BIODIVERSITAS
Volume 24, Number 12, December 2023
Pages: 6923-6933

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241254

Antibacterial activities and molecular identification of endophytic fungi isolated from mangrove *Avicennia marina*

YENI MULYANI[1,2], ASRI PENI WULANDARI[3], SISKA ELISAHBET SINAGA[4], WAHYU SAFRIANSYAH[1], AZMI AZHARI[1], SARI PURBAYA[1], FAJAR FAUZI ABDULLAH[1], KINDI FARABI[1], YOSHIHITO SHIONO[6], UNANG SUPRATMAN[1,5,♥]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Sumedang 45363, West Java, Indonesia
[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Sumedang 45363, West Java, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Sumedang 45363, West Java, Indonesia
[4]Department of Nutrition, Universitas Widya Nusantara. Jl. Untad I, Palu 94148, Central Sulawesi, Indonesia
[5]Central Laboratory, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Sumedang 45363, West Java, Indonesia. Tel.: +62-818-02122547, ♥email: unang.supratman@unpad.ac.id
[6]Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University. Tsuruoka, Yamagata 997-8555, Japan

Manuscript received: 10 October 2023. Revision accepted: 30 December 2023.

Abstract. *Mulyani Y, Wulandari AP, Sinaga SE, Safriansyah W, Azhari A, Purbaya S, Abdullah FF, Farabi K, Shiono Y, Supratman U. 2023. Antibacterial activities and molecular identification of endophytic fungi isolated from mangrove* Avicennia marina. *Biodiversitas 24: 6923-6933.* This research explored the antibacterial potential of endophytic fungi from the *Avicennia marina*'s (Forssk.) Vierh leaves, stem bark, and root, located in Blanakan, Subang District, West Java. The screening process for antibacterial activity potential was conducted using the Kirby Bauer disk diffusion method. Subsequently, the molecular screening results of the most promising fungi species were identified using Internal Transcribed Spacer (ITS) markers. The results revealed the isolation of 30 fungal isolates. Among these, seven endophytic fungi exhibited significant antibacterial activity against *Staphylococcus aureus* ATCC 29213 and *Vibrio harveyi* ATCC 5339, with inhibition zones ranging from 7.88±1.52 to 23.60±0.77 mm. Through molecular identification, 7 endophytic fungi were identified, including *Penicillium chrysogenum*, *Cladosporium anthropophilum, Trichosporon asahii, Cladosporium sphaerospermum, Fusarium verticillioides, Meyerozyma carpophila,* and *Penicillium steckii*. Notably, *C. anthropophilum* exhibited the highest inhibition zones against *S. aureus* and *V. harveyi* measuring 23.60±0.77 and 21.80±0.26 mm, and showed (Minimum Inhibitory Concentration) MIC values of 15.625±0.98 and 31.25±0.39 µg/mL, respectively. In this study, endophytic fungi isolated from different parts of *A. marina* exhibited promising antibacterial activity, with *Cladosporium anthropophilum* from the stem bark showing the highest potency against *S. aureus* and *V. harveyi*, suggesting their potential as a source of antibacterial agents. To the best of our knowledge, this is the inaugural study uncovering the isolation and antibacterial potential of endophytic fungi from the genera *Cladosporium*, *Trichosporon*, *Fusarium*, and *Meyerozyma*. These fungi were extracted from the bark, leaves, and roots of *A. marina*, situated within the unique mangrove ecosystem of Blanakan Sub-district, Subang District, West Java, Indonesia.

Keywords: *Avicennia marina, Cladosporium anthropophilum,* endophytic fungi, mangrove, *Staphylococcus aureus, Vibrio harveyi*

INTRODUCTION

The surge in vibriosis, attributed to *Vibrio harveyi*, has sent shockwaves through the marine food production industry, with an alarming 80% mortality rate observed in various shrimp species, particularly *Panaeus monodon*. Similarly, *Staphylococcus aureus* has breached established safety limits (Akerina 2016; Karimela et al. 2019), contaminating aquatic products and raising concerns due to its potential to cause severe skin infections, especially in individuals with compromised immune systems (Becker et al. 2014). The situation is further exacerbated by increasing antimicrobial resistance to antibiotics such as tetracycline, amoxicillin, erythromycin, chloramphenicol, quinolones, and sulfonamides (Junianto et al. 2020). Therefore, the urgent need is to seek more effective alternatives to combat fish diseases by discovering new drug candidates from natural sources (Kodir et al. 2017).

Endophytic fungi have gained attention as potential sources for new medicines. They are known for their capacity to produce relatively unexplored secondary metabolites, offering fresh insights for drug research and development. Interest in exploring bioactive compounds derived from endophytic fungi has surged in recent years, particularly in their roles as antibacterial and cytotoxic agents (Tiwari and Bae 2022). For example, the compound fonsecinone A exhibited antibacterial effects against *B. cereus, S. aureus, B. subtilis*, and *E. coli*, with activity observed against nearly all tested phytopathogenic fungi, and a Minimum Inhibitory Concentration (MIC) ranging from 6.25 to 50 µM (Xiao et al. 2014). Additionally, alkaloids such as fumigaclavine C and pseurotin A, produced by the endophytic fungus *Aspergillus* sp. EJC08 isolated from the medicinal plant *Bauhinia guianensis*,

were initially reported as potent broad-spectrum antibacterial agents (Pinheiro et al. 2013).

In this study, we explored the potential of endophytic fungi residing within mangrove trees as a source of novel antibacterial compounds to address these pressing challenges (Ariantari et al. 2021; Handayani et al. 2021). Of particular interest are fungi residing within the genus *Avicennia* from the Acanthaceae family, which are well-documented for their pharmacological properties in treating conditions like diabetes, HIV, hepatitis, diarrhea, and inflammation (Thatoi et al. 2016; Zhang et al. 2018). Notably, *Avicennia marina* (Forssk.) Vierh, a member of this genus, is recognized as a natural source of medicinal plants, frequently employed in traditional herbal medicine for ailments such as diarrhea, dysentery, and fever (ElDohaji et al. 2020). Extracts from *A. marina* demonstrate broad-spectrum antibacterial activity, signifying their potential as valuable sources of antibacterial agents (Okla et al. 2021; Sarkar et al. 2023). In the mangrove ecosystem, numerous endophytes, including fungal endophytes, play vital roles in facilitating mangrove adaptation (Khalil et al. 2021; Thatoi et al. 2013; Shiono et al. 2016). These endophytes have gained significant attention as potential sources of novel secondary metabolites, harnessing the rich diversity of the marine environment to produce unique compounds that contribute to the survival and adaptation of fungi in this ecosystem (de Souza Sebastianes et al. 2013; Uzma et al. 2018; Thatoi et al. 2013; Suzuki et al. 2019).

The importance of endophytic fungi residing intracellularly within plant tissues lies in their remarkable ability to produce compounds that benefit their host plants, presenting a promising and sustainable solution for the development of antimicrobial agents (Das et al. 2018; Roy et al. 2023). Previous research showed that these microorganisms were widely distributed throughout various plant tissues, such as leaves, roots, and bark, and possess the capacity to synthesize broad-spectrum antibiotics. Research on endophytic fungi extracted from mangrove plants yielded encouraging results, indicating their potential to harbor novel metabolites that could significantly benefit their host organisms (Maria et al. 2005). In a previous study conducted by Trivedi and Thumar strong antibacterial activity was reported for endophytic fungi isolated from the roots of *A. marina*, a mangrove species collected from the Gulf of Kutch in Gujarat, India (at coordinates 23˚01′58.6"N 70˚09′27.3"E) (Trivedi and Thumar 2023). As such, we embarked on a more extensive investigation into endophytic fungi isolated from the leaves, roots, and stems of *A. marina*, presenting a comprehensive overview of our findings. We successfully isolated 30 distinct endophytic fungi from various parts of the *A. marina* plant, including the roots, bark, and leaves. Our research entailed a meticulous evaluation of their antibacterial activity, with a specific focus on samples collected from Subang District in West Java, Indonesia. The results of our study highlight the considerable antimicrobial potential within these isolates, contributing significantly to our understanding of fungal endophytes in *A. marina* and their antibacterial properties.

MATERIALS AND METHODS

Study area

The sampling of *A. marina* leaves stem bark, and root was carried out in the mangrove ecosystem at Blanakan Sub-district, Subang District, West Java, Indonesia. The coordinates for Blanakan Sub-district, where the sampling sites of *A. marina* are located, are 6º16’52” S and 107º39’46” E (Figure 1).

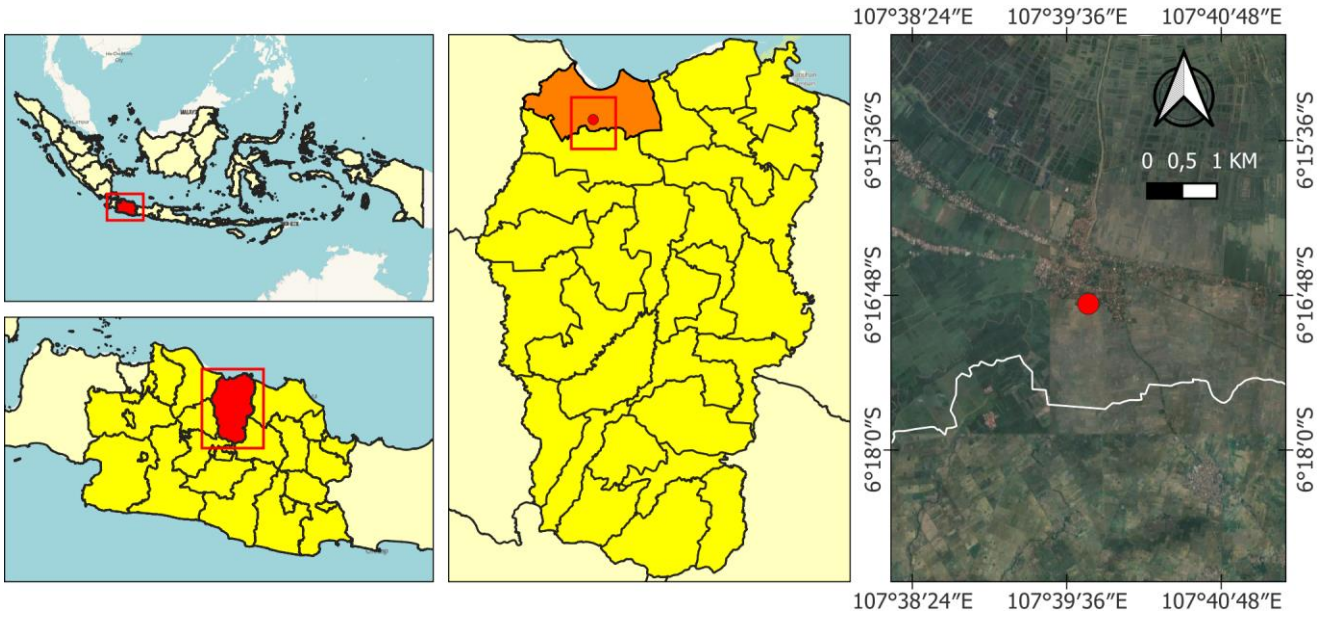

Figure 1. The location of Blanakan Sub-district, Subang District, West Java, Indonesia indicating the sampling sites of *Avicennia marina*: (red dot: 6º16’52” S, 107º39’46” E)

Procedures

Mangrove's tissue sampling

The purposive sampling technique was employed for mangrove plant sampling, entailing the intentional selection of samples based on specific requirements (healthy tissues from leaves, stem bark, and roots of *A. marina*) and the assumption that these samples could adequately represent the research site's population. Subsequently, the aforementioned healthy tissues from leaves, stem bark, and roots were excised using a sterile hacksaw and positioned within plastic ziplock bags to retard the drying process. These samples were later transported to the laboratory inside a coolbox, which maintained a temperature of 4° C (Khwaja and Arunagirinathan 2021).

Sample surface sterilization

To eliminate epiphytic bacteria, the stem bark, leaves, and roots of *A. marina* plant samples were subjected to sterilization. The process commenced with a thorough washing under running fresh water, followed by their division into small sections. Subsequently, the surfaces underwent sterilization with sterile aquades, which had been purified through autoclaving, for one minute. This was succeeded by a one-minute exposure to 70% ethanol technical grade to prevent potential contamination by epiphytic bacteria. Following this, the samples were immersed in a 5.25% sodium hypochlorite solution for five minutes, then treated with 70% ethanol technical for 30 seconds before being rinsed three times with sterile aquades. Finally, the sample surfaces were dried using whatman filter paper (Wulandari et al. 2022).

Fungal endophyte isolation and identification

After the sterilization process, each plant sample, measuring approximately 1x1 cm, was carefully positioned on individual Petri dishes containing Potato Dextrose Agar (PDA) medium (Merck). Specifically, four pieces of plant samples were placed on each Petri dish, resulting in a total of 15 plates. These plates were divided as follows, five plates for the leaves, five plates for the roots, and five plates for the bark samples. This arrangement allowed for the cultivation and isolation of microorganisms from the different parts of the *A. marina* plant while ensuring the inhibition of unwanted microbial contaminants. To inhibit bacterial growth, an additional 500 ppm of chloramphenicol was added to the PDA medium. Subsequently, the cultivated sample was then incubated for 7-21 days at room temperature (Basheer et al. 2018). To characterize the fungal endophytes, the following parameters were observed: the overall appearance of the colony, its color, edge form, and surface elevation. These observations helped in identifying and understanding the properties of the fungal endophytes present in the sample

Fermentation

The endophytic fungi were transferred to 150 mL of PDB (Potato Dextrose Broth) medium (Granulated GM403) in Erlenmeyer flasks 500 mL and incubated at 28° C. The flasks were exposed to regular daily light and dark cycles for 30 days. Following the incubation, the mycelia of the endophytic fungi were separated by filtration using Whatman filter paper, and the mycelial mass was isolated from the liquid broth through filtration. Additionally, the remaining culture broth was subjected to three rounds of partitioning with ethyl acetate at a 2:1 ratio (Khalil et al. 2021). Subsequently, the organic phase was evaporated under a vacuum and stored in vial bottles.

Screening for antibacterial activity using disk diffusion assay

In this research, *Vibrio harveyi* ATCC 5339 and *Staphylococcus aureus* ATCC 29213 from the Marine Biotechnology Laboratory, Marine Science Department, Faculty of Fisheries and Marine Science, Universitas Padjadjaran were utilized as the bacteria for the test. The bacteria used for the test was standardized to McFarland 0.5 on a 600 nm wavelength (Rosmania and Yanti 2020). The antibacterial test was conducted using the disk diffusion method. The disk diffusion method was selected for the antibacterial activity assay due to its well-established utility in evaluating the susceptibility of bacteria to various compounds. This method provides a clear and visible measurement of the inhibitory zones formed around the disks, enabling straightforward comparisons of antibacterial effectiveness among different samples. It is widely recognized and accepted in the field of microbiology, making it a practical choice for our research. The ethyl acetate fungal extract was dissolved on a 6 mm diffusion disk which was made of filter paper. The disk was then placed on top of an NA (Nutrient Agar) medium (Merck) with approximately 1 mL of bacteria test evenly spread on the surface. The selection of NA medium for evaluating antimicrobial activity was made based on its compatibility with the bacterial test strains and its established use in such assays. NA medium provides a suitable environment for bacterial growth and diffusion of antimicrobial agents. To ensure consistency, approximately 1 mL of the bacterial test suspension was evenly spread on the NA medium before placing the disk. This choice of medium and method was aimed at facilitating accurate and reproducible assessment of antimicrobial activity. Both positive control (chloramphenicol 500 ppm) and negative control (Nutrient Broth, NB Merck) were included for comparison. The resulting inhibition zone was measured after 24 hours (Sinaga et al. 2023). The presence of a clear zone, observable to the naked eye, indicated that the fungal isolated possessed antibacterial activity. Therefore, it was subjected to the Minimum Inhibitory Concentration MIC test. Effective inhibitory effects were determined based on the performance of the identified fungal isolates in the preliminary screening. Only those isolates that exhibited the most significant inhibition against the bacterial strains were selected for further testing.

Molecular identification

In the molecular identification process, validation methods were employed to confirm the accuracy and reliability of our molecular techniques. This encompassed the utilization of reference sequences and established protocols to verify the identity of the fungal isolates. The DNA extraction was

carried out following the TRIsure Bioline™ protocol from Meridian (2000). After the extraction, the DNA was amplified using the PCR (Polymerase Chain Reaction) method. The amplification primers used for DNA were ITS 1 (5' TCC GCT TAT TGA TAT GC 3') and ITS 4 (5' TCC GTA GGT GAA CCT GCG G 3'). The PCR cocktail included the GoTaq Green Master Mix. Subsequently, the resulting amplicon was visualized using the agarose electrophoresis method and measured using the 1k GeneRuler from Thermoscientific, and a 2% agarose gel with 10 µL of SYBR Gel Stain was used as the gel stain. The desired band of the fungal endophyte was within 600-700 bp (base pairs) (Schoch et al. 2012). For further analysis, the amplicon was then sent to 1st BASE for sequencing and Sanger sequencing using the BigDye method is a DNA sequencing technique that utilizes fluorescently labeled chain-terminating nucleotides (BigDye terminators) to determine the sequence of a DNA fragment. The resulting sequence was edited using BioEdit to separate bad sequence data. The edited sequence was then BLAST-ed on NCBI GeneBank. Based on the identified species, different species with top similarities were used to construct the phylogenetic tree of the fungal endophyte using MEGA-X.

Determination of MIC

The bacteria used for the test were cultured using a suitable medium. The suitable medium used in this context is Mueller Hinton Broth. It was used to culture bacteria for testing against both *S. aureus* and *V. harveyi*. The absorbance of the bacterial suspension was adjusted to 0.125 at 550 nm with sterile saline. A further 10-fold dilution of the cell suspension was performed using sterile saline, resulting in a test bacteria concentration of approximately 10^7 cfu/mL (cfu: Colony forming unit). To evaluate the sample's effect on the bacteria, various concentrations of the sample were prepared in Mueller-Hinton broth (Merck), including 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.9063, 1.9531, and 0.9766 µg/mL. Each well in the experiment received 190 µL of the sample in Mueller-Hinton broth. Subsequently, 10 µL of the organism suspension was inoculated into each well, resulting in a final cell density of 10^4 cfu/mL. The absorbance at 550 nm was subsequently measured using a spectrophotometer to assess the impact of the different sample concentrations on bacterial growth. Regarding the specific concentrations used for MIC determination, these concentrations were chosen based on preliminary testing and literature review (Alshareef 2021). Replicate experiments for the antibacterial activity assays were conducted to ensure the consistency and reproducibility of our results. This entailed the repetition of the same experiment multiple times under identical conditions to confirm the reliability of the observed outcomes.

Data analysis

The measurement of antibacterial activity was carried out based on the standard developed with categories of 0-5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and >20 mm (very strong). To analyze the macro morphology of the fungal endophyte, specific parameters were employed such as colony size, color, texture, and overall appearance, were employed to assess the macro morphology. This analysis allowed for the differentiation and characterization of different fungal endophytes based on their observable external characteristics (Mishra et al. 2021). The process of species identification involved evaluating the percent identity (more than 80%), query cover (more than 69%), bootstrap value (more than 70 points), and E-value (less than 0.0001) (Hall 2013; Pearson 2013).

RESULTS AND DISCUSSION

Characteristics of endophytic fungi derived from *A. marina*

In this research, a total of 30 fungal endophytes from different parts of *Avicennia marina* were isolated and identified. Among these, 11 isolates were obtained from the root (R1-R11), 9 were obtained from the stem bark (SB1-SB9), and 10 were obtained from the leaves (L1-L10) (Figure 2). The majority of these fungal endophytes exhibited filamentous mycelia, which radiated outward concentrically from their central point. However, the distinguishing factor between different colonies was their color. Some isolates, such as R3, R4, R5, R6, R9, R10, and R8, were white with varying hues when exposed to a light source. In contrast, isolates R1, R2, R7, and R9 exhibited unique and characteristic colors, suggesting their potential to possess significant bioactivities. Furthermore, most isolates exhibited hill-like umbonation at the center of their colonies, while R10, R3, R2, and R4 lacked this particular characteristic.

The 30 fungal endophytes exhibited a plethora of diverse characteristics and appearances, primarily attributed to variations in their classification and the metabolites or pigments they synthesized (Table 1). These fungal colonies displayed various forms, including circular, irregular, and filamentous shapes. Additionally, the colony edges also showed variation, with some being entire, filiform, and undulate in nature. In terms of texture, most colonies had a cottony texture, while ten colonies had a powdery texture. The pigments produced by the fungi exhibited a wide range of colors, including red, green, white, brownish, yellow, tosca, dark green, and greenish-yellow (Figure 2).

Endophytic fungi derived from mangrove plants exhibit diverse characteristics that can vary according to the specific source within the mangrove ecosystem. These characteristics include fungal numbers, diversity, and adaptations to the unique environmental conditions. In the context of fungal numbers, studies have shown variations in endophytic fungal populations based on the plant part from which they are isolated. For instance, the roots of mangrove plants tend to harbor a higher number of endophytic fungi compared to other plant parts like leaves and stems. This could be attributed to the nutrient-rich and anoxic conditions in the root zones, which provide a favorable habitat for fungal colonization (Maria et al. 2005).

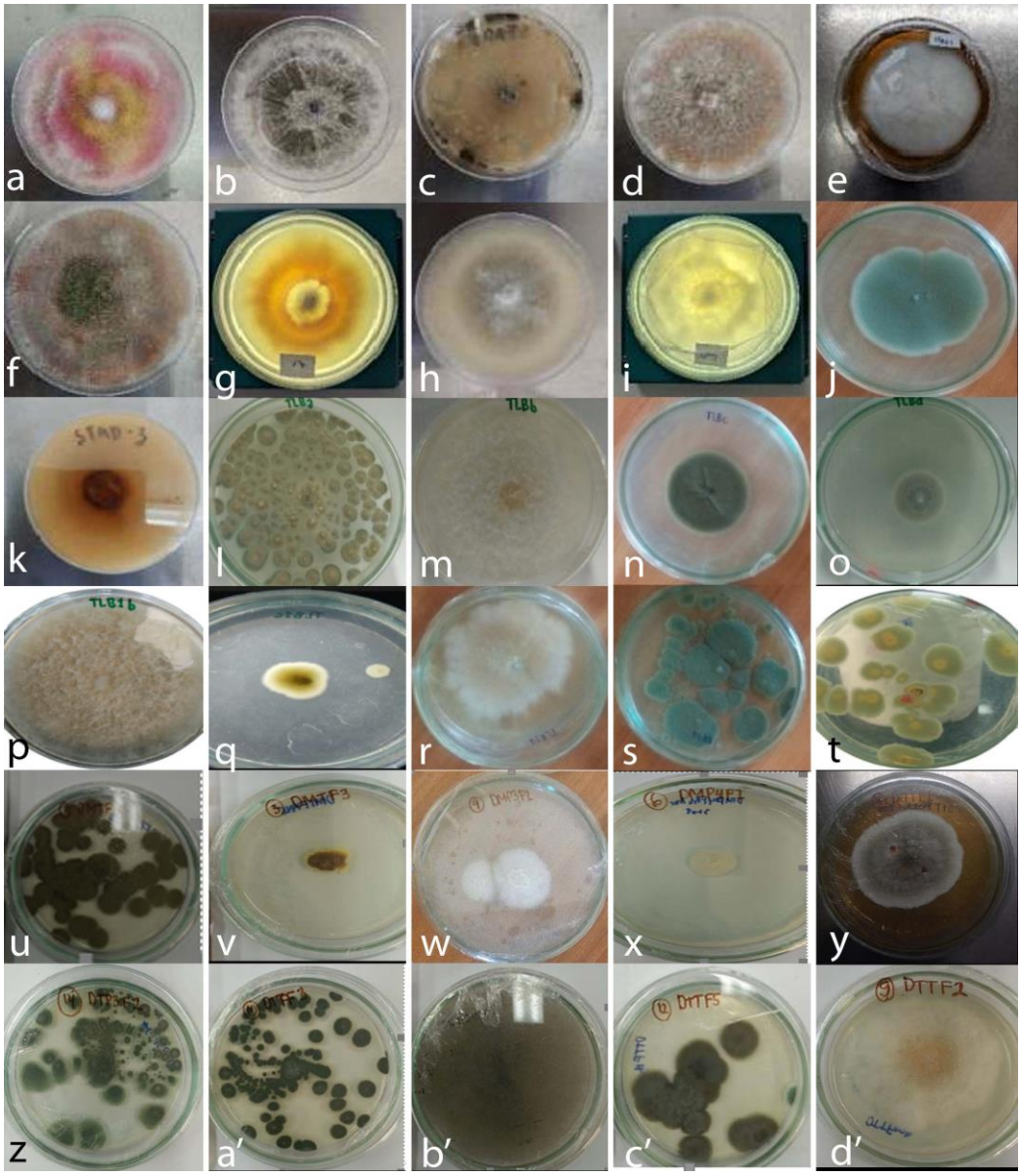

Figure 2. Morphology of *Avicennia marina* endophytic fungi which were incubated for 7-21 days at room temperature (a-k) derived from root, (l-t) stem bark, and (u-d') leaves. Isolate codes : (a) R1, (b) R2, (c) R3, (d) R4, (e) R6, (f) R7, (g) R9, (h) R8, (i) R10, (j) R11, (k) R5, (l) SB1, (m) SB2, (n) SB3, (o) SB4, (p) SB5, (q) SB6, (r) SB7, (s) SB8, (t) SB9, (u) L1, (v) L2, (w) L3, (x) L4, (y) L5, (z) L7, (a') L10, (b') L8, (c') L9, (d') L6

Endophytic fungi derived from mangrove plants exhibit diverse characteristics that can vary according to the specific source within the mangrove ecosystem. These characteristics include fungal numbers, diversity, and adaptations to the unique environmental conditions. In the context of fungal numbers, studies have shown variations in endophytic fungal populations based on the plant part from which they are isolated. For instance, the roots of mangrove plants tend to harbor a higher number of endophytic fungi compared to other plant parts like leaves and stems. This could be attributed to the nutrient-rich and anoxic conditions in the root zones, which provide a favorable habitat for fungal colonization (Maria et al. 2005).

In contrast, leaves and stems may have lower fungal populations due to the harsher environmental conditions and competition from other microorganisms. Diversity is another key characteristic that differs among endophytic fungi sourced from mangrove plants. Studies have revealed a wide variety of fungal species associated with mangrove ecosystems, with different species predominating in different plant parts. For example, studies have reported the prevalence of species from genera such as *Penicillium*, *Aspergillus*, and *Fusarium* in mangrove leaves, while roots often host unique species such as *Phomopsis* and *Glomerella* (Mulyani et al. 2023). This diversity could be influenced by factors like host specificity, niche differentiation, and microhabitat conditions. Furthermore, endophytic fungi derived from mangrove plants display remarkable adaptations to the challenging environmental conditions of mangrove ecosystems, including high salinity, low oxygen levels, and nutrient limitations. These adaptations are reflected in their metabolic capabilities, such as the production of enzymes

that facilitate nutrient acquisition from complex substrates, as well as the synthesis of bioactive compounds that may confer benefits to the host plants, including improved stress tolerance and growth promotion (Fontanez et al. 2015; Paranetharan et al. 2022).

Antimicrobial activity of endophytic fungi

The results of the antibacterial activity of endophytic fungi against two fish pathogen bacteria, namely *Vibrio harveyi* and *Staphylococcus aureus*, are presented in the Table 2. The inhibition zone diameters of the endophytic fungi against these two bacteria were measured. The positive control used in the study was chloramphenicol, an antibiotic commonly used to treat bacterial infections. Our findings reveal that certain endophytic fungi exhibited notable antibacterial activity against these pathogens, with inhibition zones ranging from moderate to very strong. Based on their antibacterial activity, we have identified the most promising endophytic fungi for further investigation, highlighting their potential as candidates for future studies. Following are the results of the antibacterial activity obtained from the thirty endophytic fungi (Table 2).

Molecular identification of endophytic fungi

Each of the obtained DNA sequences was compared against a comprehensive database using BLAST (Basic Local Alignment Search Tool) analysis, as described by Pearson (2013). The results of this analysis are summarized in Table 3, offering valuable insights into the genetic characteristics of the identified endophytic fungi (Pearson 2013). Figure 3 presents the phylogenetic tree depicting the relationships among the seven endophytic fungi. Our findings reveal that these fungi originate from different genera, including *Trichosporon*, *Cladosporium*, *Penicillium*, *Fusarium*, and *Meyerozyma*. These outcomes shed light on the evolutionary history and diversity of the endophytic fungi associated with *Avicennia marina*, emphasizing their potential as sources for the discovery of novel bioactive compounds.

Determination of MIC from the potential endophytic fungi

To establish the Minimum Inhibitory Concentration (MIC) of each of these bacterial strains, additional testing was conducted (Table 4). The results show that all seven fungal isolates exhibited potential antibacterial activity against both bacterial strains, with MIC values ranging from 15.625±0.98 to 1000±0.56 µg/mL. *Cladosporium anthropophilum* showed the lowest MIC values against *S. aureus*, while *Trichosporon asahii* showed the lowest MIC values against *V. harveyi*.

Table 1. Morphology characteristic of endophytic fungi from *Avicennia marina* which were incubated for 7-21 days at room temperature

No.	Organ of *A. marina*	Isolate code	Colony color		Radial line	Concentric circle	Colony shape	Edge of colony	Texture
			Frontside	Backside					
1	Roots	R1	Red	Red	White	Yellow	Circular	Entire	Cottony
2		R2	Green	Green	Light green	Light green	Circular	Entire	Cottony
3		R3	Opaque	Opaque			Irregular	Filiform	Cottony
4		R4	White	Pinkish			Irregular	Filiform	Cottony
5		R5	Brownish	Brownish	White		Irregular	Filiform	Cottony
6		R6	Yellowish white	Brownish			Circular	Entire	Cottony
7		R7	Brown	Brown			Irregular	Filiform	Cottony
8		R8	Brownish	White	White	Brownish	Circular	Entire	Cottony
9		R9	Yellow	Yellow	Pink	Yellow	Filamentous	Filiform	Cottony
10		R10	White	White			Irregular	Undulate	Cottony
11		R11	Green	Green	White	Green	Irregular	Undulate	Powdery
12	Stem bark	SB1	Green	Green			Irregular	Undulate	Powdery
13		SB2	White	White			Irregular	Filiform	Cottony
14		SB3	Green	Green	White	Green	Circular	Entire	Powdery
15		SB4	Green	Green	White	Green	Circular	Entire	Powdery
16		SB5	White	White			Irregular	Filiform	Cottony
17		SB6	White	White			Irregular	Undulate	Powdery
18		SB7	White	Pinkish			Irregular	Filiform	Cottony
19		SB8	Tosca	Tosca			Irregular	Undulate	Cottony
20		SB9	Greenish yellow	Greenish yellow			Irregular	Undulate	Cottony
21	Leaf	L1	Dark green	Dark green			Circular	Entire	Powdery
22		L2	Brownish-yellow	Brownish-yellow			Irregular	Undulate	Powdery
23		L3	White	White			Circular	Entire	Cottony
24		L4	Yellowish white	Yellowish white			Irregular	Filiform	Cottony
25		L5	Grey	Grey	White	Grey	Irregular	Entire	Cottony
26		L6	Yellowish white	Yellowish white			Irregular	Filiform	Cottony
27		L7	Green	Green			Circular	Entire	Powdery
28		L8	Grey	Grey			Filamentous	Filiform	Cottony
29		L9	Dark green	Dark green			Circular	Undulate	Powdery
30		L10	Green	Green			Irregular	Filiform	Powdery

Note: R: Root, SB: Stem Bark, L: Leaf

Table 2. Morphology characteristic of endophytic fungi from *Avicennia marina*

Sources of plant	Sample code	Inhibition zone (mm)		Category	
		S. aureus	***V. harveyi***	***S. aureus***	***V. harveyi***
Root (R)	R1	21.25 ± 0.56	21.03 ± 0.18	Very Strong	Very Strong
	R2	14.91 ± 1.50	9.88 ± 2.03	Strong	Moderate
	R3	12.25 ± 0.48	11.88 ± 0.67	Strong	Strong
	R4	14.90 ± 0.35	12.20 ± 0.98	Strong	Strong
	R5	16.00 ± 1.48	14.15 ± 0.81	Strong	Strong
	R6	16.45 ± 0.17	10.05 ± 0.61	Strong	Moderate
	R7	15.20 ± 1.52	13.50 ± 0.39	Strong	Strong
	R8	18.10 ± 0.26	15.35 ± 1.26	Strong	Strong
	R9	14.45 ± 0.46	11.13 ± 0.79	Strong	Strong
	R10	18.70 ± 0.26	17.68 ± 0.21	Strong	Strong
	R11	19.65 ± 0.78	18.53 ± 0.51	Strong	Strong
Stem Bark (SB)	SB1	17.12 ± 1.01	13.60 ± 0.49	Strong	Strong
	SB2	19.00 ± 0.98	13.10 ± 0.53	Strong	Strong
	SB3	23.60 ± 0.77	21.80 ± 0.26	Very Strong	Very Strong
	SB4	19.35 ± 0.55	13.30 ± 0.37	Strong	Strong
	SB5	17.95 ± 0.33	10.90 ± 0.82	Strong	Strong
	SB6	15.25 ± 0.28	10.15 ± 0.55	Strong	Moderate
	SB7	14.80 ± 0.69	12.20 ± 0.73	Strong	Strong
	SB8	19.80 ± 0.77	16.50 ± 0.66	Strong	Strong
	SB9	16.65 ± 0.22	14.12 ± 0.56	Strong	Strong
Leaves (L)	L1	18.60 ± 0.37	15.50 ± 1.16	Strong	Strong
	L2	13.68 ± 0.72	12.50 ± 0.22	Strong	Strong
	L3	15.23 ± 0.80	11.10 ± 0.32	Strong	Strong
	L4	19.20 ± 1.29	15.98 ± 0.56	Strong	Strong
	L5	11.15 ± 0.92	8.52 ± 0.77	Strong	Moderate
	L6	11.72 ± 0.44	11.75 ± 0.85	Strong	Strong
	L7	15.70 ± 0.66	7.88 ± 1.52	Strong	Moderate
	L8	11.42 ± 2.12	11.7 ± 1.17	Strong	Strong
	L9	12.16 ± 0.16	7.98 ± 0.48	Strong	Moderate
	L10	13.35 ± 0.38	10.75 ± 0.11	Strong	Strong
	+	25.10 ± 0.29	22.25 ± 0.89		
	-	0	0		

Note: + : positive control (chloramphenicol), - : negative control (Nutrient Broth)

Table 3. Result of identification of endophytic fungi species

Isolate code	Sequence length (bp)	Sequence accession number	Closest similarity	Query coverage (%)	E-value	Identity (%)	Reference accession number
L4	496	OR892619	*Trichosporon asahii*	99	0.0	100	MT482659.1
L1	506	OR892618	*Cladosporium sphaerospermum*	100	0.0	99.02	MT582795.1
SB8	609	OR892621	*Penicillium chrysogenum*	98	0.0	98.83	MN069559.1
SB3	574	OR892620	*Cladosporium anthropophilum*	99	0.0	98.60	MF472933.1
R11	616	OR900884	*Penicillium steckii*	99	0.0	98.37	KX674639.1
R1	386	OR916272	*Fusarium verticillioides*	100	0.0	100	OQ938729.1
R10	360	OR900885	*Meyerozyma carpophila*	96	0.0	91.09	MN997029.1

According to the phylogenetic (Figure 3), the sequences of these endophytic fungi show a relationship with the following species: *Penicillium chrysogenum, Cladosporium anthropophilum, Trichosporon asahii, Cladosporium sphaerospermum, Fusarium verticillioides, Meyerozyma carpophila,* and *Penicillium steckii*. The support for these relationships is strong, ranging from 59 to 100 based on bootstrap analysis.

Isolate L1 (OR892618) has a close relationship with isolate SB3 (OR892620) forming one clade in the genus *Cladosporium*, within the Division Ascomycota, Class Dothideomycetes. Likewise, isolates SB8 (OR892621) and R11 (OR900884) formed one clade in the genus *Penicillium*, within the Ascomycota, Eurotiomycetes category. Meanwhile, isolates L4 (OR892619), R1 (OR916272) and R10 (OR900885) formed separate clades consisting of the genus *Trichosporon* (Basidiomycota), *Fusarium* (Ascomycota), and *Meyerozyma* (Ascomycota).

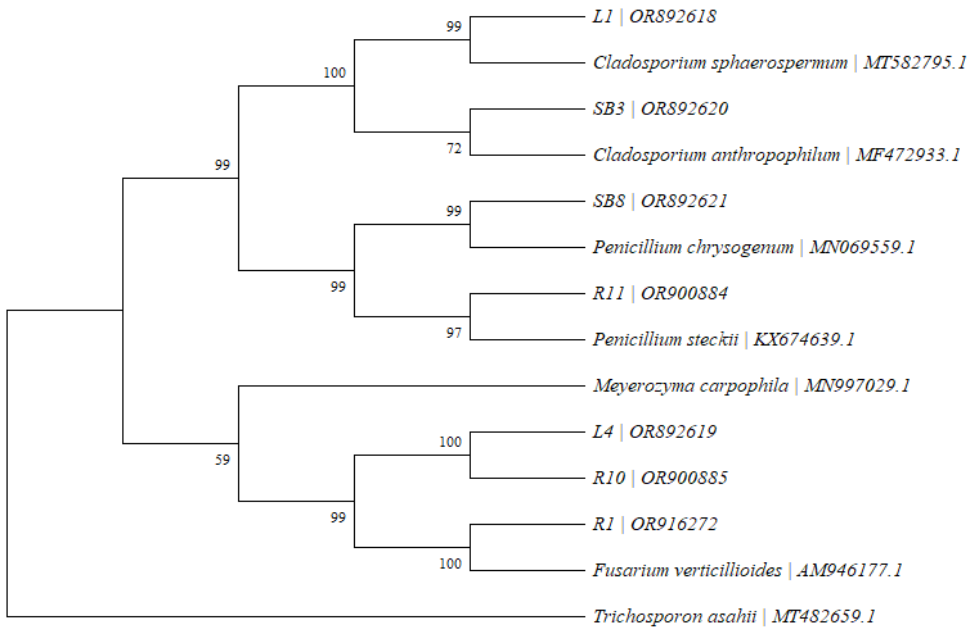

Figure 3. Phylogenetic tree illustrating the relationships among the seven endophytic fungi. The tree was constructed using the neighbor-joining method with 1000 bootstrap replicates for robust tree topology. Prior to construction, ITS gene sequences were aligned with the ClustalW algorithm to ensure accurate alignment. The best-fit nucleotide substitution model was determined through the Akaike information criterion (AIC) in MEGA7 software. The tree visualization was accomplished using FigTree software, a widely adopted tool for rendering and annotating phylogenetic trees

Table 4. Determination of MIC of 7 fungal isolates with potential antibacterial activity

Fungal species	MIC (µg/mL)	
	S. aureus	***V. harveyi***
Trichosporon asahii	1000 ± 0.56	1000 ± 0.26
Cladosporium sphaerospermum	250 ± 0.78	500 ± 0.96
Penicillium chrysogenum	250 ± 1.02	250 ± 0.82
Cladosporium anthropophilum	15.625 ± 0.98	31.25 ± 0.39
Penicillium steckii	125 ± 1.12	250 ± 0.55
Fusarium verticillioides	125 ± 0.63	250 ± 1.03
Meyerozyma carpophila	1000 ± 0.44	1000 ± 0.37

Discussion

Table 1 showed the morphology characteristics of the endophytic fungi isolated from different parts of *A. marina*. The fungal colonies displayed diverse forms, including circular, irregular, and filamentous shapes. The colony edges also showed variation, with some being entire, filiform, and undulate. In terms of texture, most colonies had a cottony texture, while ten colonies had a powdery texture. Furthermore, the pigments produced by these fungi exhibited a wide range of colors, including red, green, white, brownish, yellow, tosca, dark green, and greenish-yellow. These morphology characteristics of endophytic fungi provide important information about their taxonomic identity, ecological niche, and potential biotechnological applications. For example, the shape and texture of fungal colonies can serve as indicators of their growth rate, nutrient requirements, and interactions with other microorganisms in their environment (Nisa et al. 2015).

The color of fungal pigments can also indicate the presence of bioactive compounds, such as antibiotics, antifungals, and anticancer agents (Strobel 2003). The morphology characteristics of the endophytic fungi found in *A. marina* suggest that this mangrove species harbors a diverse and complex fungal community. The differences in colony shape, edge, and texture signify adaptations to different microhabitats within the plant, such as the roots, stem bark, and leaves. Additionally, the variation in pigment color could also indicate the presence of different secondary metabolites that could have potential biotechnological applications (Figure 2).

Previous research showed that endophytic fungi from mangrove plants can produce a wide range of bioactive compounds with potential pharmaceutical, agricultural, and industrial applications (de Souza Sebastianes et al. 2013). For example, endophytic fungi from the mangrove plant *Rhizophora mucronata* have been found to produce antimicrobial, antioxidant, and anticancer compounds. Similarly, endophytic fungi from the mangrove plant *Sonneratia alba* have been found to produce antifungal, antiviral, and insecticidal compounds. Therefore, the morphology characteristics of endophytic fungi from *A. marina* can be used as a screening tool for identifying potential sources of bioactive compounds (Buatong et al. 2011; Chaeprasert et al. 2010).

The results presented in Table 2 show the antibacterial activity of diverse endophytic fungi obtained from distinct segments of the mangrove *A. marina*, namely its roots, stem bark, and leaves. All the isolated fungi exhibited antibacterial effects against two types of bacteria, namely *S. aureus* and *V. harveyi*, with the inhibition zone ranging

from 7.88±1.52 to 23.60±0.77 mm. Among the isolated fungi, R1 and SB3 demonstrated remarkable potency, exhibiting significantly large inhibition zones of 21.25±0.56 and 23.60±0.77 mm against *S. aureus*, and 21.03±0.18 and 21.80±0.26 mm against *V. harveyi*, respectively. To further investigate these highly effective fungi, molecular identification was carried out on those with the highest inhibition zone values. Consequently, the selected fungi for molecular identification were R1, R10, and R11 from the root; SB3 and SB8 from the stem bark, and L1 and L4 from the leaves.

After a rigorous screening process for inhibitory activity, it was revealed that out of the 30 isolated endophytic fungi, only a select 7 exhibited exceptional potency, falling into the strong and very strong categories, as shown by inhibition zones exceeding our predetermined criteria of 18.60 mm against *S. aureus* and 15.50 mm against *V. harveyi*. It's important to note that other isolates classified as strong but not meeting these specific criteria were subsequently excluded from further analysis, ensuring a more focused investigation of the most promising candidates (Table 2).

The antibacterial activity of endophytic fungi has been extensively investigated due to their promising potential as sources of novel antibiotics and other bioactive compounds (Kusari and Spiteller 2015; Roy et al. 2023; Mayanti et al. 2022). It is believed that the ability of endophytic fungi to produce antibacterial compounds is an evolutionary adaptation to their intracellular existence within the host plant, where they must compete with other microorganisms for resources and space. Additionally, the antibacterial compounds produced by endophytic fungi can help protect the host plant from pathogens and herbivores, as well as enhance its growth and stress tolerance (Mulyani et al. 2023).

This research suggests the potential of endophytic fungi derived from *A. marina* to synthesize antibacterial compounds with moderate to strong activity against *S. aureus* and *V. harveyi*. The promising aspect lies in the observation that R1 and SB3 displayed exceptionally robust inhibition zones against both bacteria. This strongly suggests the existence of potent bioactive compounds within these samples. Molecular identification of these fungi could help elucidate the chemical nature of their antibacterial compounds and facilitate their further development as pharmaceuticals or agricultural agents.

Previous research showed that endophytic fungi from mangrove plants can produce a wide range of antibacterial compounds with potential pharmaceutical and agricultural applications (Khan et al. 2016). For example, endophytic fungi from the mangrove plant *Aegiceras corniculatum* have been found to produce antibacterial compounds with activity against drug-resistant strains of *S. aureus* and *E. coli* (Praptiwi et al. 2018). Similarly, endophytic fungi from the mangrove plant *Rhizophora mucronata* have been found to produce antibacterial compounds with activity against *S. aureus*, *Pseudomonas aeruginosa*, and *Vibrio cholerae* (Chaeprasert et al. 2010). Putra et al. (2023) reported that endophytic fungi isolated from *Ceriops tagal* showed antibacterial activity against *S. aureus* and *S. epidermidis*. Considering these findings, the antibacterial activity of endophytic fungi from *A. marina* could be further explored as a potential source of novel antibiotics or as a natural alternative to conventional antibiotics in aquaculture and fisheries.

The absence of antibacterial activity in certain endophytic fungi against *S. aureus* and *V. harveyi* is not surprising. The production of bioactive compounds by endophytic fungi can be influenced by various factors, such as the host plant, the microenvironment, and the stage of fungal growth (Laokor and Juntachai 2021). Moreover, the lack of activity against these two bacteria does not necessarily mean that these fungi are devoid of bioactive compounds, as they could still produce compounds with activity against other types of bacteria or other microorganisms.

Table 3 shows the results of the molecular identification of the seven endophytic fungi isolated from different parts of *A. marina*. The results of the analysis revealed that the identification of endophytic fungi at the species level is important for several reasons. First, it provides valuable insights into the diversity of endophytic fungi associated with *A. marina*, thereby enhancing comprehension of the ecological and evolutionary processes that influence the mangrove ecosystem. Second, it enables the comparison of genetic characteristics and bioactive potential of different endophytic fungi, which can aid in the selection of candidates for further biotechnological applications. Third, it facilitates the tracking of the distribution and transmission of endophytic fungi within and between different mangrove habitats, providing valuable information for conservation and management strategies.

Certain fungi showed strong potential in inhibiting all tested bacteria, with notable candidates including *Penicillium chrysogenum* (SB8) and *Cladosporium anthropophilum* (SB3) isolated from stem bark, *Trichosporon asahii* (L4), and *Cladosporium* sphaerospermum (L1) isolated from leaves, as well as *Fusarium verticillioides* (R1), *Meyerozyma guilliermondii* (R10), and *Penicillium steckii* (R11) isolated from roots. These identified species are known to be fungal endophytes found in various mangrove environments.

Out of the seven fungal isolates tested for potential antibacterial activity, *Cladosporium anthropophilum* showed the most potent antibacterial activity, as evidenced by the MIC results. The MIC for this isolate was found to be 15.625±0.98 µg/mL against *S. aureus* and 31.25±0.39 µg/mL against *V. harveyi*. This result indicates that *C. anthropophilum* is particularly effective at inhibiting the growth of both *S. aureus* and *V. harveyi* at relatively low concentrations. Previous research suggested that the high antibacterial activity of *Cladosporium* species may be attributed to the production of secondary metabolites, such as cladosporin and 5-chloro-8-hydroxyquinoline, as well as other compound groups like phenolic compounds, terpenoids, and alkaloids, which have been shown to exhibit potent antimicrobial activity (Wang et al. 2018; Hulikere and Joshi 2019; Salvatore et al. 2021; Han et al. 2021; Mulyani et al. 2023).

This research represents the first instance of *C. anthropophilum* being isolated as an endophytic fungus from *A. marina* in Blanakan Sub-district, Subang District,

West Java, Indonesia. The secondary metabolites profile of *C. anthropophilum* has not been reported previously, presenting an exciting opportunity to explore the structural diversity of secondary metabolites produced by these endophytic fungi (Salvatore et al. 2021). Additionally, *Meyerozyma guilliermondii*, known as a fungal endophyte found on the roots of mangrove *Kandelia obovata* (S., L.) Yong, has been reported to produce botryorhodines E-G, which exhibit potent α-glucosidase inhibitory activity (Chen et al. 2015; Ebrahim et al. 2020). Similarly, *Fusarium verticilliodes*, known as an endophytic fungus on *Kandelia candel*'s bark, showed higher activity against gram-positive bacteria compared to gram-negative bacteria (Aboul-Nasr and Obied-Allah 2013). Notable compounds isolated from *F. verticillioides* include fusaisocoumarin, emodin, and sesquiterpenoid phytohormone (+)-abscisic acid (ABA) (Ebrahim et al. 2020). *Trichosporon asahii* was successfully isolated from *Pluchea indica* leaves in Guandong, China. Polyketides were successfully isolated from the endophytic fungi *Cladosporium* sp. derived from the mangrove plant *Excoecaria agallocha*, as reported by Wang et al. (2018) (Gao et al. 2020; Li et al. 2018; Wang et al. 2018).

The secondary metabolites produced by these potent endophytic fungi, specifically those showing excellent antibacterial activity, hold promise as lead compounds for the development of antibiotic drugs to combat fish pathogens. Further testing, such as in vivo assays and clinical trials, will be essential to advance the development of these antibiotic drugs.

Conclusions, the study demonstrates the potential of endophytic fungi from the *Avicennia marina* plant as a source of antibacterial agents against fish pathogen bacteria. The results of the study suggest that these fungi could serve as a natural alternative to synthetic antibiotics in aquaculture. Further research is needed to identify the active compounds responsible for the observed antibacterial activity and to evaluate their safety and efficacy in vivo. Additionally, the use of endophytic fungi as a natural alternative to synthetic antibiotics in aquaculture could contribute to sustainability efforts by reducing the environmental impact of aquaculture practices.

ACKNOWLEDGEMENTS

This research was financially supported by National Postgraduate Education Scholarship (BPPDN) from the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia. The authors are grateful for the facilities from Universitas Padjadjaran, Indonesia Academic Leadership Grant, Universitas Padjadjaran, No. 1549/UN6.3.1/PT.00/2023 by Unang Supratman.

REFERENCES

Aboul-Nasr MB, Obied-Allah MRA. 2013. Biological and chemical detection of fumonisins produced on agar medium by *Fusarium verticillioides* isolates collected from corn in Sohag, Egypt. Microbiology 159 (8): 1720-1724. DOI: 10.1099/mic.0.069039-0.

Akerina FO. 2016. Analisis mikroba ikan tuna asap pada beberapa pasar di Tobelo, Halmahera Utara. In Prosiding Seminar Nasional Kemaritiman dan Sumber Daya Pulau-Pulau Kecil 2 (1): 45-50. [Indonesian]

Alshareef F. 2021. Protocol to evaluate antibacterial activity MIC, FIC and time kill method. Acta Sci Microbiol 4 (5): 2-6. DOI: 10.31080/ASMI.2021.04.0825.

Ariantari NP, Frank M, Gao Y, Stuhldreier F, Kiffe-Delf AL, Hartmann R, Höfert SP, Janiak C, Wesselborg S, Müller WE, Kalscheuer R. 2021. Fusaristatins D-F and (7S,8R)-(−)-chlamydospordiol from *Fusarium* sp. BZCB-CA, an endophyte of *Bothriospermum chinense*. Tetrahedron 85: 132065. DOI: 10.1016/j.tet.2021.132065.

Basheer MA, Mekawey AA, El-Kafrawy SB, Abouzeid MA. 2018. Antimicrobial activities of endophytic fungi of Red Sea aquatic plant *Avicennia marina*. Egypt J Microbiol 53 (1): 231-240. DOI: 10.21608/ejm.2018.3833.1082.

Becker K, Heilmann C, Peters G. 2014. Coagulase-negative *Staphylococci*. Clin Microbiol Rev 27 (4): 870-926. DOI: 10.1128/CMR.00109-13.

Buatong J, Phongpaichit S, Rukachaisirikul V, Sakayaroj J. 2011. Antimicrobial activity of crude extracts from mangrove fungal endophytes. World J Microbiol Biotechnol 27 (12): 3005-3008. DOI: 10.1007/s11274-011-0765-8.

Chaeprasert S, Piapukiew J, Whalley AJS, Sihanonth P. 2010. Endophytic fungi from mangrove plant species of Thailand: Their antimicrobial and anticancer potentials. Bot Mar 53 (6): 555-564. DOI: 10.1515/bot.2010.074.

Chen H, Daletos G, Okoye F, Lai D, Dai H, Proksch P. 2015. A new cytotoxic cytochalasin from the endophytic fungus *Trichoderma harzianum*. Nat Prod Commun 10 (4): 585-587. DOI: 10.1177/1934578X1501000412.

Das SK, Samantray D, Thatoi HN. 2018. Pharmacological applications of metabolites of mangrove endophytes: A review. Microb Biotechnol 2: 331-360. DOI:10.1007/978-981-10-7140-9_16.

de Souza Sebastianes FL, Romao-Dumaresq AS, Lacava PT, Harakava R, Azevedo JL, de Melo IS, Pizzirani-Kleiner AA. 2013. Species diversity of culturable endophytic fungi from Brazilian mangrove forests. Curr Genet 59 (3): 153-166. DOI: 10.1007/s00294-013-0396-8.

Ebrahim W, Özkaya FC, Ebada SS. 2020. Antifungal metabolites from endophytic fungus *Fusarium verticillioides* strain WF18. S Afr J Bot 133: 40-44. DOI: 10.1016/j.sajb.2020.06.029.

ElDohaji LM, Hamoda AM, Hamdy R, Soliman SSM. 2020. *Avicennia marina* natural reservoir of phytopharmaceuticals: Curative power and platform of medicines. J Ethnopharmacol 263: 113179. DOI: 10.1016/j.jep.2020.113179.

Fontanez KM, Eppley JM, Samo TJ, Karl DM, DeLong EF. 2015. Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre. Front Microbiol 6: 1-14. DOI: 10.3389/fmicb.2015.00469.

Gao Y-Q, Du S-T, Xiao J, Wang D-C, Han W-B, Zhang Q, Gao J-M. 2020. Isolation and characterization of antifungal metabolites from the *Melia azedarach*-Associated Fungus *Diaporthe eucalyptorum*. J Agric Food Chem 68 (8): 2418-2425. DOI:10.1021/acs.jafc.9b07825.

Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. Mol Biol Evol 30 (5): 1229-1235. DOI: 10.1093/molbev/mst012.

Han X, Bao X-F, Wang C-X, Xie J, Song X-J, Dai P, Chen GD, Hu D, Yao X-S, Gao H. 2021. Cladosporine A, a new indole diterpenoid alkaloid with antimicrobial activities from *Cladosporium* sp. Nat Prod Res 35 (7): 1115-1121. DOI: 10.1080/14786419.2019.1641807.

Handayani D, Mulia P, Andayani R, Wahyuni FS, Ariantari NP. 2021. Secondary metabolite from mangrove endophytic fungus *Fusarium proliferatum* AED3. Rasayan J Chem 2021: 150-155. DOI: 10.31788/RJC.2021.1456447.

Hulikere MM, Joshi CG. 2019. Characterization, antioxidant and antimicrobial activity of silver nanoparticles synthesized using marine endophytic fungus- *Cladosporium cladosporioides*. Process Biochem 82: 199-204. DOI: 10.1016/j.procbio.2019.04.011.

Junianto, Azizah SUN, Rostini I, Pratama RI. 2020. Proximate composition of cilok mackerel fish head flavor powder. Asian J Fish Aquat Res 9 (2): 1-8. DOI: 10.9734/ajfar/2020/v9i230153.

Karimela EJ, Ijong FG, Palawe JFP, Mandeno JA. 2019. Isolasi dan identifikasi bakteri *Staphylococcus epidermis* pada ikan asap pinekuhe. Jurnal Teknologi Perikanan Dan Kelautan 9 (1): 35-42. DOI: 10.24319/jtpk.9.35-42. [Indonesian]

Khalil AMA, Abdelaziz AM, Khaleil MM, Hashem AH. 2021. Fungal endophytes from leaves of *Avicennia marina* growing in semi-arid environment as a promising source for bioactive compounds. Lett Appl Microbiol 72 (3): 263-274. DOI: 10.1111/lam.13414.

Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M, Asaf S, Elyassi A, Mabood F, Shin JH, Lee IJ. 2016. Endophytic fungi from frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid. PLoS One 11 (6): e0158207. DOI: 10.1371/journal.pone.0158207.

Khwaja NUD, Arunagirinathan G. 2021. Efficacy and cardiovascular safety of alpha glucosidase inhibitors. Curr Drug Saf 16 (2): 122-128. DOI: 10.2174/1574886315666201217100445.

Kodir A, Hartono DM, Haeruman H, Mansur I. 2017. Integrated post mining landscape for sustainable land use: A case study in South Sumatera, Indonesia. Sustain Environ Res 27 (4): 203-213. DOI: 10.1016/j.serj.2017.03.003.

Kusari S, Spiteller M. 2015. Are we ready for industrial production of bioactive plant secondary. Nat Prod Rep 28: 1203-1207. DOI: 10.1039/c1np00030f.

Laokor N, Juntachai W. 2021. Exploring the antifungal activity and mechanism of action of *Zingiberaceae rhizome* extracts against *Malassezia furfur*. J Ethnopharmacol 279: 114354. DOI: 10.1016/j.jep.2021.114354.

Li S-J, Zhang X, Wang X-H, Zhao C-Q. 2018. Novel natural compounds from endophytic fungi with anticancer activity. Eur J Med Chem 156: 316-343. DOI: 10.1016/j.ejmech.2018.07.015.

Maria GL, Sridhar KR, Raviraja NS. 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of Southwest Coast of India. J Agric Technol 1: 67-80.

Mayanti T, Sinaga SE, Supratman U. 2022. Phytochemistry and biological activity of *Lansium domesticum* Corr. species: A review. J Pharm Pharmacol 74 (11): 1568-1587. DOI: 10.1093/jpp/rgac057.

Meridian. 2000. "Protocol for the Isolation of RNA Using TRIsure." Meridian Bioscience: 60-61.

Mishra P, Gupta P, Pruthi V. 2021. Cinnamaldehyde incorporated gellan/PVA electrospun nanofibers for eradicating *Candida* biofilm. Mater Sci Eng C 119: 111450. DOI: 10.1016/j.msec.2020.111450.

Mulyani Y, Sinaga SE, Supratman U. 2023. Phytochemistry and biological activities of endophytic fungi from the Meliaceae family. Molecules 28 (2): 778. DOI: 10.3390/molecules28020778.

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA. 2015. Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: A review. Microb Pathog 82: 50-59. DOI: 10.1016/j.micpath.2015.04.001.

Okla MK, Alatar AA, Al-Amri SS, Soufan WH, Ahmad A, Abdel-Maksoud MA. 2021. Article antibacterial and antifungal activity of the extracts of different parts of *Avicennia marina* (Forssk.) vierh. Plants 10 (2): 1-13. DOI: 10.3390/plants10020252.

Paranetharan MS, Thirunavukkarasu N, Rajamani T, Suryanarayanan TS. 2022. Biomass destructuring enzymes of fungal endophytes of mangrove roots. Curr Res Environ Appl Mycol 12 (1): 259-267. DOI: 10.5943/cream/12/1/16.

Pearson WR. 2013. An introduction to sequence similarity ("homology") searching. Curr Protoc Bioinforma 42 (1): 3-1. DOI: 10.1002/0471250953.bi0301s42.

Pinheiro EA, Carvalho JM, dos Santos DC, Feitosa AD, Marinho PS, Guilhon GM, de Souza AD, da Silva FM, Marinho AM. 2013. Antibacterial activity of alkaloids produced by endophytic fungus *Aspergillus* sp. EJC08 isolated from medical plant *Bauhinia guianensis*. Nat Prod Res 27 (18): 1633-1638. DOI: 10.1080/14786419.2012.75031.

Praptiwi, Raunsai M, Wulansari D, Fathoni A, Agusta A. 2018. Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. J Appl Pharm Sci 8 (8): 069-074. DOI: 10.7324/JAPS.2018.8811.

Putra IPYA, Utami KS, Hardini J, Wirasuta IMAG, Ujam NT, Ariantari NP. 2023. Fermentation, bioactivity and molecular identification of endophytic fungi isolated from mangrove *Ceriops tagal*. Biodiversitas 24 (4): 2492-2501. DOI: 10.13057/biodiv/d240565.

Rosmania R, Yanti F. 2020. Perhitungan jumlah bakteri di laboratorium mikrobiologi menggunakan pengembangan metode spektrofotometri. Jurnal Penelitian Sains 22 (2): 76-86. DOI: 10.56064/jps.v22i2.564. [Indonesian]

Roy A, Chaudhuri BN, Guchhait P, Das S. 2023. Antimicrobial activity of *Avicennia marina* flowers. EAS J Biotechnol Genet 7286 (3): 34-39. DOI: 10.36349/easjbg.2023.v05i03.001.

Salvatore MM, Andolfi A, Nicoletti R. 2021. The genus cladosporium: A rich source of diverse and bioactive natural compounds. Molecules 26 (13): 3959. DOI: 10.3390/molecules26133959.

Sarkar S, Chaudhuri BN, Guchhait P, Das S. 2023. Antibacterial and antifungal activities of *Avicennia marina* extract against various multiple drug resistant microorganisms. Saudi J Biomed Res 8 (5): 57-62. DOI: 10.36348/sjbr.2023.v08i05.002.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal Internal Transcribed Spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci 109 (16): 6241-6246. DOI: 10.1073/pnas.1117018109.

Shiono Y, Miyazaki N, Murayama T, Koseki T, Katja DG, Supratman U, Nakata J, Kakihara Y, Saeki M, Yoshida J, Uesugi S. 2016. GSK-3β inhibitory activities of novel dichroloresorcinol derivatives from *Cosmospora vilior* isolated from a mangrove plant. Phytochem Lett 18: 122-127. DOI: 10.1016/j.phytol.2016.09.007.

Sinaga SE, Fajar M, Mayanti T, Supratman U. 2023. Bioactivities screening and elucidation of terpenoid from the stembark extracts of *Lansium domesticum* Corr. cv. Kokosan (Meliaceae). Sustainability 15 (3): 2140. DOI: 10.3390/su15032140.

Strobel GA. 2003. Endophytes as sources of bioactive products. Microbes Infect 5 (6): 535-544. DOI: 10.1016/S1286-4579(03)00073-X.

Suzuki T, Ariefta NR, Koseki T, Furuno H, Kwon E, Momma H, Harneti D, Maharani R, Supratman U, Kimura KI, Shiono Y. 2019. New polyketides, paralactonic acids A-E produced by *Paraconiothyrium* sp. SW-B-1, an endophytic fungus associated with a seaweed, *Chondrus ocellatus* Holmes. Fitoterapia 132: 75-81. DOI: 10.1016/j.fitote.2018.11.017.

Thatoi H, Behera BC, Mishra RR. 2013. Ecological role and biotechnological potential of mangrove fungi: A review. Mycology 4 (1): 54-71. DOI: 10.1080/21501203.2013.785448.

Thatoi H, Samantaray D, Das SK. 2016. The genus *Avicennia*, a pioneer group of dominant mangrove plant species with potential medicinal values: A review. Front Life Sci 9 (4): 267-291. DOI: 10.1080/21553769.2016.1235619.

Tiwari P, Bae H. 2022. Endophytic fungi: Key insights, emerging prospects, and challenges in natural product drug discovery. Microorganisms 10 (2): 360. DOI: 10.3390/microorganisms10020360.

Trivedi NS, Thumar JT. 2023. Characterization and antibacterial activity of endophytic fungi isolated from *Avicennia marina*. Vegetos 36: 163-172. DOI: 10.1007/s42535-023-00582-9.

Uzma F, Mohan CD, Hashem A, Konappa NM, Rangappa S, Kamath PV, Singh BP, Mudili V, Gupta VK, Siddaiah CN, Chowdappa S. 2018. Endophytic fungi-alternative sources of cytotoxic compounds: A review. Front Pharmacol 9: 1-37. DOI: 10.3389/fphar.2018.00309.

Wang L, Han X, Zhu G, Wang Y, Chairoungdua A, Piyachaturawat P, Zhu W. 2018. Polyketides from the endophytic fungus *Cladosporium* sp. isolated from the mangrove plant *excoecaria agallocha*. Front Chem 6: 1-9. DOI: 10.3389/fchem.2018.00344.

Wulandari AP, Triani E, Sari K, Prasetyani M, Nurzaman M, Purwati RD, Ermawar RA, Nuraini A. 2022. Endophytic microbiome of *Boehmeria nivea* and their antagonism against latent fungal pathogens in plants. BMC Microbiol 22 (1): 1-16. DOI: 10.1186/s12866-022-02737-1.

Xiao J, Zhang Q, Gao Y-Q, Shi X-W, Gao J-M. 2014. Antifungal and antibacterial metabolites from an endophytic *Aspergillus* sp. associated with *Melia azedarach*. Nat Prod Res 28 (17): 1388-1392. DOI: 10.1080/14786419.2014.904308.

Zhang J, Li W, Dai Y, Shen L, Wu J. 2018. Twenty-nine new limonoids with skeletal diversity from the mangrove plant, *Xylocarpus moluccensis*. Mar Drugs 16 (1): 1-29. DOI: 10.3390/md16010038.