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Endophytic bacteria of patchouli (*Pogostemon cablin* cv. Tapaktuan) as biocontrol agents against major soil-borne pathogens

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Abstract. *Suhartono S, Husnah M, Zumaidar Z, Amalia A, Salsabila A, Muliyani A, Achza AN. 2026. Endophytic bacteria of patchouli (*Pogostemon cablin *cv. Tapaktuan) as biocontrol agents against major soil-borne pathogens. Biodiversitas 27 (5): d270527. https://doi.org/10.13057/biodiv/d270527.* Endophytic bacteria are increasingly recognized as valuable sources of biocontrol agents against soil-borne phytopathogens. The aim of this study was to evaluate the antifungal activity of 23 endophytic bacterial isolates obtained from patchouli (*Pogostemon cablin* cv. Tapaktuan) against *Sclerotium rolfsii*, *Fusarium oxysporum*, and *Phytophthora capsici* using a dual-culture assay. Antagonistic activity varied markedly among isolates, with inhibition of radial fungal growth ranging from 5.8±0.88% to 73.0±0.49%. Among all the tested isolates, DT-7 consistently exhibited the strongest antifungal activity, suppressing *P. capsici*, *S. rolfsii* and *F. oxysporum*, by 73.0±0.49%, 70.1±1.97% and 66.8±4.89%, respectively, resulting in an overall mean inhibition of 69.9±3.10%. The other two effective isolates included DT-4 and AT-7 (overall mean inhibition: 62.8±6.61% and 61.0±0.40%, respectively). Analysis of organ-associated patterns revealed that inhibition of *S. rolfsii* was generally higher among root-derived endophytes, whereas *F. oxysporum* and *P. capsici* were more effectively suppressed by leaf-derived endophytes. Statistically significant differences among plant organs were detected only for *F. oxysporum*, with root- and leaf-associated isolates showing higher inhibition than stem-associated isolates ($F(2, 20) = 3.756$; $p = 0.041$). Molecular identification based on 16S rRNA gene sequencing revealed that isolate DT-7 belonged to the genus *Stutzerimonas*, closely related to the *Stutzerimonas stutzeri* group, whereas AT-7 and DT-4 clustered within the *Enterobacter* genus. The consistent and broad-spectrum antifungal activity of DT-7 suggested its potential as a promising biocontrol candidate as a biological resource for sustainable disease management. In addition, the observed organ-specific inhibition patterns underscored the ecologically structured endophyte-pathogen interactions within patchouli tissues, providing a basis for further mechanistic and greenhouse studies. This study represents an in vitro screening with limited replication (n = 2 plates per treatment), providing a preliminary basis for subsequent greenhouse and field validation.

Keywords: Antifungal activity, biological control, endophytic bacteria, *Fusarium oxysporum*, *Pogostemon cablin*

INTRODUCTION

Patchouli (*Pogostemon cablin* Blanco Benth.) is a leading aromatic plant widely cultivated in Southeast Asia for its essential oil, which is highly demanded in the fragrance and pharmaceutical industries (Nuryani et al. 2018; Directorate General of Estates 2024). Indonesia, particularly Aceh Province, is a major producer of patchouli oil, contributing significantly to the global supply (Ernawati et al. 2021). However, patchouli cultivation is frequently constrained by fungal diseases, such as basal stem rot, damping-off, and vascular wilt, caused by *Sclerotium rolfsii*, *Phytophthora capsici*, and *Fusarium oxysporum* (Zulham and Panggeso 2021).

Sclerotium rolfsii causes basal stem rot and damping-off by producing abundant sclerotia surviving for long periods in soil, manure, and infected plant residues, making chemical control inconsistent and costly (Zulham and Panggeso 2021). Symptoms include white mycelium on stem surfaces, scorched leaves, wilting and eventual plant death (Sukamto and Wahyuno 2013). *F. oxysporum* induces vascular wilt through xylem colonization, leading to progressive yellowing, stunting, and plant death, and is difficult to eradicate once established in fields (Habibi et al. 2018). *P. capsici* causes root and collar rot and severe damping-off, especially in humid nurseries and poorly drained soils (Fenta and Mekonnen 2024; Keloth et al. 2024). Together, these pathogens cause severe yield losses and reduce oil quality, posing a major threat to patchouli production (Köhl et al. 2019; Zulham and Panggeso 2021; Directorate General of Estates 2024). Their persistence in soil and ability to infect a wide range of hosts complicate disease management strategies, which traditionally rely on synthetic fungicides. However, excessive use of chemical inputs raises environmental and health concerns, while also fostering fungicide resistance in pathogen populations (Singh et al. 2020).

Biological control using endophytic bacteria has emerged as a sustainable and eco-friendly alternative for suppressing soil-borne pathogens. These bacteria reside in the internal tissues of host plants without causing any harm and offer protection to the plant using various strategies (Gupta et al. 2020; Al-Nadabi et al. 2021; Taulé et al. 2021; Mushtaq et al. 2023; Ali et al. 2024). Among them, Gram-

negative endophytic bacteria, such as *Pseudomonas* and related taxa, are of special interest due to their capacity to suppress pathogens through the production of diffusible and volatile antifungal metabolites (such as phenazines, pyrrolnitrin, and cyclic lipopeptides), secretion of cell-wall-degrading enzymes, siderophore-mediated competition for iron, and induction of plant systemic resistance (Afzal et al. 2019; Singh et al. 2020; Mengistu 2020; Compant et al. 2021; Sriwati et al. 2022; Ali et al. 2024).

Patchouli (*P. cablin*) itself possesses notable antifungal potential. Patchouli oil inhibited the growth of several fungal pathogens, including *Candida albicans* and *Trichophyton mentagrophytes* (Setyaningrum et al. 2017), while α-guaiene, a major constituent of patchouli oil, effectively suppressed *Microsporum gypseum* (Maulani et al. 2022). Endophytes frequently exhibit host-mimicry, enabling them to produce metabolites similar to those of their hosts through long-term biochemical interactions and selective pressures within plant tissues (Venieraki et al. 2017). Therefore, patchouli endophytes may represent an underexplored reservoir of antifungal agents.

Previous studies have demonstrated that patchouli-associated rhizobacteria exhibited antifungal activities against several soil-borne pathogens, including *P. capsici*, *S. rolfsii*, *F. oxysporum*, and *Synchytrium pogostemonis* (Sukamto et al. 2019). *Bacillus* sp. obtained from stems of *P. cablin* var. Sidikalang and Patchoulina potentially inhibited the growth of *Ralstonia solanacearum* and stimulated plant growth (Yuniawati and Akhdiya 2021). Despite these advances, investigations into endophytic bacteria, particularly in the stems and leaves, remain very limited. Most existing studies have focused on rhizosphere- or root-associated microbes (Cao et al. 2020), leaving the diversity and biocontrol potential of aerial-tissue endophytes largely unexplored.

In previous work, 23 endophytic bacterial isolates were obtained from the roots, stems, and leaves of Aceh patchouli cv. Tapaktuan that have not been identified for their biocontrol potentials (Husnah et al. 2026). Endophytic bacteria isolated from different organs of *P. cablin* might exhibit organ-dependent and pathogen-specific antifungal activity, with certain isolates showing broad-spectrum inhibition against major soil-borne pathogens. Therefore, this study aimed: (i) to evaluate the antifungal activity of isolated endophytic bacteria against *S. rolfsii*, *F. oxysporum*, and *P. capsici*; (ii) to examine organ-specific antagonistic patterns to infer endophyte-pathogen interactions; and (iii) to identify promising broad-spectrum candidates for future biocontrol development in patchouli cultivation.

MATERIALS AND METHODS

Endophytic bacterial isolates

A total of 23 endophytic bacterial isolates obtained previously from the root, stem, and leaf of Aceh patchouli (*P. cablin*) of Tapaktuan cultivar were used in this study. Before the antifungal assay, the bacteria were grown and regenerated on tryptic soy agar (TSA) for 48 h at 28°C. All bacterial isolates were maintained in the Microbiology Laboratory culture collection, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Indonesia.

Fungal pathogens

Three different phytopathogenic fungi, namely *S. rolfsii*, *F. oxysporum*, and *P. capsici* were used in this study. *S. rolfsii* was obtained from the collection of the Plant Pathology Laboratory (Vie Lab), East Java, Indonesia, while *F. oxysporum* was obtained from the Indonesian Culture Collection (IPBCC), Institut Pertanian Bogor, Indonesia. *P. capsici*, was provided by the Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency (BRIN). The fungi were cultured and maintained on potato dextrose agar (PDA) at 28°C prior to the assay. All fungal cultures were incubated in the dark and routinely subcultured every 7-10 days to maintain active growth.

Antifungal activity assay and mycelial growth inhibition

The antifungal activity of endophytic bacterial isolates was assessed using a dual culture method on TSA plates (90 mm in diameter) with minor modifications (Kunova et al. 2016; Flori et al. 2020). A 6-mm diameter mycelial plug of the test pathogens (*S. rolfsii*, *F. oxysporum*, or *P. capsici*), taken from the edge of an actively growing colony (7-day-old culture), was placed at the center of each plate. Similarly, two 6-mm plugs of actively growing endophytic bacterial isolates (48-h culture), previously maintained on TSA, were positioned on the left and right margins of the plate, equidistant from the fungal inoculum. Plates were then incubated at 28° for 5-7 days until the fungus had formed a colony that nearly covered the medium on control plates (no bacterial inoculation). The experimental setup is illustrated in Figure 1.

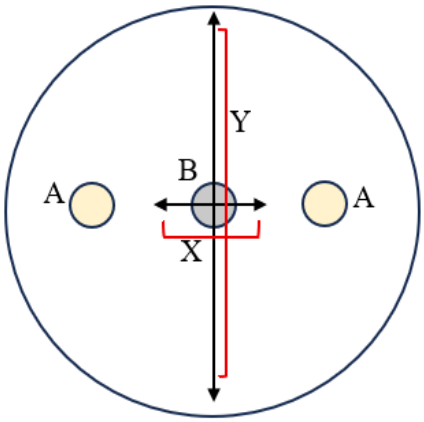

Figure 1. Schematic representation of the dual culture assay used to evaluate antifungal activity. A: endophytic bacterial colony, B: fungal colony, X: fungal colony radius toward the bacterial colony, Y: fungal colony radius in the free-growth direction without bacterial interference

Fungal growth was measured along two perpendicular axes: the vertical axis (Y), representing normal mycelial expansion without bacterial interference, and the horizontal axis (X), representing growth directed toward the bacterial colony. Each diameter was halved to obtain the corresponding radius. The percentage inhibition was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{(Y - X)}{Y} \text{ x } 100$$

Where: Y represents the fungal colony radius in the free-growth direction (without bacterial interference), and X shows the fungal colony radius in the direction of bacterial antagonism. This approach reflects the relative reduction in mycelial growth caused by the antagonistic activity of endophytic isolates. All assays were performed in duplicate, in which each isolate-pathogen combination was tested on two independent plates (biological replicates), and colony diameters were measured on each plate along two perpendicular axes. Thus, inhibition values represent the mean of two plates per treatment. The use of two plates per isolate-pathogen combination was intended for initial screening purposes, allowing rapid identification of promising antagonistic candidates rather than confirmatory efficacy assessment.

Molecular identification of selected endophytic bacteria

Molecular identification was performed for the three most active isolates (AT-7, DT-4, and DT-7) using 16S rRNA gene sequencing. These isolates were selected for molecular identification based on their consistently high and broad-spectrum antifungal activity across all tested pathogens. Genomic DNA was extracted using the Quick-DNA™ MagBead Plus Kit (Zymo Research, D4082) according to the manufacturer's instructions. Quantification of DNA concentration was performed with NanoDrop 2 000 Spectrophotometer (Thermo Scientific, USA).

Amplification of the 16S rRNA gene was performed with universal bacterial primers: 27F 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR reactions were set up in 25 µL final volumes with 12.5 µL My Taq HS Red Mix (2×) (Bioline, BIO-25048), 1 µL of each primer (10 µM), 1 µL genomic DNA template (5-100 ng), and nuclease-free water. Reaction cycling conditions were as follows: initial denaturation at 95°C for 1 min followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 52°C for 15 s, and extension at 72°C for 15 s, and final extension at 4°C. PCR products were confirmed using agarose gel electrophoresis and sequenced in both directions with Sanger sequencing.

The obtained sequences were assembled using a bioinformatics pipeline and compared with reference sequences available in the NCBI GenBank database using BLAST to determine their closest taxonomic relatives. Phylogenetic analysis was performed with MEGA software using the 16S rRNA gene sequences of the isolates and those of reference strains obtained from GenBank. A phylogenetic tree based on Neighbor-Joining algorithm with 1,000 bootstrap replications was generated to evaluate the reliability of the inferred phylogenetic tree. Taxonomic assignment was determined based on sequence similarity thresholds proposed by Stackebrandt and Goebel (1994).

Data analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS Statistics v26) and Microsoft Excel. Data are expressed as mean ± standard deviation (SD) and summarized in tables and figures. Normality (Shapiro-Wilk test) and equality of variances (Levene's test) were assessed before ANOVA. When the data followed normal distribution and homogeneity, one-way ANOVA followed by Duncan's multiple range test was used to determine difference in inhibition among organs. Conversely, when the assumptions were not met, the Kruskal-Wallis test followed by Mann-Whitney U post-hoc comparisons (with Bonferroni correction) was used. A p-value of ≤ 0.05 was considered statistically significant different.

RESULTS AND DISCUSSION

Morphological characteristics of endophytic bacteria

The morphological and Gram-staining characteristics of all 23 endophytic bacterial isolates were summarized in Table 1 with more detailed descriptions were reported in our previous work (Husnah et al. 2026). In general, all isolates exhibited circular colonies with entire margin, while variations were observed in elevation, pigmentation, and optical properties. The majority of isolates formed glistening and opaque colonies. Both cocci- and bacilli-shaped cells were observed, and Gram staining revealed predominantly Gram-positive bacteria.

Among these, the three most promising isolates (AT-7, DT-4, and DT-7), selected based on their strong antifungal activity, were further characterized in detail and are presented in Figure 2. These isolates produced creamy-white, circular colonies with smooth surfaces and entire margins after 48 hours of incubation on TSA at 28°C. Microscopic observations under 1000× magnification revealed that all isolates consistently exhibited a Gram-negative reaction. This interpretation was confirmed after calibration with Gram controls processed in parallel (*Staphylococcus aureus* as Gram-positive and *Escherichia coli* as Gram-negative), which verified that the isolates' staining pattern matched the Gram-negative reference. Morphologically, isolate AT-7 displayed coccus-shaped cells arranged singly or in small clusters, whereas DT-4 and DT-7 were rod-shaped, appearing singly or in pairs.

Table 1. The morphological characteristics of 23 endophytic bacteria isolated from patchouli

Isolates	Colony elevation	Colony texture	Optical property	Pigmentation	Gram staining	Cell shape
AT-01	Raised	Glistening	Opaque	Cream	+	Cocci
AT-02	Raised	Glistening	Opaque	Cream	+	Cocci
AT-03	Convex	Glistening	Opaque	Cream	+	Cocci
AT-04	Umbonate	Glistening	Opaque	Cream	+	Cocci
AT-05	Convex	Glistening	Opaque	Tan	-	Bacilli
AT-06	Convex	Glistening	Opaque	Cream	-	Cocci
AT-07	Convex	Dull	Opaque	Tan	-	Cocci
BT-01	Convex	Glistening	Opaque	Tan	+	Bacilli
BT-02	Pulvinate	Glistening	Opaque	Yellow	+	Bacilli
BT-03	Raised	Glistening	Opaque	Tan	+	Cocci
BT-04	Raised	Glistening	Opaque	Tan	+	Bacilli
BT-05	Raised	Glistening	Opaque	Yellow	+	Cocci
BT-06	Flat	Glistening	Translucent	Yellow	+	Cocci
BT-07	Raised	Glistening	Translucent	Cream	+	Cocci
BT-08	Convex	Glistening	Opaque	Tan	+	Bacilli
DT-01	Convex	Glistening	Translucent	Yellow	-	Bacilli
DT-02	Raised	Glistening	Opaque	Yellow	-	Bacilli
DT-03	Raised	Glistening	Opaque	Yellow	-	Bacilli
DT-04	Raised	Glistening	Opaque	Tan	-	Bacilli
DT-05	Raised	Glistening	Opaque	Tan	+	Bacilli
DT-06	Convex	Glistening	Translucent	Yellow	-	Bacilli
DT-07	Convex	Glistening	Opaque	Tan	-	Bacilli
DT-08	Convex	Dull	Opaque	Tan	+	Bacilli

Note: (+): Gram positive; (-): Gram negative

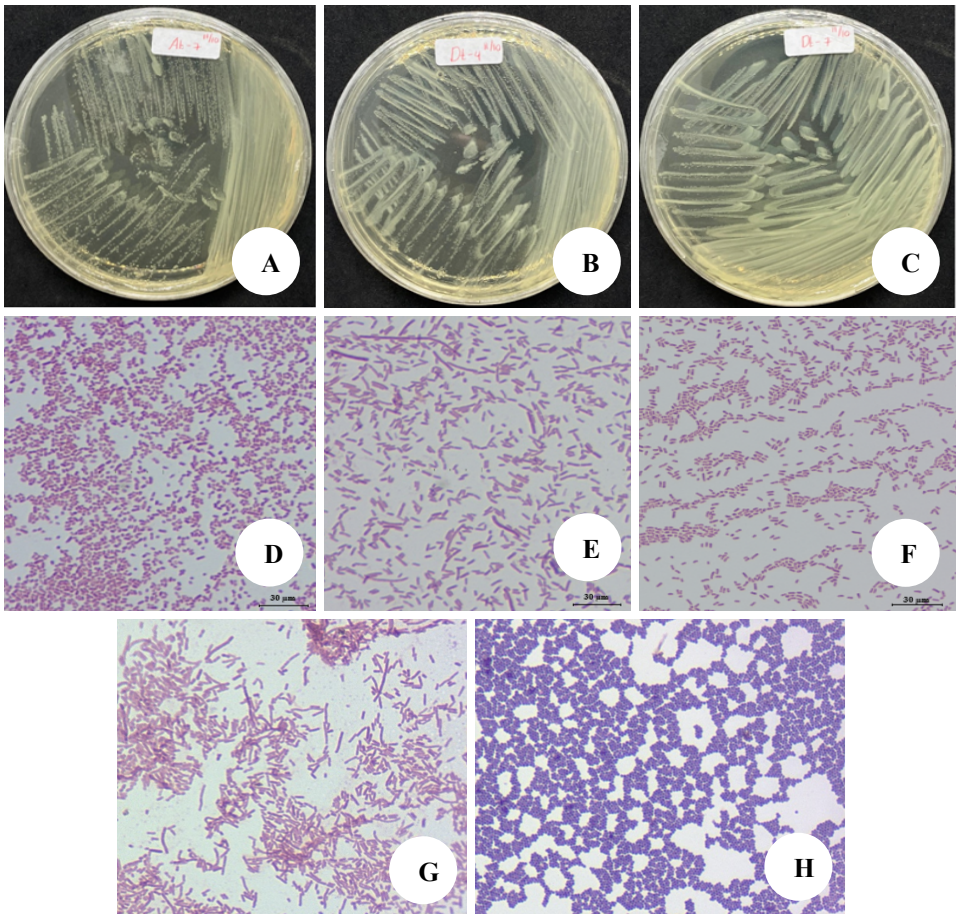

Figure 2. Morphological characteristics of bacterial endophyte isolates. A. Colony morphology of AT-7, B. Colony morphology of DT-4, and C. Colony morphology of DT-7, E. Gram staining reaction of AT-7, F. Gram staining reaction of DT-4, G. Gram staining reaction of DT-7, G. *Escherichia coli* (Gram-negative), H. *Staphylococcus aureus* (Gram-positive) as Gram control. Magnification 1000×. Scale bars: 30 µm

Antifungal activity of endophytic bacteria

Antifungal activities of individual endophytic bacterial isolates from *P. cablin* var. Tapaktuan against each pathogen is presented in Table 2. In this analysis, all 23 isolates were evaluated separately to determine their inhibitory effect on each fungal pathogen. All isolates exhibited measurable antagonistic activity, with average inhibition of mycelial growth ranging from 5.8±0.9 to 73.0±0.5%. Among individual isolates, DT-7 demonstrated the strongest inhibition across all pathogens, followed by DT-4 and AT-7. DT-7 also showed the highest pathogen-specific inhibition, particularly against *P. capsici* and *S. rolfsii* (Table 2; Figure 3). Overall, the highest-performing isolates were obtained from root and leaf tissues, indicating that these organs may harbor endophytes with stronger antifungal potential.

Antifungal activities based on the plant organ of origin

The antifungal activities of endophytic bacteria were further analyzed based on the plant organ of origin (root, stem, and leaf) to assess whether isolates from different tissues exhibited distinct antagonistic patterns against each pathogen. Numerically, different inhibition patterns were observed among organs, but these trends were not always statistically significant and depended on the fungal pathogen tested (Figure 4). For *S. rolfsii*, root-derived isolates showed the highest inhibition followed by stem and leaf. Conversely, inhibition of *F. oxysporum* and *P. capsici* was strongest for isolates from the leaf, followed by the root and stem. Statistical analysis using One-way ANOVA revealed that organ-related differences were not significant for *S. rolfsii* ($F(2, 20) = 0.659$; $p = 0.528$) and *P. capsici* ($F(2, 20) = 1.190$; $p = 0.325$) (Figure 4.A and Figure 4.C, respectively). However, a significant organ effect was detected for *F. oxysporum* ($F(2, 20) = 3.756$; $p = 0.041$), where leaf- and root-derived isolates showed higher inhibitory activity against the pathogen compared to those from stems (Figure 4.B).

Antifungal activities based on pathogens

To further interpret the overall antagonistic performance, the data were further analyzed by grouping all isolates (n = 23) based on the target pathogens, regardless of their plant organ of origin. This approach was used to compare the relative susceptibility of each pathogen to the endophytic bacterial community as a whole. The result revealed that antifungal activity of overall endophytic bacteria (irrespective of organ source) also displayed distinct patterns (Figure 5). A significant difference in percent inhibition among fungal species was detected by the Kruskal-Wallis analysis ($H(2) = 7.410$; $p = 0.025$). Among the three pathogens, *S. rolfsii* was the least susceptible, with mean inhibition of 46.6±18.4%. In contrast, markedly higher inhibition was observed against *F. oxysporum* (58.6±10.0%) and *P. capsici* (58.2±6.6%). Further analysis using the pairwise Mann-Whitney U test confirmed that the antagonistic activity of the isolates against *S. rolfsii* was significantly lower than that against *F. oxysporum* ($U = 165.0$; $Z = -2.186$; $p = 0.029$) and *P. capsici* ($U = 150.5$; $Z = -2.505$; $p = 0.012$). However, no significant difference in percent inhibition between *F. oxysporum* and *P. capsici* ($U = 247.0$; $Z = -0.385$; $p = 0.701$).

Table 2. Antifungal activity of endophytic bacteria from the root, stem, and leaf of *P. cablin* against *S. rolfsii*, *F. oxysporum*, and *P. capsici* (n = 23)

Endophytes	Percent inhibition of mycelial growth (%) (mean±SD)			Average (across all pathogens)
	Sclerotium rolfsii	*Fusarium oxysporum*	*Phytophthora capsici*	
AT-1	52.2±7.4	48.8±3.9	66.0±1.5	55.7±9.1
AT-2	56.0±0.7	61.8±2.8	53.5±2.1	57.1±4.3
AT-3	50.8±2.6	61.9±5.0	61.3±1.0	58.0±6.2
AT-4	55.2±11.2	67.5±1.8	50.5±0.4	57.7±8.8
AT-5	50.3±0.2	56.7±3.3	51.8±2.5	52.9±3.4
AT-6	31.8±3.0	62.6±2.5	59.5±5.0	51.3±17.0
AT-7	60.5±5.5	61.3±2.2	61.1±1.7	61.0±0.4
BT-1	12.7±8.0	49.3±2.9	55.5±0.7	39.2±23.1
BT-2	40.3±11.0	62.1±2.0	53.4±3.3	51.9±11.0
BT-3	58.2±6.8	47.1±3.4	57.3±2.8	54.2±6.1
BT-4	51.7±1.3	39.8±2.4	58.2±1.0	49.9±9.3
BT-5	48.4±0.6	35.9±2.5	59.7±0.7	48.0±11.9
BT-6	67.6±7.2	36.6±3.5	53.5±4.9	52.6±15.5
BT-7	55.5±0.4	65.2±4.2	61.9±3.5	60.9±4.9
BT-8	55.5±4.1	65.4±3.3	48.3±1.4	56.4±8.6
DT-1	48.3±10.7	61.6±0.6	61.7±1.6	57.2±7.7
DT-2	11.5±1.9	58.9±1.4	51.0±5.9	40.5±25.4
DT-3	66.3±0.9	47.9±0.4	57.4±1.6	57.2±9.2
DT-4	58.6±5.1	59.4±4.1	70.4±3.5	62.8±6.6
DT-5	16.3±1.5	65.4±7.6	56.1±2.0	46.0±26.0
DT-6	5.8±0.9	66.9±1.0	67.7±2.0	46.8±35.5
DT-7	70.1±2.0	66.8±4.9	73.0±0.5	69.9±3.1
DT-8	47.5±15.2	64.8±1.4	50.3±3.1	54.2±9.3
Average (towards each pathogen)	44.6±18.4	57.1±10.0	58.2±6.6	

Note: SD: standard deviation; AT: Tapaktuan roots; BT: Tapaktuan stems; DT: Tapaktuan leaves

Molecular identification of bacterial endophytes

Analysis of 16S rRNA gene sequences successfully identified the three most effective endophytic isolates. Based on the phylogenetic analysis (Figure 6), isolate AT-7 and DT-4 showed the highest sequence similarity to members of the genus *Enterobacter*, closely related to *Enterobacter sichuanensis* and *Enterobacter hormaechei,* respectively. Meanwhile, isolate DT-7 clustered within the *Stutzerimonas* group, showing high similarity to *Stutzerimonas stutzeri*.

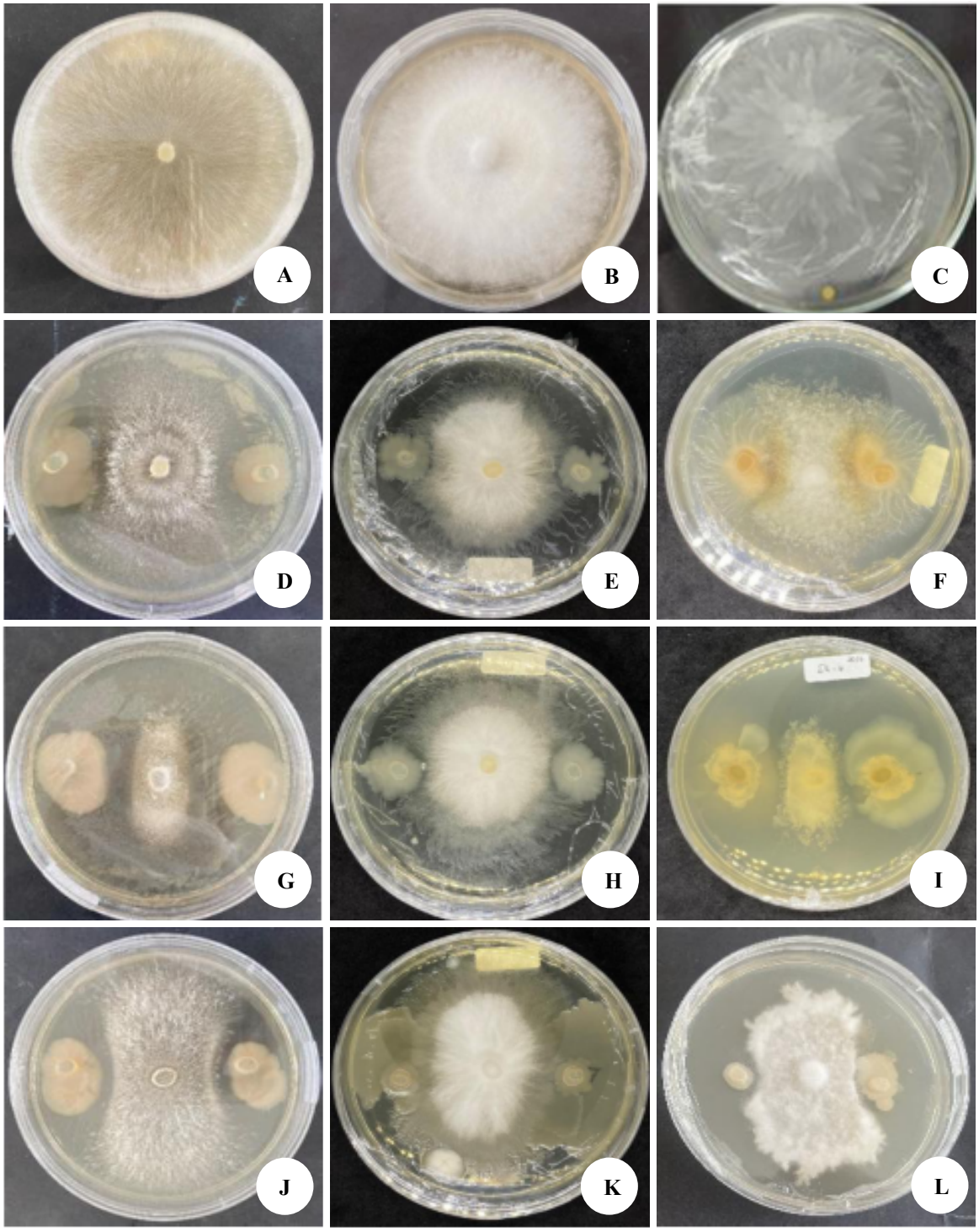

Figure 3. Antifungal activity on day 7 after incubation. A. Control against *S. rolfsii*, B. Control against *F. oxysporum*, C. Control against *P. capsici*, C. Isolate AT-7 against *S. rolfsii*, D. Isolate AT-7 against *F. oxysporum*, E. Isolate AT-7 against *P. capsici*, G. Isolate DT-4 against *S. rolfsii*, H. Isolate DT-4 against *F. oxysporum*, I. Isolate DT-4 against *P. capsici*, J. Isolate DT-7 against *S. rolfsii* K. Isolate DT-7 against *F. oxysporum* L. Isolate DT-7 against *P. capsici*. Diameter of plates: 9 cm

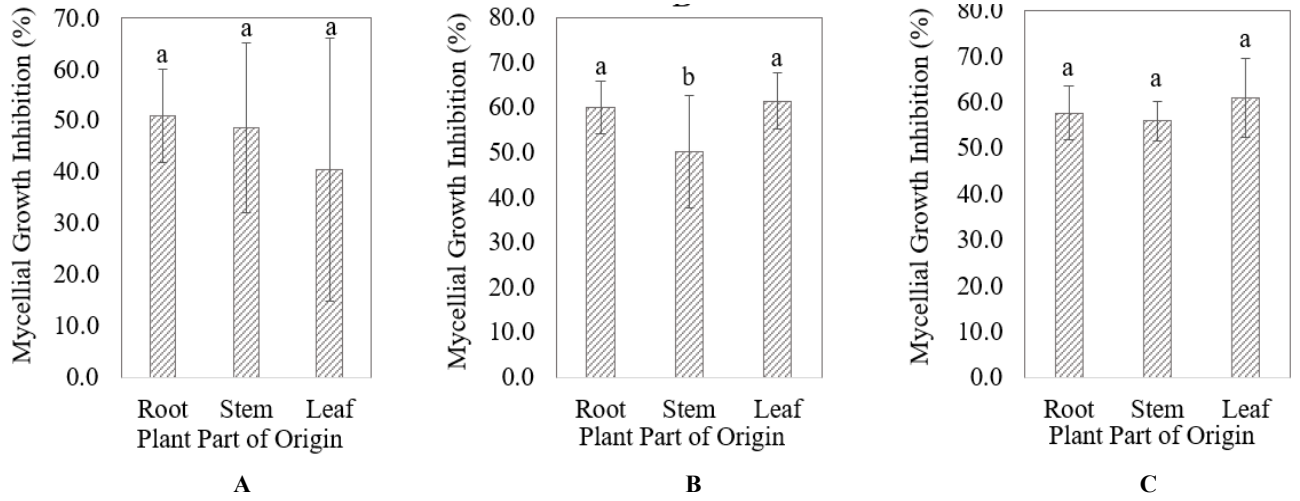

Figure 4. Mycelial growth inhibition (%) of three phytopathogenic fungi (A. *S. rolfsii*, B. *F. oxysporum*, C. *P. capsici*) by endophytic bacteria isolated from different plant organs (root, stem, and leaf) of *Pogostemon cablin* cv. Tapaktuan (n = 23). Bars represent mean±standard deviation. Different letters above the bars indicate significant differences among organs of origin according to ANOVA followed by Duncan's multiple range test ($p<0.05$)

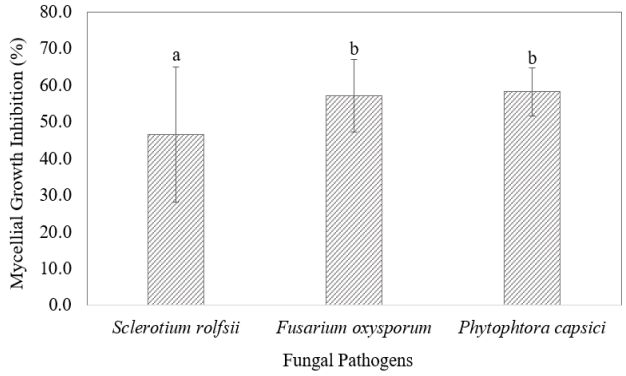

Figure 5. Antifungal activities of all endophytic bacteria (irrespective of plant organs) against *S. rolfsii*, *F. oxysporum*, and *P. capsici* (n = 23). Bars represent mean±standard deviation. Different superscript letters above the columns indicate significant differences among pathogens as determined by the Kruskal-Wallis test, followed by pairwise Mann-Whitney U tests ($p<0.05$)

Discussion

The results of this study demonstrated that patchouli (*P. cablin* cv. Tapaktuan) harbors diverse endophytic bacteria with significant antifungal activity against major soil-borne pathogens. All 23 isolates inhibited fungal growth in vitro with varying antagonistic capacity ranging from weak to strong (percent inhibition: 5.8±0.9 to 73.0±0.5%), indicating substantial functional diversity within the patchouli endosphere. Such variability is consistent with previous studies on medicinal plant-associated bacterial endophytes, where inhibition often differ markedly among isolates due to variation in secondary metabolite production and ecological adaptation (Erjaee et al. 2019; Al-Nadabi et al. 2021; Mushtaq et al. 2023; Ali et al. 2024). This diverse array of antagonistic performance among the endophytic bacteria might be influenced by specific isolate, plant organ of origin, or fungal target, supporting the concept of niche-specific functional specialization. Wu et al. (2021) suggested that endophyte communities are shaped by tissue-specific microhabitats and selective pressures that favor different defensive traits.

Among all isolates, isolate DT-7 exhibited the strongest and most consistent broad-spectrum activity, suppressing *S. rolfsii*, *F. oxysporum*, and *P. capsici* by 70.1±2.0%, 66.8±4.9%, and 73.0±0.5%, respectively, yielding an overall mean inhibition of 69.9±3.1%. Two additional isolates, DT-4 and AT-7, also showed high antagonistic potential, with mean inhibition values of 62.8±6.6% and 61.0±0.4%, respectively. This level of inhibition is comparable to that reported for effective biocontrol endophytes in other crops, particularly against *Fusarium* spp. (Kumar et al. 2021; Khanna et al. 2022), indicating their potential for further evaluation as biocontrol agents against soil-borne fungal pathogens in patchouli. The superior performance of these isolates was consistent with previous findings that certain endophytic bacteria, particularly those belonging to Proteobacteria, can exhibit broad-spectrum antifungal activity (Compant et al. 2021; Ali et al. 2024).

The results obtained from the study of plant organ origin revealed clear numerical trends emerged, but significance was pathogen-dependent. For *S. rolfsii*, isolates from the root showed higher inhibition, whereas for *F. oxysporum* and *P. capsici*, isolates from the leaf were more effective. Stem isolates, on the other hand, tended to exhibit lower inhibition compared to root- or leaf-associated isolates, particularly against *F. oxysporum* and *P. capsici*. This pattern is ecologically plausible because endophytes in roots and leaves occupy contrasting niches and experience different microbial competitors. Root endophytes are continuously exposed to soil-borne fungi and may therefore evolve targeted suppression against pathogens such as *S. rolfsii*, which persist as sclerotia in soil (Paparu et al. 2020; Keloth et al. 2024). In contrast, leaf endophytes face diverse aerial microbes and fluctuating humidity, potentially selecting for broader or more aggressive antifungal chemistry

effective against vascular wilt and oomycete pathogens such as *F. oxysporum* and *P. capsici*, respectively. Tissue-structured endophyte functions have been documented in multiple medicinal and crop hosts, supporting the view that organ microhabitats act as filters shaping defensive phenotypes (Wu et al. 2021). A relatively lower inhibition shown by stem isolates may indicate weaker selective pressure for antifungal persistence in stems, or a community might be oriented more toward growth-promotion traits than pathogen suppression, as also suggested in other plant systems (Kandel et al. 2017). Based on the findings, it can be stated that long-term microbial exposure and selective pressure in root and leaf tissues favor the persistence of endophytes with stronger antifungal traits, particularly against soil-borne and vascular pathogens.

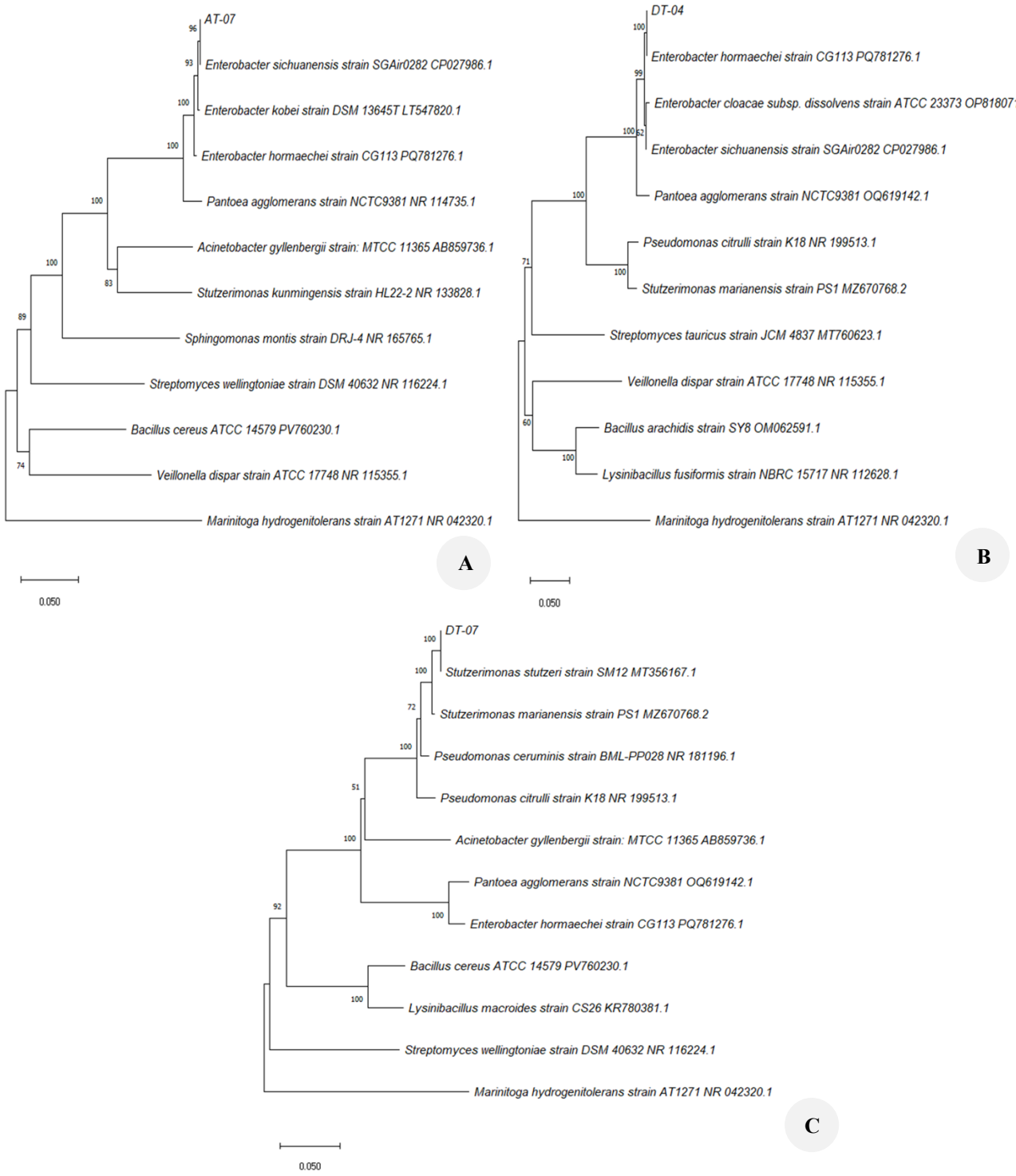

Figure 6. Phylogenetic tree based on 16S rRNA gene sequences showing the position of isolates A. AT-07, B. DT-04, and C. DT-07 among closely related bacterial taxa

Organ-structured endophyte functionality has been repeatedly observed across plant hosts, where roots typically select for competitively robust endophytes adapted to soil pathogen pressure, while leaves select for defense-oriented endophytes exposed to variable aerial microbiota (Liu et al. 2019). Stems often harbor intermediate or lower antagonistic activity because they are more buffered from direct pathogen exposure and act as conduits for endophyte movement. The patterns found in the present study, therefore fit a broader ecological framework of tissue-dependent endophyte specialization, strengthening the inference that patchouli organs represent distinct reservoirs of defensive microbiota.

Statistical analysis showed no significant difference in inhibition level among organs of origin towards *S. rolfsii* and *P. capsici*. In contrast, a significant organ effect was detected for *F. oxysporum*, where root- and leaf-derived isolates showed significantly stronger antagonistic activity than those from the stem. The significant contrast between stem-derived isolates and those from root and leaf against *F. oxysporum* likely reflects unequal selection pressures among plant organs. As a soil-borne vascular wilt fungus, *F. oxysporum* initiates infection through roots and subsequently colonizes xylem tissues, making the root endosphere a primary infection court where endophytes are repeatedly exposed to *Fusarium* challenge and intense soil microbial competition (Begum et al. 2025). These conditions are expected to favor the persistence of root endophytes with strong antagonistic capacities, including antibiosis, siderophore-mediated competition, and lytic enzyme production (Dita et al. 2018; Singh et al. 2020). Leaves, although not the initial entry point of *Fusarium*, experience frequent exposure to diverse aerial microbes and abiotic stresses, which can select for endophytes with broad-spectrum antifungal traits that are also effective against vascular pathogens (Kandel et al. 2017; Al-Nadabi et al. 2021; Wu et al. 2021). In contrast, stem tissues are relatively protected and often function as a transitional niche for endophyte movement within the plant, resulting in weaker pathogen-driven selection for high antifungal activity (Kandel et al. 2017). Consequently, root- and leaf-derived endophytes can display similarly high inhibition of *F. oxysporum* in vitro, whereas stem endophytes show significantly lower activity. Such patterns emphasized that the efficacy of endophytes is not only isolate-specific but also shaped by the ecological origin within the host plant and the pathogen types it combats. Moreover, leaf-associated microbiota may represent valuable sources of biocontrol agents for vascular wilt pathogens caused by *F. oxysporum*, aligning with recent reports of aerial endophytes exhibiting strong antifungal traits (Mohamad et al. 2020; Al-Nadabi et al. 2021).

The findings of antifungal activities based on pathogens demonstrated that both *F. oxysporum* and *P. capsici* were inhibited significantly more strongly than *S. rolfsii*, regardless of the organ source. The relatively lower suppression of *S. rolfsii* may be attributed to its ability to form sclerotia, robust survival structures that provide resilience against antifungal metabolites, which pose a well-known challenge in biological control (Paparu et al. 2020; Keloth et al. 2024). In contrast, the higher susceptibility of *F. oxysporum* and *P. capsici* is likely associated with general antifungal mechanisms reported for endophytic bacteria, such as antibiosis, siderophores-mediated competition, and enzymatic degradation, as described in previous studies (Shastri et al. 2020; Kumar et al. 2021; Khanna et al. 2022; Patel et al. 2024). The comparable inhibition levels observed between these two pathogens further suggest that the endophytes possess broad-spectrum antifungal mechanisms that are equally effective against multiple non-sclerotial fungi.

Isolate DT-7 consistently exhibited the highest and most stable inhibition against all tested pathogens, indicating its superior antifungal potential among the endophytic bacteria evaluated in this study. Molecular identification revealed that DT-7 belongs to the genus *Stutzerimonas*, most closely related to *S. stutzeri*, a lineage previously classified within the *Pseudomonas stutzeri* phylogenetic group (Gomila et al. 2022). Bacteria affiliated with the *S. stutzeri* group have increasingly been recognized as effective antifungal and biocontrol agents. Previous studies have reported that *S. stutzeri*-related strains suppress a wide range of phytopathogenic fungi, including *Bipolaris oryzae*, *Fusarium solani*, and *Aspergillus flavus*, through mechanisms such as volatile-mediated inhibition, production of antifungal metabolites, and competitive interactions (Gong et al. 2022; Zhao et al. 2022; Metwally et al. 2025). Although the specific antifungal mechanisms were not directly investigated in this study, the strong and broad-spectrum antifungal activity of DT-7 observed in the present study is presumably associated with functional traits commonly reported for members of *S. stutzeri*-related endophytic bacteria.

Isolates AT-7 and DT-4, on the other hand, were identified as members of the genus *Enterobacter*. These bacteria are commonly reported as plant-associated endophytes with antagonistic activity against soil-borne fungal pathogens. In patchouli-associated systems, an *Enterobacter* sp. has previously been shown to inhibit *S. rolfsii* and *F. oxysporum* in vitro through the biosynthesis of phenolic-related antifungal compounds (Sukamto et al. 2019). More broadly, *Enterobacter* endophytes isolated from medicinal plants have been reported to suppress multiple fungal pathogens through combined antibiosis and nutrient competition (Compant et al. 2021; Ali et al. 2024). In addition, medicinal-plant endophytes are known to co-evolve within metabolite-rich tissues and may contribute to or mimic host secondary metabolites, thereby enhancing antimicrobial functions that complement host defenses (Nguyen et al. 2023). In this study, the observed inhibition patterns of AT-7 and DT-4 were comparable to those reported for *Enterobacter* and other Gram-negative endophytic Proteobacteria, although the underlying mechanisms for these isolates remain to be elucidated and are proposed here as hypotheses requiring further validation.

These findings carry considerable implications for sustainable agriculture. Soil-borne pathogens such as *S. rolfsii*, *F. oxysporum,* and *P. capsici* are notorious for causing severe yield losses in economically important crops worldwide. Reliance on chemical fungicides is increasingly unsustainable due to resistance development, environmental contamination,

and food safety concerns (Köhl et al. 2019; De Mio et al. 2024; Fenta and Mekonnen 2024). Endophytic bacteria, particularly broad-spectrum antagonists like DT-7, represent promising eco-friendly candidates that could potentially be integrated into biocontrol programs or combined with other disease management practices. Their ability to colonize host tissues offers additional advantages, including stable associations with crops and long-term protection against pathogens. In practice, DT-7 (alone or formulated with complementary strains such as DT-4 or AT-7) could be further explored for potential deployment as a nursery or seedling bioinoculant to improve stand establishment, followed by soil or foliar applications at transplanting to stimulate plant growth and defense mechanisms against pathogens. For practical biocontrol development, DT-7 could be advanced through a stepwise validation process. Collectively, these findings provide a strong foundation for future mechanistic studies and greenhouse validation toward sustainable disease management strategies.

This study possesses several limitations that should be acknowledged. First, inhibition was assessed only in vitro using a dual-culture assay, which did not fully represent complex rhizosphere and in planta interactions. Such assays were essential for screening but may over- or under-estimate effectiveness under complex rhizosphere conditions. Second, although key isolates were successfully identified at the genus and species levels based on 16S rRNA sequencing, deeper functional characterization of antifungal metabolites and colonization dynamics remains to be addressed. Third, replication was limited to two plates per isolate-pathogen combination, which is acceptable for a screening study but should be expanded for efficacy validation. These limitations aligned with common constraints in early biocontrol discovery and reinforce the need for follow-up work. Despite these limitations, the findings provide a reliable preliminary basis for the identification of promising biocontrol candidates, especially against soil-borne fungal pathogens, and support further validation under greenhouse and field conditions.

In conclusion, this study demonstrated that endophytic bacterial communities of patchouli (*Pogostemon cablin* cv. Tapaktuan) are functionally diverse and possess in vitro activity against several important soil-borne fungal pathogens. In terms of antagonistic activity against these pathogens, activity differed by isolate as well as showing patterns of organ-specificity and pathogen selectivity, with isolates originating from leaves and roots displaying the greatest suppression activity against *F. oxysporum and P. capsici* in particular. Specifically, isolate DT-7 (*Stutzerimonas* sp., closely related to the species group *S. stutzeri*) exhibited the greatest broad-range antifungal activity of the 23 isolates tested, followed by two *Enterobacter* isolates DT-4 and AT-7. Although these initial results are limited by low replication inherent to dual-culture screening assays, they nonetheless suggest an ecological structure to the interaction between endophytic bacteria and target pathogens within patchouli plants. With this in mind, DT-7 proved to be a promising biocontrol candidate and should serve as a solid foundation for future mechanistic studies as well as greenhouse and field trials for biocontrol of patchouli diseases.

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