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Isolation and Characterization of Fungal Endophytes from Mangrove Ceriops tagal (Perr.) C. B. Rob

Anggun Sophia[1,2], Anthoni Agustien[1]*, Syamsuardi[1], Chairul[1]

[1]Departement of Biology, Fakulty of Mathematics and Natural Sciences, Universitas Andalas. Jl. Unand, Kampus Limau Manis, Padang 25163, West Sumatra, Indonesia

[2]Medical Laboratory Technology, Faculty of Health Sciences, Universitas Perintis Indonesia, Indonesia

*Email: aagustien@gmail.com

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Abstract— Ceriops tagal is a mangrove plant species with potential as a source of endophytic fungal isolates. Known for its resilience in extreme environments, such as high salinity, Ceriops tagal provides an ideal habitat for endophytic fungi. The endophytic fungi isolated from Ceriops tagal are capable of producing bioactive compounds with promising potential. This study aims to isolate and characterize endophytic fungi in Ceriops tagal tissues. Conducted in August 2024, the research follows an exploratory descriptive method. The study stages include surface sterilization of samples, isolation of endophytic fungi, purification of fungal isolates, and morphological identification of endophytic fungi macroscopically and microscopically. The results reveal that isolation and characterization of endophytic fungi from the roots, twigs, and leaves of Ceriops tagal yielded four isolates, two of which belong to the genus Aspergillus, one to the genus Curvularia one to the genus Pestalotiopsis.

Keywords – Ceriops tagal, endophytic fungi, mangrove, morphology.

I. INTRODUCTION

Endophytic fungi are microorganisms that live within plant tissues without causing disease [1]. Endophytic fungi in various plant species indicate a symbiotic relationship that can benefit the host plant by providing protection against pathogens, enhancing tolerance to environmental stress, and contributing to the plant's metabolic processes [2]. Endophytic fungi are a rich potential source of enjoyable biological activities and exhibit high diversity [3]. Exploring endophytic fungi from plants is valuable for discovering fungi with unique and specific capabilities

Ceriops tagal is a mangrove plant species with potential as a source of endophytic fungal isolates. Known for its ability to withstand extreme environments, such as high salinity [4], *Ceriops tagal* provides an ideal habitat for endophytic fungi. The endophytic fungi isolated from *Ceriops tagal* are capable of producing bioactive compounds that are similar or even nearly identical to those of their host plant, often in relatively high quantities [5]. The presence of endophytic fungi in mangrove plants has attracted researchers' attention due to their ability to adapt to extreme conditions, which drives them to produce specific metabolites essential for their survival [1]. This makes endophytic fungi from *Ceriops tagal* a rich source of novel bioactive compounds with significant potential [6].

Further research on the isolation and characterization of endophytic fungi from *Ceriops tagal* is crucial to uncover the potential bioactive compounds they produce, which remain largely unexplored. Given their resilience in extreme environmental conditions,

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these endophytic fungi could be a source of unique bioactive compounds, such as antimicrobial, antifungal, or antioxidant agents. This research may open new opportunities for developing biotechnology, pharmaceutical, and health products.

II. MATERIALS AND METHODOLOGY

The method used in this study is an exploratory approach aimed at gathering data and information on the endophytic fungi residing in the mangrove plant *Ceriops tagal*. This research is conducted in two stages. The first stage involves isolating healthy plant organs and purifying fungal isolates. The second stage characterizes the fungal isolates through macroscopic and microscopic observations.

Sample Collection

Endophytic fungi were isolated from the fresh and infection-free root, branch and leaves of *C. tagal,* collected from Sungai Pisang, Bungus, Padang, West Sumatra, Indonesia, in August 2024. The collected plant parts were transported immediately to the laboratory for further processing.

Procedures

Preparation of PDA Medium

39 g of Potato Dextrose Agar (PDA) medium is dissolved in 1 liter of distilled water and heated using a hot plate and magnetic stirrer. The PDA medium is then sterilized in an autoclave at 121°C for 15 minutes [7] .

Sample surface sterilizatin

The sample surface is sterilized by rinsing with tap water to remove minute debris, then cut into 1×1 cm pieces using a sterile knife. The sample pieces are sterilized by soaking in 70% ethanol for 1 minute, then in 3.5% NaOCl solution for 5 minutes, and finally rinsed again in 70% ethanol for 30 seconds [8]. The sterilized pieces are placed on sterile tissue and left to air dry until the ethanol has evaporated.

Endophytic fungi isolation

Each sample is planted using tweezers into a petri dish containing PDA medium by splitting the plant part and placing it with the cut surface in contact with the PDA medium [8]. The samples are then incubated at 27°C for 5-7 days [9]. The petri dishes are periodically checked to monitor the development of endophytic fungal colonies.

Purification of endophytic fungal isolates

Each grown fungal isolate is transferred to a PDA medium using an inoculating needle in a separate petri dish. Macroscopic observation of the colonies is conducted based on color, surface, and colony edge characteristics. Colonies with similar traits are considered the same isolate [8]. Each colony with distinct morphology is separated as an individual isolate. Morphological observation is repeated after 5-7 days [10]. If further macroscopic differences in colony growth are observed, additional separation is conducted until a pure isolate is obtained [8].

Morphological endophytic fungi identification

Morphological identification was made using both macroscopic and microscopic features [11]. For macroscopic characteristics, endophytes were characterized based on colony appearance, mycelial growth pattern, margin, and pigmentation, while microscopic analysis involved mycelia preparation on slides and staining them with the lactophenol-cotton blue reagent [12]. Features such as

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hyphal type, septate or aseptate features, spores type, sporangia, conidia, and arrangement of sporangiophores and conidiophores were observed.

III. Result and Discussion

Result

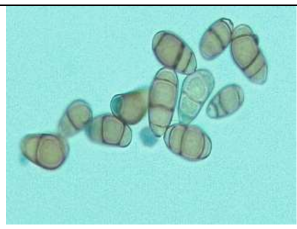
Isolation of endophytic fungi

Four endophytic fungal strains were successfully isolated from mangrove *C. tagal* and coded as CTA1, CTD1, CTD2 and CTR1. The fungal isolates CTA1 originated from the roots, CTD 1 and CTD2 from the leaves and CTR1 from the twigs. The isolates were identified macroscopically and microscopically. Morphological and other characteristics are presented in Table 1, Table 2 and Table 3.

Table 1: Macroscopic characterization of endophytic fungi isolated from root[1], leaves[2], and twigs[3]

Isolates	Surface color	Reverse color	Texture	Colony shape	Edge Colony
CTA1	Brownish	Gray	Cottony	Circular	Undulate
CTD1	Dark gray	Dark gray	Cottony	Irregular	Entire
CTD2	White at the edges with a black center	White at the edges with a black center	Granular	Circular	undulate
CTR1	Green with white edges	Greenish white	Cottony	Circular	Undulate

Table 2: Morphology of *C. tagal* endophytic fungi which were incubated for 7 day at room temperature

Isolate code	Organ of *C. tagal*	Macroscopic		Microscopic
		Surface	Reverce	
CTA1	Root			
CTD1	Leaves			

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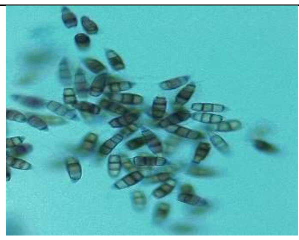
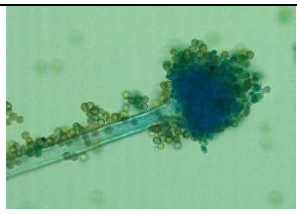
CTD2	Leaves			
CTR1	Twigs			

Table 3. Microscopic characterization of endophytic fungi isolated from root[1], leaves[2], and twigs[3]

Isolates	Spora type	Spora froms	Hyphae	Specific characteristic	Genus
CTA1	Conidia	Globase	Septa	Conidiophores erect	Aspergillus
CTD1	Conidia	Globase	Septa	Conidia are typical curved	Culvularia
CTD2	Conidia	Globase	Septa	Conidia fusiform	Pestalotiopsis
CTR1	Conidia	Globase	Septa	Uniseriate, contiguous phialides	Aspergillus

The results of microscopic observations compared with Samson's (2010) identification book showed that 2 isolates of fungi belonged to the genus Aspergillus, 1 isolate of Culvularia and 1 isolate Pestalotiopsis.

Discussion

The composition of endophytic fungi isolated from roots, branches, and leaves showed differences. This indicates that each tissue has a distinct microenvironment and function. Plant-associated habitats are dynamic environments, and many factors can influence the composition of endophytic fungi. The presence of endophytic fungi in plants appears to be influenced by ecological and physiological factors such as geographical location, plant age, and host tissue specificity [13].

The genus Aspergillus is commonly found in the mangrove plant *Ceriops tagal*. The fungus is highly adaptive, can survive in extreme environmental conditions, and can colonize host tissues [12]. Additionally, *Aspergillus* has excellent tolerance to high salinity, making it a dominant genus in *Ceriops tagal*. Besides Aspergillus, the fungus *Curvularia* was also found in the mangrove plant *Ceriops tagal*. Curvularia is a fungal genus that grows in various environmental conditions, including those with high salinity. Although less common than Aspergillus, Curvularia, Pestalotiopsis can colonize host tissues and adapt well to extreme conditions.

Aspergillus is the dominant genus found in *C. tagal* [14]. Previously, [15] reported that Aspergillus was also found in the leaves of *C. tagal*, while a study by [16] found that, in addition to Aspergillus, Curvularia, Pestalotiopsis was also present in the same plant. These findings indicate the fungal diversity in the *C. tagal* mangrove species, which can adapt to extreme environmental conditions. The presence of both fungi in *C. tagal* suggests their ability to survive in harsh environmental conditions.

IV. CONCLUSION

Based on the research, the isolation and characterization of endophytic fungi from the mangrove plant *Ceriops tagal* resulted in the identification of 5 isolates that could grow on PDA medium. Macroscopic and microscopic observations suggested that two isolates belong to the genus Aspergillus, one isolate Pestalotiopsis and one isolate belongs to the genus Curvularia.

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