbadje et al., 2021). However, contamination with microorganisms that may act as endophytes, saprophytes, or asymptomatic pathogens is commonly observed (Cassells, 1991). Contamination may emanate from the plant's plant tissues (endophytes) and surfaces (Nair and Padmavathy, 2014). Proper growth media and explant sterilizations can easily control microbes on the explant's surface and those carried from the environment (Sivanesan et al., 2021). In contrast, endophytes are challenging to control because they are inside the plant tissue and are tolerant to surface sterilization (Hardoim et al., 2015).

Endophytes like bacteria, fungi, and actinomycetes can colonize healthy living tissues and establish a symbiotic relationship with the host plant (Nair and Padmavathy, 2014). Host plants benefit from endophytes through plant growth promotion (Afzal et al., 2019), pathogen and insect attack defence (Sturz and Matheson, 1996; Azevedo et al., 2000; Pieterse et al., 2014; Martínez-Hidalgo et al., 2015; Oukala et al., 2021), and increased tolerance to abiotic factors including salinity (Ali et al., 2014), low temperature (Subramanian et al., 2015), and heavy metals (Rajkumar et al., 2009). Bacterial endophytes of bananas are known plant growth promoters and biocontrol agents. For instance, several bacterial endophytes from diverse communities form an antagonistic relationship against Fusarium oxysporum f.sp. cubense (Foc) (Jie et al., 2009). Plant growth promotion in banana cv. Prata Ana has also been demonstrated in shoot tip cultures colonized with endophytic Klebsiella pneumoniae (Fernandes et al.,

2013). Thus, the utilization of endophytes could improve the banana production system. However, any new endophytes found from a plant must undergo pathogenicity testing to ensure that they do not cause infection to the host once removed from their natural system (within the host).

This study identified and characterized an endophytic bacterium isolated from healthy tissuecultured banana 'Grand Nain' and 'Saba.' We hypothesize that endophytes from healthy banana plants do not harm host plants but function as plant growth promoters. Thus, these endophytes may be used directly or indirectly as potential bioinoculants under a green and sustainable agriculture production system.

2. Materials and Methods

Isolation and storage of the bacterial contaminants

White-to-yellow pigmented bacteria were observed in multiple shoot cultures of tissue-cultured banana cultivars Grand Nain and Saba. A loopful of bacterial cells growing from the stems was transferred onto a nutrient agar (NA) medium. Plates were stored at room temperature (28-30°C) for two days (with 14 hours of light in 24 hours cycle) (Cruz and Balendres, 2021). The bacterium was then purified and further characterized (see succeeding section). A loopful of the bacterium from a 48-hourold culture was transferred to a fresh NA plate and incubated using the abovementioned conditions. Cultures were stored in microcentrifuge tubes containing 1 mL of sterile distilled water. The cultures were deposited at the Bacteria Repository of the Institute of Plant Breeding, Agriculture and Food Science College, University of the Philippines Los Baños, Laguna, Philippines.

Morphocultural characterization and PCR assay

The bacterial morphology of 48 to 72-hour-old cultures was assessed under a light microscope (Olympus CX23, Japan), and the colony characteristics were recorded. The bacterial genomic DNA was extracted using Chen and Kuo's procedure (Chen and Kuo, 1993) for molecular analyses. The isolated genomic DNA was standardized to 30 ng/µL and was subsequently used as a template for the succeeding polymerase chain reaction (PCR) assay, which amplifies the 16S ribosomal gene region. The PCR assay was performed in MyCycler[™] Thermal

Cycler (Bio-Rad, USA) in a 15-uL reaction volume (Cruz and Balendres, 2021). The PCR cocktail mix consisted of 1x PCR Buffer (Invitrogen), 2.0 mM MgCl2 (Invitrogen), 0.2 mM dNTPs (Invitrogen), 0.2 (27F. 5'μM each of the forward AGAGTTTGATCCTGGCTCAG-3') and reverse (1492R, 5'-GGTTACCTTGTTACGACTT-3') primers (Lane, 1991), one U Tag DNA Polymerase (Invitrogen), one µL of the bacterial genomic DNA, and DEPC-water to volume. The thermal cycling conditions were as follows: initial denaturation at 95°C for two min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for two min, and final extension at 72°C for seven min. The PCR products were resolved by gel electrophoresis in 1.0% Agarose (Vivantis) and 0.5X Tris-Acetate-EDTA (TAE) buffer and were sent to

Apical Scientific Sdn. Bhd. (Malaysia) for DNA sequencing.

Molecular characterization and phylogenetic analysis

A consensus DNA sequence was derived from the resultant forward and reverse sequences using Geneious software. Sequence similarity analysis was performed in the NCBI BLASTN program (Zhang and Madden, 1997). Sequences were analysed based on the highest percent similarity, e-value, and query cover. The authentic 16S rDNA sequences of five species of Luteibacter available in Genbank (Table 1) were compared with the consensus sequences of the three *Luteibacter yeojuensis* SbM36C, GN11-20, SabaM36A isolates obtained from this study. The phylogenetic distance of the three bacterial isolates to eight *Luteibacter yeojuensis* isolates from other

Table 1 - Luteibacter species with the closest similarity to the 16S rDNA region of the bacterium were isolated in this study

Species	Strain	Source	Locality	16S Genbank Accession	Reference	
Luteibacter yeojuensis	IHB B 6856	Aquilaria agallocha	India	KF668474.1	NCBI GenBank	
Luteibacter jiangsuensis	JW-64-1	Soil	China	NR_132709.1	Wang <i>et al.</i> (2011)	
Luteibacter anthropi	CCUG 25036	Human blood sample	Sweden	NR_116911.1	Kampfer <i>et al.</i> (2009)	
Luteibacter rhizovicinus	LJ96	Hordeum vulgare	Denmark	NR_042197.1	Johansen et al. (2005)	
Luteibacter pinisoli	MAH-14	Soil	South Korea	KY964279.1	Huq and Akter (2017)	
Burkholderia vietnaminensis	LMG 10929	Oryza sativa	Vietnam	NR_041720.1	LiPuma <i>et al</i> . (1999)	

IHB= Institute of Himalayan Bioresource Technology, Post Box No. 6, Palampur, Himachal Pradesh 176061, India;

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MAH= Food and Nutrition, Chung-Ang University, 4726, Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi-do 17546, South Korea; LMG= Ghent University, Belgium.

Table 2 - Luteibacter yeojuensis strains from other countries compared with the strains isolated in this study

Species	Strain	Source	Locality	16S Genbank Accession	Reference
Luteibacter yeojuensis	IHB B 6856	Aquilaria agallocha	India	KF668474.1	NCBI GenBank
Luteibacter yeojuensis	T-79	Curcuma longa	India	KM589043.1	Kandan <i>et al.</i> (2014)
Luteibacter yeojuensis	NBRC 106387	Not Available	Japan	AB682403.1	Nakagawa et al. (2011)
Luteibacter yeojuensis	HBU 72524	Not Available	China	MW365223.1	Lv (2020)
Luteibacter yeojuensis	R2A16-10	Soil	Korea	NR_043618	Kim <i>et al</i> . (2006)
Luteibacter yeojuensis	RT27	Oryza sativa	China	MK014251.1	NCBI GenBank
Luteibacter yeojuensis	OsEnb_ALM_B9	Oryza sativa	India	MN889326.1	Kumar <i>et al.</i> (2020)
Luteibacter yeojuensis	Z51	Rock	China	KM019785.1	Zhang (2014)
Burkholderia vietnaminensis	LMG 10929	Oryza sativa	Vietnam	NR_041720.1	LiPuma <i>et al.</i> (1999)

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countries (Table 2) was further determined. Burkholderia vietnaminensis (LMG 10929) was used as the outgroup in all analyses (LiPuma *et al.* 1999). The generated 16S rDNA sequences were aligned using CLUSTALW in MEGA X software (Kumar *et al.* 2018). The phylogenetic trees were constructed using the Tamura Nei model (Tamura and Nei 1993) with uniformly distributed rates and 1,000 bootstrap replicates.

Gram test using KOH

A potassium hydroxide (KOH) test was performed following Buck's procedure (Buck, 1982) to identify the gram reaction of the bacterial isolates. Therefore, a micropipette placed 10 μ L of 3% KOH on the top of a clean microscope slide. A loopful of bacteria from a forty-eight to 72-hour-old was then transferred to the drop of KOH using a sterile wire loop. The mixture was constantly stirred for 15 seconds and observed for viscosity and formation of mucoidal string. Gram-negative bacteria turn vicious and form a mucoidal string. In contrast, Gram-positive bacteria do not turn viscous with no formation of mucoidal string. Three replicates were used for each isolate.

In-vitro pathogenicity testing and morphometric assessment

The pathogenicity of the bacterial isolates in tissue-cultured banana plantlets was assessed in vitro using a pseudostem injection assay; the four-weekold banana 'Lakatan' (AA), 'Grand Nain' (AAA), and 'Saba' (ABB) were surface-disinfected using 70% ethanol and air-dried. Pseudostems were cut and inoculated by injecting 200 µL of bacterial suspension (0.5, OD₆₀₀) in wounded and unwounded tissues. The pseudostems were injected with sterile distilled water (SDW) for the control treatment. Treated plantlets were maintained in the plant regeneration medium (basal Murashige and Skoog, 3 mg·l⁻¹ 6benzylaminopurine, 3% sucrose, 7 g·l⁻¹ agar, pH 5.7) and exposed to 14-hour fluorescent light cycle at 20±5°C temperature (Murashige and Skoog, 1962). Four replicate plantlets were used for each isolate and variety. Symptom development was assessed at 14 days post-inoculation. Morphometric characteristics - plant height, number of shoots, and roots formed - were evaluated from all treatments. Analysis of Variance (ANOVA) was performed using the IRRI Statistical Tool for Agricultural Research (STAR Nebula) software with a 95% confidence. The experiment was performed twice.

3. Results

Identity of the bacterial contaminants

Bacteria that are white to yellow were isolated from heathy-looking tissue-cultured bananas cv. Grand Nain and Saba (Fig. 1a to 1c). Colonies are white to yellow in color, round, and slightly raised, with the entire margin in NA medium. Cell shapes were bacilli, 1-2 µm x 7-10 µm size, containing monotrichous, amphitrichous, or lophotrichous flagella (Fig. 1d to 1f). The isolate colony and morphology resembled that of Luteibacter spp. The isolates were gram-negative based on the KOH test (Fig. 1 g to 1i). The bacterial isolates' identity was further validated through molecular analysis of the 16S ribosomal DNA region. The bacterial isolates had high similarity (>99%) to Luteibacter yeojuensis in BLASTN analysis and were grouped within the Luteibacter yeojuensis clade in the constructed phylogenetic tree (Table 3, Fig. 2). A distance tree also revealed that the three Philippine isolates have the closest similarity to Luteibacter yeojuensis strain IHB B 6856 from India (Fig. 3).



Fig. 1 - Morphological and cultural characteristics of the bacterial contaminants. Cultural characteristics of GN11-20 (a), SbM36A (b), and SbM36C (c) strains of 48-hourold cultures grown in nutrient agar (NA) medium. The white bar represents 2 cm. Bacterial cell morphology for each isolate was also shown (d, e, and f). The black bar at the upper left represents 10 μ m. Gram reaction of the bacterial contaminants using potassium hydroxide (KOH) test (g, h, and i) indicated the isolates were gramnegative through viscous string formation.

Isolate	Species	16S rRNA (Accession)
SbM36A	Luteibacter yeojuensis IHB B 6856	99.09% (KF668474.1)
GN11-20	Luteibacter yeojuensis IHB B 6856	99.64% (KF668474.1)
SbM36C	Luteibacter yeojuensis IHB B 6856	99.16% (KF668474.1)

Table 3 - Percentage similarities of the three bacterial isolates associated with tissue-cultured banana based on BLASTN search



Fig. 2 - The phylogenetic position of the 16S rDNA of the three Luteibacter yeojuensis strains was isolated in this study with other bacterial species. The tree was constructed using the Tamura-Nei model (Tamura and Nei, 1993) with 1,000 bootstrap replicates. Burkholderia vietnamiensis (LMG 10929) served as an outgroup.



Fig. 3 - The distance tree of the 16S rDNA sequences of *Luteibacter yeojuensis* GN11-20, SbM36A, and SbM36C was isolated in this study with other strains from Asia. The tree was constructed using the Tamura-Nei model (Tamura and Nei, 1993) with 1,000 bootstrap replicates. *Burkholderia vietnamiensis* (LMG 10929) served as an outgroup.

Morphometric characters of the banana plants

Results demonstrated the potential of L. yeojuensis for increased plant height and shoot proliferation on three. Both L. yeojuensis GN11-20 and L. yeojuensis SbM36A increased plant height in 'Lakatan' plants (Table 4, Fig. 4). More shoots were consistently recorded in banana cv. Grand Nain inoculated with the three L. yeojuensis isolates compared to the control treatment. One isolate, Luteibacter yeojuensis GN11-20, significantly increased shoot proliferation of tissue-cultured 'Grand Nain', the cultivar where the bacterium was initially isolated (Table 4, Fig. 4). On the other hand, no significant differences were observed in the number of shoots formed in banana 'Lakatan' and 'Saba' were inoculated with the three L. yeojuensis isolates compared to the control treatment. The plant height, the number of shoots, and the roots of the three genotypes inoculated with L. yeojuensis SbM36C were not significantly different from the control treatment.

Non-pathogenicity of bacteria to tissue-cultured bananas

In-vitro pathogenicity tests showed that the three Luteibacter yeojuensis strains were non-pathogenic to the three banana cultivars (Fig. 4 and 5) in wounded and unwounded assays. There were no adverse effects observed in plants inoculated with Luteibacter yeojuensis SbM36C, GN11-20, and SbM36A as compared to the control treatments (Fig. 5). No stunting and wilting in any of the test banana plants were observed. When corm tissues were dissected, there was no browning in any inoculated plants, and the appearance of the corm was similar to that of the control treatment.

4. Discussion and Conclusions

Little is known about the endophytes of banana plants. This study isolated bacterial endophyte *L*.

Genotype	Trootmont	Plant height (cm)		Number of shoots emerged		Number of roots formed	
	Heatment	Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value
Grand Nain	SbM36C	2.7 ± 0.5	0.7855 NS	4.3± 2.5 ab	0.0278 *	2.5 ± 1.9	0.4148 NS
	GN11-20	2.4 ± 0.6		7.3 ± 0.5 a		2.5 ± 1.3	
	SbM36A	2.4 ± 0.4		3.0 ± 2.7 b		4.3 ± 1.7	
	Control	2.7 ± 0.7		2.5 ± 1.7 b		2.5 ± 1.9	
Lakatan	SbM36C	2.3 ± 0.6 b	0.0224 *	5.0 ± 3.4	0.6799 NS	2.0 ± 0.8	0.0659 NS
	GN11-20	3.2 ± 0.2 a		3.8 ± 1.0		5.0 ± 0.8	
	SbM36A	3.5 ± 0.3 a		3.5 ± 0.6		2.5 ± 1.3	
	Control	2.9 ± 0.7 ab		5.0 ± 2.7		3.8 ± 2.5	
Saba	SbM36C	2.9 ± 0.5	0.5947 NS	4.3 ± 0.5	0.0851 NS	2.8 ± 1.7	0.2367 NS
	GN11-20	2.4 ± 0.3		2.5 ± 1.7		2.5 ± 1.3	
	SbM36A	2.6 ± 0.8		4.3 ± 0.5		2.0 ± 1.2	
	Control	2.5 ± 0.6		2.8 ± 1.3		1.3 ± 0.5	

Table 4 - Effect of inoculation of Luteibacter yeojuensis SbM36C, GN11-20, SbM36A strains on in-vitro shoot and root production of

Plantlets inoculated with water served as control. Asterisks (*) indicate significant differences between the treatments and the corresponding control by Least Significant Difference (LSD) test at α = 0.05;

Ns= not significant. Different letters in the mean values for each genotype indicate significant differences between the treatments.



Fig. 4 - Pathogenicity of *Luteibacter yeojuensis* SbM36C, GN11-20, and SbM36A strains isolated in this study at 14 days post-inoculation (dpi). Data shows two plantlets for each treatment. Control plantlets (treated with sterile distilled water) were also shown (d). The bar at the upper left represents 2 cm.

yeojuensis isolates SbM36C, GN11-20, and SbM36A from healthy tissue-cultured banana cultivars Saba and Grand Nain. While the colony characteristics (size and pigmentation) of the three bacterial isolates varied among the isolates, all three isolates belonged to the same species, as confirmed by the 16S rDNA



Fig. 5 - Morphometric characteristics of tissue-cultured banana plantlets inoculated with *Luteibacter yeojuensis* SbM36A, GN11-20, and SbM36C strains isolated in this study. Plantlets inoculated with sterile distilled water served as control. The plant height (a), number of shoots that emerged (b), and number of roots formed (c) of the three genotypes were collected 14 days post-inoculation. Different letters in each bar indicate significant differences (p<0.05).</p>

sequence analyses. Bacterial species under the order Xanthomonadales, such as Luteibacter sp., are gramnegative, aerobic, and carotenoid-producing species that provide the yellow-orange-red colour of the cultures (Saddler and Bradbury, 2005). The carotenoid pigments in Xanthomonadales are lipidsoluble and play a significant role in culture survival under low-temperature conditions and against UV radiation (Azman et al., 2018). The bacterial isolates from this study were obtained from different sources of banana genotypes: GN11-20 was isolated from 'Grand Nain,' SbM36C and SbM36A were isolated from 'Saba,' respectively. These genotypes were of different ages from the time of isolation of the endophytes. Hence, they had a potentially varying exposure to temperature and UV radiation. This genotypic and environmental variation might have affected the levels of carotenoids found in the endophytes isolated in this study, thereby affecting pigmentation despite belonging to the same species (Dieser et al., 2010). This study also highlights the importance of molecular assays, analyzing the 16S rDNA sequences, in identifying species when phenotypes of the bacteria are influenced by their response to the environment.

None of the *L. yeojuensis* isolates from this study resulted in infection in tissue-cultured banana 'Lakatan,' 'Grand Nain,' and 'Saba.' However, there were recorded differences in the morphometric characteristics of the three banana cultivars as influenced by the inoculation of three endophytes. Both L. yeojuensis GN11-20 and L. yeojuensis SbM36A have growth-promoting properties in tissuecultured banana plants at 14 days post-inoculation; this positively affects plants and may increase if treatments are extended for a longer incubation time (e.g., a month). The study supports the hypothesis that bacterial endophytes from healthy plants benefit their host plant. Nevertheless, not all endophytes within the same species have plant growthpromoting properties, as demonstrated by L. yeojuensis SbM36C.

Two isolates - *L. yeojuensis* SbM36A and *L. yeojuensis* GN11-20 - significantly enhanced plant height in 'Lakatan'. The *L. yeojuensis* GN11-20 further improved shoot emergence in 'Grand Nain' plants. These results suggest a symbiotic relationship between the two *Luteibacter yeojuensis* isolates and bananas. On the other hand, *L. yeojuensis* SbM36C did not significantly affect the growth of banana plants regarding plant height, number of shoots, and

roots. Several factors may have affected the performance of the three bacterial isolates or the endophytes' successful colonization in the plant host. These factors include plant genotype and tissue type (Hardoim et al., 2015). The longevity of exposure to the endophyte could also have an effect. Extending the inoculation period from 2 weeks to 4 weeks might increase the plant growth-promoting activity of the endophyte. None of the L. yeojuensis isolates increased the number of roots in the three genotypes tested. Previous reports have recorded the potential of Luteibacter rhizovicinus for enhanced root development in barley, specifically leading to higher weight and length of the roots (Guglielmetti et al., 2013). Hence, investigating the potential of the endophytes isolated for root development might also lead to a further understanding their plant growthpromoting activities.

The improved plant height and shoot production by L. yeojuensis strains isolated in this study might be stimulated by the increased IAA synthesis (Pieterse et al., 2009). Luteibacter sp., as an endohyphal bacterium (endophyte that forms a symbiotic relationship with a fungus) of Platycladus orientalis, increased indole-acetic acid (IAA) production, resulting in significantly higher seedling and root length (Hoffman et al., 2013). The antagonistic property of *L. yeojuensis* to plant pathogens has also been associated with high indolic compound production by L. yeojuensis. However, further investigation is needed since endophytic bacteria can utilize several other mechanisms (e.g., phosphate solubilization, gelatinase, and chitinase production) for plant growth improvement (Liu et al., 2017; Tang et al., 2020).

Several Luteibacter species are endophytes of economically important crops such as rice (Raj et al., 2019), and apple (Piagnini et al., 2007) acting either as plant-growth promoters or biocontrol agents. In bananas, Luteibacter sp. has been previously detected as an endophyte of 'Gros Michel' (Köberl et al., 2015) and was later found to have an antagonistic relationship with Fusarium oxysporum f. sp. cubense TR4 (Foc TR4) that causes severe wilt disease in bananas (Köberl et al., 2017; Nakkeeran et al., 2021). This study isolated and identified three strains of Luteibacter species, specifically L. yeojuensis, from healthy banana plants. This is the first confirmed report of L. yeojuensis as an endophyte of banana 'Lakatan', 'Grand Nain', and 'Saba'. The cultivar Grand Nain, a Cavendish

banana cultivar group member, is susceptible to Foc TR4. Hence, it would be worthwhile to test the bioactivity of the three *L. yeojuensis* strains in this study to Foc TR4 strains found in the country.

This study reports three *Luteibacter yeojuensis* strains as banana endophytes for the first time. It further demonstrates the growth-promoting potential of two *L. yeojuensis* strains in three tissue-cultured banana plants 'Grand Nain', 'Saba', and 'Lakatan'. The bacterial strains could be used to develop bioinoculants to improve plant growth in the future. However, its effect on other banana cultivars not used in this study should be further explored. Investigating the role of isolated endophytes as potential biological agents of diseases in bananas would be worthwhile research.

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