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CLUSTER ANALYSIS OF ENDOPHYTIC BACTERIA IN PATCHOULI (*POGOSTEMON CABLIN* BENTH.) CULTIVARS: INSIGHTS INTO PLANTS AND MICROBIAL RELATIONSHIPS

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SUMMARY

The presented study investigated the diversity and phenetic relationships of endophytic bacterial communities isolated from three patchouli (*Pogostemon cablin* Benth.) cultivars found in Aceh, Indonesia, viz., Tapaktuan, Sidikalang, and Lhokseumawe. The obtained isolates totaled 77, coming from roots, stems, and leaves of the patchouli cultivars and entailing characterization based on morphological features. These include colony shape, margin, elevation, appearance, optical property, pigmentation, Gram reaction, and cell shape. The results demonstrated considerable morphological diversity across cultivars and plant organs, with Lhokseumawe exhibiting the highest diversity of isolates. Most isolates were Gram-positive, distributed mainly in the stem tissues. Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) and the Numerical Taxonomy System (NTSYS)-pc software revealed no distinct grouping of isolates by plant cultivar and its parts. However, organ-specific tendencies were evident. Cluster I consisted mostly of root-derived isolates (15 out of 27 isolates, 55.6%) (non-pigmented Gram-positive cocci), whereas cluster II comprised predominantly of stem- and leaf-associated bacteria (brighter-pigmented Gram-positive bacilli and Gram-negative bacilli, respectively). Although morphology-based traits provided useful preliminary insights, their limitations underscored the need for molecular approaches for accurate characterization. Overall, these findings offer a valuable basis for harnessing native endophytes in sustainable patchouli cultivation and disease management.

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Keywords: Patchouli (*P. cablin* Benth.), cultivars, endophytic bacteria, isolates, cluster analysis, morphological features, microbial diversity, organ-specific tendencies

Key findings: The 77 endophytic bacterial isolates obtained from three patchouli (*P. cablin* Benth.) cultivars were morphologically diverse and did not cluster strictly by plant cultivar and plant parts. However, organ-specific tendencies were notable. The findings highlighted the limitations of morphological traits for bacterial classification and suggested that organ-specific microenvironments influence the diversity of bacterial endophytes.

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) is a commercially important aromatic plant known for its essential oil, with a wide use by the fragrance and pharmaceutical industries (Directorate General of Estates, 2022–2024). In Indonesia, particularly in Aceh Province, various local cultivars of patchouli, such as Tapaktuan, Sidikalang, and Lhokseumawe, undergo cultivation, with each observed with unique morphological, chemical, and ecological characteristics (Nuryani *et al.*, 2018). The varietal variations may be partly due to the influence of both environmental factors and their associated microbial communities, particularly endophytic bacteria that inhabit internal plant tissues without causing harm to the plants (Mengistu, 2020; Raihannisa *et al.*, 2024).

Endophytic bacteria isolation can proceed from different parts of the patchouli plants, including the roots and stems, with varying population densities, depending on the plant variety and location. Halimursyadah *et al*. (2021) reported the presence of diverse rhizobacteria isolates from a patchouli plantation in Nagan Raya, Aceh, Indonesia, including *Necercia* sp., *Pseudomonas cepacia*, *Bacillus firmus*, *Bacillus badius*, *Bacillus alvei*, and *Bacillus stearothermophilus*, with potential plant growth-promoting properties. Another study successfully isolated bacterial endophytes from patchouli stems of Sidikalang and Patchoulina cultivars, mostly representing the genus of *Bacillus*, exhibiting similar properties to the root-associated isolates (Yuniawati and Akhdiya, 2021). However, information on the diversity of endophytes in different *P. cablin* cultivars, such as Tapaktuan and Lhokseumawe, particularly those from the leaves, remains scarce.

Endophytic bacteria play a crucial role in plant health, growth, and development; suppress the population of root nematode (*Pratylenchus brachyurus*); enhance stress tolerance; and provide protection against various pathogens, boosting the patchouli oil yield and the availability of essential soil nutrients (Taulé *et al.*, 2021; Mushtaq *et al.*, 2023). Endophytic bacteria can also promote plant growth through various mechanisms. They can produce phytohormones, which are plant growth regulators, and augment essential soil nutrients' availability (Wu *et al*., 2021). This can result in increased plant fresh weight and overall productivity. By harnessing the beneficial effects of these bacteria, farming communities can potentially improve patchouli yields and oil quality in a more environmentally friendly way.

However, the presence of specific endophytic bacterial communities within plants may vary based on host genotype, tissue types, and the existing environmental conditions (Papik *et al.*, 2020). Therefore, it is crucial to understand how the distribution of these bacteria occurs across different patchouli cultivars and their plant parts, which can offer valuable insights into plant-microbe interactions and potential applications in agriculture. Furthermore, it could also provide early insight into the genetic relationship between plant cultivars, since plant genetics influences the endophytic microbes (Pinski *et al.*, 2019; Wu *et al.*, 2021).

The following study aimed to characterize the morphological diversity of endophytic bacterial isolates from three patchouli cultivars and analyze their phenetic relationship using cluster analysis. By applying the Numerical Taxonomy System (NTSYS), the investigations explored whether endophyte clustering acquired considerable influences more from the plant cultivar or the specific plant parts, such as roots, stems, and leaves. This approach offers novel insights into the organ-specific distribution of bacterial endophytes in patchouli and lays the groundwork for their future application in plant-growth promotion and biocontrol strategies.

MATERIALS AND METHODS

Sample collection

Collection of healthy plant samples of three different patchouli (*P. cablin* Benth.) cultivars, viz., Tapaktuan, Lhokseumawe, and Sidikalang, aged 6–7 months, came from Lhong, Aceh Besar District, Aceh, Indonesia (5°15′22.4″N, 95°16′00.9″E). Samples of fresh roots, stems, and green leaves (10 g each), when collected, used a sterile knife before storing in plastic bags for their processing within 24 hours. The stem samples included the main stem and branches, whereas the root encompassed the primary and lateral roots to ensure representative sampling. The samples remained in a chamber with ice to maintain freshness. The samples' identification employed a taxonomist at the Department of Biology, Syiah Kuala University (USK) (identification number: 759/UN11.F8.4/TA.00.03/2025), before being deposited in the Acehense Herbarium.

Sample preparation and surface sterilization

The plant samples incurred thorough washing under running water, then air-drying on tissue paper before being subjected to surface sterilization. The plant parts entailed cutting into smaller pieces (±4 cm) and surface sterilization by immersion in 5% sodium hypochlorite (NaOCl) for 5 min. After being rinsed with distilled water, the samples' immersion in 70% ethanol took 5 min, followed by subsequent rinsing three times with sterile distilled water. Surface sterility verification ensued by inoculating 100 µL of the final rinse water onto tryptic soy agar (TSA) medium and incubating it at 28 °C for 72 h (Cardoso *et al.*, 2020).

Isolation and purification of endophytic bacteria

The isolation of endophytic bacteria commenced by grinding 1 g of each plant part in a mortar and diluting it with sterile distilled water to a dilution factor of 10^{-2}. A 100 µL suspension from each dilution reached inoculation onto TSA medium supplemented with 30 µL/mL nystatin as an antifungal agent. The plates received incubation at 28 °C for 72 h, with daily observation. The selection of colonies exhibiting distinct morphological characteristics succeeded, followed by their purification on TSA medium using the quadrant streaking technique. Pure isolates culturing continued, followed by partially storing (48–72 hours old) in 10% sterile glycerol solution (750 µL in microtubes) at -20 °C. Additionally, isolates also attained storage on a slant TSA medium at 4 °C for short-term preservation until used (Nxumalo *et al.*, 2020).

Morphological identification of bacterial isolates

Data on the morphological characteristics of endophytic bacterial colonies, including colony shape, margin, elevation, color, and appearance, underwent recording. Microscopic observations, such as Gram staining and bacterial cell shape, also received documentation. The isolates bore codes based on their distinct morphological features (Whitman *et al.*, 2015).

Construction of the morphological characterization matrix

Categorical traits assigned into numerical codes transpired for uniform data representation. These traits included colony shapes (circular: 1; irregular: 2; and circular with raised edge: 3), margins (entire: 1; scalloped: 2; ciliate: 3; undulate: 4; filiform: 5), elevations (flat: 1; raised: 2; and convex [convex, umbonate, and pulvinate]: 3), pigmentation (non-pigmented [cream, gray, and beige]: 1; tan: 2; and bright hues [yellow, orange, pink, and peach]: 3), appearance (glistening: 1; dull: 2); optical property (translucent: 1; opaque: 2), Gram staining reactions (positive: 1; negative: 2), and bacterial cell shapes (cocci: 1; bacilli: 2; coccobacilli: 3). The said data's compilation formed a matrix for comparative analysis. Each row in the matrix represented the bacterial isolates, while each column corresponded to a specific morphological characteristic (Rohlf, 2005).

Cluster analysis and dendrogram construction using NTSYS

The compiled morphological data underwent phenetic analysis using the NTSYS-pc software (version 2.10e). The simple matching coefficient (SMC), as employed, quantified the morphological resemblance among the isolates. The similarity matrix generation proceeded from the encoded dataset, with the unweighted pair group method with arithmetic mean (UPGMA) applied to construct a dendrogram illustrating the relationship among the isolates based on morphological similarity (Rohlf, 2005).

Statistical analysis

Conducting all analyses used the NTSYS-pc software (version 2.10e). The software facilitates the computation of similarity indices, clustering, and dendrogram construction. The resulting dendrogram gained analysis to identify clusters, with the distribution of isolates within clusters examined to determine potential relationships based on plant cultivar (Tapaktuan, Sidikalang, and Lhokseumawe) and plant parts such as roots, stems, and leaves.

RESULTS AND DISCUSSION

Bacterial endophytes

Bacterial endophytes obtained totaled 77 from three different cultivars of patchouli: Tapaktuan (23 isolates), Sidikalang (27 isolates), and Lhokseumawe (27 isolates), found in Aceh Province, Indonesia. Based on plant organs, 22 isolates came from the roots, 32 from the stems, and 23 from the leaves. Morphological characterization revealed most of the endophytic bacterial colonies were circular with entire margins and exhibited convex or raised elevations (Tables 1–3). However, some bacterial colonies were flat, umbonate, and pulvinate. Colony pigmentation ranged from neutral tones (cream, gray, beige, and tan) to bright hues, such as yellow, peach, pink, and orange, with tan as the most dominant. Microscopically, the isolates displayed diverse cell morphologies, including cocci, bacilli, and coccobacilli, particularly found among the isolates collected from the patchouli cultivars Sidikalang and Lhokseumawe. These results highlighted diverse morphological patterns among the endophytic bacterial isolates of the Aceh patchouli.

Gram staining showed a predominance of Gram-positive bacteria (44 isolates, 57.1%), particularly obtained from the stems of Tapaktuan and Sidikalang cultivars (Tables 1 and 2). This finding was in line with a previous study report, suggesting a prevalence of *Bacillus* spp. in the stems of Sidikalang and Patchoulina cultivars (Yuniawati and Akhdia, 2021). The predominance of Gram-positive forms in stems may be in relation to their thicker cell walls, which confer resistance to oxidative stress and desiccation in aerial tissues (Li *et al*., 2022). This adaptation enhances their survival in environments with fluctuating moisture and exposure to UV light. Ecologically, such resilience proposes that stem-associated Gram-positive endophytes (e.g., *Bacillus* spp. and *Staphylococcus*) can play key roles in producing antifungal metabolites and enhancing host defense (Emitaro *et al*., 2024).

Table 1. Morphological characteristics of endophytic bacteria derived from the cultivar Tapaktuan of *P. cablin*.

Isolate	Macroscopic							Microscopic	
	Shape	Margin	Elevation	Appearance	Optical Property	Pigmentation*	Pantone Code	Gram	Cell Shape
AT-01	Circular	Entire	Raised	Glistening	Opaque	Cream	PMS 453	+	Cocci
AT-02	Circular	Entire	Raised	Glistening	Opaque	Cream	PMS 454	+	Cocci
AT-03	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 454	+	Cocci
AT-04	Circular	Entire	Umbonate	Glistening	Opaque	Cream	PMS 460	+	Cocci
AT-05	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 4755	-	Bacilli
AT-06	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 468	-	Cocci
AT-07	Circular	Entire	Convex	Dull	Opaque	Tan	PMS 155	-	Cocci
BT-01	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 466	+	Bacilli
BT-02	Circular	Entire	Pulvinate	Glistening	Opaque	Yellow	PMS 458	+	Bacilli
BT-03	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 4665	+	Cocci
BT-04	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 4675	+	Bacilli
BT-05	Circular	Entire	Raised	Glistening	Opaque	Yellow	PMS 461	+	Cocci
BT-06	Circular	Entire	Flat	Glistening	Translucent	Yellow	PMS 106	+	Cocci
BT-07	Circular	Entire	Raised	Glistening	Translucent	Cream	PMS 467	+	Cocci
BT-08	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 4545	+	Bacilli
DT-01	Circular	Entire	Convex	Glistening	Translucent	Yellow	PMS 102	-	Bacilli
DT-02	Circular	Entire	Raised	Glistening	Opaque	Yellow	PMS 121	-	Bacilli
DT-03	Circular	Entire	Raised	Glistening	Opaque	Yellow	PMS 107	-	Bacilli
DT-04	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 468	-	Bacilli
DT-05	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 4685	+	Bacilli
DT-06	Circular	Entire	Convex	Glistening	Translucent	Yellow	PMS 128	-	Bacilli
DT-07	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 481	-	Bacilli
DT-08	Circular	Entire	Convex	Dull	Opaque	Tan	PMS 489	+	Bacilli

* Colony color determination used the Pantone Matching System (PMS) through visual comparison with the Pantone Color Chart (https://www.cal-print.com/InkColorChart.htm).

Table 2. Morphological characteristics of endophytic bacteria derived from the cultivar Sidikalang of *P. cablin*.

Isolate	Macroscopic							Microscopic	
	Shape	Margin	Elevation	Appearance	Optical Property	Pigmentation*	Pantone Code	Gram	Cell Shape
AS-01	Circular	Entire	Pulvinate	Glistening	Opaque	Tan	PMS 4685	-	Cocci
AS-02	Circular	Entire	Pulvinate	Glistening	Opaque	Tan	PMS 155	-	Bacilli
AS-03	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 4545	+	Cocci
AS-04	Circular	Entire	Raised	Glistening	Opaque	Cream	PMS 482	+	Cocci
AS-05	Circular	Scalloped	Raised	Glistening	Opaque	Yellow	PMS 1365	-	Cocci
AS-06	Circular	Ciliate	Raised	Glistening	Opaque	Tan	PMS 4685	-	Cocci
AS-07	Irregular	Scalloped	Raised	Dull	Opaque	Cream	PMS 568	-	Cocci
AS-08	Circular	Entire	Pulvinate	Glistening	Opaque	Yellow	PMS 1215	-	Cocci
BS-01	Circular	Entire	Convex	Dull	Opaque	Cream	Cool gray 1	-	Bacilli
BS-02	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 4685	+	Bacilli
BS-03	Irregular	Undulate	Flat	Dull	Opaque	Cream	PMS 454	+	Cocci
BS-04	Circular	Entire	Flat	Dull	Opaque	Tan	PMS 4685	+	Bacilli
BS-05	Circular	Entire	Convex	Glistening	Opaque	Yellow	PMS 134	+	Cocci
BS-06	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 148	+	Bacilli
BS-07	Circular	Entire	Flat	Dull	Opaque	Cream	PMS 454	+	Cocci
BS-08	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 454	+	Bacilli
BS-09	Circular	Entire	Raised	Glistening	Opaque	Yellow	PMS 123	+	Cocci
BS-10	Circular	Entire	Flat	Glistening	Opaque	Tan	PMS 4745	+	Cocci
BS-11	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 4685	+	Bacilli
BS-12	Circular	Entire	Raised	Glistening	Translucent	Tan	PMS 155	+	Cocci
BS-13	Circular	Entire	Pulvinate	Glistening	Opaque	Tan	PMS 5035	+	Coccobacilli
DS-01	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 454	+	Cocci
DS-02	Circular	Entire	Convex	Glistening	Opaque	Pink	PMS 503	-	Bacilli
DS-03	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 155	+	Cocci
DS-04	Circular	Undulate	Flat	Glistening	Opaque	Orange	PMS 130	+	Bacilli
DS-05	Circular	Entire	Convex	Glistening	Opaque	Pink	PMS 178	+	Bacilli
DS-06	Circular	Entire	Convex	Glistening	Opaque	Orange	PMS 1555	+	Bacilli

* Colony color determination used the Pantone Matching System (PMS) through visual comparison with the Pantone Color Chart (https://www.cal-print.com/InkColorChart.htm).

Table 3. Morphological characteristics of endophytic bacteria derived from the cultivar Lhokseumawe of *P. cablin*.

Isolate	Macroscopic							Microscopic	
	Shape	Margin	Elevation	Appearance	Optical Property	Pigmentation*	Pantone Color	Gram	Cell Shape
AL-01	Circular	Entire	Flat	Glistening	Opaque	Tan	PMS 149	-	Cocci
AL-02	Circular	Entire	Flat	Glistening	Translucent	Beige	PMS 481	-	Cocci
AL-03	Circular	Entire	Flat	Dull	Translucent	Gray	Warm gray 2	+	Bacilli
AL-04	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 155	-	Cocci
AL-05	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 155	+	Bacilli
AL-06	Circular	Entire	Convex	Glistening	Opaque	Tan	PMS 148	+	Coccobacilli
AL-07	Circular	Entire	Flat	Glistening	Opaque	Gray	PMS 400	-	Cocci
BL-01	Circular with raised edge	Entire	Flat	Dull	Opaque	Cream	PMS 454	+	Bacilli
BL-02	Circular with raised edge	Entire	Convex	Glistening	Opaque	Yellow	PMS 134	+	Bacilli
BL-03	Circular with raised edge	Entire	Flat	Opaque	Opaque	Cream	PMS 4535	-	Bacilli
BL-04	Circular with raised edge	Entire	Flat	Glistening	Translucent	Yellow	PMS 123	-	Cocci
BL-05	Circular	Entire	Flat	Glistening	Opaque	Cream	PMS 155	-	Bacilli
BL-06	Circular	Entire	Flat	Glistening	Opaque	Peach	PMS 162	-	Bacilli
BL-07	Circular	Entire	Flat	Dull	Opaque	Cream	PMS 454	-	Bacilli
BL-08	Circular	Entire	Convex	Glistening	Opaque	Beige	PMS 434	-	Bacilli
BL-09	Circular	Entire	Convex	Glistening	Opaque	Yellow	PMS 135	+	Cocci
BL-10	Circular	Entire	Raised	Glistening	Opaque	Orange	PMS 1575	+	Coccobacilli
BL-11	Circular	Entire	Raised	Glistening	Opaque	Yellow	PMS 1215	-	Bacilli
DL-01	Circular	Entire	Raised	Glistening	Opaque	Tan	PMS 482	+	Bacilli
DL-02	Circular	Entire	Umbonate	Glistening	Opaque	Tan	PMS 489	-	Bacilli
DL-03	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 482	+	Cocci
DL-04	Circular with raised edge	Entire	Raised	Glistening	Opaque	Tan	PMS 4755	-	Bacilli
DL-05	Circular	Entire	Convex	Glistening	Translucent	Yellow	PMS 1225	+	Bacilli
DL-06	Circular	Entire	Convex	Glistening	Translucent	Tan	PMS 149	+	Bacilli
DL-07	Circular	Entire	Convex	Glistening	Translucent	Tan	PMS 148	-	Bacilli
DL-08	Circular	Entire	Convex	Glistening	Opaque	Cream	PMS 454	-	Bacilli
DL-09	Circular	Entire	Convex	Dull	Opaque	Gray	Cool gray1	-	Bacilli

* Colony color determination used the Pantone Matching System (PMS) through visual comparison with the Pantone Color Chart (https://www.cal-print.com/InkColorChart.htm).

Although Gram-positive endophytes were dominant, Gram-negative bacteria were also prevalent across various plant parts, mainly in the roots of Sidikalang and the leaves of Tapaktuan cultivars. In fact, in contrast to our findings, previous studies suggested a predominance of Gram-negative bacteria in patchouli plants. Shi *et al*. (2024) reported the abundance of *Pseudomonas* spp. in the roots, stems, and leaves of patchouli plants growing in Guangdong, China. Another study showed the presence of *Pseudomonas putida* in the rhizosphere of patchouli plants in Jorhat, India, showing antibacterial and growth-promoting activities (Borah *et al*., 2018). Other studies on soft tissues of different aromatic plants also reported Gram-negative dominance in the leaves (Maulidia *et al.*, 2021; Mahlangu and Tai, 2022). The occurrence of Gram-negative isolates in roots might be due to their metabolic flexibility and stronger interaction with root exudates, which provide a nutrient-rich microenvironment conducive for colonization (Cordovez *et al*., 2019).

Such differences in bacterial distribution patterns among patchouli plants may arise from cultivar-specific traits, host genotypic factors, and accession history. Likewise, these could be due to existing environmental conditions, such as soil types and climates, across different regions that modulate bacterial colonization and their survival (Cordovez *et al.*, 2019; Malacrinò *et al.*, 2022). In Aceh, the relatively humid environment and diverse soil microbiota may have influenced the observed distribution. The differences also highlight that plant genotype interacts with ecological context to shape endophytic communities.

Cluster analysis of endophytic bacteria

The dendrogram generated using NTSYS based on morphological characteristics of 77 isolates from the roots, stems, and leaves of three patchouli cultivars (Tapaktuan, Sidikalang, and Lhokseumawe) did not exhibit distinct clustering patterns from plant cultivars and organs (Figure 1). Isolates scattering across the dendrogram indicated that phenotypic traits alone were insufficient to delineate consistent clusters. These results aligned with previous findings regarding the limitations of morphology-based classification for bacterial identification and clustering (Sousa *et al.*, 2013; Hugerth and Andersson, 2017). Additionally, the bacterial phenotypes are also typically highly plastic and can vary significantly due to environmental factors, such as nutrient availability, pH, and host-microbe interactions (Van-Teeseling *et al.*, 2017). In endophytic contexts, where microenvironments can differ substantially across the tissues, this plasticity was even more noticeable and likely contributes to the absence of well-defined clustering (Cordovez *et al.*, 2019).

With the similarity coefficient of 0.57, the isolates underwent division into two major clusters: Cluster I (27 isolates) and Cluster II (42 isolates). However, a few isolates from the cultivars Sidikalang (AS-07, BS-05, and DS-04) and Lhokseumawe (AL-03, BL-01, BL-04, BL-07, and DL-09) remained ungrouped, being categorized as outliers. These morphologically divergent isolates exhibited unique combinations of colony shapes, pigmentation, and cell forms, suggesting the highest degree of intravarietal phenotypic diversity. This phenomenon may result from fine-scale environmental heterogeneity, organ-specific microenvironments, and stochastic colonization events, as also reported in previous studies (Papik *et al.*, 2020; Compant *et al.*, 2021; Huang *et al.*, 2024).

Interestingly, although overall cluster grouping did not correlate with either plant cultivar or the organs, the closer examination of each cluster revealed organ-specific tendencies. Dominating Cluster I were the root-derived isolates (15 out of 27 isolates, 55.6%), characterized mainly by non-pigmented (cream-colored) colonies and Gram-negative cocci (Figure 2). The results suggested a more selective ecological niche in the rhizosphere, potentially influenced by root exudates and reduced environmental fluctuations. In contrast, Cluster II contained more stem- (19 out of 42 isolates, 45.2%) and leaf-derived (18 out of 42 isolates, 42.9%)

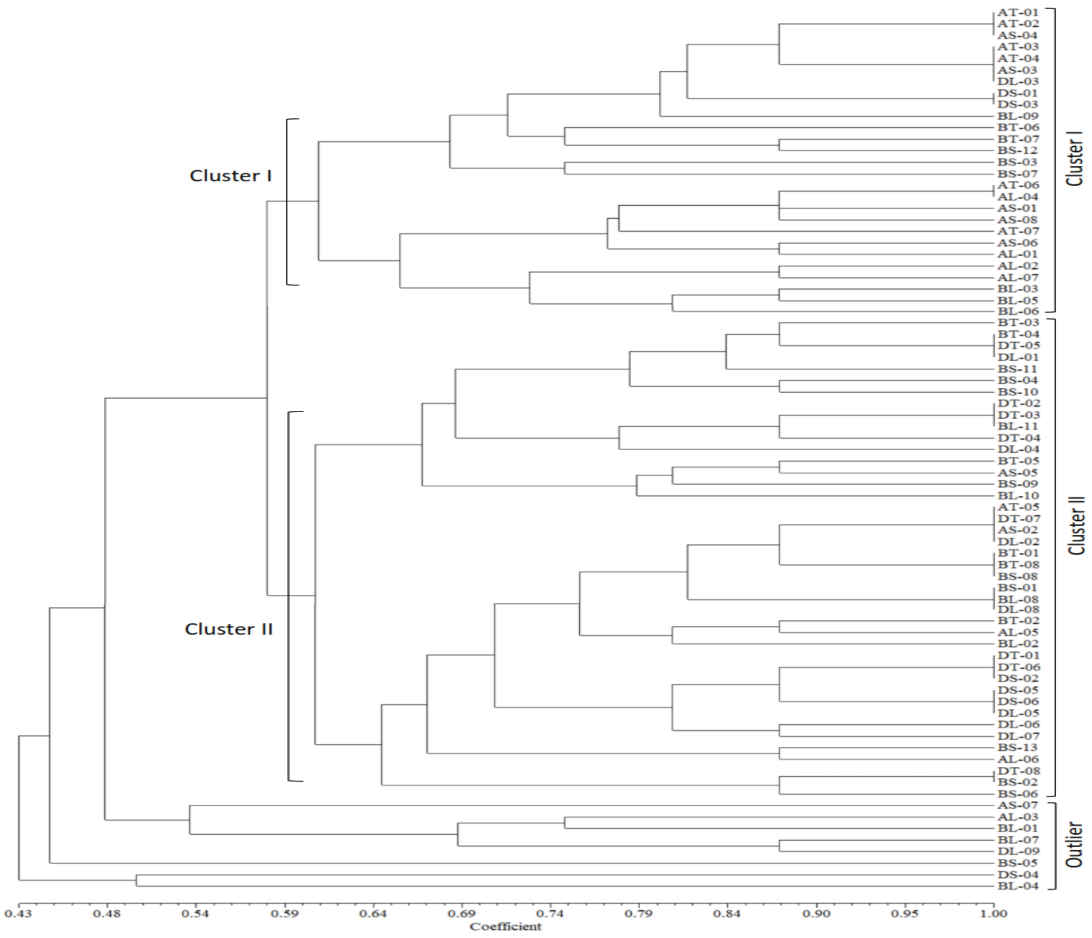

Figure 1. Dendrogram of endophytic bacteria isolated from the root, stem, and leaf of three Aceh patchouli cultivars (Tapaktuan, Sidikalang, and Lhokseumawe). AT: Tapaktuan root; BT: Tapaktuan stem; DT: Tapaktuan leaf; AS: Sidikalang root; BS: Sidikalang stem; DS: Sidikalang leaf; AL: Lhokseumawe root; BL: Lhokseumawe stem; DL: Lhokseumawe leaf, generated based on morphological traits.

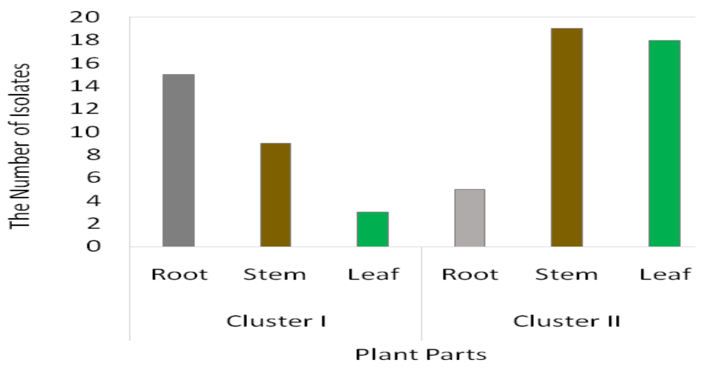

Figure 2. Bar graph illustrating the distribution of isolates based on plant parts (root, stem, leaf) in each of the two main dendrogram clusters (defined at a similarity coefficient of 0.57), regardless of plant cultivar.

bacteria with brighter pigmentation (yellow, orange, pink, and dominant tan), with stem-associated isolates being mostly Gram-positive bacilli, whereas leaf-derived isolates were Gram-negative bacilli. The results indicated a more heterogeneous above-ground bacterial community, likely shaped by the dynamic conditions of aerial plant compartments, such as light and humidity. These results supported the growing evidence that plant organ compartmentalization, rather than plant genotype, exerts a considerable influence on endophytic community structure, assembly, and the phenotype (Li *et al.*, 2023; Santos *et al.*, 2024).

In the current study, such compartment-specific differentiation aligned with previous studies, showing an obvious distinction between root and aerial tissue microbiomes, both in composition and in dominant morphotypes (Zhang *et al.*, 2023; Mahadeo *et al.*, 2024). For the presented study, a clear organ-specific distribution pattern was evident in the Sidikalang cultivar, showing the predominance of Gram-negative bacteria in the root and Gram-positive forms in above-ground tissues (Table 1). In contrast, the Lhokseumawe cultivar exhibited the greatest diversity of isolates across all organs, presumably related to its genetic background and ecological adaptability, which together provide broader niches for microbial colonization. These findings suggested that Lhokseumawe could serve as a valuable reservoir of beneficial endophytes, offering potential for development as biocontrol agents and plant-growth promoters in patchouli breeding and sustainable cultivation.

The said distribution trend of endophytic bacteria highlighted the intricate interplay between host microenvironments and microbial adaptation. Root-associated isolates may harbor traits favorable for plant growth and development and soil interaction. However, the more diverse assemblages in stems and leaves offered potential sources of novel biocontrol agents (Trivedi *et al.*, 2020; Wu *et al.*, 2021). Furthermore, the observed distribution patterns reflected biochemical interactions between host tissues and microbes. Plant-derived metabolites, such as phenolics and terpenoids in patchouli, can act as selective pressures influencing bacterial colonization (Trivedi *et al.,* 2020). Root exudates are common to recruit specific microbial taxa, while aerial metabolites can limit colonization to stress-tolerant groups (Mahadeo *et al*., 2024). Therefore, the organ-specific tendency observed in this study may represent an outcome of plant-microbe biochemical interactions that differentially shape bacterial community assembly. Understanding the distribution of endophytic bacteria could aid in the selection of beneficial strains for biocontrol and improvement in plant health.

Implications and future directions

Although this study relied solely on morphological data, the observed phenotypic diversity and compartmental distribution of endophytic bacteria provided valuable insights into patchouli-microbe interactions. The detection of phenotypically distinct isolates, especially those ungrouped in the dendrogram, further underscored the necessity of incorporating molecular techniques, such as 16S rRNA gene sequencing, multi-locus sequence analysis (MLSA), and whole-genome-based metrics (ANI GTDB), to achieve robust taxonomic resolution and infer functional relationships (López-Hermoso *et al.*, 2017; Parks *et al.*, 2022; Saudah *et al.*, 2022; Wang *et al.*, 2022, 2023; Zumaidar *et al.*, 2025).

CONCLUSIONS

The results revealed a high morphological diversity among the endophytic bacterial communities isolated from three patchouli cultivars. The findings suggested plant organ microenvironments exert greater influence on bacterial community structure than the host genotype. The presence of morphologically distinct and ungrouped isolates further underscored the complexity and plasticity of endophytic populations. The morphological data offered preliminary insights, integrating molecular tools essential for accurate taxonomic identification and understanding functional potential. Overall, this study provided a valuable basis for the future exploration and utilization of endophytic bacteria in sustainable patchouli cultivation and biocontrol strategies.

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