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RESEARCH ARTICLE

Endophytic bacteria isolated from the medicinal plant kratom (*Mitragyna speciosa*): Morphological, biochemical, and molecular characteristics, and their potential use as plant growth promoters

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Abstract

Endophytic bacteria live within and colonize plant tissues, forming symbiotic interactions that often enhance host growth, health, and stress tolerance without causing harm. This study aimed to isolate endophytic bacterial strains from the medicinal plant 'kratom' (*Mitragyna speciosa*), characterize their biological functions by analyzing morphological, biochemical, and molecular properties, and evaluate their plant growth-promoting potential. The research followed standard procedures for isolating and analyzing the production of plant growth-promoting compounds, including indole-3-acetic acid, 1-aminocyclopropane-1-carboxylate deaminase, siderophores, cellulase, nitrogen fixation, and phosphate solubilization, and for assessing their effects on soybean in planta, including bacterial root colonization and plant growth. Of the isolates tested by 16S rDNA sequencing, five were successfully identified, belonging to *Chromobacterium alkanivorans* (2), *Bacillus methylotrophicus* (1), *Stenotrophomonas maltophilia* (1), and *Chromobacterium violaceum* (1). Quantitative assays showed that the bacterial isolates produced 13.35 – 24.11 g mL^{-1} of indole acetic acid, 20.77 – 24.95 µg mL^{-1} of inorganic phosphate solubilization activity, 63.34 – 84.29 U mL^{-1} of acid phosphatase, and 2.92 – 4.86 U mL^{-1} of cellulase. Qualitative tests confirmed that each isolate produced siderophores, nitrogenase, 1-aminocyclopropane-1-carboxylate deaminase, indole acetic acid, cellulase, and both organic and inorganic phosphate solubilization. Greenhouse experiments demonstrated that all bacterial isolates promoted plant growth, increasing soybean shoot length by 17.71% to 54.17%, root length by 14.36% to 39.34%, and total dry weight by 2.24% to 56.71% compared to controls. These findings support the potential application of endophytes as biofertilizers in sustainable agriculture, highlighting their capacity as plant growth-promoting bacteria.

Keywords: Endophytic bacteria; kratom; Mitragyna speciosa; plant growth-promoting; qualitative and quantitative assays.

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1. Introduction

Endophytic bacteria inhabit and colonize the internal plant tissues, and they possess a mutually beneficial interaction with their host without causing any harmful effects. Bacteria have beneficial effects by assisting in the absorption of nutrients and protecting plants from pathogens, which helps them compete in nature. Similarly, plants serve as a food source for endophytes, completing the cycle. Several types of endophytic microorganisms with high population densities are found in some tissues, with a density of 1 × 10^{2} to 1 × 10^{9} endophytic bacteria per gram of plant tissue. Geographical location, genotype, cultivation methods, cultivar, interactions with other organisms, and other environmental factors significantly impact this density (Abdillah et al., 2014). Endophytes usually enter plant tissue mainly through stomata or cracks in primary and lateral roots (Zhang et al., 2022). They act as biological control agents and promoters of plant growth in agriculture by their ability to inhibit harmful microbes.

Different endophytic bacteria can stimulate plant growth through direct or indirect mechanisms. The indirect mechanism involves inhibiting pathogenic bacteria (as antibiotics) or insects (as pesticides) (Ramanuj & Shelat, 2019). The direct mechanism includes producing growth hormones or supplying essential nutrients that support plant growth and development. One such growth hormone that promotes plant growth is indole acetic acid (IAA), which causes root cells to elongate or divide. Phosphate-solubilizing bacteria (PSB) play a key role in making available phosphate for plant use. The complex form of P in soil prevents plants from absorbing it directly. Therefore, PSB helps mineralize complex P through two mechanisms, depending on the P types (Tariq & Ahmed, 2022). PSB solubilizes inorganic P by producing organic acids i.e; malic acid, oxalic acid, and phenolic acid (Maharana & Dhal, 2022). Mineralization of organophosphate esters (OPEs) by PSB involves several enzymes, including alkaline phosphomonoesterases (ACPs) and phytase. The gene alkaline phosphatase D (*phoD*) is one of the alkaline phosphatases (ALP)-encoding genes commonly found in PSB (Xu et al., 2021). Nitrogen (N) is another essential macronutrient for plant growth and development. Plants can meet their N requirements through symbiotic interactions with endophytic bacteria, which can fix atmospheric N and convert it into inorganic N compounds like ammonia that plants can use directly (Zhang et al., 2022). N-fixing bacteria fix and convert atmospheric N using nitrogenase enzymes encoded by the *nif* gene, including *nifH*, which encodes nitrogenase reductase (Sepp et al., 2023). The main N-fixing bacteria are rhizobia, primarily found in leguminous plants. However, endophytic N-fixing bacteria can also inhabit non-leguminous plants without forming nodules, living in roots, leaves, and stems (Rana et al., 2023).

Medicinal kratom (*Mitragyna speciosa*), a herbaceous Southeast Asia ethnobotanical tree (Indonesia, Malaysia, and Thailand), has been grown in low to medium-land forests (Auld et al., 2013). In Indonesia, it is a typical plant found in Putusibau, West Kalimantan. Kratom, or local name known as ‘purik’ leaves, has been used by the surrounding community as an herbal medicine for treating diarrhea, relieving muscle pain, and reducing fever. Furthermore, the pharmacological effects have been reported to be antinociceptive, antioxidant, antibacterial, and antidepressant. Generally, this medicinal plant is consumed by chewing, smoking, brewing like tea, making into juice, and processed into powder (Brown et al., 2017). The traditional medicinal kratom plant, valued for its psychoactive alkaloids (e.g., mitragynine), faces cultivation challenges including variable growth, alkaloid yield influenced by environmental factors, and potential for microbial enhancement. Many types of medicinal plants have tissues (roots, stems, leaves, fruits, and flowers) from which endophytic bacteria can be isolated. A large number of plants live in association with bacteria, such as the genera *Pseudomonas*, *Pantoea*, *Serratia*, *Stenotrophomonas*, *Bacillus*, *Paenibacillus*, and *Chromobacterium* (Ramanuj & Shelat, 2019; Aydi Ben Abdallah et al., 2018; Zhao et al., 2015; Anillo et al., 2016).

Endophytic bacteria of medicinal plants like *M. speciosa* offer promise for improving plant performance through plant growth-promoting (PGP) traits, although studies remain limited compared to other hosts (Liyuwardi, 2024). Recent isolations from *M. speciosa* leaves have yielded novel actinobacterial endophytes (e.g., *Streptomyces mitragynae* sp. nov.) with antimicrobial activity, highlighting the plant's rich endomicrobiome (Pitiwittayakul et al., 2026).

Although a lot of research has looked into endophytic bacteria from various medicinal plants for PGP properties, there has been little research into the endophytic bacterial communities associated with kratom. Despite kratom's documented pharmacological qualities and broad traditional use, the variety, functional features, and biotechnological possibilities of its endophytic bacteria are still completely unknown. In particular, no systematic investigation has examined whether kratom-associated endophytes have PGP mechanisms such as IAA generation, P solubilization, N fixation, siderophore activity, and ACC deaminase production (Semenzato & Fani, 2024; Sharma et al., 2025), whereas broader evaluations across multiple economically important crops, such as soybean. are needed to determine the scalability and efficacy of these fungi. Recent reviews highlight their applications as eco-friendly bioinoculants, with diverse genera (e.g., *Bacillus*, *Stenotrophomonas*, *Chromobacterium*) demonstrating multifaceted PGP traits and modulation of bioactive compounds in hosts such as *Lessertia frutescens*, basil, and others (Sharma et al., 2025; Ercole et al., 2026). These advancements underscore endophytes' potential to address cultivation challenges in high-value medicinal species amid climate variability and reductions in chemical inputs. Despite these progresses in medicinal plant endomicrobiomes, substantial knowledge gaps remain for the kratom tree valued for its psychoactive alkaloids (e.g., mitragynine) with emerging cultivation needs in regions like Indonesia. Direct studies on bacterial endophytes' isolation, molecular characterization, and PGP functions (e.g., IAA production, nutrient mobilization, or contributions to plant growth and alkaloid stability) in kratom are notably limited or absent, with most microbial research centering on fungal bioactive potential, gut microbiota modulation by kratom extracts, or abiotic factors affecting alkaloid variability (Zhang et al., 2025). This scarcity represents a critical gap, particularly for tropical medicinal crops facing environmental stresses and sustainable production demands.

Most current studies emphasize endophytes from cultivated crops or medicinal plants, neglecting the untapped bioactivity of fungi associated with kratom, especially from biodiversity hotspots like West Kalimantan. Therefore, the objective of this study was to characterize endophytic bacteria isolated from the medicinal plant kratom, focusing on exploring their PGP traits and potential applications in sustainable agriculture. Specifically, the study aimed i) to evaluate their ability to produce key biologically active compounds such as IAA, ACC deaminase, siderophores, cellulase, and to perform N fixation and P solubilization; and ii) to investigate their effects on seed germination and early growth of soybean (*Glycine max*), an economically important crop. By addressing this knowledge gap, the study contributes to microbial bioprospecting from underutilized medicinal plants and supports the development of eco-friendly bioinoculants for agriculture.

2. Methodology

2.1.Isolation and molecular identification of endophytic bacteria

The study was conducted at the Laboratory of Mycology and the greenhouse (GH) facility (6°29'30.9" South latitude; 106°50'43.3" East latitude) of the Research Center for Applied Microbiology in Bogor, West Java, Indonesia.

Healthy kratom plants (*M. speciosa*) were collected from plantations in Pontianak, West Kalimantan, Indonesia. Leaves, stems, and roots were washed under running water to remove adhering soil and debris, then cut into 1–2 cm segments. Surface sterilization was performed by immersion in 70% ethanol for 1 min, followed by 3 min in sodium hypochlorite (NaOCl), and rinsing three times with sterile distilled water. Sterilized tissues were placed on nutrient agar (NA) medium (5.0 g peptone, 1.5 g yeast extract, 5.0 g NaCl, 20 g agar; pH 7.2) and incubated at room temperature for 3 days. Emerging colonies were subcultured repeatedly to obtain pure isolates, which were maintained on NA slants at 4 °C for further analysis.

Identification of bacterial isolates was performed using PCR amplification of 16S rDNA with primers 27 forward (F): 5'-AGA GTT TGA TCC TGG CTC AG-3` and primer 1492 reverse (R): 5'-GGT TAC CTT GTT ACG ACT T-3`. To determine the sequence of the strands, the PCR products were subsequently sequenced using the Sanger method on an ABI Prism 3130 genetic analyzer (Chen et al., 2014). The BioEdit software was used to trim and assemble the sequences. The Basic Local Alignment Search Tool (BLAST) was employed to compare the sequences with genomic data registered at the National Center for Biotechnology Information (NCBI) (Auld et al., 2013). Additionally, the EzBioCloud database was utilized to identify the taxon or species with the highest and closest molecular homology or similarity (Masrukhin et al., 2021).

2.2 Screening of plant growth–promoting (PGP) traits

2.2.1 Indole-3-acetic acid (IAA) production

IAA production was assessed using both qualitative and quantitative methods. For qualitative screening, bacterial isolates were cultured on solid tryptic soy (TS) medium supplemented with L-tryptophan and incubated for 24 h, followed by staining with Salkowski's reagent.

For quantitative determination, isolates were inoculated into 20 mL liquid TS medium containing 200 μg mL^{-1} L-tryptophan and incubated for 72 h under shaking conditions. Cultures without bacterial inoculation served as controls. After incubation, 1 mL of culture supernatant was mixed with 4 mL of Salkowski's reagent and incubated for 20 min in the dark. Absorbance was measured at 530 nm, and IAA concentration was calculated using a standard calibration curve (Jatav et al., 2017; AL Kahtani et al., 2020).

2.2.2 ACC deaminase activity

Endophytic bacteria were inoculated on a Davis and Fink (DF) salt minimal medium to qualitatively test the production of ACC deaminase with 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.2 g $MgSO_47H_2O$, 2.0 g glucose, 2.0 g gluconic acid, and 2.0 g citric acid, with trace elements with 1.0 mg $FeSO_4.7H_2O$, 10 mg H_3BO_3, 11.19 mg $MnSO_4.H_2O$, 124.6 mg

$ZnSO_4.7H_2O$, 78.22 mg $CuSO_4.5H_2O$, 10.0 mg MoO_3, pH 7.2 added 3 mM ACC and $(NH_4)_2SO_4$ as a N source. The bacteria that grew on the media during the three days of incubation at room temperature showed that ACC deaminase was being produced (Moon & Ali, 2022).

2.2.3 Inorganic and organic phosphate solubilization

The endophytic bacteria obtained were then tested for qualitative P solubilization activity in vitro on solid Pikovskaya media (0.5 g YE; 10.0 g dextrose; 5.0 g Ca3 $(PO4)_2$; 0.5 g $(NH_4)_2SO_4$; 0.2 g KCl; 0.1 g MgSO4; 0.0001 g $MnSO_4.H_2O$; 0.0001 g $FeSO_4.7H_2O$; 15.0 g agar; 1.0 L distilled water; pH 7.0 ± 0.2 before sterilization). It was evident that endophytic bacteria could solubilize P because a halo zone surrounded the colony. Determination of the phosphate solubilization index (PSI) was done using Eq. 1 (Doilom et al., 2020):

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

Furthermore, bacteria with clear zone indications were measured for their activity in solubilizing inorganic P in liquid Pikovskaya media using the molybdate blue color method. The amount of P available was calculated using the standard KH_2PO_4, and as a control, Pikovskaya medium was used without inoculation. The activity of organic P solubilization was determined by measuring the acid phosphatase using para-nitrophenyl phosphate disodium (pNPP) 0.115 M as the substrate, and a spectrophotometer at 400nm wavelength was used to measure the yellow absorbance of the present para-nitrophenol (pNP). A pNP solution with a concentration of 1–10 ppm was utilized as a standard, and distilled water was used as the blank (Behera et al., 2017).

2.2.4 Nitrogen fixation potential

A qualitative assessment was made of the bacteria's capacity to fix N using semi-solid nitrogen-free bromothymol blue (NFB) media (5.0 g malic acid, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.02 g NaCl, 0.1 g $CaCl_2$, 2.0 mL bromthymol blue, 4.0 mL Fe, EDTA 1.64%, 4 g KOH, 1.0 mL vitamins (Biotin, Pyridoxol HCL), 2.0 mL microelement solution ($CuSO_4$, $ZnSO_4.7H_2O$, H_3BO_3, $NaMoO_4.2H_2O$, $MnSO_4.H_2O$) and distilled water. Furthermore, semi-solid NFB media were used to inoculate bacterial cultures, which were then incubated for 72 h at room temperature. Nitrogenase activity is characterized by the formation of round pellicles like white rings under the surface of the medium (Harahap et al., 2023).

2.2.5 Cellulase activity

The cellulase activity of bacteria was conducted qualitatively and quantitatively. The qualitative test was calculated based on the cellulase solubilization index on carboxymethyl cellulose (CMC) agar plates, using Eq. 2 (Tang et al., 2020):

$$Cellulase\ soluble\ index\ (CSI) = \frac{clear\ zone\ diameter + colony\ diameter}{colony\ diameter} \quad \text{(Eq. 2)}$$

Endophytic bacteria were inoculated on CMC media containing Congo red, by preparing materials such as 0.5 g YE, 0.5 g CMC, 500 mL Aquadest, 9.0 g pure agar, 0.5 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4$, 0.5 g $MnSO_4$, and 0,5 g $FeCl_3$. Furthermore, incubation was performed for five days at room temperature. Subsequently, the culture was flooded with the Congo red solution. The development of a clear zone surrounding the colony was indicative of cellulase activity (Sharma et al., 2020).

The cellulase activity was determined quantitatively by measuring the endo-β-1,4-glucanase activity following the 3,5-dinitrosalicylic acid (DNS) method. The quantity of enzyme that can hydrolyze CMC and release 1 micromole (µmol) of glucose in a minute of reaction is known as one unit of endo-β-1, 4-glucanase activity.

2.2.6 Siderophore production

Hexadecyltrimethylammonium bromide (HDTMA) and chromeazurol S (CAS) media were used as indicators in the siderophore production process on blue agar (Lipková et al., 2021). After being inoculated on CAS agar, all of the obtained bacterial isolates were incubated for 24 h at 28 °C. An orange-yellow zone formed around the colony, indicating siderophore activity.

2.2.7 Detection of functional genes (*phoD* and *nifH*)

To find the genes encoding the enzymes nitrogenase and phosphatase, the PCR method was employed. The genes targeted in this study were phosphatase D (*phoD*) and nitrogenase (*nifH*). The *phoD* gene was detected using the primers *phoD*-F 733 (5'-TGG GAY GAT CAY GAR GT-'3) and *phoD*-R 1083 (5'-CTG SGC SAK SAC RTT CCA-3') (Ragot et al., 2015). Nitrogenase genes were detected using the primers *nifH* F (5'-GGCAAGGGCGGTTATCGGCAAGTC-'3) and *nifH* R (5'-CCATGGTGATCGGGTCGGGATG-'3) (Sulistiyani & Meliah, 2017). The amplified *phoD* gene was obtained using PCR at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 seconds (sec.), 58 °C for 30 sec, and 72 °C for 30 sec, followed by 10 min at 72 °C. The *nifH* gene was amplified using 30 cycles of denaturation at 94 °C for 60 sec, annealing at 57

°C for 60 sec, elongation at 72 °C for 60 sec, and final extension at 72 °C for 5 min. The PCR product was stained with GelRed (Thermo Scientific), separated using agarose gel electrophoresis, and then examined under a UV lamp. DNA fragment size was determined by comparing it to DNA Ladder 1 kb (Geneaid).

2.3 Greenhouse (GH) experiment

2.3.1 Preparation of bacterial inoculum

Bacterial cultures were prepared before colonization testing. Five bacterial culture isolates were prepared by inoculating 1 mL of each bacterial isolate (BM, DM, BP, DP, and LBI) in 250 mL Erlenmeyer flasks containing nutrient broth (NB) media. Incubation on the shaker was performed for three days at 120 rpm. Following a 10 min centrifugation of the bacterial culture at 12,000xg, the pellets were dissolved in distilled water to create a bacterial cell suspension with a density of 10^8 colony-forming units per milliliter (CFU mL^{-1}).

2.3.2 Root colonization assay

Seed germination was prepared before colonization testing and planting in the GH. Sterilized soybean seeds were soaked in 1% NaOCl for 30 sec, rinsed five times with sterile distilled water, and allowed to germinate for two days in a Petri dish lined with filter paper. Five chosen endophytic bacterial isolates were examined for their capacity to colonize soybean root tissues. The seeds were incubated for two days at room temperature after being sterilized as previously mentioned. Germinated seeds were soaked in each culture suspension for 30 min. Furthermore, the inoculated germinated seeds were sown aseptically in a glass tube containing 100 g of sterile sand. Germination was performed for 8 days, and irrigation was conducted with 10 mL of sterile Hoagland nutrient solution. Each bacterial treatment was repeated four times. The bacterial ability to colonize root tissues was achieved by re-isolating the bacteria from the roots. Re-isolating was conducted by removing the sprouts and cleaning the roots from the sand. The sprouts were later sterilized using 70% alcohol and 1% NaOCl. Subsequently, five rinses with distilled water were performed on the roots. The roots were then mashed, and 1 g of root tissue extract was diluted with physiological NaCl in a series of 10^{-1} to 10^{-7} dilutions. After inoculating 100 µL aliquots on sodium agar medium, the colonies were counted after three to five days of room temperature incubation.

2.3.3 Effect of endophytic bacteria on soybean growth

Experiments were performed using pots in a GH facility at the National Research and Innovation Agency, Cibinong, West Java, Indonesia. A completely randomized design with one factor was used in this study. The treatment was inoculation using five endophytic bacteria (code isolates: BP, DM, BM, and LBI), and without inoculation as a control. Every treatment was carried out three times. The 2-day-old soybean sprouts were soaked with bacterial culture (density 10^8 CFU mL^{-1}) for 60 min. The seeds were soaked with 25 mL of sterile distilled water instead of the control. Then, 300 g of a soil and sand mixture was added to the pot. Soybean sprouts inoculated with each endophytic bacterium were transferred to a pot containing two sprouts. The application of the inoculant was repeated one week after sawn. The plant was watered using tap water during the experiment. After 30 days, the plants were harvested, and the following variables were noted: total dry weight, shoot length, and root length.

2.4 Statistical analysis

All assays were conducted in triplicate. Results were expressed as mean ± standard deviation. One-way analysis and the analysis of variance (ANOVA) test were used to compare different samples in the statistical analysis of the data using Statistical Package for the Social Sciences (SPSS) software for Windows version 23.

3. Results and discussion

3.1 Isolation of endophytic bacteria

Nine endophytic bacterial strains were isolated from the stems, leaves, and roots of *Mitragyna speciosa* (kratom). These isolates are coded as: BB and BM (red stems), BW and BP (white stems), DR and DM (red leaves), DB and DP (white leaves), and LBI (roots). The isolation of endophytic bacteria from *M. speciosa* in this study using standard surface sterilization and molecular identification mirrors recent efforts yielding antibacterial strains from Kalimantan kratom and a novel *Streptomyces mitragynae* study reported in Thailand. These highlight *M. speciosa's* endophytic diversity, often dominated by actinobacteria with bioactive potential, which could extend to PGP traits like IAA production or nutrient solubilization screened.

In the present study, nine endophytic bacterial strains were isolated from *M. speciosa*, five of which, *S. maltophilia* (BM), *C. alkanivorans* (BP), *B. methylotrophicus* (DM), *C. violaceum* (DP), and an unclassified root isolate (LBI), exhibited multiple PGP traits. These included IAA production, phosphate solubilization, siderophore production, nitrogen fixation, cellulase activity, and ACC deaminase activity. Quantitative assays confirmed strong IAA production, P solubilization, and cellulolytic activity, all of which contribute to improved plant growth and health.

Several studies have highlighted the potential of medicinal plants as reservoirs of growth-promoting endophytic bacteria. For instance, endophytes have been isolated from *Zingiber officinale* (Jasim et al., 2014), *Lonicera japonica* (Zhao et al., 2015), and *Mussaenda roxburghii* (Afzal et al., 2019), exhibiting traits beneficial for PGP. Bacterial strains from *M. roxburghii* capable of thriving in nitrogen-free media and exhibiting siderophore production, phosphate solubilization, and indole-3-acetic acid (IAA) synthesis (Afzal et al., 2019). In line with this study, it was reported that the isolation of endophytes from various plant tissues possessed multiple PGP traits, including IAA and siderophore production, phosphate solubilization, and the secretion of enzymes such as cellulase and ACC deaminase (Ramanuj & Shelat, 2019). These are consistent with other findings that several growth-promoting endophytes, including *Stenotrophomonas maltophilia*, *Pseudomonas geniculata*, *B. amyloliquefaciens*, and *B. subtilis*, were identified from tomato plants (Aydi Ben Abdallah et al., 2018), whereas *S. maltophilia* and *Bacillus* spp. were isolated from the roots of Zingiberaceae plants (Jasim et al., 2014). Similarly, it was also reported that 67 endophytes belonging to the genera *Bacillus*, *Chromobacterium*, and *Stenotrophomonas* could be isolated from Colombian coffee plants (Anillo et al., 2016), underscoring the widespread presence of these genera in diverse hosts.

3.2 Identification

The five isolates with the highest plant growth-promoting traits, i.e., BM, BP, DM, DP, and LBI isolates, were identified using 16S rDNA sequencing. This result was clarified by the evolution or proximity of the bacterial species on the phylogenetic tree (Table 1; Figure 1). Phylogenetic study revealed that these isolates share 99% similarity with *S. maltophilia, C. alkanivorans*, *B. methylotrophicus*, and *C. violaceum*. These taxa appear frequently in medicinal plants. The construction results showed that the isolate codes BM, BP, DM, DP, and LBI are closely related and have similarities to the species groups *Stenotrophomonas maltophilia* ATCC 13637, *C. alkanivorans* IITR-71, *B. methylotrophicus* CBMB205, and *C. violaceum* ATCC 12472 in one phylogenetic tree with bootstraps of 87, 96, 96, 85, and 100%, respectively.

The isolation of endophytic bacteria from *M. speciosa* tissues, including *C. alkanivorans*, *B. methylotrophicus*, *S. maltophilia*, and *C. violaceum*, represents a significant addition to the limited knowledge of kratom's endomicrobiome. While recent studies have explored endophytes in other medicinal plants for PGP and bioactive roles, direct reports on bacterial endophytes in kratom remain sparse, often focusing on antimicrobial fungi or root-associated microbes rather than PGP bacteria. The molecular characterization in this study (16S rRNA) and PGP screening align with contemporary methods, revealing traits like IAA production and phosphate solubilization that could enhance kratom's biomass, stress tolerance, and alkaloid production amid cultivation challenges in Southeast Asia.

Among the isolates, *B. methylotrophicus* exhibited strong PGP potential, consistent with recent genomic analyses of related *Bacillus* spp. as endophytes in medicinal plants. For instance, *B. subtilis* 10-4, a PGP endophyte, harbors genes for nutrient acquisition (nitrogen, phosphorus, iron), phytohormone synthesis (auxins, salicylic acid), and stress tolerance (glutathione, osmoprotectants), leading to improved crop growth under abiotic stresses in lab and field trials. Similarly, *Bacillus* endophytes from *Lessertia frutescens* demonstrated hydrolytic enzymes and biofertilizer efficacy in pot trials, enhancing seedling height and biomass (Tsipinana et al., 2024). In kratom, this genus may fill a gap, as prior work on *M. speciosa* microbes focuses on gut modulation rather than endophytic PGP. In this study, endophytic isolates obtained could optimize alkaloid biosynthesis and plant resilience, warranting field studies in Indonesian contexts.

Table 1
BLAST results with the largest and closest molecular similarity

Isolate code	Species	Strain	Accession number	Similarity
BM	*Stenotrophomonas maltophilia*	NCTC10257 (T)	NCTC10257	99%
BP	*Chromobacterium alkanivorans*	IITR-71(T)	NR 153735.1	99%
DM	*Chromobacterium alkanivorans*	IITR-71(T)	NR 153735.1	99%
DP	*Bacillus methylotrophicus*	KCTC 13613 (T)	KY 643639.1	99%
LBI	*Chromobacterium violaceum*	ATCC 12472	NR 074222.1	99%

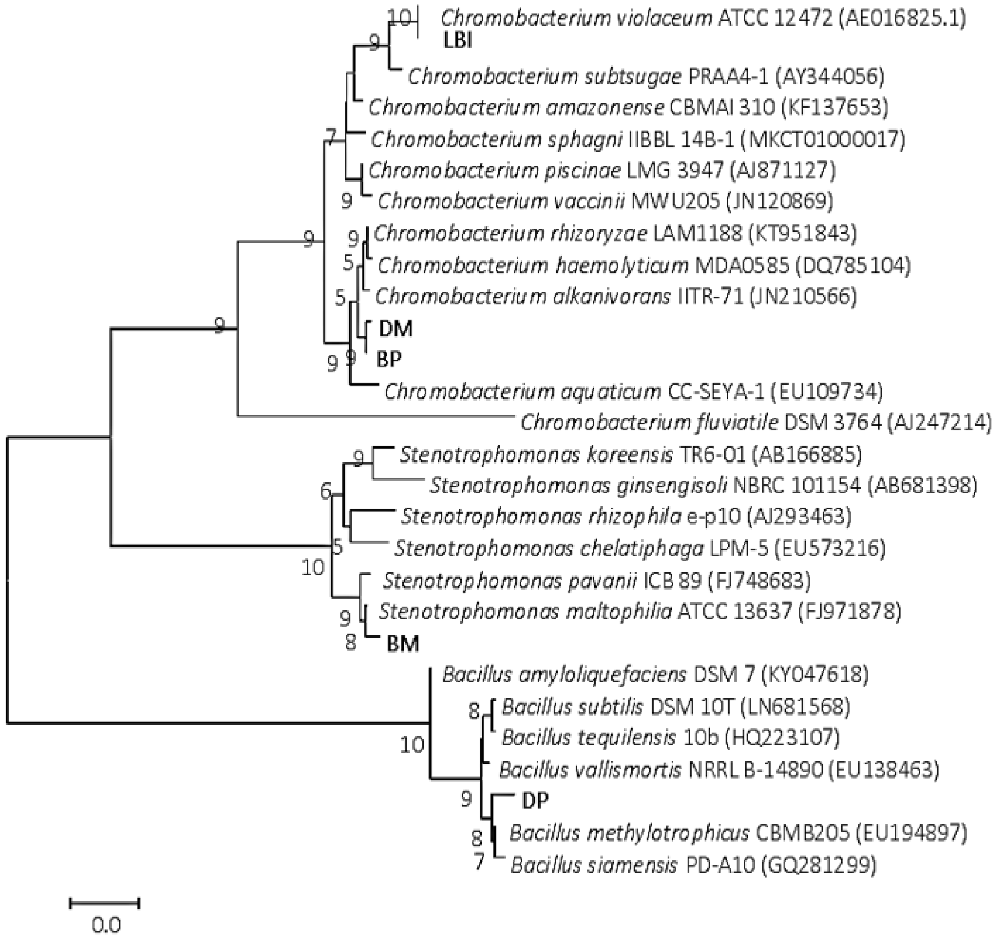

Figure 1. Phylogenetic trees based on 16S rRNA gene sequences of three bacterial genera, *Stenotrophomonas*, *Chromobacterium*, and *Bacillus*, were constructed using the neighbor-joining (NJ) method with 1000 replications. Bootstraps > 50% were shown, and Aquifex was used as the out-group.

S. maltophilia, often viewed as opportunistic but increasingly recognized as a beneficial endophyte, showed PGP traits in this study, mirroring recent findings in medicinal and stress-prone plants. In *Fagonia indica* and *Dicoma anomala*, *S. maltophilia* produced antimicrobial metabolites (esters, fatty acids, terpenes) with broad-spectrum activity against pathogens, aiding plant defense (Rahman et al., 2022). A novel PGP strain, *S. maltophilia* SaRB5, facilitated phytoremediation and growth in *Salix suchowensis* by cadmium absorption and stress alleviation. Multi-omics of *S. maltophilia* S-11 revealed Pb^{2+} bioremediation potential via enhanced survival and bioinoculant traits. Its presence in kratom suggests dual roles in growth promotion and heavy metal tolerance, valuable for sustainable cultivation in contaminated tropical soils (Lin et al., 2025; Zhao et al, 2024).

The *Chromobacterium* (*C. violaceum* and *C. alkanivorans*) isolates are noteworthy for their rarity as endophytes in medicinal plants, with recent studies highlighting their shift from pathogens to PGP agents (Venkatramanan & Nalini, 2024). In *Artemisia nilagirica*, *C. violaceum* WVAT6 displayed antagonistic activity against human pathogens via intracellular metabolites, supporting plant health. Bioprospecting revealed *C. violaceum*'s PGP potential as a rhizobacterium, promoting growth beyond its violacein/antibiotic roles (Khan et al., 2024). Virulence regulation studies (e.g., quorum sensing, biofilm, T3SS) underscore controlled beneficial applications. While in *C. alkanivorans* suggest hydrocarbon degradation and stress tolerance could extend to kratom, enhancing alkaloid stability and environmental adaptation, novel for *M. speciosa*, where microbial influences on mitragynine are emerging but underexplored. These genera collectively bolster kratom's endophytic diversity, offering eco-friendly alternatives to chemical fertilizers for improving growth parameters, bioactive yields, and microbial preservation in medicinal cultivation. Compared to endophytes in *Panax notoginseng* or

Sedum alfredii, the isolates in this study highlight kratom's potential as a reservoir for multifunctional bacteria (Zhang et al., 2025). Future work should include pot/field trials in different conditions to validate PGP effects on mitragynine content and stress resistance, addressing gaps in kratom-specific endophyte research.

3.3 Qualitative assay for endophytic bacteria

All isolates were tested for physiological features that promote plant growth (Table 2), such as IAA production, ACC deaminase activity, N fixation, siderophore formation, P solubility, and cellulase activity. All isolates produced IAA, as evidenced by a color change from yellow to pink.

A color shift from yellow to brown indicated a qualitative assay of ACC deaminase in the DF medium. Endophytic bacterial isolates BM, BB, BP, DR, DM, DB, DP, and LBI showed a color change from yellow to brown, which means that these isolates had ACC deaminase activity. On the other hand, there was no color change in the media for the BW isolate.

All tested isolates on CAS agar formed orange circular halos around the colony, indicating siderophore activity. Endophytic bacteria produce siderophores, which are Fe-chelating secondary metabolites that help plants grow by supplying Fe. The clear zone surrounding the colony demonstrated the ability to solubilize P on solid Pikovskaya media supplemented with tricalcium phosphate ($Ca_3(PO4)_2$). The presence of organic and inorganic acids released by PSB is what causes inorganic P to become soluble. Except for isolates BB and BW, which tested negative, nearly every isolate tested was able to solubilize P, with a PSI ranging from 2.11 to 2.20. On semi-solid NFB media, all isolates that were tested were able to develop and produce white veil-like pellicles beneath the surface.

Cellulolytic microbes are crucial to the decomposition process, especially when it comes to breaking down complex polysaccharides into simple sugars. The formation of a clear zone surrounding the colony as a result of the cellulase enzyme's hydrolytic activity was a characteristic of cellulase activity. The hydrolysis product was a simple sugar monosaccharide that could not make a complex bond with Congo red, producing a clear zone. Eight isolates formed clear zones with a diameter of about 1.50 – 4.00 cm and a CSI between 4.60 and 16.00, while the BW isolate did not show a similar result.

3.4 Quantitative assay

A quantitative assay of endophytic bacteria on IAA activity, cellulases, and inorganic and organic P solubilization was performed on identified isolates.

3.4.1 Indole acetic acid (IAA)

After 72 h of incubation, the concentration of IAA generated by the chosen bacteria varied between 19.37 and 24.10 µg mL^{-1} (Figure 2). The highest concentration was obtained by isolate *C. alkanivorans* DM (24.10 µg mL^{-1}) and was not appreciably different from *B. methylotrophicus* DP (23.91 µg mL^{-1}) but very different from *C. alkanivorans* BP (20.25 µg mL^{-1}), *C. violaceum* LBI (19.81 µg mL^{-1}), and *S. maltophilia* BM (19.37 µg mL^{-1}).

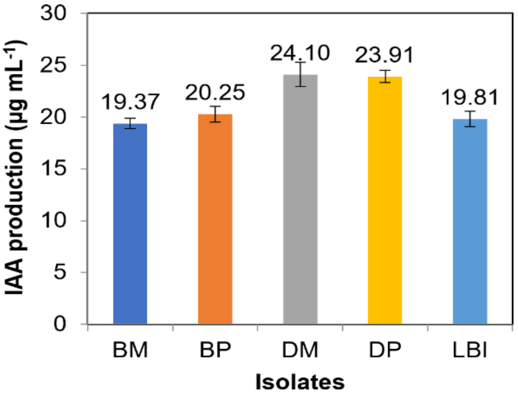

Figure 2. Production of IAA by endophytic bacteria isolated from kratom (*M. speciosa*). The vertical bar corresponds to standard deviation (SD).

Table 2

Characterization of endophytic bacteria for plant growth promotion

Isolates	IAA	ACC deaminase	Nitrogenase	Sidero-phore	Phosphate solubilization			Cellulase		
					HØ	CØ	PSI	HØ	CØ	CSI
BM	+	+	+	+	4.00	3.50	2.14	30.00	2.00	16.00
BB	+	+	+	+	-	4.00	-	15.00	4.00	4.75
BW	+++	*-	+	+	-	5.00	-	-	5.00	-
BP	++	+	+	+	5.00	4.50	2.11	23.00	3.00	6.67
DR	++	+	+	+	7.00	6.00	2.17	33.00	3.00	12.00
DM	+	++	+	+	7.50	6.50	2.15	40.00	5.00	9.00
DB	++	++	+	+	8.00	7.00	2.14	18.00	5.00	4.60
DP	+	+	+	+	6.00	5.00	2.20	28.00	4.00	8.00
LBI	++	+++	+	+	8.00	7.00	2.14	25.00	4.00	5.25

* - = not detected, + = low, ++ = moderate, +++ = high, HØ= halo diameter (cm), CØ = colony diameter (cm), SI Solubilization Index = (HØ+CØ)/CØ.

The production of IAA by endophytes is particularly important in enhancing root architecture and nutrient uptake. IAA stimulates root elongation and the proliferation of lateral root hairs, thereby improving nutrient acquisition (Afzal et al., 2019). It was reported that IAA production ranged from 11.50 to 49.20 mg L^{-1} by *Bacillus* and *Paenibacillus* isolates (Zhao et al., 2015), while IAA synthesis was documented by *S. maltophilia* C29b at 12.38 ppm even in the absence of tryptophan (Tang et al., 2020).

3.4.2 Inorganic and organic P

The capacity of endophytic bacteria to quantitatively dissolve both organic and inorganic P was investigated using Pikovskaya media containing $Ca_3(PO_4)_2$ as a source of *p*NPP 0.115 M as a substrate. The results showed that all isolates could solubilize inorganic and organic phosphate (Figure 3). The inorganic phosphate solubilization activity was determined based on the available phosphate concentration in the medium. In this study, it was found that the inorganic phosphate solubilization activity ranged from 20.77 to 24.95 µg mL^{-1}, and *C. alkanivorans* BP had the highest phosphate solubilization activity, while *C. violaceum* LBI came in second. Organic phosphate was determined by acid phosphatase activity, which ranged from 63.34 to 84.29 U mL^{-1}.

In terms of phosphorus mobilization, numerous studies report the capability of endophytes, *Bacillus*, *Stenotrophomonas*, and *Chromobacterium* to mineralize organic phosphorus and solubilize inorganic forms. For example, *B. subtilis* LK14 produced a clear halo zone of 56.71 mm and phosphatase activity of 8.4 nM mg.min^{-1} after five days of incubation (Khan et al., 2016), while *Bacillus safensis* ZY16 solubilized up to 151.5 mg L^{-1} of P in liquid media (Wu et al., 2019). P solubilization in a liquid medium was obtained 1.87 to 45.6 mg L^{-1} by *Bacillus* sp. Endophytic bacteria of the genus *Stenotrophomonas*, such as *S. maltophilia* PM22, have also been reported to produce phosphatases and solubilize phosphate efficiently (Zhao et al., 2015).

3.4.3 Cellulases

The production of cellulase enzymes from endophytic bacteria tested ranged from 2.92 to 4.86 U/mL (Figure 4). The largest cellulase activity (4.86 U mL^{-1}) was shown by *S. maltophilia* BM and was not significantly different from *C. alkanivorans* DM (4.72 U mL^{-1}) and *B. methylotrophicus* DP (4.11 U mL^{-1}). However, it was significantly different from *C. violaceum* LBI (3.22 U mL^{-1}) and *C. alkanivorans* BP (2.92 U mL^{-1}).

Several isolates also displayed cellulase activity, detected using Congo red, which binds specifically to β-1,4-glycosidic bonds in polysaccharides (Sharma et al., 2020). It was found that 167 endophytic strains showed cellulolytic activity, with halo zones ranging from 10 to 30 mm (Zaghloul et al., 2016). Cellulase activity indices (CSI) were reported to range between 2.57 and 2.86 (Al Kahtani et al., 2020), and it was observed that even higher CSI values (3.59–10.41) (Tang et al., 2020). These enzymes facilitate host colonization by hydrolyzing the plant's outer cell layers and establishing symbiosis. There have been reports of endophytic bacteria, including *Bacillus* and *Paenibacillus*, having cellulase activity (Zhao et al., 2015). Cellulase enzymes of 2.00 - 4.50 and 4.30 U mL^{-1} were produced by endophytic bacteria from the genera *Bacillus* and *Stenotrophomonas* that were isolated from mangroves (Ntabo et al., 2018).

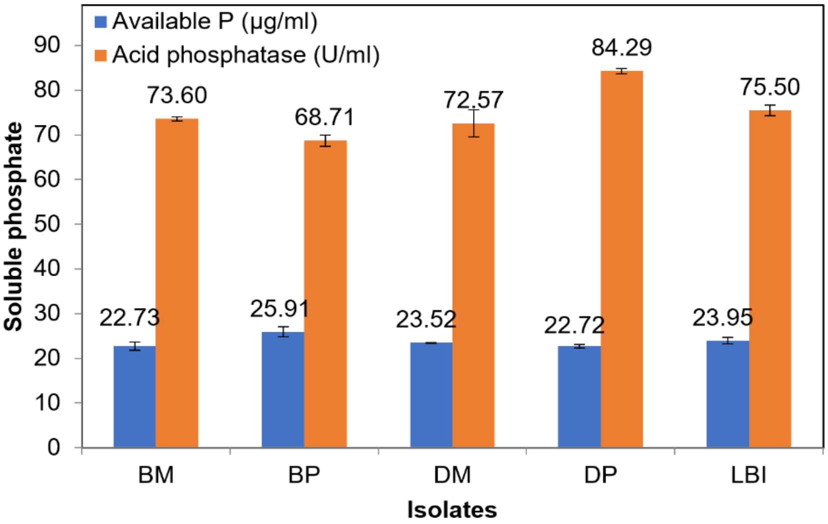

Figure. 3. Phosphate solubilization by endophytic bacteria isolated from kratom (*M. speciosa*). The vertical bar corresponds to SD.

Furthermore, 44 isolates obtained from legume and non-legume plants produced cellulase between 0.50 - 0.98 U mL^{-1} (Zaghloul et al., 2016). Certain lytic enzymes, like cellulases, are secreted by endophytic microbial strains in advantageous locations. These enzymes help bacterial strains enter plant tissue by hydrolyzing the outer layer, allowing the endophyte and host to establish a symbiotic relationship.

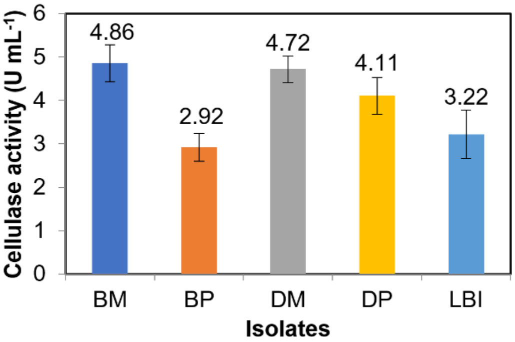

Figure 4. Cellulase production by endophytic bacteria isolated from kratom (*M. speciosa*). The vertical bar corresponds to SD.

3.4.4 Detection of *phoD* and *nifH* genes

The PCR method used to detect the *phoD* and *nifH* genes on the five selected bacterial isolates yielded negative results. Although all isolates demonstrated activity in dissolving P and fixing N, this is very likely to occur. ACC deaminase, a cytoplasmic enzyme produced by various endophytes, alleviates ethylene-induced stress in plants, thereby promoting growth under adverse conditions (Naing et al. 2021). Iron chelation via siderophores is indicated by a color change in the dark blue CAS agar assay (Zhao et al., 2015), while atmospheric nitrogen fixation is often demonstrated by a color shift in N-free media (Mir et al., 2021).

Attempts to amplify *nifH* and *phoD* genes in this study were unsuccessful. This aligns with other results that reported gene absence in certain isolates despite evident functional activity (Sulistiyani & Meliah, 2017). The *phoD* gene and two additional phosphatase genes, *phoX* and *phoA*, encode a phosphatase involved in P dissolution (Nishihara et al., 2018). In this study, only one of three bacterial isolates tested yielded positive results for the *nifH* gene detection. The complexity of detecting these genes arises from primer specificity and gene diversity. It was also emphasized that no universal primer exists for *nifH*, and successful detection often depends on the primer's compatibility with the isolate's gene sequence (Sulistiyani & Meliah, 2017). The primers that are currently available are intended to cover only specific groups (Angel et al., 2018). It is suggested that degenerate primer design and primer pair combinations may improve target gene detection success (Angel et al., 2018). Although molecular screening for *nifH* and *phoD* genes was unsuccessful, the observed N-fixing and N-solubilizing abilities in vitro point to the presence of alternative gene variants or mechanisms, emphasizing the importance of whole-genome sequencing or metagenomic analysis of promising isolates in future research, which can reveal new genes and pathways involved in PGP processes, stress tolerance, and endophytic colonization, resulting in a better knowledge of plant-microbe interactions. Despite the lack of molecular confirmation for *nifH* and *phoD*, functional assays suggest these strains may possess alternative nitrogen fixation and phosphorus solubilization mechanisms. This highlights the need for whole-genome or metagenomic approaches to uncover novel genes and pathways involved in PGP and stress resilience.

3.5 The GH experiment

3.5.1 Effect of bacterial inoculations on root colonization and soybean growth

BP showed the highest bacterial colonization of soybean roots isolate with a population of 600 x 10^{3} CFU g^{-1} roots (BP strain), followed by DP isolate (467 x 10^{3} CFU g^{-1} roots), BM (340 x 10^{3} CFU g^{-1} roots), DM (313 x 10^{3} CFU g^{-1} roots), and LBI (257 x 10^{3} CFU g^{-1} roots) (Figure 5). This finding revealed that the inoculated bacteria were able to survive in soybean root tissue.

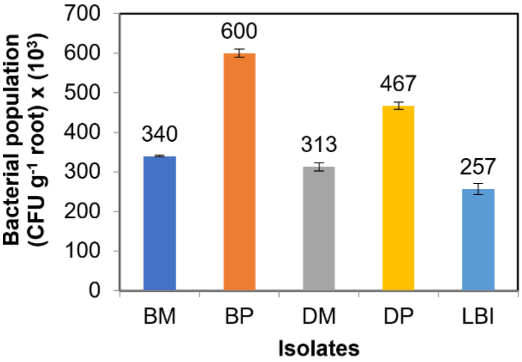

Figure 5. Endophytic bacteria colonization in soybean root tissue. The vertical bar corresponds to SD.

3.5.2 Growth of soybean on shoot length, root length and plant dry weight

This study found that all five isolates tested significantly improved the growth of soybean plants from 17.71 to 54.17%, the root length from 14.36 to 39.34%, and the dry weight from 2.24 to 56.71% compared to the control (Table 3).

Table 3
Effect of endophytic bacterial inoculation on the growth of soybeans

Treatment	Shoot length (cm)	Root length (cm)	Plant dry weight (g)
BM*	36.57^{cd} ± 1.30	10.67^{a} ± 1.20	1.70^{ab} ± 0.05
BP	34.33^{bc} ± 1.20	11.00^{a} ± 1.50	1.46^{a} ± 0.12
DM	32.17^{b} ± 1.60	13.00^{a} ± 1.30	1.37^{a} ± 0.04
DP	42.33^{e} ± 1.00	12.33^{a} ± 0.90	2.10^{b} ± 0.31
LBI	39.50^{de} ± 3.20	12.00^{a} ± 1.00	1.67^{ab} ± 0.13
C	27.33^{a} ± 1.20	9.33^{a} ± 0.30	1.34^{a} ± 0.07

*Means in the same row followed by the same letter in each column are not significantly different ($p \leq 0.05$) as determined by the least significant difference (LSD) test. Values represent mean ± standard deviation (n=3), C= plant without inoculant (control).

The application of different bacterial isolates showed significant effects on soybean growth, as measured by shoot length, root length, and plant dry weight. Among the isolates tested, DP demonstrated the most pronounced growth-promoting effect, producing the longest shoot length (42.33 cm), which was significantly higher than all other treatments. It also resulted in the highest plant dry weight (2.10 g), indicating its strong potential as a plant growth-promoting bacterium. Isolates BM and LBI also enhanced shoot length (36.57 cm and 39.50 cm, respectively) and plant dry weight (1.70 g and 1.67 g, respectively), although these values were not significantly different from each other and were lower than those observed in the DP treatment. In contrast, the control treatment showed the lowest shoot length (27.33 cm), root length (9.33 cm), and plant dry weight (1.34 g), underscoring the beneficial effects of bacterial inoculation. While root length did not differ significantly among treatments (ranging from 9.33 cm to 13.00 cm), all bacterial isolates slightly increased root length compared to the control, with the DM isolate achieving the highest root length (13.00 cm), albeit not significantly.

Inoculation studies further substantiate the growth-promoting potential of these bacteria. *B. subtilis* LK14 enhanced shoot and root biomass and chlorophyll content in *Solanum lycopersicum* (Khan et al., 2016). *Stenotrophomonas* and *Bacillus* strains isolated from ginger could improve maize nutrient uptake (N, P, K) and root parameters (Zhang et al., 2018), also linked IAA production to increased root system size, enhancing water and nutrient absorption (Behera et al., 2017). Root exudation, a key factor in microbial recruitment, is enhanced by endophyte-derived IAA. Numerous studies on the potential of endophytic bacteria to stimulate plant growth have also been published. Inoculation of *B. subtilis* LK14, which produces ACC deaminase, IAA, and soluble P, increased the chlorophyll (a and b) content of *S. lycopersicum* plants, along with increased shoot and root biomass (Khan et al., 2016). Several endophytic bacteria of the genera *Stenotrophomonas* and *Bacillus* were isolated from ginger, which can produce IAA (1.02 – 49.66 µg mL^{-1}) and P-solubilizing activity (9.3 – 233.05 µg mL^{-1}), enhance the development of maize plants, and increase N, P, and Potassium (K) uptake (Zhang et al., 2018). IAA-producing bacteria directly affects root parameters such as length and volume, number of fibers, and dry weight (Behera et al., 2017). This plant's ability to absorb water and nutrients is enhanced by its larger root system. The secretion of plant root exudates, which directly provides bacteria with energy, increased when endogenous bacteria produced IAA.

The finding revealed that the inoculated bacteria were able to survive in soybean root tissue. The colonization increased significantly up to 8 days after inoculation. The rise in colonization over time shows successful root endophytic establishment, which is consistent with other findings that reported TN10 isolates, which had a population of 6.4 × 10^5 CFU g^{-1} roots, had the highest bacterial colonization on faba bean roots (Zaghloul et al., 2016). The colonization ability of endophytic bacteria is due to the compatibility between bacteria and their host without damaging the tissue. This result was consistent with the findings that discovered eight endophytic bacteria isolated from healthy tomato plants could increase the maximum root length by 17.3 - 28.3% and the plant height by 30.5 – 35.9% when compared to the control (Aydi Ben Abdallah et al., 2018). Moreover, they reported bacteria identified as *S. maltophilia* CT12, *S. maltophilia* CT13, *S. maltophilia* CT16, *Pseudomonas geniculata* CT19, *B. amyloliquefaciens* CT32, *B. subtilis subsp. inaquosorum* CT43, *B. licheniformis* SV4, and *B. subtilis* SV5 promote growth. Soybean root colonization assays confirmed the ability of these isolates to persist in planta, with bacterial populations increasing significantly up to 8 days post-inoculation. This finding supports effective endophytic establishment and robust colonization by isolate TN10 in faba bean roots (Zaghloul et al., 2016). Similarly, it was demonstrated that endophytic *Stenotrophomonas*, *Pseudomonas*, and

Bacillus strains significantly enhanced root and shoot growth in tomato (Aydi Ben Abdallah et al., 2018).

The endophytic strains found in this study, particularly those from a unique medicinal plant such as *M. speciosa*, can be turned into environmentally benign biofertilizers to reduce soybean cultivation's reliance on chemical inputs. While GH studies reveal promising results. In greenhouse trials, soybean plants inoculated with these isolates, particularly *C. violaceum* (DP), showed marked improvements in shoot and root development, confirming their potential as effective bioinoculants. The DP isolate consistently outperformed others in all growth parameters, indicating its strong candidacy for biofertilizer development conditions.

The observed PGP traits, including ACC deaminase and siderophore production, suggest these isolates may also enhance plant resilience under abiotic and biotic stresses. Future research should explore these stress-alleviation mechanisms and evaluate isolate performance under field conditions and in diverse cropping systems. Ultimately, harnessing endophytic strains from unique medicinal plants like *M. speciosa* represents a promising strategy for developing eco-friendly biofertilizers, reducing reliance on chemical inputs, and supporting sustainable agriculture. The DP isolate showed the best performance across all measured parameters and can be considered the most effective in promoting soybean growth under the tested.

The PGP effects observed (e.g., enhanced growth parameters) are consistent with broader medicinal plant studies, and in *M. speciosa*, root-associated microbes have been linked to modulated alkaloid profiles (e.g., mitragynine accumulation). This suggests endophytes could serve dual roles in promoting vegetative growth while potentially optimizing psychoactive compound yields for sustainable harvesting. Given kratom's regional importance in Indonesia, such bacteria represent eco-friendly tools for improving cultivation amid environmental stresses. Limited prior PGP-focused studies on *M. speciosa* endophytes underscore the value of this work in expanding knowledge toward bioinoculant applications, similar to *Bacillus/Citrobacter/ Serratia* isolates from other medicinal plants, enhancing rosemary or basil (Sharma et al., 2025). Future research could explore field trials in tropical settings like Indonesia. The compatibility of these endophytes with other economically important crops can expand their benefits to a broader range of farming systems. In addition, it should focus on field validation of these isolates under various climatic conditions to confirm the consistency, efficiency, and scalability of these endophytes, as well as investigate their role in enhancing plant tolerance to biotic and abiotic stresses in different cropping systems.

4. Conclusions

This study successfully isolated and characterized endophytic bacteria associated with the medicinal plant kratom (*M. speciosa*), demonstrating their high potential as PGP. Molecular identification using 16S rDNA sequencing revealed the presence of several bacterial genera, which are known to have beneficial plant-microbe interactions, including *C. alkanivorans*, *B. methylotrophicus*, *S. maltophilia*, and *C. violaceum*.

Quantitative studies indicated that these isolates produce considerable amounts of IAA, acid phosphatase, and cellulase, as well as the ability to solubilize inorganic P. Qualitative evaluations revealed that endophytic bacteria produce siderophores, nitrogenase, and ACC deaminase, all of which are associated with enhanced nutrient acquisition, stress tolerance, and overall plant vigor. GH trials with soybean as a model crop showed that inoculation with these endophytes resulted in significant gains in shoot and root length, as well as overall plant biomass, demonstrating their ability to positively influence plant growth under controlled settings. These findings highlight the ecological and biotechnological importance of endophytic bacteria from *M. speciosa*, not only for improving the understanding of endophyte-host dynamics in medicinal plants, but also for their potential use as biofertilizers in sustainable agriculture.

Further studies are required to validate the performance of these endophytic bacteria under field conditions and across different crops and environments. In addition, the molecular mechanisms underlying plant–endophyte interactions and in planta colonization remain unclear. Future research should also focus on biosafety assessment and formulation optimization to ensure stability and effectiveness for practical biofertilizer applications. Addressing these gaps will be essential for translating the biotechnological potential of kratom-associated endophytic bacteria into reliable and scalable biofertilizer applications.

Conflict of interest

The authors express no conflict of interest associated with this study.

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Authors' contribution

Suliasih, Sri Widawati, Mat Aidi: Conceptualization, Methodology, Investigation, Supervision, Project administration. **Suliasih, Sri Widawati**: Writing – Original Draft, Validation. **Yadi Suryadi, Dwiningsih Susilowati, Agus Muharam**: Writing – Original Draft, Writing – Review & Editing. Idris: Formal analysis, Validation. **Titin Yulineri**: Investigation, Resources. **Arwan Soegiarto, Rini Handayani, Lidia Kristina Panjaitan, Neni Gunaeni, Eli Korlina**: Resources, Writing – Review & Editing.

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