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Isolation and Identification of Endophytic Fungi from Leaves and Petioles of Salam Plants (*Syzygium polyanthum* Wight) as Potential Antibiotic Producers

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Abstract

Synthetic antibiotics have long been used to stimulate livestock growth; however, their use has raised concerns due to potential negative impacts. Therefore, alternative sources of antibiotics are needed. Microorganisms, particularly fungi, are known producers of natural antibiotics. Endophytic fungi are microorganisms that live within plant tissues without causing harm to the host and are capable of producing secondary metabolites, including antibiotics. This study serves as a preliminary investigation aimed at isolating and identifying endophytic fungi with antibiotic-producing potential. The primary objective was to isolate and identify endophytic fungi from the leaves and petioles of Salam (*Syzygium polyanthum* Wight). Leaf and petiole samples were obtained from traditional markets. Isolation was conducted using the direct planting method with a spread technique, followed by morphological identification. The results showed that, from three replications, five fungal isolates were obtained from leaves and seven isolates from petioles. The isolates from leaves were identified as *Mycelia sterilia*, *Acremonium* sp., and *Fusarium* sp., while those from petioles included *Mycelia sterilia*, *Fusarium* sp., *Aspergillus* sp., and unidentified fungi.

Keywords: Antibiotics, endophytic fungi, leaves, petioles, *Syzygium polyanthum*

Introduction

Antibiotic growth promoters (AGPs) are feed additives that have long been used to enhance animal growth performance. For many years, these additives were applied widely in livestock production systems worldwide. However, prolonged use of AGPs has been associated with negative impacts on animal health and potential risks to humans as consumers. For this reason, several countries, including Indonesia, have banned the use of AGPs. In response to this concern, alternative feed additives are needed to improve intestinal health and animal performance without adverse effects. Antibiotics can generally be classified into two types: synthetic antibiotics, which are industrially manufactured, and natural antibiotics, which are produced by microorganisms, typically as secondary metabolites. Various microbes, including fungi such as endophytic fungi, are known producers of these bioactive compounds.

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According to Vandna and Mahendra (2023), endophytic microorganisms are defined as organisms that colonize the internal tissues of plants throughout all or part of the plant life cycle, regardless of whether the interaction is beneficial, harmful, or neutral to the host. Jendri et al. (2022) reported that endophytic microbes are associated with approximately 300,000 plant species distributed globally. In addition, endophytic bacteria and fungi have co-evolved with plants and established specialized relationships with their hosts, inhabiting healthy plant tissues and forming an important component of plant micro-ecosystems. Singh et al. (2020) emphasized that endophytic fungi obtain nutrients and protection from host plants while living intracellularly within healthy tissues, which can induce the production of secondary metabolites.

Akmalasari et al. (2013) further noted that endophytic microbes play an important role in protecting host plants from pathogens and predators and in enhancing resistance to disease infections. This relationship has been described by Rodriguez et al. (2009) as a form of symbiotic mutualism, in which both organisms benefit. Endophytic fungi promote host plant growth by producing secondary metabolites that increase resistance to biotic and abiotic stresses (Juan et al., 2022). Kumala and Siswanto (2007) reported that endophytic fungi infect specific tissues of healthy plants and are capable of producing secondary metabolites such as mycotoxins, enzymes, and antibiotics. Similarly, Rashmi et al. (2019) stated that endophytic fungi are symptomless inhabitants of plant tissues and are involved in the production of antibiotics and other compounds with therapeutic importance.

Syzygium polyanthum Wight is widely distributed in Southeast Asian countries, including Malaysia, Thailand, Indonesia, and Singapore. This plant commonly grows in hilly areas and forests but is also cultivated in fields and home gardens near residential areas in rural regions. The leaves, fruits, and bark of this plant have long been used for various medicinal and non-medicinal purposes (Ismail and Ahmad, 2019). *S. polyanthum*, which belongs to the Myrtaceae family, is especially well known for its leaves, which are widely used as a culinary spice and as traditional medicine for diabetes, diarrhea, and hypertension. The leaves contain various bioactive compounds, including essential oils, tannins, and flavonoids (Dewijanti et al., 2019). As a medicinal plant, *S. polyanthum* produces a range of secondary metabolites that are valuable for pharmaceutical applications (Hakim et al., 2015). These metabolites are not only synthesized by the plant itself but are also produced by microorganisms that inhabit its tissues.

This study was a preliminary investigation aimed at isolating and identifying endophytic fungi found in the leaves and petioles of *S. polyanthum.*

Research Methods

The plant parts selected as samples were the petioles and leaves of *S. polyanthum*. The collected samples were placed in sterile polybags and stored in a refrigerator until isolation was performed. Sterilization, isolation, and purification of endophytic fungi followed a modified method described by Irdawati et al. (2017).

Isolation of endophytic fungi was carried out using the direct planting with spread method on potato dextrose agar (PDA) medium supplemented with 1% chloramphenicol to inhibit bacterial growth. Petiole and leaf samples were first washed under running water for 10 minutes to remove adhering debris. The samples were then surface-sterilized by immersion in 70% ethanol for 3 minutes, followed by immersion in distilled water for 3 minutes, and a final immersion in 70% ethanol for 1 minute. After sterilization, the samples were drained on sterile filter paper and cut into 1–2 cm segments using a sterilized knife. Each sample was replicated three times.

The sample segments were placed onto PDA medium in Petri dishes, with the cut surface in direct contact with the medium. All Petri dishes

were incubated at room temperature for 14 days. Fungal colony growth was observed daily throughout the incubation period. Colonies that exhibited distinct morphological characteristics were separated and subcultured onto fresh PDA medium to obtain pure isolates. Each Petri dish contained only a single fungal isolate after purification. Identification of the obtained isolates was conducted based on macroscopic characteristics, including colony morphology observed from the upper and lower surfaces of the Petri dishes, and microscopic characteristics examined under 400× magnification.

Research Results and Discussion

Results

The isolation of endophytic fungi from the leaves and petioles of *S. polyanthum* yielded twelve isolates. The number of isolates obtained from the leaves was five, while seven isolates were obtained from the petioles (see Table 1).

Table 1
Number of Endophytic Fungal Isolates from Parts of S. polyanthum

Part of Plant	Number of Isolates
Leaves	5 (Fig. 1, 2, 3, 4, 5)
Petioles	7 (Fig. 6, 7, 8, 9, 10, 11, 12)

All isolates were then identified. Five isolates from the leaves were identified as three species, namely *Mycelia sterilia* (see Figure 1 and Figure 4), *Acremonium* sp. (see Figure 2), and *Fusarium* sp. (see Figure 3 and Figure 5). Seven isolates from the petioles were identified as three species, while the remaining isolates were classified as unidentified fungi. The species identified were *M. sterilia* (see Figure 6 and Figure 9), *Fusarium* sp. (see Figure 8), *Aspergillus* sp. (see Figure 11), and unknown fungi (see Figure 7, Figure 10, and Figure 12).

The macroscopic and microscopic characteristics of five endophytic fungal isolates from the leaves were as follows.

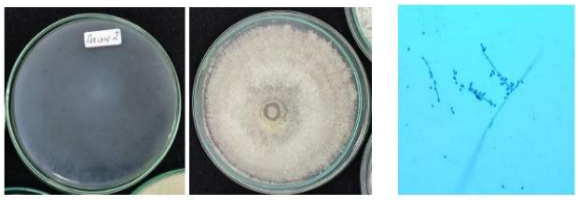
Isolate number 1 (*Mycelia sterilia*). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a yellow reverse, and the texture was cottony. The microscopic character (b): The hyphae were hyaline, septate, and had a smooth surface (see Figure 1).

Figure 1. ***Mycelia sterilia***
(a) Macroscopic and (b) microscopic characters


(a) (b)

Isolate number 2 (*Acremonium* sp.). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white to grey, with a black reverse, and the texture was cottony. The microscopic character (b): Microconidia were observed, with smooth and hyaline surfaces. The hyphae were hyaline, non-septate, and smooth (see Figure 2).

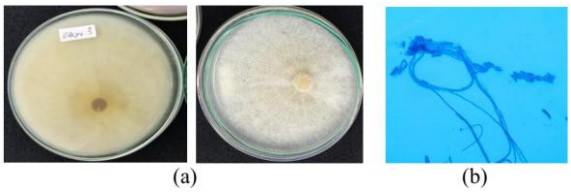
Figure 2. ***Acremonium*** **sp.**
(a) Macroscopic and (b) microscopic characters


(a) (b)

Isolate number 3 (*Fusarium* sp.). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a yellow reverse, and the texture was cottony. The microscopic character

(b): Macroconidia were observed, smooth and hyaline. The hyphae were hyaline, septate, and smooth (see Figure 3).

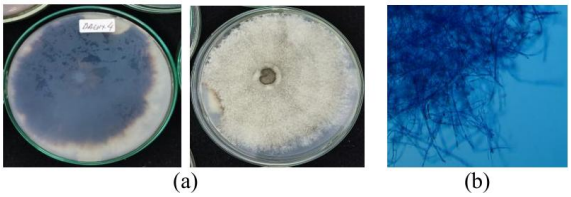
Figure 3. *Fusarium* sp.
(a) Macroscopic and (b) microscopic characters



Isolate number 4 (*Mycelia sterilia*). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a blackish-yellow reverse, and the colony texture was velvety. The microscopic character (b): The hyphae were hyaline, septate, and smooth (see Figure 4).

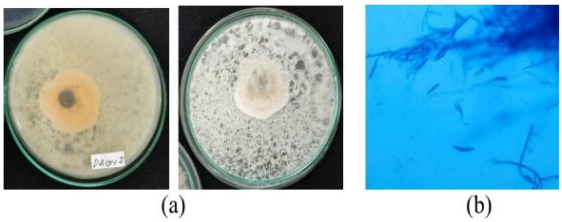
Figure 4. *Mycelia sterilia*

(a) Macroscopic and (b) microscopic characters



Isolate number 5 (*Fusarium* sp.). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a yellow reverse, and the texture was cottony. The microscopic character (b): Macroconidia were observed, smooth and hyaline. The hyphae were hyaline, non-septate, and smooth (see Figure 5).

Figure 5. *Fusarium* sp.
(a) Macroscopic and (b) microscopic characters

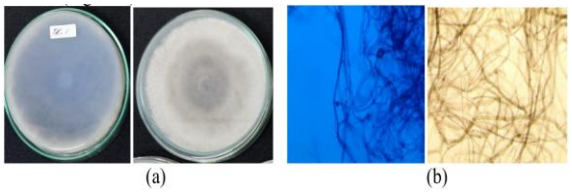


The macroscopic and microscopic characteristics of seven endophytic fungal isolates from petioles were as follows:

Isolate number 6 (*Mycelia sterilia*). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a blackish-yellow reverse, and the texture was cottony. The microscopic character (b): The hyphae were brown, septate, and smooth (see Figure 6).

Figure 6. *Mycelia sterilia*

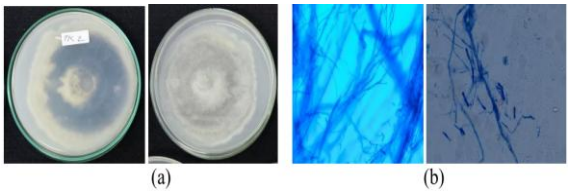
(a) Macroscopic and (b) microscopic characters



Isolate number 7 (Unknown fungus). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a yellow reverse, and the texture was cottony. The microscopic character (b): Macroconidia were observed, smooth and hyaline. The hyphae were hyaline, septate, and smooth (see Figure 7).

Figure 7. Unknown fungus

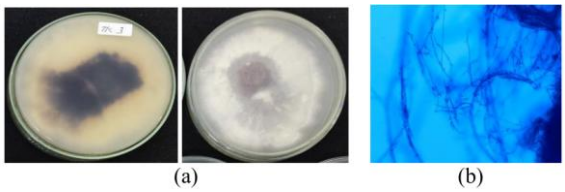
(a) Macroscopic and (b) microscopic characters



Isolate number 8 (*Fusarium* sp.). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a violet reverse, and the texture was cottony. The microscopic character (b): Macroconidia were observed, smooth and hyaline. The hyphae were hyaline, septate, and smooth (see Figure 8).

Figure 8. *Fusarium* sp.

(a) Macroscopic and (b) microscopic characters

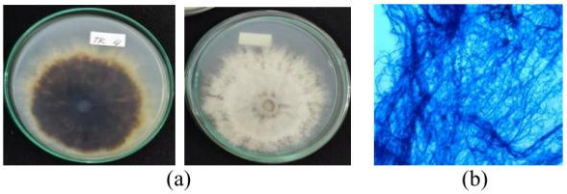


(a) (b)

Isolate number 9 (*Mycelia sterilia*). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a black reverse, and the texture was powdery. The microscopic character (b): The hyphae were hyaline, non-septate, and smooth (see Figure 9).

Figure 9. *Mycelia sterilia*

(a) Macroscopic and (b) microscopic characters

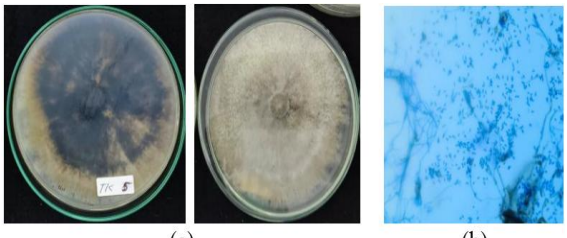


(a) (b)

Isolate number 10 (Unknown fungus). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was yellowish-white, with a black reverse, and the texture was velvety. The microscopic character (b): Microconidia were observed, smooth and hyaline. The hyphae were hyaline, non-septate, and smooth (see Figure 10).

Figure 10. Unknown fungus

(a) Macroscopic and (b) microscopic characters



(a) (b)

Isolate number 11 (*Aspergillus* sp.). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was green, with a yellow reverse, and the texture was granular. Radial furrows were observed. The microscopic character (b): Microconidia/sporangia were globose and smooth. The hyphae were hyaline, non-septate, and smooth (see Figure 11).

Figure 11. *Aspergillus* sp.

(a) Macroscopic and (b) microscopic characters

(a) (b)

Isolate number 12 (Unknown fungus). The macroscopic character (a): The color of the colony grown on potato dextrose agar in a Petri dish was white, with a black-and-white reverse, and the texture was velvety. A growth zone was also observed. The microscopic character (b): Microconidia/sporangia were observed, smooth and hyaline. The hyphae were hyaline, non-septate, and smooth (see Figure 12).

Figure 12. Unknown fungus

(a) Macroscopic and (b) microscopic characters

(a) (b)

Table 2

Identification of Endophytic Fungal Species from Parts of S. polyanthum

Part of Plant	Number of Isolates	Species
Leaves	1	*Mycelia sterilia*
	2	*Acremonium* sp.
	3	*Fusarium* sp.
	4	*Mycelia sterilia*
	5	*Fusarium* sp.
Petioles	6	*Mycelia sterilia*
	7	Unknown Fungus
	8	*Fusarium* sp.
	9	*Mycelia sterilia*
	10	UnknownFungus
	11	*Aspergillus* sp.
	12	Unknown Fungus

Discussion

In this study, PDA medium was used. This medium was selected because it supports the growth of a wide range of microorganisms, particularly fungi. To inhibit bacterial growth, the medium was supplemented with the antibiotic chloramphenicol. The samples used in this study were petioles and leaves of *S. polyanthum* Wight. From all samples, a total of twelve endophytic fungal isolates were obtained.

These results indicate that plant internal tissues can harbor microorganisms, commonly referred to as endophytic microbes. This finding is consistent with previous studies. Simlai et al. (2014) successfully isolated endophytic microbes from various parts of the mangrove plant *Sonneratia caseolaris*. Similarly, Restiani et al. (2016) isolated endophytic microorganisms from *S. polyanthum* using leaf, root, and bark samples.

All endophytic microbes isolated in the present study were fungi. According to Illarya (2014) and Yahya et al. (2017), the majority of endophytic microorganisms are fungi, which are able to live within plant tissues without causing negative effects on their host. Akmalasari et al. (2013) also reported that one major group of endophytic microbes is fungi. In their study, endophytic fungi were isolated from mangosteen tissues, yielding eleven fungal isolates identified as *Phoma* sp., *Acremonium* sp., two isolates of *Penicillium* sp., *Geotrichum* sp., *Pestalotiopsis* sp., *Botryosphaeria* sp., *Colletotrichum* sp., *Chrysosporium* sp., *Aspergillus* sp., and *Blastomyces* sp., while two isolates remained unidentified. In contrast, Murdiyah (2017) explored endophytic fungi from two medicinal plants, *Kesambi* (*Schleichera oleosa*) and *ketapang* (*Terminalia catappa*), and obtained three fungal isolates.

The number of endophytic fungal isolates obtained from each part of *S. polyanthum* differed. Seven isolates were obtained from the petioles, whereas five isolates were obtained from the leaves. Singh et al. (2020) reported that endophytic fungi isolated from *Argemone mexicana* (family Papaveraceae) showed varying distribution between plant parts. In their study, *Aspergillus*, *Penicillium* sp., *Aspergillus oryzae*, *Aspergillus niger*, and *Aspergillus flavus* were abundant in roots and shoots. Meanwhile, *Aspergillus versicolor*, *Aspergillus sydowii*, and *Penicillium chrysogenum* were found only in roots, whereas *Aspergillus striatus*, *Aspergillus tubingensis*, *Emericella qinqixianii*, and *Emericella striata* were found exclusively in shoots. Differences in the number of isolates may be influenced by variations in the ability of each endophytic fungus to adapt to host plant conditions (Devi et al., 2021). Another possible factor is the location and position of different plant parts, which create distinct microhabitats and influence microenvironmental conditions for endophytic microbial growth. Faeth and Fagan (2002) explained that each plant section differs in physical, chemical, and nutritional characteristics, as well as in sunlight exposure. This may explain why fewer endophytic fungal isolates were obtained from leaves compared to petioles. Leaves are more exposed to sunlight, which can increase tissue desiccation and create conditions less favorable for fungal growth compared to the more protected petiole tissues.

Aini et al. (2016) and Hakim et al. (2015) state that plants are naturally associated with microbes, and this relationship is predominantly positive. This association is commonly referred to as symbiotic mutualism, meaning that both organisms produce products that are mutually beneficial. As reported by Abdel-Motaal et al. (2010), many endophytic fungi produce bioactive compounds as secondary metabolites that enhance host resistance against pathogenic attacks. Rashmi et al. (2019) further confirmed that these secondary metabolites are not only produced by the plant itself but also by microbes that inhabit plant tissues. Thus, both plants and endophytic microbes can produce similar bioactive compounds with mutually beneficial effects. However, this ability is not shared by all fungi, which explains why not all fungi can function as endophytic microbes. This may account for the limited number of endophytic fungi identified in the present study. Four types of endophytic fungi were isolated, with some genera found in multiple plant organs. *Mycelia*

sterilia (genus *Mycelia*) and *Fusarium* sp. (genus *Fusarium*) were found in leaves and petioles, whereas *Acremonium* sp. was detected only in leaves, and *Aspergillus* sp. only in petioles (see Table 2). According to Rashmi et al. (2019), dominant endophytic fungal genera include *Alternaria*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium*, and *Phoma*. Similarly, Wen et al. (2022) identified *Fusarium* from *Avicennia lanata* and *Cassia alata*, and *Aspergillus* from *Acanthospermum australe* and *Nyctanthes arbor-tristis* Linn. Indrawati et al. (2021) isolated isolated four endophytic fungi from *Syzygium cumini* fruit, namely *Candida guilliermondii* (genus *Candida*), *Penicillium* sp. (genus *Penicillium*), *Mycelia sterilia* (genus *Mycelia*), and *Aspergillus* sp. These findings indicate that several endophytic fungi identified in the present study are consistent with those reported by other researchers.

As previously explained by Aini et al. (2016) and Hakim et al. (2015), the association between plants and microbes is generally beneficial to both parties. Such interactions are commonly observed in medicinal plants, which are defined as plants that contain substances in one or more organs that can be used for therapeutic purposes. In this study, *S. polyanthum* was used as the host plant. As a medicinal plant, it was proven to harbor endophytic fungal isolates, indicating a close relationship between medicinal plants and endophytic microbes. This relationship has also been reported by Akmalasari et al. (2013) in mangosteen (*Garcinia mangostana* L.) and by Murdiyah (2017) in *Kesambi* (*Schleichera oleosa*) and *Ketapang* plants, where fungal endophytes were isolated from internal plant tissues. The association between medicinal plants and endophytic fungi may be attributed to the presence of bioactive compounds that enhance host resistance to pathogens. These bioactive compounds are produced not only by the plant but also by endophytic microbes. Jha and Sit (2022) stated that various bioactive compounds are typically localized within plant tissues. Rashmi et al. (2019) further explained that endophytic fungi infect healthy plant tissues and are capable of producing mycotoxins, enzymes, and antibiotics without causing harm to the host plant. Many of these compounds contribute to increased resistance against pathogenic attacks. In addition, Restiani et al. (2016) and Wen et al. (2022) isolated broad-spectrum antibiotic-producing microbes from *S. polyanthum* using leaves, roots, and bark as samples.

Conclusion

This study successfully isolated endophytic fungi from different parts of *Syzygium polyanthum* Wight. A total of twelve isolates were obtained, consisting of five isolates from the leaves and seven isolates from the petioles. The fungal isolates from the leaves were identified as *Mycelia sterilia*, *Acremonium* sp., and *Fusarium* sp., whereas those from the petioles were identified as *Mycelia sterilia*, *Fusarium* sp., *Aspergillus* sp., and several unidentified fungi.

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