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Identification of Endophytic Fungi on the Leaves of *Rhyzophora stylosa* Griff Mangrove in the Coastal Area of Bokonusan Semau Village

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Abstract

Rhyzophora stylosa Griff is a mangrove plant that grows on the coast of Bokunusan village, Semau District, Kupang Region. Endophyte fungi are found in plant tissues and can function as agents for controlling pathogenic pests. The activity of compounds produced by endophytic fungi is usually greater than the activity of compounds of their host plants (Strobel et al., 2004).The aim of research was to isolate and identify endophyte fungi found in the young and mature leaf of R. stylosa Griff at the Mangrove forest, Bokunusan Village, Semau District, East Nusa Tenggara. This research was conducted from November to December 2023 at the Microbiology Laboratory; Biology Study Program, Artha Wacana Christian University (AWCU). The method used was the Potato Dextrose Agar (PDA) media and fungal identification based on macroscopic and microscopic characteristics. Based on the results of the study, nine isolate was isolated from young and mature leaf of R. stylosa Griff. Two endophyte fungi were obtained from R. stylosa Griff leaves. The endophyte fungi found were genus Aspergillus and Trichoderma

Keywords: *Aspergillus, Macroscopic, Microscopic, Potato dexrose agar, Trichoderma*

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INTRODUCTION

The potential of the diversity of natural resources, especially plants, until now still needs to be researched for its benefits. When viewed from the perspective of chemistry, plants have an indispensable source of bioactive compounds. According to Putri et al., (2018) sources of bioactive compounds obtained from plants, animals, microbes and marine organisms are continuously explored, along with the emergence of new diseases detected.

Organisms that include endophytic microbes include endophytic fungi (Strobel, 2003). Endophytic fungi are a group of fungi that partially or all of their lives are in plant tissue and are usually not harmful to their hosts (Hasiani et al., 2015). The relationship between endophytic microbes and host plants is a form of mutualism symbiosis, which is a form of mutually beneficial relationship (Akmalasari et al., 2013).

In plant tissues that contain endophytic fungi can produce compounds that have the same properties as the host plant, although the types of compounds are different. The activity of compounds produced by endophytic fungi is usually greater than the activity of compounds of their host plants (Strobel et al., 2004). One of the many types of plants contains bioactive compounds produced by endophytic fungi, namely mangrove plants. Mangroves are plants that live between sea and land, in the form of shrubs and trees and at high tide, the roots of these mangrove plants will be flooded by water and at low tide the roots will be seen (Noor et al., 2012). One of the species of mangroves is Rhizophora stylosa which can be found in almost all mangrove forests. This plant has a high adaptability, and is able to live in environmental conditions that are quite extreme, namely high salt content.

Rhyzophora stylosa Griff is used by the community as a building material and also as firewood. In addition, this plant also has a pharmacological role because the results of secondary metabolites contain compounds that have the ability to treat several diseases such as diarrhea, dysentery, vomiting, rheumatism, muscle pain, internal injuries, tuberculosis, new wounds, lumbago, bone pain, joint pain, and stop bleeding (Abubakar et al., 2019).

To date, many researchers have managed to isolate endophytic fungi and secondary metabolites from many plants. However, researchers who isolate endophytic fungi from mangrove plants and information about mangrove endophytic fungi from *R. stylosa* as a producer of natural compounds are still limited in Indonesia, especially in mangrove ecosystems in Kupang Regency, Because of this limited information, research on the isolation and identification of endophytic fungi on *R. stylosa* mangrove leaves was carried out in the mangrove forest area of Bokunusan Village, Semau District, Kupang Regency, East Nusa Tenggara.

METHOD

Sample Preparation

Leaf sampling of both young and mature mangrove leaves of *R. stylosa* Griff was conducted in the mangrove forest of Bokonusan Semau, during the period of November to December 2021. The sampling protocol involved the use of sterilized scissors to separate the leaves from the stem, and the samples were immediately transferred to sterile zip lock bags to ensure minimal contamination. The samples were stored in a cool box with ice packs to maintain low temperatures during transport to the Microbiology Laboratory of the Biology Education Study Program at AWCU. Upon arrival, the samples were further processed for further analysis.

Sterilization and Media Preparation

All equipment used in this study underwent sterilization prior to use. Glassware was sterilized in an oven at 160˚C - 170˚C for a period of 1 hour, while heat-sensitive equipment was subjected to sterilization in an autoclave at 121˚C for 15 minutes. Needle

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holders were sterilized using direct heat from a Bunsen burner until they reached a temperature at which they became red-hot (Nuramalia, 2016).

Potato Dextrose Agar (PDA) constitutes a widely employed media type utilized for the growth and cultivation of different microorganisms, including bacteria, fungi, and other living cells. Seawater was utilized as the isolation media for fungi. Media preparation was initiated by accurately weighing 3.9 grams of PDA media on an analytical balance, which was then carefully poured into an Erlenmeyer flask and mixed with 100 mL of seawater. The mixture was carefully stirred using a magnetic stirrer on a hotplate to ensure adequate homogenization of the media. Once the media was homogenized, it was sterilized using an autoclave at 121˚C, and dispensed into Petri dishes in 15 mL aliquots (Pitarini, 2014).

Fungal Isolation

Mangrove leaves were washed with running water, and then sterilized with 70% alcohol. The leaves were aseptically lifted with sterile forceps and cut into four pieces using sterile scissors to obtain square-shaped segments. The leaf segments were then sterilized again with 70% alcohol and placed onto Potato Dextrose Agar (PDA) medium. The plates were then stored at 25°C for approximately 24-48 hours to allow for fungal growth (Nuramalia, 2016).

Fungal Purification

Purification was performed on individual fungal colonies that were considered different based on macroscopic morphology, including colony color and shape, which grew on the mangrove leaves. This purification aimed to separate colonies with distinct morphologies to be used as individual isolates (Ariyanto et al., 2013). The inoculation loop was heated until the wire glowed and then cooled for approximately 8-10 seconds before use. A small piece of fungal mycelia was taken from the surface of the original agar plate and transferred to a new PDA plate (Sanjaya et al., 2010). The isolates were stored at room temperature for approximately 24-48 hours until fungal growth was observed (Nuramalia, 2016).

RESULTS AND DISCUSSION

Fungal Endophyte Purification

The total of 9 fungal endophyte isolates was obtained, originating from young leaves (4 isolates) and mature leaves (5 isolates). Macroscopic morphological characteristics included colony mycelium that varied in color, ranging from white and black, to dark green, brown, with a central brown part, with rough, smooth, fibrous surface colonies, with even, uneven, serrated, and finely granular colony edges (Table 1).

Identification of Endophytic Fungi

Following the purification process, the identification of fungi was conducted through macroscopic observations of colony color, shape of colony edge and surface, and microscopic examination of rhizoids, hyphae, conidiophores, conidia, and phili (Fig. 1), in accordance with the guidelines outlined by (Gherbawy & Voigt, n.d.; Rafael &

Calumpong, 2019), and of the nine endophytic fungal isolates, two genera were identified as Aspergillus (six isolates) and Trichoderma (three isolates) (Fig. 2).

Table 1. Identification of Endophytic fungi isolated from *R. stylosa* leaves in Bokonusan Semau Coastal area

Isolate Code	Macroscopic character			Microscopic character	Fungal Genus
	Colony Color	Colony surface	Colony edges		
1.DM-Rs-01-AR	Grey	Rough	even	Conidia, sporangia and Conidiophore.	*Trichoderma*
2.DM-Rs-02-AR	Black	Finely-grained	Uneven	Rhizoid, acaulose, single, elongated conidiophore, with sporangia and conidia	*Aspergillus*
3.DM-Rs-03-AR	Light green	Soft	Uneven	Septate hyphae with fine branching, present stolon and spores	*Aspergillus*
4.DM-Rs-04-AR	White	Soft	Uneven	Rhizoid, acaulose, single, elongated conidiophore, with sporangia and conidia	*Trichoderma*
5.DT-Rs-01-AR	Grey	soft	Flat	Spores, sporangia, fine and non-septate, and stolon	*Aspergillus*
6.DT-Rs-02-AR	White	soft	Uneven	Septate hyphae with fine branching, present stolon and spores	*Aspergillus*
7.DT-Rs-03-AR	White	soft	Uneven	Septate hyphae with fine branching, present stolon and spores	*Trichoderma*
8.DT-Rs-04-AR	Black	soft	Uneven	Rhizoid, acaulose, long conidiophore, with sporangia, conidia, and stolon	*Aspergillus*
9.DT-Rs-05-AR	Green	Rough	Uneven	Rhizoid, acaulose, single, elongated conidiophore, with sporangia and conidia	*Aspergillus*

Notes: DM= Young leaf; DT= mature leaf

The samples used in this research were young and mature leaves of *R. stylosa* Griff mangrove, from which nine endophytic fungal isolates were obtained, with four isolates originating from young leaves and five isolates from mature leaves (Table 1). The greater number of endophytic fungal isolates obtained from mature leaves than from young leaves can be attributed to the higher concentration of secondary metabolites in mature leaves compared to young leaves (Putri et al., 2018).

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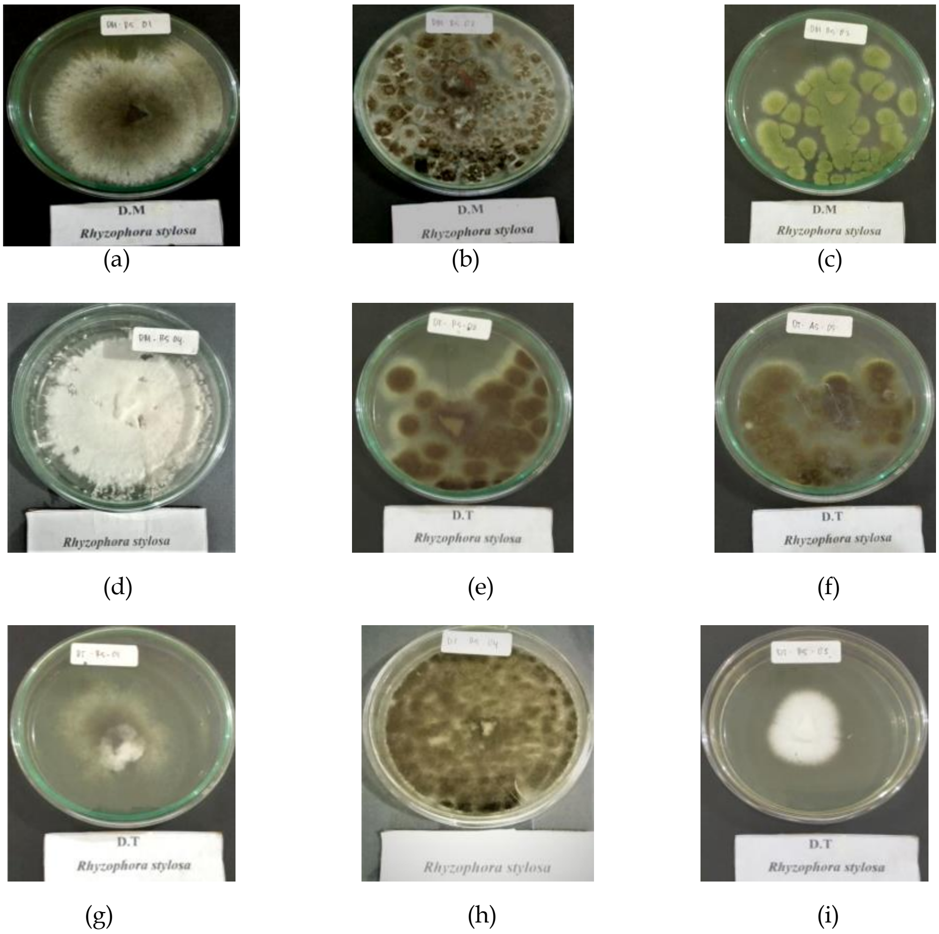
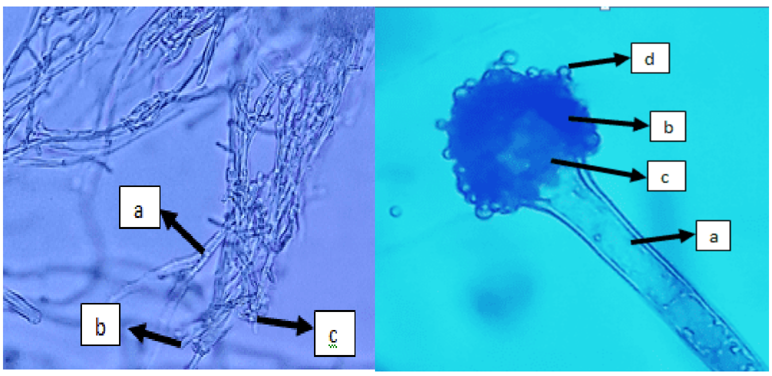


Figure 1. Endophyitic fungus isolated from young and mature leaf of *R. stylosa* Griff
Notes: (a) Isolate code DM-Rs-01-AR; (b) Isolate code DM-Rs-02-AR; (c) Isolate code DM-Rs-03-AR; (d) Isolate code DM-Rs-04-AR;(e) Isolate code DT-Rs-01-AR; (f) Isolate code DT-Rs-02-AR; (g) Isolate code DT-Rs-03-AR; (h) Isolate code DT-Rs-04-AR; (i) Isolat code DT-Rs-05-AR;



(a) **(b)**

Figure 2. Microscopic structure of genus Trichoderma (A) and Aspergillus (B) with a magnification of 400× (Notes: a: conidiophores; b: phialide; c: conidia; d: vesicle)

The production of secondary metabolites in plants is known to be an adaptive response to unfavorable environmental conditions and serves various purposes such as defense against predators, attraction of pollinators, and signal molecules (Rasyid, 2012). The levels of secondary metabolites in plants can vary depending on both environmental and endogenous factors. The age and maturity of a plant are important factors that influence the levels of active secondary metabolites.

Previous studies have shown that *Rhizophora mucronata* has antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, while extracts from *R. apicullata* leaves have antibacterial and antifungal properties against *Candida albicans.* Phytochemical analysis of *R. mucronata* has revealed the presence of various compounds such as tannins, alkaloids, flavonoids, terpenoids, and saponins (Ernawati & Hasmila, 2015). The secondary metabolites found in mangrove plants, as well as their antioxidant activities, have been used as a basis for investigating the content of endophytic fungal isolates. The variation in secondary metabolite content in plants is influenced by the age of the sample and the environmental conditions in which the plant is grown, although qualitatively, the secondary metabolite content is similar.

CONCLUSION

Endophytic fungi that have been isolated from the mangrove leaves of *R. stylosa* Griff consist of 9 isolates of microscopic fungi and are identified from 2 genera, namely the genus Trichoderma (3 isolates), and Aspergillus (6 isolates). Each genus has different macroscopic and microscopic characteristics. These findings contribute to the understanding of the diversity of endophytic fungi associated with mangrove plants, and may have potential applications in the discovery of new bioactive compounds with pharmaceutical and agricultural significance. Further research on the metabolic and genetic diversity of these fungal isolates may provide insights into their ecological and evolutionary roles within the mangrove ecosystem.

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Conflict of interest	:	The authors declare that they have no conflicts of interest.
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