Jurnal Sains Akuakultur Tropis
Departemen Akuakultur
Fakultas Perikanan and Ilmu Kelautan – Universitas Diponegoro
Jl. Prof. Soedarto, SH, Tembalang,Semarang 50275
Telp. (024) 7474698, Fax.: (024) 7474698
Email: sainsakuakulturtropis@gmail.com, sainsakuakulturtropis@undip.ac.id

Molecular Identification and Antibacterial Activity of Fungi Isolated from Mangrove Sediments Against *Vibrio harveyi*

Amelia Rahmawati[1*], Aninditia Sabandingsih[1], Sarjito[2], Dion Saputra[3]

[1]Postgraduate program on Aquatic Resource Management, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, S.H, Tembalang, Semarang, Jawa Tengah
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, Indonesia
[3]Laboratory of Genome Science, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
* Corresponding author: rahmawatiamelia@students.undip.ac.id

Abstract

Vibrio harveyi is one of the major bacterial pathogens causing vibriosis in shrimp aquaculture, leading to significant reductions in production and economic losses. This study aimed to molecularly identify a fungal isolated from sediment mangrove and evaluate its antibacterial activity against *V. harveyi*. The research was conducted experimentally to assess the antibacterial activity of fungi isolated from mangrove sediments against *Vibrio harveyi* using the agar plug method. The fungal isolate exhibiting the largest inhibition zone was selected for molecular identification based on the Internal Transcribed Spacer (ITS) region using the universal primers ITS1 and ITS4. The obtained sequence was analyzed using the Basic Local Alignment Search Tool (BLAST), followed by phylogenetic analysis to assess its evolutionary relationship with related fungal taxa. BLAST analysis revealed that isolate MS.02 exhibited 100% sequence similarity to *Trichoderma yunnanense*, while phylogenetic analysis further confirmed the close phylogenetic relationship between the isolate and this species.

Antibacterial testing showed that isolate MS.02 inhibited the growth of *V. harveyi* with a moderate inhibition category, producing a clear inhibition zone of 8.45 ± 3.65 mm. The formation of the inhibition zone indicated the production of bioactive compounds with antibacterial properties. These findings indicate that fungi isolated from mangrove sediments exhibit antibacterial activity against *V. harveyi* and warrant further investigagtion as potential source of antibacterial compounds. The study provides scientific evidence supporting the utilization of mangrove-derived fungi as environmentally friendly alternatives for disease management in shrimp aquaculture. In conclusion, isolate MS.02 possesses promising antibacterial activity against *V. harveyi* and may serve as a potential biocontrol agent for sustainable aquaculture practices.

Keywords: mangrove sediment, antibacterial activity, *Vibrio harveyi, Trichoderma*

INTRODUCTION

Vibrio harveyi is an opportunistic pathogenic bacterium and one of the primary causative agents of vibriosis in shrimp aquaculture (Dwyana & Murniati, 2020). Infection by this bacterium can lead to reduced growth performance, decreased survival rates, and substantial declines in aquaculture productivity. Vibriosis is considered one of the most economically significant bacterial diseases in shrimp farming, as it can infect all developmental stages of shrimp, including larvae, post-larvae, juveniles, subadults, and adults. Under severe outbreak conditions, mortality rates may reach up to 100%, resulting in significant economic losses to the aquaculture industry (Sarjito *et al.,* 2016). In intensive shrimp culture systems, bacterial diseases caused by the genus Vibrio are among the most frequently encountered health problems. In particular, *V. harveyi* has been

reported to cause mass mortality of up to 80% in shrimp larvae and juveniles before they reach three months of culture, highlighting the high susceptibility of early developmental stages to infection (Sarjito *et al*., 2020).

Mangrove ecosystems are recognized as hotspots of microbial diversity, harboring a wide range of microorganisms with significant ecological and biotechnological potential (Rangkuti *et al*., 2017; Yulma *et al*., 2019). The search for environmentally friendly natural antibacterial compounds has become an important focus in sustainable aquaculture research. Microorganisms, particularly fungi, are known to produce a variety of secondary metabolites with diverse biological activities, including antibacterial, antifungal, and antioxiandt properties. Microorganisms associated with mangrove ecosystems can generally be classified into three groups: rhizospheric microorganisms, which inhabit the sediment and root-surrounding zone of mangrove plants; endophytic microorganisms, which live within plant tissues without causing harm to the host; and phyllospheric microorganisms, which colonize the surface of mangrove leaves and other aerial plant parts (Millatia *et al.,* 2024). Fungi isolated from mangrove sediments are therefore commonly categorized as rhizospheric fungi. Rhizospheric fungi play important ecological roles in nutrient cycling and in maintaining both aerobic and anaerobic microenvironments within mangrove sediments. In addition, these fungi are capable of producing a wide range of bioactive secondary metabolites, including antibacterial compounds, β-glucans, chitosan-derived compounds, flavonoids, and terpenoids (Cordova *et al*., 2024). Among them, species belonging to the genera *Trichoderma*, *Aspergillus*, and *Penicillium* are frequently reported from mangrove environments and have attracted considerable attention due to their potential as producers of antimicrobial compounds (Saravanakumar *et al*., 2016). These fungi have been reported to exhibit important biological activities, including antibacterial properties and biocontrol capabilities against various pathogens (Sulaiha *et al*., 2022).

Although several studies have reported the antibacterial activity of fungi derived from mangrove environments (Mulyani *et al*., 2024; Filizola *et al*., 2019), information regarding the molecular identification and antibacterial activity of fungi isolated from mangrove sediment against *Vibrio harveyi* remains limited, particularly for isolates obtained from Indonesian mangrove ecosystems. In addition, most previous studies have primarily focused on endophytic fungi isolated from mangrove tissues such as leaves, stems, and roots, as well as their general antibacterial activities (Trianto *et al*., 2023; Sukarno, 2023). Therefore, further studies integrating molecular identification and antibacterial assays are required to provide a more comprehensive understanding of the potential of fungi from mangrove sediments as natural sources of antibacterial agents.

This study aimed to molecularly identify a fungal isolate from mangrove sediments using the Internal Transcribed Spacer (ITS) marker and to evaluate its antibacterial activity against *Vibrio harveyi* using the agar plug method. The results of this study are expected to provide information on the potential of fungi isolated from mangrove sediments as natural sources of antibacterial compounds that may be further developed to support sustainable disease management in shrimp aquaculture.

MATERIALS AND METHODS

Location

The study was conducted at the Tropical Marine Biotechnology, Faculty Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia. Twenty-two fungal isolates from mangrove sediments were obtained from the culture collection of the Tropical Marine Biotechnology Laboratory, Diponegoro University, Semarang, Indonesia. The test bacterium, *Vibrio harveyi*, was obtained from the culture collection of the Brackish Water Aquaculture Fisheries Center (BBPBAP), Jepara, Indonesia, and was used as the target pathogen for antibacterial activity assays.

Exploration study

This study employed an experimental research design to explore the antibacterial potential of fungi isolated from mangrove sediments against *Vibrio harveyi*. A total of 22 fungal isolates were reactivated and subsequently screened for antibacterial activity against *Vibrio harveyi* using the agar plug method with three replications (Sibero *et al*. 2019; Sabandingsih *et al*. 2017). The isolate exhibiting the strongest antibacterial activity, as indicated by the largest inhibition zone, was selected for molecular identification. Genomic DNA was amplified using the universal fungal primers ITS1 and ITS4 targeting the Internal Transcribed Spacer (ITS) region. The obtained DNA sequence was then analyzed using the Basic Local Alignment Search Tool (BLAST) to determine its closest taxonomic affiliation, followed by phylogenetic tree construction to assess its evolutionary relationship with related fungal taxa.

Fungal activation

Fungal activation was performed by transferring fungal isolates from slant cultures onto fresh agar medium to enable the observation of their morphological characteristics. The isolates were activated on Potato Dextrose Agar (PDA) using the point inoculation method described by Millatia *et al.* (2022). A small amount of fungal hyphae from the surface of the culture medium was aseptically collected using an inoculating needle and inoculated onto the surface of fresh PDA plates. The cultures were incubated at room temperature (27°C) for 3 days until uniform fungal growth with consistent colony color and morphology was observed on the PDA medium (Sabandingsih *et al.,* 2016).

Morphological identification

Macroscopic identification was conducted to determine the colony characteristics of the fungal isolates (Michelle *et al*., 2017). Fungal colonies grown on Potato Dextrose Agar (PDA) were directly observed for their macroscopic features, including colony surface color, reverse colony color, and colony morphology (Putra *et al*., 2021). Microscopic identification was performed using a light microscope at 400× magnification to examine fungal structures, including hyphae, spores, and conidia (Prakash & Bhargava, 2016; Abboudi, 2021). The slide culture method was employed for microscopic observation. According to Tjampaka *et al*. (2024), the slide culture technique provides better results than other fungal observation methods. In this method, the fungus was cultured on a small PDA block placed on a glass slide, covered with a coverslip, and incubated in a Petri dish at room temperature (27°C) for three days before microscopic examination.

Antibacterial activity assay

The pathogenic bacterium *V. harveyi* was cultured in Nutrient Broth (NB) and adjusted to a turbidity equivalent to 0.5 McFarland standard. The bacterial suspension was incubated on a shaker for 24 h, after which it was evenly spread onto the surface of Mueller–Hinton Agar (MHA) plates using a sterile cotton swab. Fungal isolates that had been cultured for 48 h were cut into small agar plugs and placed onto the surface of MHA plates previously inoculated with *V. harveyi*. The plates were then incubated at 27°C for 24 h. The inhibition zone was measured according to the method described by Ayunigrum *et al*. (2019) by determining the diameter of the clear zone formed around the fungal plug using a digital caliper. Based on the classification proposed by Ayunigrum *et al*. (2019), inhibition activity was categorized as moderate (12–16 mm), strong (17–21 mm), and very strong (>21 mm). Alternatively, according to Pratiwi *et al*. (2024), inhibition zones can be classified as very sensitive (>20 mm), sensitive (10–20 mm), moderate (5–10 mm), weak (<5 mm), and resistant (0 mm). The assay was performed using the agar plug method following the procedures described by Sibero *et al*. (2019) and Sabandingsih *et al*. (2017).

Molecular identification

Molecular identification of fungal isolates was initiated by DNA extraction using the Chelex 20% DNA MiniPrep method. Prior to extraction, samples were immersed in Phosphate Buffered Saline (PBS) solution for 24 h to facilitate the release of fungal cells or biomass from the substrate and to reduce potential contamination. DNA amplification was performed using the Polymerase Chain Reaction (PCR) technique in a thermal cycler. The internal transcribed spacer (ITS) region was amplified using the primer pair ITS1 (5'-TCT GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') Sandy *et al*., (2015). The PCR reaction was carried out in a total volume of 25 µL, consisting of 12.5 µL GoTaq Green Master Mix, 1 µL ITS1 primer, 1 µL ITS4 primer, 9.5 µL nuclease-free water (ddH_2O), and 1 µL DNA template. The PCR products were subsequently analyzed by electrophoresis on a 1% agarose gel and visualized under a UV transilluminator to observe the presence of DNA bands (Chrisnawati *et al*., 2023).

The PCR amplification products were sent to PT Genetika Science for sequencing of the 18S rRNA gene. The obtained sequence data were then analyzed and edited using MEGA 11 software (Chrisnawati *et al*., 2023). The resulting DNA sequences were further compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to determine sequence homology and identify the closest related species (Renner *et al*., 2024).

Phylogenetic tree analysis

Phylogenetic analysis was conducted to determine the evolutionary relationships of the fungal isolates with reference species retrieved from the GenBank database. Reference sequences were selected based on the highest similarity obtained from the BLAST search results. All sequences were aligned using ClustalW implemented in MEGA version 11. This process aimed to align homologous nucleotide positions so that similarities and differences among sequences could be accurately compared. The resulting alignment was used as the basis for constructing the phylogenetic tree (Taribuka *et al*., 2016).

The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with the Kimura 2-Parameter (K2P) substitution model. This method is commonly applied in phylogenetic analyses based on the Internal Transcribed Spacer (ITS) marker and is appropriate for the samples used in this study. The robustness of the tree topology was evaluated using bootstrap analysis with 1,000 replicates, and bootstrap values were displayed on each branch to indicate the reliability of the inferred relationships (Jati *et al*., 2023). *Saccharomyces cerevisiae* was used as an outgroup to root the phylogenetic tree. The selection of the outgroup was based on its taxonomic distance at the genus and species level, representing a more distantly related lineage compared to the analyzed fungal isolates.

Data analysis

Data obtained from morphological characterization, ITS-based molecular identification, and antibacterial activity assays were analyzed descriptively and presented in the form of tables, figures, and narrative descriptions to illustrate the characteristics of the fungal isolate and its antibacterial potential. Data processing and analysis included DNA sequence analysis using MEGA version 11 software and statistical analysis using SPSS software. DNA sequences obtained from fungal isolates were analyzed and edited using MEGA version 11. The resulting sequences were then compared with reference sequences available in the

National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to determine sequence homology and identify the closest related species.

RESULTS AND DISCUSSION

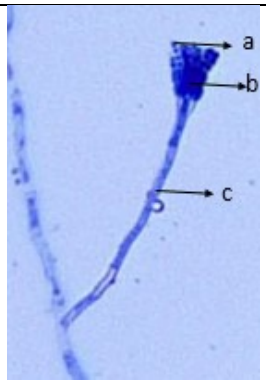
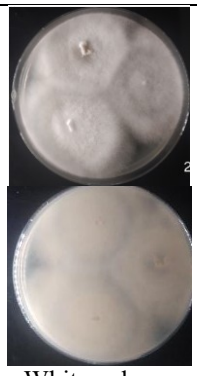
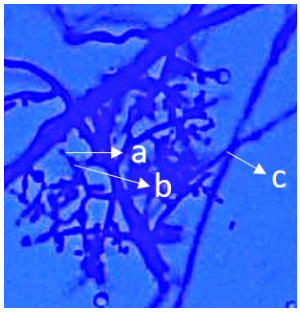
Results

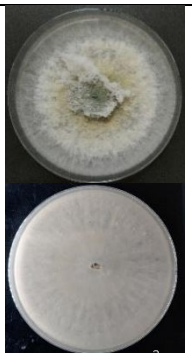
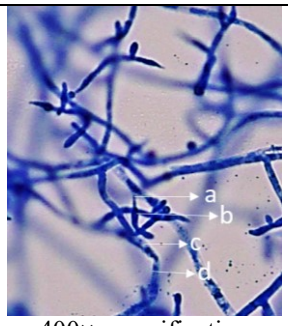
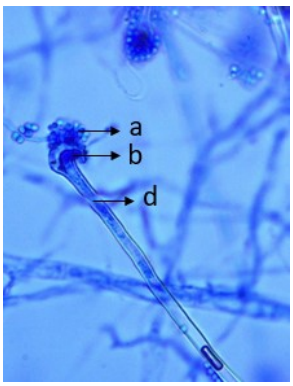
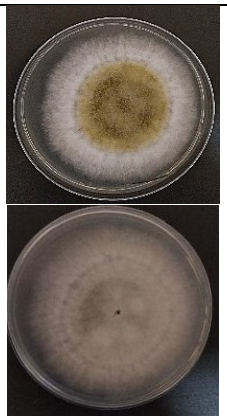
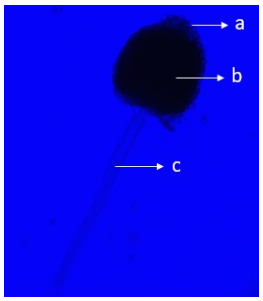
Morphological identification

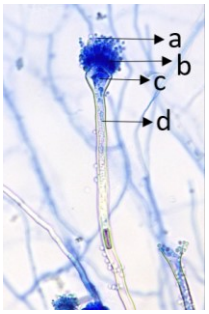
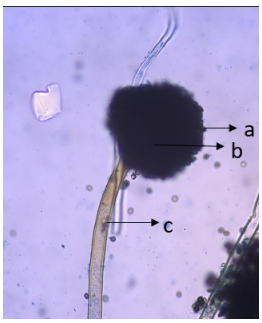
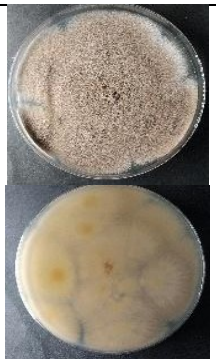
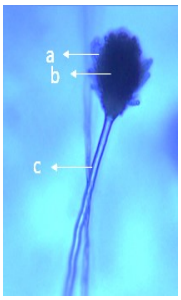
A total of 22 fungal isolates were succesfully recovered and subjected to morphological characterization. Preliminary identification was carried out based on both macroscopic and microscopic features, which remain important criteria for fungal taxonomy and classification. Macroscopic observations included colony color, texture, margin, elevation, growth pattern, and pigmentation on Potato Dextrose Agar (PDA), whereas microscopic observations focused on the morphology of hyphae, conidiophores, vesicles, phialides, conidia, and other reproductive structures. These characteristics were compared with published taxonomic descriptions to facilitate the identification of the isolates.

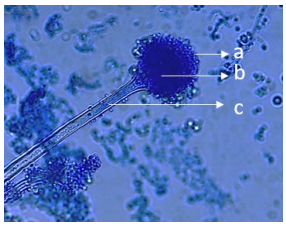
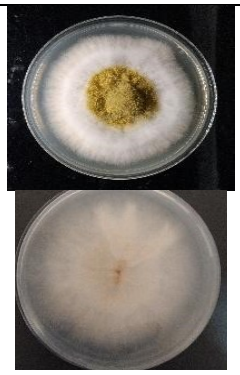
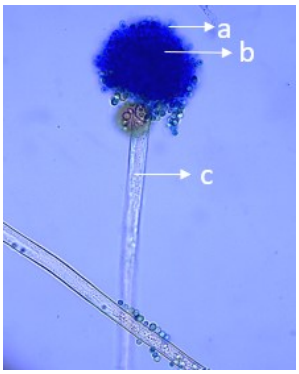
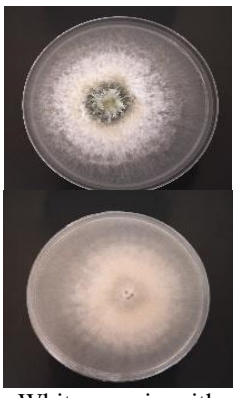
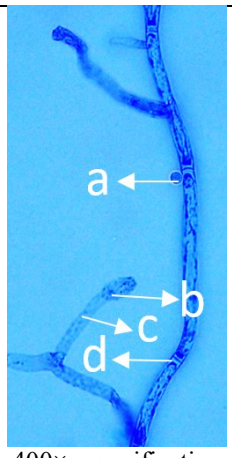
Considerable morphological variation was observed among the fungal isolates, indicating the presence of diverse fungal taxa. Differences in colony appearance, pigmentation, hyphal structure, and spore morphology provided important diagnostic features for distinguishing individual isolates. Based on their macroscopic and microscopic characteristics, the 22 fungal isolates were classified into three genera: *Aspergillus* (15 isolates), *Trichoderma* (3 isolates), and *Penicillium* (4 isolates) while others showed distinct morphological traits that required further confirmation through molecular analysis. The morphological identification presented in Table 1 and several isolates presented in Figure 1.

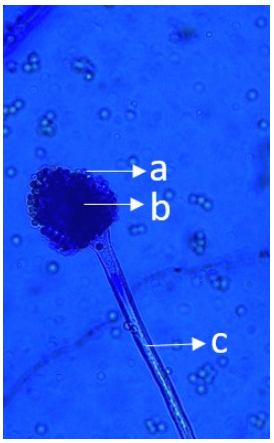
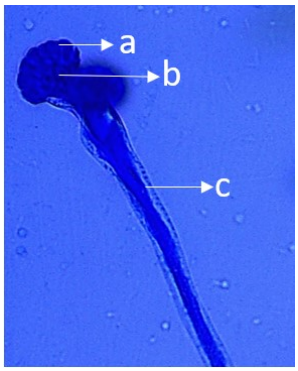
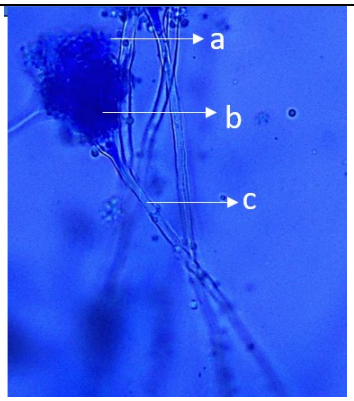
Tabel 1. Microscopic and macroscopis chatracteristics of fungal isolates

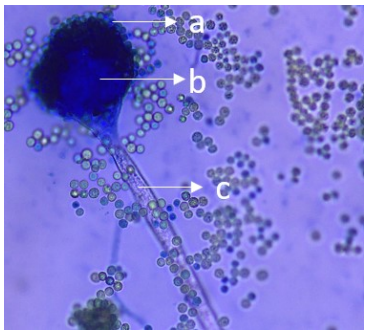
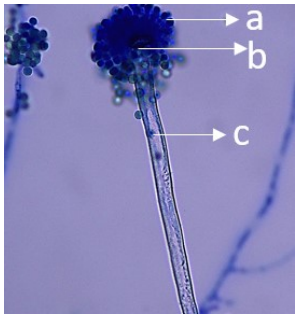
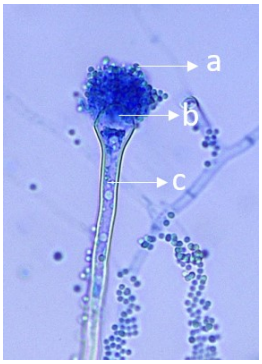
Isolate code	Morphological identification	Macroscopic characteristics	Microscopic characteristics	Reference
MS.01	*Penicillium*	 Grayish green and cream colony, circular, velvety texture with filamentous margin, flat elevation.	 400× magnification a. Conidia : globose b. Fialida: flask-shaped / ampulliform c. Conidiophore: penicillus	Ogórek (2018)
MS.02	*Trichoderma*	 White colony, circular, cottony texture, flat elevation.	 400× magnification a. Conidia : globose b.Fialida : flask-shaped c.Conidiophore : irregularly branched	Samuel *et al*. (2010)

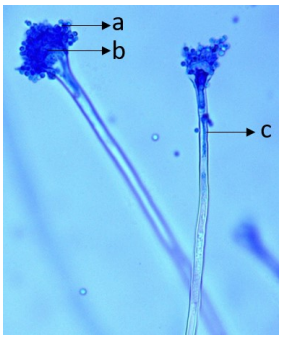
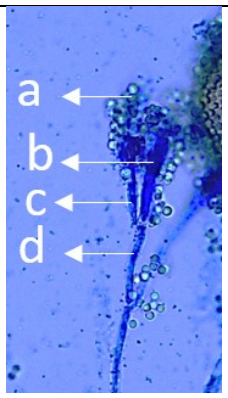
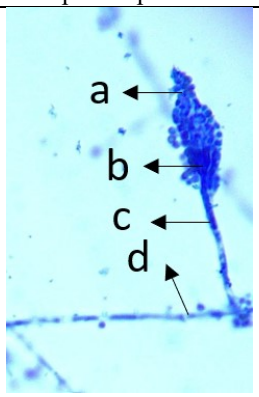
MS.03	*Trichoderma*	 White, yellow, and green colony, circular, fluffy texture with filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Fialida : flask-shaped c. Conidiophore : irregularly branched d. Hypha : bersepta hyaline	Dawy *et al*. (2025); Prasetyo *et al*. (2024)
MS.04	*Aspergillus*	 White colony with pink margin, circular, velvety texture with cottony-filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : unbranched	Klich (2002); Padmavathi *et al*. (2012)
MS.05	*Aspergillus*	 White margin with green center, circular, powdery texture with cottony-filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Vesicle : globose c. Conidiophore : unbranched	Klich (2002); Putri *et al*. (2022)

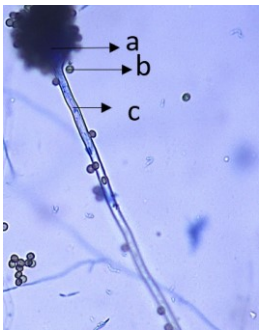
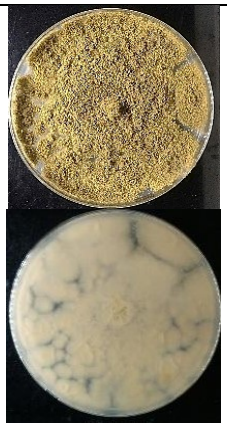
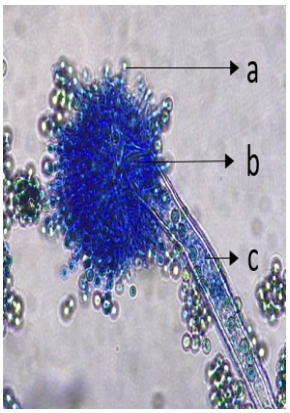
MS.06	*Aspergillus*	 White margin with brown center, circular, powdery center and velvety margin, raised elevation.	 400× magnification a. Conidia : globose b. Fialida : ampulliform/flask-shaped c. Conidiophore : hyaline and unbranched	Klich (2002)
MS.07	*Aspergillus*	 White margin with black center, irregular colony, powdery center with filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Vesicle : spheroid c. Conidiophore : hyaline and unbranched	Klich (2002); Nguyen *et al*. (2023)
MS.08	*Aspergillus*	 White margin with brown center, irregular colony, powdery center with filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Vesicle : oval c. Conidiophore: hyaline and unbranched	Dugan (2005)

MS.09	*Aspergillus*	 White margin with black center, circular colony, velvety texture with filamentous margin, raised elevation.	 400× magnification a. Conidia : globose b. Vesicle : globose c. Conidiophore : hyaline and unbranched .	Klich (2002); Abbas *et al.* (2021)
MS.10	*Aspergillus*	 White margin with light green center, circular colony, powdery center with cottony-filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : hyaline and unbranched	Klich (2002); Putri *et al.* (2022)
MS.11	*Trichoderma*	 White margin with dark green center, circular colony, powdery center with cottony-filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Fialida : flask-shaped c. Conidiophore : hyaline and branched d. Hypha : bersepta hyaline	Surma *et al.* (2025)

MS.12	*Aspergillus*	 White margin with light green center, irregular colony, powdery center with cottony-filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : hyaline and unbranched	Wibowo *et al*. (2017); Klich (2002)
MS.13	*Aspergillus*	 White margin with light green center, irregular colony, powdery center with cottony-filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : hyaline and unbranched	Wibowo *et al*. (2017); Klich (2002)
MS.14	*Aspergillus*	 White margin with brown center, circular colony, powdery center with cottony-filamentous margin, flat elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : hyaline and unbranched	Dugan (2005); Zain *et al*. (2009)

MS.15	*Penicilium*	 White margin with dark green center, irregular colony, velvety texture, umbonate elevation.	 400× magnification a. Conidia : globose b.Vesicle : subglobose c. Vesicle : hyaline and unbranched	Visagie *et al*. (2014)
MS.16	*Aspergillus*	 Gray colony, circular, velvety center with filamentous margin, flat elevation.	 400× magnification a. Conidia : globose b. Vesicle : subglobose c. Conidiophore : hyaline and unbranched	Ogórek (2018)
MS.17	*Aspergillus*	 Cream center with brown and white margin, circular colony, velvety center with filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : clavate c. Conidiophore : hyaline and unbranched	Ogórek (2018)

MS.18	*Aspergillus*	 Brown center with white margin, circular colony, powdery center with velvety margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : clavate c. Conidiophore : hyaline and unbranched	Klich (2002)
MS.19	*Penicilium*	 Gray center with white margin, irregular colony, velvety texture, flat elevation.	 400× magnification a. Conidia : globose b. Fialida: flask-shaped / ampulliform c. Metulae : elongated d. Conidiophore: penicillus.	Ogórek (2018)
MS.20	*Penicilium*	 Gray center with white margin, irregular colony, velvety texture, flat elevation.	 400× magnification a. Conidia : globose b. Fialida: flask-shaped/ampulliform c. Metulae : elongated d. Conidiophore: penicillus	Ogórek (2018)

MS.21	*Aspergillus*	 Black center with white margin, irregular colony, powdery center with velvety-filamentous margin, umbonate elevation.	 400× magnification a. Vesicle : globose b. Conidia : globose c. Conidiophore : hyaline and unbranched	Klich (2002)
MS.22	*Aspergillus*	 Green center with white margin, irregular colony, powdery center with filamentous margin, umbonate elevation.	 400× magnification a. Conidia : globose b. Vesicle : globose c. Conidiophore : hyaline and unbranched	Wibowo *et al*. (2017)

Antibacterial activity assay

The antibacterial activity assay demonstrated that fungal isolates recovered in this study exhibited varying degrees of inhibitory effects against *Vibrio harveyi*. Among the 22 isolates tested, several isolates produced clear inhibition zones, indicating the presence of antibacterial metabolites capable of suppressing bacterial growth, while others did not exhibit any detectable activity. Based on the diameter of the inhibition zones, the isolates were categorized into different activity levels ranging from weak to moderate inhibition. Based on the antibacterial activity assay, isolates exhibiting the largest inhibition zones against *Vibrio harveyi* were considered to possess the strongest antibacterial potential. Therefore, the isolate with the highest inhibitory activity was selected for subsequent molecular identification through ITS sequence analysis and phylogenetic tree construction to determine its taxonomic identity and evolutionary relationship with closely related fungal species. These findings suggest that certain fungal isolates possess promising antibacterial potential and may serve as candidates for further bioactive compound screening. The mean inhibition zone diameters and activity categories of all isolates are summarized in Table 2.

Table 2. Antibacterial assay of fungal isolates against *V. harveyi*

Sample code	Mean±SD (mm)	Category
MS.01	0 ± 0	-
MS.02	8.45 ± 3.65	++
MS.03	5.11 ± 1.50	++
MS.04	7.22 ± 1.92	++
MS.05	2.77 ± 4.81	+
MS.06	2 ± 3.46	+
MS.07	0 ± 0	-
MS.08	0 ± 0	-
MS.09	5.77 ± 2.34	++
MS.10	0 ± 0	-
MS.11	0 ± 0	-
MS.12	2.6 ± 4.61	+
MS.13	2.7 ± 4.81	+
MS.14	1.66 ± 2.88	+
MS.15	2.66 ± 4.61	+
MS.16	1 ± 1.73	+
MS.17	1 ± 1.73	+
MS.18	2.77 ± 4.81	+
MS.19	0 ± 0	-
MS.20	2.22 ± 3.84	+
MS.21	0 ± 0	-
MS.22	2.77 ± 4.81	+

Activity categories were classified according to Pratiwi *et al.* (2024): very sensitive (>20 mm), sensitive (10–20 mm), moderate (5–10 mm), weak (<5 mm), and resistant (0 mm).

Molecular identification

Among the 22 fungal isolates tested, MS.02 exhibited the highest antibacterial activity against *V. harveyi*, with a mean inhibition zone diameter of 8.45 ± 3.65 mm, which was classified as moderate activity according to the criteria of Pratiwi *et al*. (2024). The superior inhibitory effect displayed by MS.02 compared to the other isolates suggests a greater potential for the production of antibacterial metabolites. Therefore, MS.02 was selected for subsequent molecular identification and phylogenetic analyses.

PCR amplification of the fungal isolate MS.02 successfully generated a DNA fragment corresponding to the targeted ribosomal region. The amplified product was subsequently analyzed by agarose gel electrophoresis to verify the presence and approximate size of the PCR amplicon. Visualization under UV illumination revealed a distinct DNA band, indicating successful amplification of the target sequence. The PCR amplification of the fungal isolate MS.02 produced a clear DNA fragment ranging from 500 to 700 bp (Fig.1), consistent with the expected size of the ITS rDNA region commonly used for fungal identification. This result confirms the successful amplification of the target region and indicates that the PCR product was suitable for downstream sequencing and phylogenetic analyses.

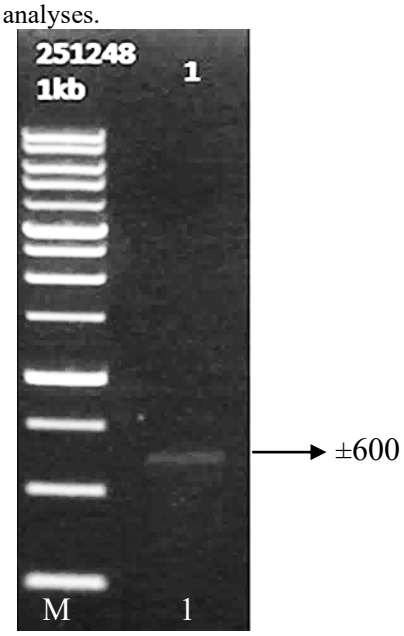


Figure 2. Agarose gel electrophoresis of the PCR-amplified ITS region of the fungal isolate. Lane M: 1 kb DNA ladder; Lane 1: PCR product showing a DNA fragment of approximately 500–700 bp

To determine the taxonomic identity of the most active fungal isolate, the ITS sequence obtained from isolate MS.02 was compared with reference sequences available in the NCBI database using the Basic Local Alignment Search Tool (BLAST). Sequence similarity analysis was performed to identify the closest related species based on query cover, E-value, percentage identity, and accession number. The BLAST results revealed a high degree of similarity between isolate MS.02 and reference sequences in the GenBank database, providing preliminary molecular evidence for species-level identification. The detailed results of the BLAST analysis are presented in Table 3.

Table 3. BLAST analysis of fungal isolate MS.02

Sample Code	Closest species	Query cover	E-value	Percent identitiy	Access number
MS.02	*Trichoderma yunnanense*	95%	0.0	100%	NR 134419.1

Phylogenetic tree analysis

To further confirm the taxonomic position of isolate MS.02, a phylogenetic analysis was performed based on ITS rDNA sequences using the Neighbor-Joining method with the Kimura 2-Parameter (K2P) model. Reference sequences representing closely related *Trichoderma* species were retrieved from the GenBank database and included in the analysis. *Mucor cerradoensis* was used as an outgroup to root the phylogenetic tree. The resulting phylogeny showed that isolate MS.02 clustered within the *Trichoderma* clade and exhibited a close relationship with *Trichoderma yunnanense* (NR 134419.1) supported by a bootstrap value of **97**. Bootstrap values above 70 are generally considered to indicate reliable phylogenetic groupings, suggesting that MS 02 is closely related to, or potentially belongs to the *T. yunnanense* lineage. This close relationship implies that both taxa share a relatively recent common ancestor compared with other *Trichoderma* species included in the analysis. The **Trichoderma** clade also comprised several related species, including *Trichoderma songyi*, *T. dorotheae*, *T. taiwanense*, *T. hispanicum*, *T. koningii*, *T. trixiae*, *T. junci*, and *T. caerulescens*. However, the internal nodes among these species were supported by bootstrap values ranging from **40 to 80**, indicating different levels of genetic divergence and varying confidence in their phylogenetic relationships. In contrast, the **Aspergillus** species formed a distinct monophyletic clade containing *Aspergillus tibetensis*, *A. cylindricus*, *A. telluris*, *A. guangxiensis*, *A. marneyi*, and *A. pseudoalabamensis*. The phylogenetic relationship of isolate MS.02 with related reference species is presented in Figure 3.

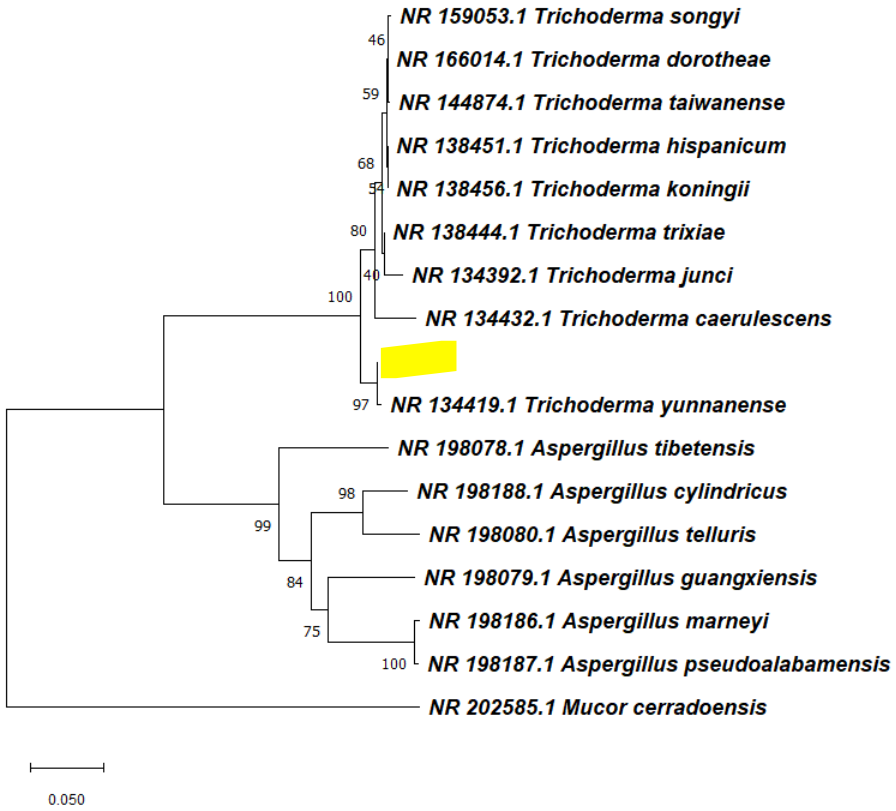


Figure 3. Phylogenetic tree of fungal isolate MS.02

Discussion

The result of this study, morphological characterization revealed that the fungal isolates recovered from mangrove sediment were predominantly affiliated with the genus *Aspergillus*, *Penicillium*, and *Trichoderma*. This finding is consistent with previous studies reporting that these genera are commonly associated with mangrove ecosystems and are frequently recovered from mangrove sediments. In particular, *Aspergillus* and *Penicillium* have been reported among the most frequently isolated fungal genera from mangrove sediments, while *Trichoderma* is also commonly detected in mangrove-associated fungal communities (Li *et al*., 2023; Oliveira *et al.,* 2025).These genera are commonly reported from mangrove ecosystems due to their adaptability to fluctuating salinity, nutrient availability, and environmental stress conditions (Gao *et al*., 2021). Previous studies have demonstrated that mangrove-associated fungi are prolific producers of bioactive compounds, including antibacterial, antifungal, antioxiandt, and anticancer metabolites (Trianto *et al*., 2021). Fungi are generally classified into two main groups, namely molds and yeasts. Molds are multicellular fungi characterized by spreading colony growth accompanied by the presence of hyphae and conidia. Macroscopically, mold colonies typically exhibit cottony, velvety, or powdery textures, whereas microscopically they possess hyphae and reproduce through spore formation. Alfanda (2020) reported that molds are multicellular fungi with hyphal structures and the ability to produce mycotoxins that may pose risks to human health. This characteristic distinguishes them from yeasts. Yeasts are unicellular fungi that lack true hyphae (Pratiwi & Akhdiya, 2020). Yeast colonies are generally round with smooth, glossy surfaces and are typically white or cream in color. Microscopically, yeast cells are spherical or oval in shape and reproduce through budding (Akbar *et al*., 2019). Based on the fungal observations conducted in this study, all identified isolates were classified as molds. This classification was supported by the presence of hyphae and asexual reproductive structures in the form of conidia. Furthermore, microscopic examination revealed septate and hyaline hyphae, as well as globose conidia. The macroscopic and microscopic characteristics observed were compared with previous literature to support the identification of the fungal isolates at the genus level.

The antibacterial activity assay demonstrated considerable variation among the fungal isolates in their ability to inhibit the growth of *Vibrio harveyi*. Among the 22 isolates tested, isolate MS.02 exhibited the strongest antibacterial activity, producing the largest inhibition zone (8.45 ± 3.65 mm). Although classified as moderate activity according to Pratiwi *et al*. (2024). The differences in inhibition zone diameters observed in this study may be attributed to the varying abilities of fungal isolates to produce and secrete secondary metabolites with antibacterial properties. In addition, these differences may be influenced by variations in the

diffusion capacity of active compounds within the medium and differences in fungal growth during the incubation period. Compounds with lower molecular weights and higher solubility generally exhibit better diffusion through agar media, thereby potentially producing larger inhibition zones. Setiawan and Warsito (2025) stated that several factors influence the formation of inhibition zones, including compound concentration, assay method, active compound content, incubation time and temperature, as well as the pH and type of medium used. Similarly, Lestari *et al*. (2016) reported that antibacterial activity is affected by four main factors, namely extract concentration, metabolite composition, diffusion capacity, and the type of bacteria being inhibited. Therefore, these factors may account for the variation in inhibition zone diameters observed among fungal isolates, even when they belong to the same genus. This finding indicates the capability of fungi from sediment mangrove to produce metabolites that suppress pathogenic bacteria. *V. harveyi* is one of the most important bacterial pathogens in marine aquaculture and is frequently associated with mass mortality in shrimp and fish farming systems (Putra *et al.,* 2025).

Molecular identification based on ITS rDNA sequencing revealed that isolate MS.02 shared 100% sequence identity with *Trichoderma yunnanense*. The ITS region remains the most widely accepted molecular marker for fungal species identification because of its high discriminatory power among closely related taxa (Shapkin *et al*., 2026). The high sequence similarity observed in this study confirms the morphological identification and indicates that isolate MS.02 belongs to *T. yunnanense*. Phylogenetic analysis further supported the molecular identification results by clustering isolate MS.02 within the *Trichoderma* clade and showing a closest evolutionary relationship with *T. yunnanense* with strong bootstrap support (97%) based on 1,000 bootstrap replicates. This close phylogenetic relationship indicates that isolate MS.02 shares a recent common ancestor with *T. yunnanense* and exhibits a high degree of genetic relatedness. Previous studies have shown that *T. yunnanense*, as well as closely related *Trichoderma* species, produces hydrolytic enzymes and bioactive secondary metabolites that contribute to antimicrobial, antifungal, and immunomodulatory activities (Prismantoro *et al.,* 2024; Song and Ji, 2024). Therefore, the close evolutionary relationship observed in this study suggests that isolate MS.02 may possess similar biological characteristics. However, further physiological and biochemical analyses are required to verify these functional properties.

Conclusion

A total of 22 fungal isolates were successfully recovered from mangrove sediment, comprising 15 isolates of *Aspergillus*, 3 isolates of *Trichoderma*, and 4 isolates of *Penicillium*. Among these, isolate MS.02 exhibited the strongest antibacterial activity and was classified as having moderate antibacterial activity against *Vibrio harveyi* based on the inhibition zone classification. Molecular identification revealed that isolate MS.02 shared 100% ITS rDNA sequence identity with *Trichoderma yunnanense*, and phylogenetic analysis further confirmed its close evolutionary relationship with *T. yunnanense*, supported by a bootstrap value of 97% based on 1,000 bootstrap replicates. These findings demonstrate that mangrove sediment harbors diverse fungal communities with antibacterial potential and highlight *T. yunnanense* isolate MS.02 as a promising source of natural antibacterial compounds for the control of vibriosis in aquaculture.

Acknowledgments

This researchers is fully funded by Master Thesis Research Scheme (Basis of Information on Research and Community Service / BIMA), with contract number 601-118/UN7.D2/PP/VI/2024 and also extended gratitude to Tropical Marine Biotechnology Laboratory and Aquaculture Laboratory,, Universitas Diponegoro for facilitating the field as well as laboratory equipment.

References

Abboudi, M. 2021. Bacterial growth on recycled agar medium media used in different cultures. Journal of Agroalimentary Processes and Technologies 27(2).

Akbar, G.S., E. Kudiyantini, and Wijanarka. 2019. Isolation and morphological and biochemical characterization of yeasts from honey pineapple (*Ananas comosus* L.) peel waste for bioethanol production [In Indonesian]. *Berkala Bioteknologi*, 2(2).

Alfanda, M.Y. 2020. Identification and enumeration of molds on postharvest apple peels from Batu, Malang [In Indonesian]. Jurnal SainsHealth. 4 (1).

Ayuningrum, D., Liu, Y., Riyanti., Sibero, M. T., Kristiana, R., Asagabaland, M. A., Wuisan, Z. G., Trianto, A., Radjasa, O. K., Sabdono, A., & Schaberle, T. F. 2019a. Tunicateassociated bacteria show a great potential for the discovery of antimicrobial compounds. PLOS ONE 14(3): 1-14.

Chrisnawati, S.D., A. Sabandingsih, O.E. Jati, and D. Ayuningrum. 2023. Isolation and molecular identification of rhizosphere bacteria from *Rhizophora* sp. mangrove sediments in the Tapak mangrove ecosystem, Semarang [In Indonesian]. *Jurnal Kelautan*, 16(2).

Cordova-A.I.C., G.A. Guzman, D.M. Felix, C.E. Cesena, J.G. Armenta and J.V. Chavez. 2024. Immunochemical Response And Gene Expression in Juvenile Shrimp (*Litopenaeus vannamei*) Exposed to Microorganisms Isolated From Marine Sediment. Veterinaria Mexico. 11.

Dwyana, Z., Murniati. (2020). Uji Sensitivitas Bakteri Probiotik terhadap *Vibrio harveyi* Penyebab Vibriosis Secara In Vitro. *Jurnal Ilmu Alam and Lingkungan.* 11 (2), 15

Dwyana, Z., and Murniati. 2020. In vitro sensitivity test of probiotic bacteria against *Vibrio harveyi*, the causative agent of vibriosis [In Indonesian]. *Jurnal Ilmu Alam dan Lingkungan*, 11(2): 15.

Filizola, P.R.B., M.A.C. Luna, A.F. Souza, I.L. Coelho, D. Laranjeira, and G.M. Campos-Takaki. 2019. Biodiversity and phylogeny of novel *Trichoderma* isolates from mangrove sediments and potential of biocontrol against *Fusarium* strains. *Microbial Cell Factories*, 18: 89.

Gao, H., Y. Wang, Q. Luo, L. Yang, X. He, J. Wu, K. Kachanuban, P. Wilaipun, W. Zhu, and Y. Wang. 2021. Bioactive metabolites from acid-tolerant fungi in a Thai mangrove sediment. *Frontiers in Microbiology*, 11: 609952.

Harni, R., W. Amaria, and Supriadi. 2017. Potential of *Trichoderma* spp. as biological control agents against plant diseases [In Indonesian]. *Jurnal Litbang Pertanian*, 36(1): 1–10.

Jati, W.W., A. Kristini, A.S. Mulyatni, and A.L. Abadi. 2023. Molecular identification of *Trichoderma* sp. T10 isolate from ISRI as a biological control agent against *Xylaria* root and basal stem rot disease [In Indonesian]. *Indonesian Sugar Research Journal*, 3(1): 25–32.

Lestari, Y., P. Ardiningsih, and Nurlina. 2016. Antibacterial activity against Gram-positive and Gram-negative bacteria of extracts and fractions from nipa palm (*Nypa fruticans* Wurmb.) leaves collected from the Sungai Kakap coastal area, West Kalimantan [In Indonesian]. *Jurnal Kimia Khatulistiwa*, 5(4): 1–8.

Li, M., Raza, M., Song, S., et al. (2023). *Application of culturomics in fungal isolation from mangrove sediments*. Microbiome, 11, 272.

Li, M.F., G.H. Li, and K.Q. Zhang. 2019. Non-volatile metabolites from *Trichoderma* spp. *Metabolites*, 9(3): 58.

Linayati, Syakirin, M.B., Supito, Z. Arifin. 2026. Edukasi tentang Bahaya Resistensi Antibiotik pada Tambak Uandg di Kabupaten Malang. *Mestaka: Jurnal Pengabdian Kepada Masyarakat.* 5 (2), 207.

Michelle, V., E.D. Quiljoe and S.B. Rondonuwu. 2017. Identifikasi Bakteri pada Pegangan Eskalator di Salah satu Pusat Perbelanjaan di Kota Manado. Jurnal Ilmiah Sains. 17 (01).

Millatia, Z., A. Sabandingsih, and M.R. Mukananfola. 2022. Isolation and characterization of fungi from Tapak mangrove sediments, Semarang. *Jurnal Pasir Laut*, 6(2).

Mulyani, Y., A.P. Wulandari, S.E. Sinaga, W. Safriansyah, et al. 2024. Antibacterial activities and molecular identification of endophytic fungi isolated from mangrove *Avicennia marina*. *Biodiversitas Journal of Biological Diversity*, 25(12).

Narasswati, N., R. Oktavia, N. Nenci, Y. Eryati, T.T.T. Nugroho, and Y. Nurulita. 2017. Potential of secondary metabolites from *Trichoderma* sp. LBKURCC22 isolated from secondary peat forest soil as antibiotics [In Indonesian]. *Chimica et Natura Acta*, 5(2).

Oliveira, J. B. M., Corrêa Junior, D., Parente, C. E. T., & Frases, S. (2025). *Fungi in Mangrove: Ecological Importance, Climate Change Impacts, and the Role in Environmental Remediation*. Microorganisms, 13(4), 878.

Prakash, P. Yegneswaran and B. Kanika. 2016. A Modified Micro Chamber Agar Spot *Slide culture* Technique For Microscopic Examination Of Filamentous Fungi. Journal of Microbiological Methods. 123 : 126–129.

Pratiwi, R. and A. Akhdiya. (2020). Morphology and Biochemistry Diversity of Rhizospheric and Endophytic Yeasts of Rice Plant. Bul. Plasma Nutfah 26(1):39–50.

Prismantoro, D., Jefferson, T. A., Akbari, S. I. (2024). In vitro inhibition mechanisms of *Trichoderma yunnanense* TM10 against *Pyricularia oryzae* and *Rhizoctonia solani* causing blast and sheath blight diseases in rice (*Oryza sativa* L.). *All Life*.

Putra, I.K.V.D., I.P. Sudiarta, and N.W. Suniti. 2021. Morphological identification of fungal contaminants on lontar manuscripts [In Indonesian]. *Jurnal Agroteknologi Tropika*, 10(4).

Putra, R.B.D.S., A.M.S. Hertika, J.A. Inggrida, A.N.A. Putra, F. Setiawan, and A.M.S. Achmadi. 2025. Analysis Of Nano-Chitosan-Based Extracellular Products Of *Vibrio harveyi* Enriched With *Caulerpa Lentillifera* Extract As An Oral Vaccine For Hybrid Grouper Against *Vibrio harveyi* Infection. Journal of Aquatropica Asia. 10 (02).

Rangkuti, A. M., Reza, M., Ani, C., Yulma, R., and Hasan, E. A. (2017). Ekosistem Pesisir and Laut Indonesia. Bumi Aksara, Jakarta.

Renner, S., M.D. Scherz, C.L. Schoch, M. Gottschling and M. Vences. 2024. Improving the Gold Standard in NCBI GenBank and Related Databases: DNA Sequences From Type Specimens And Type Strains. Syst. Biol. 73 (2).

Sabandingsih, A., O. Christianawati, M.T. Sibero, H. Nuryadi, O.K. Radjasa, A. Sabdono and A. Trianto. 2016. Screening Antibacterial Agent from Crude Extract of Marine Derived Fungi Associated with Soft

Corals against MDR *Staphylococcus haemolyticus*. IOP Conf. Series: Earth and Environmental Science 55.

Sandy, Y.A., S. Djauhari, and A.W. Sektiono. 2015. Molecular identification of antagonistic fungus *Trichoderma harzianum* isolated from agricultural soil in Malang, East Java [In Indonesian]. *Jurnal HPT*, 3(3).

Sarjito, A.H.C. Haditomo, K. Erlinda, Desrina, and S.B. Prayitno. 2020. Role of *Gracilaria verrucosa* extract in the feed as immunostimulant of white shrimp (*Litopenaeus vannamei*) infected *Vibrio harveyi*. *Advances in Animal and Veterinary Sciences, 8*(12), 1427–1434.

S, M., M. Apriliani, and D. Afriani. 2016. Causative agents of vibriosis in intensively cultured white shrimp (*Litopenaeus vannamei*) in Kendal. *Jurnal Kelautan Tropis*, 18(3): 189–194.Setiawan, B. and K. Warsito. (2025). Potential of Endophytic Bacteria as Biocontrol of Colletotrichum coffeanum F.Noack Fungus Causing Anthracnose in Arabica Coffee (Coffea arabica L.) in Vitro. Jurnal Biologi Tropis, 25 (3): 4492 – 4498.

Shapkin, V., T. Zelenka, T. Větrovský, M. Kostovčík, I. Eichlerová, P. Kohout, L. Žifčáková, J. Borovička, M. Tomšovský, S. Adamčík, P. Baldrian, and M. Kolařík. 2026. Limitations of common molecular markers in fungal biodiversity analysis and the benefits of their synergistic use. *Molecular Ecology Resources*, 26(3): e70123.

Sibero, M.T., A. Sabandingsih, O.K. Radjasa, A. Sabdono, A. Trianto, and Subagiyo. 2019. Characterization of bioactive compounds from the marine fungus *Trichoderma asperellum* MT02 with anti-extended spectrum β-lactamase (ESBL) *Escherichia coli* activity. *Jurnal Kelautan Tropis*, 22(1).

Song, Y.-P., & Ji, N.-Y. (2024). Chemistry and biology of marine-derived *Trichoderma* metabolites. *Natural Products and Bioprospecting, 14*, 14.

Sukarno. (2023). Antibacterial activity profile of mangrove endophytic fungi isolated from Berau Regency, Indonesia. Jurnal Bioteknologi & Biosains Indonesia, 9(1), 86–99.

Sulaiha, S., D. Mustikaningtyas, T. Widiatningrum, and P. Dewi. 2022. Bioactive compounds of *Trichoderma erinaceum* and *Trichoderma koningiopsis* and their antibacterial potential [In Indonesian]. *Life Science*, 11(2): 120–131.

Sulistiyono, F.D., L. Soesanto, and N.I. Ratnaningtyas. 2021. Protease activity of four *Trichoderma* spp. isolates from rhizosphere soil [In Indonesian]. *Chimica et Natura Acta*, 9(3).

Taribuka, J., C. Sumardiyono, S.M. Widyastuti, and A. Wibowo. 2016. Exploration and identification of endophytic *Trichoderma* from banana plants [In Indonesian]. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, 16(2): 115–123.

Tjampaka, C.R., R. Agustini, I. Baihaki, S. Noor, and A. Bustani. 2024. Slide culture as an indirect microscopic method for mold identification [In Indonesian]. *Jusindo*, 6(1).

Trianto, A., O.K. Radjasa, Subagiyo, A.A. Isabella, M.S. Bahry, H. Purnaweni, R. Djamaludin, A. Tjoa, I. Singleton, D.M. Evans, and K. Diele. 2023. Molecular identification and biotechnological potential of *Cerithidea cingulata*- and *Lottia scabra*-associated fungi as extracellular enzyme producer and anti-vibriosis agent. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, 28(2): 136–147.

Yulma, G.I. Santriani, Awaludin, B. Ihsan, B. Pratiwi. 2019. Bacteria Diversity in Sediment From Mangrove and Bekantan Conservation Area, Tarakan City. *Aquasains: Jurnal Ilmu Perikanan and Sumberdaya Perairan.* 7 (2). 698.