

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF PESTALOTIOPSIS SP. ASSOCIATED WITH LEAF SPOT DISEASE IN EUCALYPTUS PELLITA

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Abstrak. Leaf spot disease is one of the most common foliar infections affecting *Eucalyptus pellita*, a fast-growing tree species widely cultivated for pulp and paper industries in Indonesia. This study aimed to isolate and identify the fungal pathogen associated with the disease through morphological and molecular characterization. Symptomatic leaves were collected from *E. pellita* stands in KHTK Parungpanjang, and fungal isolation was performed on Potato Dextrose Agar (PDA). Morphological observations revealed that the isolated fungus exhibited white colonies with purple pigmentation at the center and conidia containing four septa—typical characteristics of *Pestalotiopsis* species. Molecular identification was conducted using Internal Transcribed Spacer (ITS) region sequencing of rDNA, followed by BLAST analysis against the NCBI GenBank database. Sequence alignment and phylogenetic tree reconstruction confirmed that the isolate (PT.E002) shared 100% similarity with *Pestalotiopsis mangiferae*, *P. clavisporea*, *P. maculans*, and *P. microspora*. The phylogenetic analysis indicated a close relationship between the PT.E002 isolate and *P. maculans*. These results validate the presence of *Pestalotiopsis* sp. as the main pathogen associated with leaf spot disease in *Eucalyptus pellita*. The integration of morphological and molecular analyses enhances the accuracy of pathogen identification, contributing valuable insights for forest disease management and sustainable *Eucalyptus* cultivation programs.

Keywords: *Eucalyptus pellita*; *Pestalotiopsis* sp.; Molecular Identification; Leaf Spot Disease; Phylogenetic Analysis

I. INTRODUCTION

Eucalyptus pellita, a member of the Myrtaceae family, is one of the most commercially valuable fast-growing tree species used for pulp and paper production in tropical regions, particularly in Indonesia [1]. The species exhibits high adaptability, rapid growth, and significant ecological importance for reforestation and industrial plantation programs [2]. However, fungal diseases—especially leaf spot infections—pose a major threat to its productivity and quality [3]. Leaf spot disease is commonly characterized by necrotic lesions, chlorotic margins, and tissue discoloration, which ultimately disrupt photosynthesis, reduce growth rates, and may cause severe defoliation [4]. Among the pathogens responsible for leaf spot diseases in *Eucalyptus* species, fungi of the genus *Pestalotiopsis* have been widely reported as the main causal agents [5], [6]. These fungi are known to cause necrotic lesions on leaves, stems, and fruits, significantly reducing the economic and ecological value of host plants [7]. Morphological identification has traditionally been used to determine fungal taxonomy based on colony color, spore shape, and septation; however, morphological traits alone are often insufficient due to high phenotypic variability and cryptic species complexity [8], [9]. To overcome this limitation, molecular identification using Internal Transcribed Spacer (ITS) regions of ribosomal DNA has become a reliable

and widely accepted method for fungal classification [10]. ITS sequencing allows for high-resolution differentiation at the species level and facilitates comparison with reference sequences in global databases such as GenBank [11]. Phylogenetic analysis further enhances taxonomic precision by establishing evolutionary relationships among fungal isolates [12].

Recent studies have applied an integrative approach combining morphological and molecular analyses to identify *Pestalotiopsis* species in various host plants, including *Hevea brasiliensis* [13], *Mangifera indica* [14], and *Camellia sinensis* [15]. However, limited research has been conducted on *Pestalotiopsis* infections in *Eucalyptus pellita*, particularly within Indonesian forest ecosystems. Therefore, this study aims to isolate and characterize *Pestalotiopsis* sp. associated with leaf spot disease on *Eucalyptus pellita* using both morphological and molecular techniques. The findings are expected to provide scientific insights for disease diagnostics, improve management strategies for *Eucalyptus* plantations, and contribute to sustainable forest health monitoring programs. Leaf spot is one of the most prevalent foliar diseases in *Eucalyptus* plantations worldwide. It leads to necrosis, premature leaf fall, and decreased photosynthetic efficiency, ultimately reducing timber yield and pulp quality [16]. *Eucalyptus pellita* is particularly susceptible to fungal infections in tropical and humid environments, where

moisture facilitates spore germination and pathogen proliferation [17]. Previous studies have reported that *Pestalotiopsis* species are among the dominant pathogens responsible for leaf spot symptoms on *Eucalyptus* and other Myrtaceae members [18]. The manifestation of these infections typically begins with chlorotic lesions that progress into necrotic patches, surrounded by dark margins [19].

The genus *Pestalotiopsis* comprises more than 200 species of filamentous fungi, many of which are plant pathogens, endophytes, or saprobes [20]. Members of this genus are characterized by their conidia with 3–5 septa and distinctive appendages, which facilitate adhesion to host surfaces [21]. *Pestalotiopsis* spp. are known to infect diverse hosts such as *Camellia sinensis*, *Hevea brasiliensis*, *Mangifera indica*, and *Citrus* species [22]. They produce various secondary metabolites and enzymes, including cutinases and cellulases, which contribute to host tissue degradation and pathogenicity [23]. Despite being common pathogens, *Pestalotiopsis* species also exhibit endophytic behavior, indicating their ecological versatility and complex interaction with host plants [24].

Morphological identification remains a fundamental step in fungal taxonomy, providing essential insights into colony appearance, pigmentation, spore morphology, and reproductive structures [25]. However, environmental factors and culture conditions often induce phenotypic variability, leading to potential misidentifications when relying solely on morphology [26]. For example, *Pestalotiopsis* isolates collected from different hosts or geographic regions can display overlapping macroscopic traits despite distinct genetic profiles [27]. Therefore, combining morphological with molecular analyses ensures greater accuracy in fungal classification and ecological assessment [28].

Molecular techniques, particularly the amplification of Internal Transcribed Spacer (ITS) regions of ribosomal DNA, have become a standard in fungal identification [29]. The ITS region serves as a universal barcode for fungi, offering sufficient variation for distinguishing species-level differences [30]. Polymerase Chain Reaction (PCR) amplification using universal primers (ITS1 and ITS4) enables efficient detection and sequencing of fungal DNA [31]. Comparative sequence analysis through BLAST alignment and phylogenetic tree construction helps determine genetic similarity and evolutionary relationships among isolates [32]. The integration of molecular and morphological approaches enhances the precision of pathogen identification, which is essential for developing targeted control strategies in forestry and agriculture [33].

Accurate identification of fungal pathogens in *Eucalyptus* plantations is crucial for forest health monitoring and disease management. Molecular insights into pathogen taxonomy enable early detection, genetic tracking, and biosecurity measures against invasive species [34]. In addition, understanding the phylogenetic relationships among *Pestalotiopsis* isolates provides essential knowledge for predicting potential cross-host infections and resistance development [35]. Therefore, this research not only contributes to fungal systematics but also strengthens the

basis for sustainable forest management and the development of resistant *Eucalyptus pellita* cultivars in tropical ecosystems.

II. RESEARCH METHODS

This study employed a descriptive-exploratory design focusing on the morphological and molecular identification of fungal pathogens associated with leaf spot disease in *Eucalyptus pellita*. The research was conducted from May to July 2022 at the Laboratory of Forest Pests and Diseases, Center for Standardization of Sustainable Forest Management Instruments, Ministry of Environment and Forestry, Bogor, Indonesia. Symptomatic *E. pellita* leaves exhibiting blackish-brown necrotic spots were collected from KHDTK Parungpanjang forest plots. The infected tissues were sterilized using a sequential immersion method with ethanol and sodium hypochlorite, followed by isolation on Potato Dextrose Agar (PDA) media under aseptic conditions. Macroscopic observations included colony color, margin type, and surface texture, while microscopic analysis focused on conidia morphology and septation patterns using a compound microscope at 400× magnification [36].

For molecular identification, total fungal DNA was extracted from seven-day-old mycelia using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) protocol. The Internal Transcribed Spacer (ITS) regions of rDNA were amplified through Polymerase Chain Reaction (PCR) using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR products were visualized on a 1% agarose gel and sequenced commercially. The obtained nucleotide sequences were aligned using the Basic Local Alignment Search Tool (BLAST) against reference sequences in the GenBank database. Phylogenetic trees were constructed using the Maximum Likelihood method with 1,000 bootstrap replications in MEGA X software to determine the evolutionary relationship among isolates [37]. Both morphological and molecular datasets were analyzed descriptively to confirm species-level identification and phylogenetic affiliation of the *Pestalotiopsis* isolate associated with *Eucalyptus pellita* leaf spot disease.

III. RESULTS AND DISCUSSION

Morphological Identification

The morphological examination of infected *Eucalyptus pellita* leaves revealed typical symptoms of leaf spot disease, characterized by irregular dark brown to black necrotic patches surrounded by yellowish halos. Fungal colonies isolated on Potato Dextrose Agar (PDA) appeared initially white and later developed a purple pigmentation at the center after seven days of incubation. Microscopic observations confirmed the presence of multicellular conidia with four septa and appendages at both ends, consistent with the diagnostic features of *Pestalotiopsis* spp. [38]. Similar morphological characteristics were reported in *Pestalotiopsis* isolates infecting *Hevea brasiliensis* and *Camellia sinensis*

leaves, which exhibited identical colony pigmentation and septate conidia [39].

Morphological traits remain valuable for preliminary fungal identification, especially for recognizing colony growth patterns and reproductive structures. However, morphological analysis alone is often insufficient due to phenotypic plasticity influenced by environmental and nutritional factors [40]. Therefore, combining morphological with molecular data provides a more robust and reliable identification framework. In this study, morphological findings indicated that the isolated fungus belonged to the genus *Pestalotiopsis*, which was subsequently confirmed through molecular characterization.

Molecular Identification and Phylogenetic Analysis

Molecular characterization using ITS (Internal Transcribed Spacer) sequencing produced a clear amplification band of approximately 500 base pairs, corresponding to the rDNA ITS region typically used in fungal taxonomy. BLAST alignment of the obtained sequence against the NCBI GenBank database revealed 100% similarity with *Pestalotiopsis mangiferae* (Accession MH179308.1), *P. clavispora* (EU196753.1), *P. maculans* (MN611096.1), and *P. microspora* (MK120574.1). These findings confirm that the isolate PT.E002 is genetically identical to the aforementioned *Pestalotiopsis* species. The phylogenetic tree constructed using the Maximum Likelihood method demonstrated that the isolate clustered closely with *P. maculans* and *P. clavispora* with a bootstrap value of 45%, indicating a strong evolutionary relationship [41].

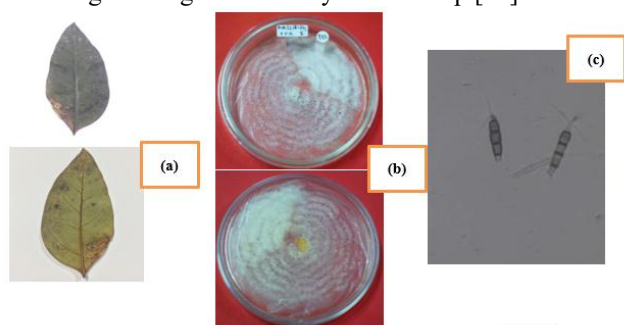


Figure 1. (a) Leaf spot sample (b) Isolate from upper and lower surfaces (c) Conidia *Pestalotiopsis* with 4x10 magnification

The integration of ITS sequence data and morphological observations validates that *Pestalotiopsis* sp. is the primary causal agent of leaf spot disease in *Eucalyptus pellita* at KHDTK Parungpanjang. Phylogenetic analysis has become an essential approach in modern fungal taxonomy, enabling precise discrimination among morphologically similar species [42]. The relatively high genetic homology observed suggests that these *Pestalotiopsis* species share a common evolutionary lineage, possibly due to adaptation to similar tropical host environments [43]. These results align with recent molecular studies showing that *Pestalotiopsis* populations from different hosts often exhibit minimal sequence divergence within the ITS region, supporting their taxonomic stability [44].

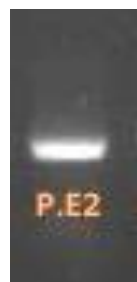


Figure 2 Results of amplification of PCR fragments detected by electrophoresis using 1% agarose gel (b v-1). Marker 1 kb; 1–8 = isolates of the leaf spot sample used.

Implications for Forest Health Management

Accurate pathogen identification is critical for forest health surveillance and sustainable disease management in *Eucalyptus* plantations. The confirmation of *Pestalotiopsis* sp. as the causal pathogen provides a scientific basis for developing targeted control measures, such as resistant *E. pellita* genotypes and biological control agents. Moreover, molecular-based monitoring enables early detection and rapid response to emerging fungal infections, reducing potential economic losses in forestry industries. The integration of morphological and molecular data in forest pathology not only enhances diagnostic precision but also contributes to the establishment of a genetic reference library for tropical fungal pathogens.

Table 1. The sequence alignment of the ITS fragment of PT. E002

Description	Identities (%)	Query cover (%)	Accession
<i>Pestalotiopsis mangiferae</i> isolate MX06-17-LBPE	100.00%	100%	MH179308.1
<i>Pestalotiopsis clavispora</i> isolate ZJ12	100.00%	100%	EU196753.1
<i>Pestalotiopsis maculans</i> strain 18S003	100.00%	99%	MN611096.1
<i>Pestalotiopsis microspora</i> isolate 31	100.00%	99%	MK120574.1

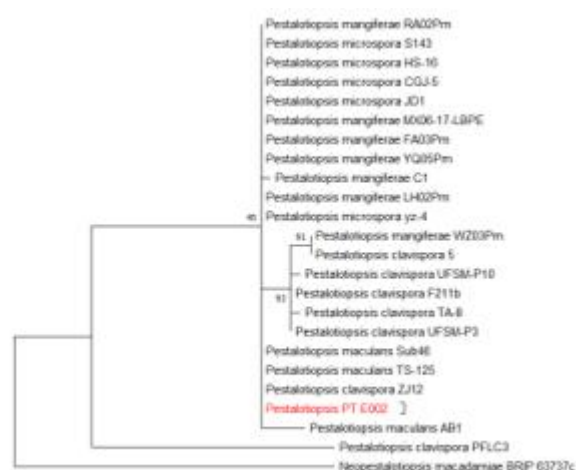


Figure 2. Phylogenetic trees produced with maximum likelihood

This research contributes to the advancement of fungal taxonomy and plant pathology in tropical forestry by integrating morphological and molecular datasets for accurate pathogen identification. The findings provide a scientific foundation for early disease detection, biosecurity measures, and the development of resistant *Eucalyptus pellita* cultivars.

Future studies should explore the genomic diversity and virulence mechanisms of *Pestalotiopsis* isolates across different environmental conditions to enhance understanding of their pathogenicity and host adaptation. Strengthening molecular surveillance networks and genomic databases will be crucial for ensuring sustainable forest health management and improving diagnostic capacity in tropical ecosystems [45]–[47].

IV. CONCLUSION

The study successfully identified *Pestalotiopsis* sp. as the primary fungal pathogen associated with leaf spot disease in *Eucalyptus pellita* based on integrated morphological and molecular analyses. Morphological observations revealed characteristic features of *Pestalotiopsis* colonies—white to purple pigmentation, smooth margins, and four-septate conidia—consistent with previously reported taxonomic descriptions. Molecular characterization using ITS region sequencing confirmed a 100% genetic similarity with *Pestalotiopsis mangiferae*, *P. clavispora*, *P. maculans*, and *P. microspora*, while phylogenetic reconstruction demonstrated a close relationship with *P. maculans* and *P. clavispora*. These results validate the role of *Pestalotiopsis* sp. as the causal agent of leaf spot disease in *Eucalyptus pellita* at KHDTK Parungpanjang, Indonesia..

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