IJCR - Indonesian Journal of Chemical
p. ISSN: 2354-9610, e. ISSN:2614-5081
Vol. 11, No. 1, Hal. 158-173 (July 2026)

Preliminary Analysis of Forensic Microbial Profiling Through the Isolation of Endophytic Bacteria Associated with Datura metel L.

Pra Analisis *Forensic Microbial Profiling* Melalui Isolasi Bakteri Endofit Khas *Datura metel L.*

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Submitted: 12 May 2026, Revised: 15 June 2026, Accepted: 11 July 2026

Abstract

This preliminary study aimed to isolate and characterize culturable endophytic bacteria from young leaves of Datura metel L. as an initial step to explore their potential relevance to forensic microbial profiling. Endophytic bacteria are non-pathogenic microorganisms that inhabit plant tissues and may produce a variety of bioactive compounds. This study employed a laboratory experimental approach using Nutrient Agar (NA) medium for bacterial isolation, followed by purification using a streak plate technique and macroscopic observation of colony morphology. Young leaf samples were divided into four anatomical tissue sections, namely leaf petiole (TD), leaf stem (BD), veined leaf section (DT), and non-veined leaf section (D), yielding four isolates designated as I-TD, I-BD, I-DT, and I-D. The isolates were incubated and observed for up to 72 h. The results showed that isolates I-TD and I-DT exhibited bacterial growth and were successfully purified after a single streaking step, whereas isolates I-BD and I-D showed no observable colony growth. The purified isolates were characterized by circular colonies, white to yellowish-white colonies, smooth to slightly mucoid surfaces, and entire margins. These findings provide baseline information regarding the tissue-specific distribution of culturable endophytic bacteria in Datura metel L. leaves and may support future studies on forensic microbial profiling. However, this study was limited to culture-based isolation and macroscopic colony characterization without microscopic examination or molecular identification of the bacterial isolates.

Keywords*: Endophytic bacteria, Datura metel L., isolation, colony morphology, forensic microbial profiling*

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Abstrak

Penelitian pendahuluan ini bertujuan untuk mengisolasi dan mengkarakterisasi bakteri endofit dari daun muda *Datura metel L*. sebagai langkah awal untuk mengeksplorasi potensi keterkaitannya dengan *forensic microbial profiling*. Bakteri endofit merupakan mikroorganisme non-patogen yang hidup di dalam jaringan tanaman dan berpotensi menghasilkan berbagai senyawa bioaktif. Penelitian ini menggunakan pendekatan eksperimental laboratorium dengan media Nutrient Agar (NA) untuk isolasi bakteri, dilanjutkan dengan pemurnian menggunakan teknik *streak plate* dan pengamatan morfologi koloni secara makroskopis. Sampel daun muda dibagi menjadi empat bagian jaringan, yaitu tangkai daun (TD), batang daun (BD), daun bertulang (DT), dan daun tanpa tulang (D), sehingga diperoleh empat isolat yang diberi kode I-TD, I-BD, I-DT, dan I-D. Isolat diinkubasi dan diamati hingga 72 jam. Hasil penelitian menunjukkan bahwa isolat I-TD dan I-DT mengalami pertumbuhan bakteri dan berhasil dimurnikan setelah satu kali proses *streaking*, sedangkan isolat I-BD dan I-D tidak menunjukkan pertumbuhan koloni yang dapat diamati. Isolat yang berhasil dimurnikan memiliki karakteristik koloni berbentuk bulat, berwarna putih hingga putih kekuningan, permukaan halus hingga sedikit berlendir, serta tepi koloni rata. Temuan ini memberikan informasi dasar awal mengenai distribusi bakteri endofit yang dapat dikultur pada jaringan daun *Datura metel* dan berpotensi mendukung penelitian lanjutan terkait *forensic microbial profiling*. Namun demikian, penelitian ini masih terbatas pada isolasi berbasis kultur dan karakterisasi morfologi koloni secara makroskopis tanpa identifikasi mikroskopis maupun molekuler terhadap isolat bakteri yang diperoleh.

Kata Kunci: Bakteri endofit, *Datura metel L.*, Isolasi, Morfologi koloni, Forensic Microbial Profiling

INTRODUCTION:

For more than a century, microbiology has played a relatively limited role in forensic science (Robinson *et al*., 2021). However, since the early 2000s, the emergence of bioterrorism threats stimulated the development of microbial forensics, defined as "the scientific discipline dedicated to analyzing evidence from bioterrorism acts, biological crimes, or the accidental release of microorganisms or toxins for attribution purposes" (Robinson *et al*., 2021). This discipline emerged as a scientific response to increasingly complex biological threats, highlighting the importance of microorganisms in forensic and biomedical investigations.

Datura metel L. is an annual herbaceous plant belonging to the Solanaceae family and is commonly known as devil's weed, devil's cucumber, and devil's trumpet. The plant commonly grows as a wild species and is characterized by trumpet-shaped flowers ranging in color from white to purple, as well as distinctive spiny fruits. Traditionally, *Datura metel L.* has been used in herbal medicine for the treatment of asthma, rheumatic pain, and skin disorders. These medicinal properties are attributed to the presence of secondary metabolites, including tropane alkaloids, flavonoids,

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saponins, and tannins, which exhibit potential bioactive activities (Samuel *et al*., 2024). Despite the pharmacological importance of *Datura metel L.*, the presence of endophytic bacteria associated with the plant, particularly in young leaves, has been rarely reported. Studies investigating endophytic microorganisms associated with *Datura* leaves may provide valuable baseline scientific data for future exploration and evaluation of their bioprospective potential. Endophytic bacteria are non-pathogenic microorganisms that inhabit healthy plant tissues without causing disease symptoms, morphological alterations, or harmful effects on their host plants (Suhandono & Utari, 2014). These bacteria are known to enhance plant health and productivity through the inhibition

of pathogenic microorganisms and the stimulation of plant growth. Endophytic bacteria can be found in various plant species, particularly medicinal plants. One notable example is *Datura metel L.*, which is known to contain tropane alkaloid secondary metabolites. In addition, endophytic bacteria may produce bioactive compounds similar to those of their host plants, thereby offering promising applications in the health and pharmaceutical sectors. Endophytic bacteria inhabiting plant tissues occupy a relatively protected environment and obtain an adequate nutrient supply from their host plants. These microorganisms play important roles in maintaining plant health and resilience (Malnova, 2013). Their beneficial effects include the production of siderophores, abscisic acid, indole-3-acetic acid (IAA), and other metabolites that support plant growth and development (Tian *et al*., 2015). This mutualistic association provides advantages for both the bacteria and the host plant. Previous studies have primarily focused on the utilization of extracts from *Datura metel L*. For instance, Akharaiyi (2011) investigated the ethanol extracts of the bark, stem, and root of the plant, while Lim *et al*. (2020) evaluated the antibacterial activity of its fruit extract. In contrast, the exploration of endophytic bacteria associated with this plant remains scarce. Despite the increasing interest in endophytic microorganisms from medicinal plants, studies focusing on endophytic bacteria associated with *Datura metel L.* are still limited, particularly studies examining tissue-specific distribution in young leaves. Most previous investigations have concentrated on the phytochemical constituents and pharmacological activities of *Datura metel L.* extracts, whereas the diversity and localization of its endophytic bacterial communities remain poorly

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understood. The novelty of the present study lies in the comparative isolation and macroscopic characterization of endophytic bacteria from distinct young leaf tissues, including the petiole (TD), leaf stem (BD), veined leaf section (DT), and non-veined leaf section (D). Furthermore, this work provides a preliminary contribution to forensic microbial profiling by establishing baseline information on tissue-associated endophytic bacterial populations in *Datura metel L.*

Microbial profiling has emerged as a promising forensic approach in forensic investigations because microbial communities associated with biological materials may provide unique microbial signatures useful for identification and source attribution. Endophytic bacteria inhabiting internal plant tissues may exhibit tissue-specific distribution influenced by host characteristics and microenvironmental conditions. However, information regarding culturable endophytic bacteria associated with different tissues of young *Datura metel L.* leaves remains scarce. Although this study does not attempt to establish forensic biomarkers, the isolation and preliminary characterization of endophytic bacteria may provide baseline microbiological data that could support future investigations into the forensic relevance of plant-associated microbial communities.

Therefore, this study aimed to isolate culturable endophytic bacteria from different tissues of young *Datura metel L.* leaves and to characterize their colony morphology macroscopically as preliminary baseline data for future microbiological and forensic investigations.

MATERIAL AND METHODS:

Materials

The equipment used in this study consisted of an Esco laminar air flow (LAF) cabinet, an All-American autoclave, a Memmert incubator, disposable Onemed Petri dishes, Pyrex test tubes, beakers, and Erlenmeyer flasks, forceps, scissors, inoculating loops, a Bunsen burner, droppers, an Eppendorf micropipette, a Thermo Scientific vortex mixer, spatulas, an analytical balance, an IKA hot plate stirrer, and Pyrex graduated cylinders. The materials used in this study included fresh young leaves of

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Datura metel L. collected from Sidorejo Village, Sekampung Udik District, East Lampung Regency, Nutrient Agar (NA) medium from Oxoid, sterile distilled water, 70% ethanol from Onemed, 5% sodium hypochlorite solution from Bayclin, cotton, aluminum foil, labeling paper, tissue paper, and plastic wrap used to seal the Petri dishes during incubation.

Research Design

This study employed a laboratory-based experimental design with a descriptive approach. The study focused on the isolation of endophytic bacteria from young leaves of *Datura metel L.* using culture techniques on Nutrient Agar (NA) medium. The obtained isolates were subsequently purified by repeated streaking methods, followed by macroscopic characterization of colony morphology. No intervention involving human or animal subjects was performed.

Time and Place of Study

This study was conducted during April 2026 at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung.

Sample Collection and Identification

The samples used in this study consisted of young leaves of *Datura metel L*. Young leaves were defined as their light green color, smaller size compared to mature leaves, and their position within the first to fourth leaves from the shoot apex (Mulangsari, 2018). Samples were collected in Sidorejo Village, Sekampung Udik District, East Lampung Regency. Plant identification was performed at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung.

Sterilization of Equipment and Media Preparation

I. Equipment Sterilization

All equipment used in this study was thoroughly washed and air-dried prior to the sterilization process. Disposable Petri dishes used for endophytic bacterial isolation were immersed in 96% ethanol for 2 min and subsequently exposed to ultraviolet (UV) irradiation for 30 minutes.

Other laboratory equipment, including spatulas, stirring rods, and scissors, were also immersed in 96% ethanol for 2 min followed by UV exposure for 30 minutes.

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Test tubes and droppers were sealed with cotton plugs and individually wrapped in newspaper prior to sterilization. These materials were sterilized using a hot-air oven at 160 °C for 1 hour (Sartika *et al*., 2023). The mouths of Erlenmeyer flasks were covered with cotton, while graduated cylinders were sealed with cotton and individually wrapped in newspaper. Both types of glassware were autoclaved at 121 °C for 15 minutes. Forceps, inoculating loops, and microscope slides were sterilized by flaming until red-hot (Sartika *et al*., 2023).

II. Preparation of Nutrient Agar Medium

Nutrient Agar (NA) medium was prepared by dissolving 11.2 g of NA powder in 400 mL of sterile distilled water in an Erlenmeyer flask. Then, 0.08 g of ketoconazole powder was added to the medium to prevent fungal contamination. The mixture was heated with continuous stirring on a hot plate stirrer for 10–15 minutes until the medium was completely dissolved. The prepared medium was subsequently autoclaved at 121 °C for 15 minutes, with the Erlenmeyer flask sealed with aluminum foil (Zulkifli *et al*., 2018).

Isolation and Purification of Endophytic Bacteria from *Datura metel L.* Leaves

The isolation and purification of endophytic bacteria from *Datura metel L.* leaves began by washing the leaf samples under running water. Surface sterilization was subsequently performed by cutting the leaf tissue into sterile 2 × 2 mm sections using sterilized scissors. Leaf sections were prepared from the leaf petiole, leaf stem, veined leaf lamina, and non-veined leaf lamina.

The leaf sections were surface-sterilized by immersion in 70% ethanol for 1 minute, followed by immersion in 5% sodium hypochlorite solution for 2 minutes, and finally rinsed with sterile distilled water. The sterilization procedure was intended to eliminate contaminating bacteria present on the leaf surface, thereby ensuring that the isolated bacteria originated from the internal leaf tissues (Ompusunggu et al., 2019). One limitation of this study is that surface sterilization effectiveness was not validated through final rinse water plating or additional sterility controls.

Thereafter, each sample section was placed into four different Petri dishes containing Nutrient Agar (NA) medium. All Petri dishes

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were incubated at 37 °C and monitored at incubation periods of 0, 24, 48, and 72 hours.

Macroscopic Identification of Endophytic Bacteria from *Datura metel L.* Leaves

The macroscopic identification of endophytic bacterial isolates from *Datura metel L.* leaves was performed based on colony morphology characteristics. Colony shape was observed from the upper surface and classified as circular or oval. Colony elevation was observed from the side view and classified as flat or raised. Colony margins were examined from the upper view and classified as undulate or spreading. Colony pigmentation was also recorded, as certain bacteria produce characteristic pigments during growth on culture media, including white, yellowish-white, or milky-colored colonies (Faradiska, 2012).

Macroscopic colony characterization is a fundamental and widely accepted preliminary approach in microbiological investigations for distinguishing culturable bacterial isolates and assessing observable phenotypic diversity. In the present study, variations in colony shape, pigmentation, margin characteristics, surface texture, and growth patterns provided evidence of phenotypic differences among isolates recovered from different tissues of young *Datura metel L.* leaves. Although these observations do not permit definitive

taxonomic identification, they constitute valuable baseline microbiological information regarding the occurrence and distribution of culturable endophytic bacteria within the host plant. Furthermore, the successful isolation and purification of distinct bacterial colonies demonstrate the presence of tissue-associated endophytic bacterial populations and provide a foundation for subsequent phenotypic, biochemical, and molecular investigations. To achieve higher taxonomic resolution and validate isolate identity, future studies should incorporate Gram staining, biochemical profiling, and 16S rRNA gene sequencing. Nevertheless, the current findings remain scientifically relevant as an initial contribution toward understanding the

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diversity of culturable endophytic bacteria associated with young *Datura metel L.* leaves, for which detailed microbiological information remains limited.

Data Analysis

The observational data were presented in the form of tables and figures accompanied by descriptive explanations to illustrate differences in colony characteristics among BD, TD, DT, and D isolates. This study was designed as a preliminary exploratory investigation using one young *Datura metel L.*

leaf sample divided into four tissue sections, namely leaf petiole (TD), leaf stem (BD), veined leaf section (DT), and non-veined leaf section (D). Each tissue section was processed once for bacterial isolation and purification; therefore, no biological or technical replication was performed. Consequently, the findings were analyzed qualitatively based on observable colony growth and macroscopic morphological characteristics, including colony shape, elevation, margin, surface texture, and pigmentation. The results are presented as descriptive baseline observations and should not be interpreted as statistically validated conclusions. Future studies employing larger sample sizes and replicated experimental designs are required to evaluate reproducibility and statistical significance. This analysis aimed to provide an overview of the presence and diversity of endophytic bacteria based on the isolation and purification results obtained in this study.

RESULTS AND DISCUSSION

The young leaf samples of *Datura metel L.* used as the source of endophytic bacteria are presented in Figure 1.

Figure 1. Young leaves of *Datura metel* L. used in this study

Based on the samples used in this study, the plant material consisted of leaves of *Datura metel L.* that had undergone taxonomic identification at the Botany Laboratory, Faculty of Mathematics and Natural Sciences University of Lampung. The identification results confirmed that the plant sample was correctly identified as *Datura metel L.* belonging to the Solanaceae family. Morphologically, the leaves exhibited broad

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laminae with pointed apices, relatively smooth to slightly wavy margins, and clearly visible pinnate venation on the leaf surface. The green coloration of the leaves indicated that the samples were fresh and healthy, thereby fulfilling the criteria for endophytic bacterial isolation.

Isolation of Endophytic Bacteria

The colony morphology of endophytic bacteria isolated from *Datura metel L.* leaves was observed macroscopically. Purification was performed on isolates obtained from the leaf stem (BD), leaf petiole (TD), veined leaf section (DT), and non-veined leaf section (D). Repeated streaking techniques were carried out to obtain single colonies or pure isolates, thereby facilitating the macroscopic observation of bacterial colony morphology. The purification results of isolates I BD, I TD, I DT, and I D are presented in Figure 2.

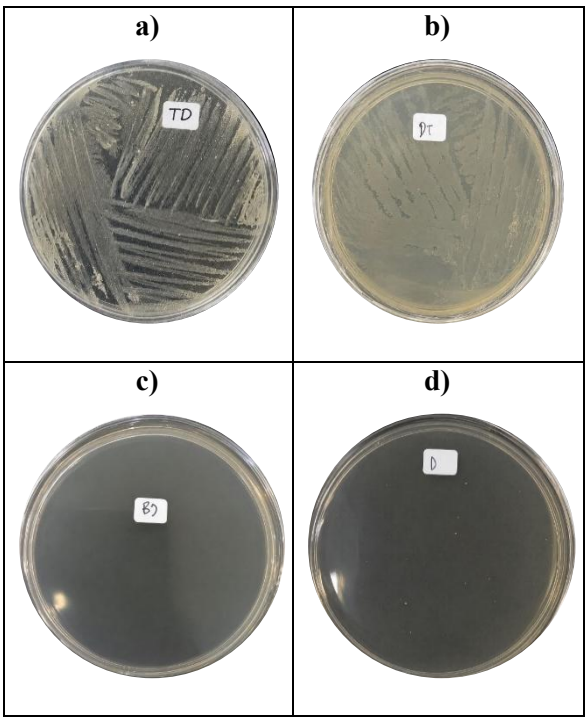

Figure 2. Purified endophytic bacterial isolates obtained from different young leaf tissues of *Datura metel L.* after incubation on Nutrient Agar (NA) medium for 72 h: (a) I- TD (leaf petiole), (b) I-BD (leaf stem), (c) I-DT (veined leaf section), and (d) I-D (non-veined leaf section).

Morphological analysis showed that bacterial colonies obtained from isolates I BD, I TD, I DT, and I D are presented in Table 1.

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Table 1. Results of Macroscopic Morphological Observation of Endophytic Bacteria

No	Isolate	Growth	Morphology	Description
1.	I-TD	Growth	Circular colonies, creamy white color, smooth surface, entire margins	Successfully purified
2.	I-DT	Growth	Circular colonies, yellowish-white color, convex and smooth surface, slightly mucoid, entire margins	Dominant growth
3.	I-BD	No growth	-	-
4.	I-D	No growth	-	-

Based on the isolation of endophytic bacteria from *Datura metel L.* leaves incubated for up to 72 hours, several bacterial isolates were successfully obtained and subsequently purified through repeated streaking methods. This purification process aimed to separate bacterial cells in order to obtain single colonies (pure isolates), which were characterized by distinct colony growth and uniform morphological characteristics. The purified isolates obtained in this study were coded as I-TD, I-BD, I-DT, and I-D.

Based on the observations presented in Table 1, the endophytic bacterial isolates generally exhibited circular colony shapes, relatively flat surfaces with slight elevations, and colony pigmentation ranging from translucent white to yellowish-white. These findings are consistent with the description reported by Faradiska (2012), which stated that endophytic bacterial colonies commonly display circular or oval shapes, white to yellowish pigmentation or milky textures, and colony margins with wavy or spreading characteristics.

The observational results demonstrated that each isolate exhibited distinct macroscopic colony morphology characteristics. These differences included variations in colony color, shape, size, surface texture, elevation, and colony margin patterns. Furthermore, the findings obtained in this study are consistent with the report by Gary Strobel and Daisy Castillo (2003), which stated that endophytic bacteria exhibit high morphological diversity, reflecting species diversity and adaptation to host plant tissues. The observed morphological variations indicate that the isolates obtained in this study were likely derived from different types of endophytic bacteria.

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Isolate I-TD exhibited relatively distinct and well-separated colony growth following the purification process. The colonies were generally circular in shape with small to medium sizes, cream to yellowish-white pigmentation, and smooth, slightly convex surfaces. The colony margins appeared regular and even, indicating stable growth without irregular spreading patterns.

Isolate I-DT exhibited more dominant colony growth compared to the other isolates. The colonies appeared more abundant and distinct, particularly along the streaking lines. Morphologically, the colonies were circular in shape with cream-white coloration, smooth to slightly mucoid surfaces, and convex elevations. Most colony margins were regular; however, several areas appeared slightly irregular due to the dense colony growth. The predominance of isolates obtained from the veined leaf section (DT) may be associated with the anatomical characteristics of leaf veins, which provide a favorable microenvironment for endophytic bacterial colonization. Leaf veins are closely connected to the vascular system and facilitate the transport of water, nutrients, and metabolites throughout plant tissues. These conditions may support a higher abundance and diversity of endophytic bacteria compared with other leaf regions. In addition, the spatial distribution of endophytic bacteria within plant tissues is influenced by tissue structure and nutrient availability, resulting in different colonization patterns among the petiole, leaf stem, veined leaf section, and non-veined leaf section.

These morphological variations indicate that the obtained isolates were likely derived from different types of endophytic bacteria. This finding is consistent with the report by Hallmann et al. (1997), which stated that endophytic bacterial communities within a plant are complex and heterogeneous, thereby exhibiting phenotypic variations that can be observed through colony morphology characteristics.

No bacterial colony growth was observed in the BD and D samples, either during the initial isolation stage or after purification through repeated streaking methods. Purification attempts were still performed on the BD and D samples to further confirm the presence of endophytic bacteria; however, observational results demonstrated that no single colonies developed on the culture media following further incubation.

The findings of this study indicate that the BD and D sections of *Datura metel L.* leaves

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possibly did not contain culturable endophytic bacteria, or that the bacterial population within the tissues was present in very low abundance and therefore undetectable using culture-based methods. The absence of bacterial growth in isolates I-BD and I-D may be attributed to several factors. First, endophytic bacterial populations within these tissues may have been present at very low abundance, making them difficult to detect using conventional culture-based techniques. Second, the surface sterilization procedure involving ethanol and sodium hypochlorite, although necessary to eliminate epiphytic microorganisms, may have reduced the viability of some sensitive endophytic bacteria. In addition, Nutrient Agar (NA) medium may not provide optimal nutritional requirements for all endophytic bacterial species, particularly fastidious microorganisms that require specific growth factors. Furthermore, incubation conditions such as temperature and incubation duration may not be equally suitable for all endophytic bacterial populations. Therefore, the absence of visible colonies in BD and D samples does not necessarily indicate the complete absence of endophytic bacteria, but may reflect methodological limitations associated with culture-dependent isolation approaches.

Endophytic bacteria generally enter plant tissues through the roots; however, plant parts directly exposed to air, such as flowers, stems, leaves (through stomata), and cotyledons, may also serve as entry routes for these microorganisms. Once inside the plant tissues, endophytic bacteria may either colonize specific localized sites or spread throughout the entire plant. These microorganisms are capable of inhabiting vascular tissues as well as intercellular spaces (Zinniel *et a*l., 2002), including roots, stems, leaves, and fruits (Simarmata *et al*., 2007; Bacon & Hinton, 2006). The exact population of endophytic bacteria within plant tissues cannot be determined precisely; however, their presence can be detected through isolation on agar media.

In this study, Nutrient Agar (NA) medium was used for the isolation of endophytic bacteria. NA is considered a nutrient-rich medium containing yeast extract, peptone, NaCl, and agar. Endophytic bacteria are able to grow on NA medium due to its complex composition, which likely resembles the nutritional conditions found within plant tissues.

The interaction between endophytic bacteria and plants represents a form of symbiosis that may be neutral, mutualistic, or

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commensal (Bacon & Hinton, 2006). In mutualistic associations, endophytic bacteria obtain nutrients derived from plant metabolic processes while simultaneously contributing to plant defense against pathogens. In return, the host plant benefits from nutritional derivatives and bioactive compounds produced by the endophytic bacteria that support plant survival and development (Simarmata *et al*., 2007).

The identification of bacterial isolates in this study was limited to culture-based isolation and macroscopic colony morphology characterization. Additional analyses such as Gram staining, biochemical characterization, and 16S rRNA gene sequencing were not performed. Therefore, the taxonomic identity of the isolates could not be determined with certainty. Future studies should include molecular and phenotypic identification methods to provide more comprehensive characterization of the isolated endophytic bacteria.

The absence of microscopic observations does not diminish the value of the present findings, as the primary objective of this study was to conduct a preliminary exploration of culturable endophytic bacteria associated with young *Datura metel L.* leaves through isolation, purification, and macroscopic characterization. Colony morphology assessment, including colony shape, pigmentation, margin characteristics, elevation, and surface texture, is widely recognized as an essential first step in microbiological investigations for distinguishing bacterial isolates and evaluating microbial diversity. The successful recovery and purification of isolates I-TD and I-DT demonstrate the presence of culturable endophytic bacterial populations within specific leaf tissues and provide baseline information regarding their distribution patterns. Nevertheless, because morphological characteristics alone cannot accurately determine bacterial taxonomy, further studies incorporating microscopic examination, Gram staining, biochemical profiling, and molecular identification using 16S rRNA gene sequencing are necessary to achieve species-level characterization. Therefore, the present study should be regarded as an initial descriptive contribution that establishes a foundation for future investigations on endophytic bacterial communities associated with *Datura metel L*.

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CONCLUSIONS

The present study preliminarily isolated and characterized culturable endophytic bacteria from young leaves of *Datura metel L*. Differences in colony morphology among isolates indicate the presence of diverse endophytic bacterial populations inhabiting different leaf tissues, with isolates from the veined leaf section (DT) showing the most dominant growth. These findings provide baseline information regarding endophytic bacterial diversity associated with *Datura metel L.* and may support future studies on their ecological roles and potential applications. Further research involving biochemical characterization and molecular identification, such as 16S rRNA gene sequencing, is recommended to achieve more accurate bacterial identification and a deeper understanding of endophytic bacterial communities.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to all parties who contributed support to the completion of this research. Special appreciation is extended to the academic supervisors for their guidance, valuable suggestions, and constructive input throughout the research process and the preparation of this article. The authors also acknowledge the contributions of colleagues who assisted in sample collection, laboratory experiments, and the preparation of this scientific manuscript. The authors realize that this study could not have been successfully completed without the assistance and support of various individuals and institutions; therefore, sincere appreciation is extended to everyone who contributed to this work.

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