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Culturable marine fungal biodiversity from the south coast of Jember, East Java, Indonesia

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Abstract. *Puspitasari E, Senjarini K, Dewanti IDAR, Febrianti V, Hasanah FM, Labes A. 2025. Culturable marine fungal biodiversity from the south coast of Jember, East Java, Indonesia. Biodiversitas 26: 3000-3010.* Marine fungi with their unique environment are a promising source for drug discovery. Their distinct natural products, different from those of terrestrial fungi, give them potential bioactivity. However, not all marine fungi are culturable. This research aimed to determine the biodiversity of culturable marine fungi from Jember south coast, East Java, Indonesia. The sampling was conducted on samples available on the Jember south coast, East Java, Indonesia, including seawater, sand, wood remains, green algae, and red algae. The isolation of marine fungi using fungal basal media. Once a single colony with different morphotypes was obtained, it was characterized based on its morphology (macroscopically and microscopically using bright field and scanning electron microscope) and molecularly. The fungal morphological features were described based on the colony's and cell's appearance. The molecular characteristics were carried out using DNA barcoding based on the internal transcribed spacer. The results revealed 17 isolates of culturable marine fungi from Jember south coast. They were dominated by the genus *Penicillium* (47.059%), followed by *Scopulariopsis* and *Curvularia* (17.647%), *Aspergillus* (11.765%), and *Cladosporium* (5.882%); members of phylum Ascomycota. The results showcase the rich diversity of species that have potential as drug candidates. Further studies are needed to reveal their bioactivity.

Keywords: Biodiversity, Jember South Coast, marine fungi, molecular characteristics, morphological characteristics

Abbreviations: Ga: Green algae, ITS: Internal Transcribed Spacer, Ra: Red algae, Sa: Sand, SEM: Scanning Electron Microscope, Wa: Seawater, and Wo: Wood. GPY: Glucose Peptone Yeast, H: Hastings media, HS: Hastings media + streptomycin, K2P: Kimura-2-Parameter, Mag: Magnification, NJ: Neighbor-Joining, P: Potato dextrose agar, PS: Potato dextrose agar + streptomycin

INTRODUCTION

Fungi, a dominant microorganism in marine ecosystems, have been extensively studied (Imhoff et al. 2011; Reich and Labes 2017; Stiebeling and Labes 2022; Labes 2023). Marine fungi stand out with their potential to revolutionize drug discovery. Their bioactivities and beneficial for therapy have been well-documented (Silber et al. 2016; Deshmukh et al. 2018; El-Bondkly et al. 2021; Zhang et al. 2021a; Ameen et al. 2021, 2023; Ji et al. 2023; Kempken 2023). With a high growth rate, marine fungi could potentially resolve raw material supply issues (Puspitasari et al. 2024). Their unique growing environment, distinct secondary metabolic pathways, and immense biodiversity enable marine fungi to produce natural products with potential bioactivity distinct from their terrestrial counterparts (Ji et al. 2023).

Yet, not all marine fungi are culturable. Most isolates identified through metagenomic analysis are viable. However, many marine fungi are considered 'unculturable'; they cannot be cultivated despite being alive and potentially metabolically active (Zhang et al. 2021b). According to Zhang et al. (2024), standard approaches using standard basal media and adjusted environmental conditions can successfully cultivate only 1.2% of marine fungi. Nevertheless, isolation of marine fungi is a crucial step to ensure that they can be up-scaled into industrial-scale production once they are proven to have a targeted bioactivity (Pham et al. 2021). The site-specific environmental condition will influence the biodiversity of marine fungi and their natural products, resulting in the divergence of their bioactivity (Vetaas et al. 2019; Bayona et al. 2022; Rini et al. 2023; Puspitasari et al. 2024). In order to mimic their natural habitat, the condition of culture in the laboratory setting has to be adjusted as closely as possible to their natural conditions (Puspitasari et al. 2024), including those from Jember south coast, East Java, Indonesia.

Currently, the potential development of this area has only focused on tourism and pond farming (Pemkab Jember 2023a, 2023b, 2023c). It is even included in the development of national tourism (Pemkab Jember 2023b). The exploration of its biodiversity is mostly on its

macroorganisms, and not much data is available on the microorganisms (Nugraha et al. 2023). However, with a high diversity of organisms, especially microorganisms, this location is a potential source that needs to be explored, mainly for drug discovery. The exploration of the culturable marine fungi from Jember south coast, East Java, Indonesia, is the first step of a new drug candidate development originating from this area. Species identification is crucial to elaborate their potential activity and characterization further, leading to precise identification in exploring the biodiversity related to their potential use for drug discovery (Mori et al. 2023; Mandal et al. 2024).

The determination of culturable marine fungi biodiversity can be conducted not only based on their morphological characteristics, but also by molecular characteristics using DNA barcoding. DNA barcoding is easier, more precise, and can be used to identify species at any stage of life (Antil et al. 2023). There are some gene markers available for fungi's DNA barcoding, including the internal transcribed spacer (ITS), 28S rRNA, 18S rRNA, *RPB1*, *RPB2*, etc. (Reich and Labes 2017).

ITS is usually used for fungi DNA barcoding (Prastowo et al. 2022; Guerra-Mateo et al. 2023). ITS is the major fungal DNA barcode because in a wide variety of fungi, interspecies variability outweighs intraspecies variability, and PCR success rates are high (Reich and Labes 2017). Therefore, the International Fungal Barcode Consortium endorsed ITS as the principal fungal barcode in 2012 (Bradshaw et al. 2023). This research aimed to determine the biodiversity of culturable marine fungi from Jember south coast, East Java, Indonesia. The species were determined based on both morphological and molecular characteristics.

MATERIALS AND METHODS

Study area

The sampling campaign was conducted on some samples available on Jember South Coast, East Java, Indonesia, between the GPS coordinates at 8°25'60"S, 113°32'59.8"E and 8°25'60"S, 113°32'59.7"E (Figure 1). The sampling location consisted of 2 stations, with a distance of 5 m, and 3 plots were made at each station. Abiotic factors or environmental factors at each sampling station were measured, including water salinity, pH, and temperature. The samples taken were coastal and marine water, sediment/benthos (sand, wood remains), and organic living matter (green algae and red algae). The samples were then labeled Wa (seawater), Sa (sand), Wo (wood), Ga (green algae), and Ra (red algae). Seawater samples were taken at the surface with a maximum depth of 30 cm, then 1 mL was put into a sterile tube. Sand substrate samples were taken into a Falcon tube up to 1 mL. Wood samples were taken from locations that were still flooded with seawater, then cut into small pieces and put into a Falcon tube. Samples of green algae attached to the rocks were taken using a sterile cotton swab by rubbing it on the surface of the rocks that were still flooded with seawater and then inserted into the Falcon tube. Red algae samples were taken from the sea and then put into the Falcon tube. The sampling procedure was carried out aseptically, and all equipment was used in sterile conditions. The samples were then immediately cultured on the media to be used (FUAS 2022).

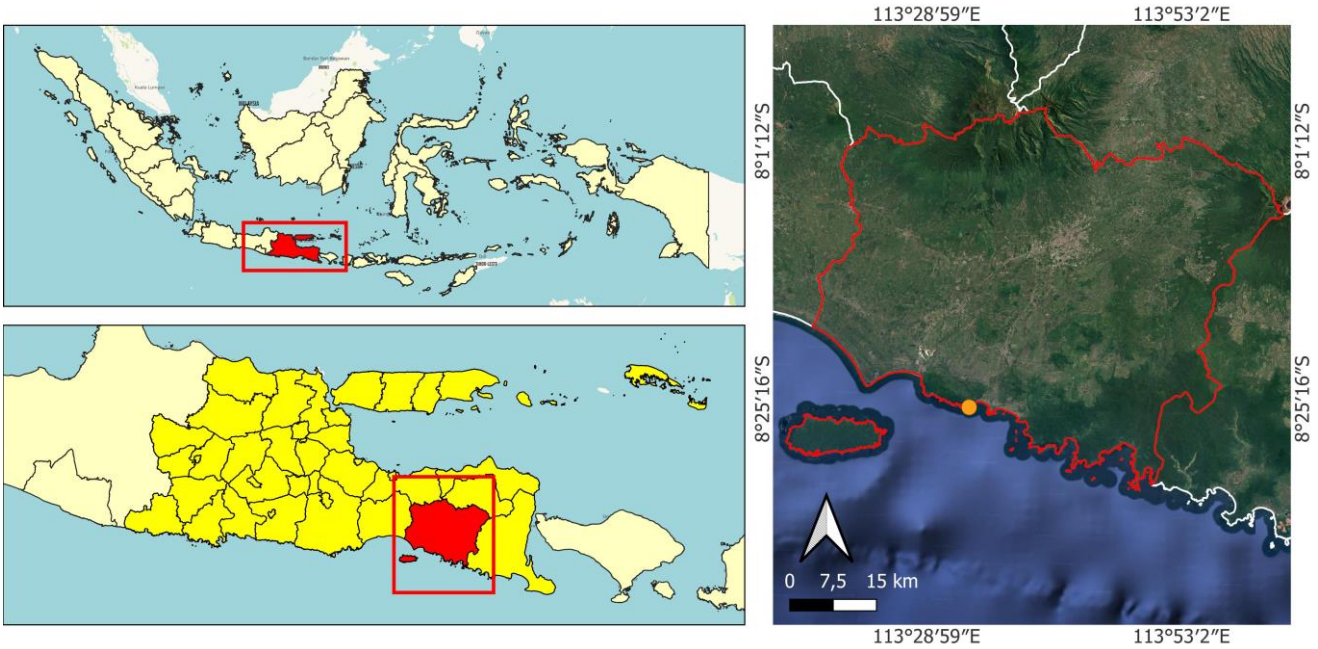

Figure 1. Sampling location of marine fungi in Jember south coast, East Java, Indonesia

Procedures

Marine fungi isolation

The marine fungal samples originating from Jember south coast were diluted in sterile distilled water from 10^{-1} to 10^{-10} dilutions. The marine fungi, then, were cultured using fungal basal media, i.e., Hastings media, potato dextrose agar (PDA), Hastings media + antibacterial (streptomycin), and PDA + antibacterial (streptomycin). The media used were labeled as H (Hastingsmedia), HS (Hastingsmedia + streptomycin), P (potato dextrose agar), and PS (potato dextrose agar + streptomycin) (FUAS 2022).

The diluted samples were then cultured using the spread plate method. The dilution level is adjusted to the number of colonies that grew, i.e., at a dilution level that can still produce separated colonies. Cultured samples were incubated at room temperature for 3-4 days. Samples that have grown were carried out to the next stage, i.e., marine fungal isolation, using the streak plate method until a single colony is formed. The culture condition was adjusted to their original conditions, including environmental temperature (29°C), pH (7.9), and salinity (3.3%). They were isolated and differentiated based on their morphotype. Isolation was carried out multiple times until a single colony was obtained. The isolates obtained were then named according to the source of the sample and the media used, with the addition of a sequential number behind it, e.g., SaH 1 as isolate number 1 from the sand sample grown on Hastingsmedia, WoHS 3 as isolate number 3 from the wood sample grown on Hastingsmedia + streptomycin media, RaP 2 as isolate number 2 from red algae sample grown on potato dextrose agar, etc. (FUAS 2022).

Culturable marine fungi characterization

The characterization of culturable marine fungi was done on its morphology and molecular approaches.

Morphological characterization

The morphological characterization was done macroscopically and microscopically. The macroscopic fungal morphological features were described based on their colony appearance observed from the top and bottom views of the plate. The microscopic characterization was analyzed by slide culture observed using a bright field (Olympus BX53) and scanning electron microscope (SEM, Hitachi TM 3000) to show the characteristics with different magnifications (Mag.). These microscopes allow the hyphae, conidiophores, and arrangement of conidia (spores) observation. During morphological characteristics visualization, the culturable marine fungi were cultured using glucose peptone yeast (GPY) agar for a clearer media background (FUAS 2022).

Molecular characterization

The molecular characteristics were carried out using DNA barcoding based on ITS (Guerra-Mateo et al. 2023). A loop of an isolated colony of marine fungi (obtained from the marine fungi isolation procedure) was disrupted, and their DNA was isolated using a Nucleospin Microbial DNA kit (Macherey Nagel, cat no. 740235.250). Then, it was mixed with PCR Master Mix (Promega) and ITS primers consisting of forward primer: ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') and reverse primer: ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Ibrahim et al. 2023). The PCR reaction mixture contained 25 µL of PCR master mix 2X, 2.5 µL of ITS 1 F (10 µM), 2.5 µL of ITS 4 R (10 µM), 1 µL of DNA template, and 19 µL of ddH_2O. The DNA amplification was done in a thermal cycler (BioRad) with the following conditions: initial denaturation at 95°C for 1 minute, denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 1 minute. The cycle was repeated 34 times, and the PCR results were stored at 4°C. The PCR products were visualized using agarose 1.5% gel electrophoresis for confirmation and then purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). The purified PCR products were then sent to PT. Genetika Science Indonesia, Tangerang, Indonesia, for sequencing analysis.

Possible bioactivity for culturable marine fungi

The culturable marine fungi isolates obtained from Jember south coast were then searched for their bioactivity using a literature review.

Data analysis

The macroscopic morphology was described qualitatively based on its color, shape, edge, elevation, texture, pigment, and exudate. These macroscopic morphological data were observed from the top and bottom views of the plate. The microscopic observation was described by its hyphae, conidiophores, and conidia. Published work was used to establish the identification of culturable marine fungi, such as George et al. (2019), Chang et al. (2020), Saif et al. (2020), El-Dawy et al. (2021), FUAS (2022), Nwobodo et al. (2022), Sklenář et al. (2022), Widjajanti et al. (2022), and Arifah et al. (2023).

The respective ITS sequence obtained from the PCR product was analyzed by sequencing. The rough sequencing data was edited using Chromas Pro version. 2.1.0. The results of data editing will obtain a complete DNA sequence, and the data is compared with DNA sequences that have been previously documented in the GenBank using BLAST (Basic Local Alignment Search Tool) online software at www.ncbi.nlm.nih.gov/Blast to identify species. The BLAST result sequences then went through the sequence alignment process using CLUSTAL W with the help of the MEGA 11 application for phylogenetic tree construction. Then, the fungi species were confirmed using the database from https://www.mycobank.org/. Species that have been identified were then continued with phylogenetic tree reconstruction to see their taxonomy. The reconstruction used a consensus form from chromatogram editing results. The phylogenetic tree was reconstructed using the Neighbor-Joining (NJ) tree method with the Kimura-2-Parameter (K2P) model and 1,000 bootstraps. Both morphological and molecular characterization results will be used to determine the biodiversity of culturable marine fungi from Jember south coast (FUAS 2022). Marine fungi that have been identified were then analyzed with a literature study to determine the possibility of their bioactivity. These

preliminary data are important for the exploration of marine fungi bioactivity as the next drug candidate.

RESULTS AND DISCUSSION

Marine fungi isolation

Based on the morphotypes, 47 marine fungi isolates were obtained from Jember south coast, East Java, Indonesia. There were 6 isolates from WaP, 2 isolates from WaPS, 1 isolate from SaH, 1 isolate from SaP, 3 isolates from SaPS, 10 isolates from WoH, 10 isolates from WoHS, 5 isolates from WoP, 2 isolates from GaP, 2 isolates from RaHS, and 5 isolates from RaP.

The differences in colony morphotypes were used as the basis for isolation. Colony morphotypes are unique because although the sub-culture technique with the streak method is identical, the shape and color of the colonies that grow can be different. For instance, SaH 1 colonies showed a characteristic in the form of cream color rhizoid powder texture with dark brown pigment (Figure 2.A); WoH 3.1 colonies showed greenish-brown color filamentous with powder texture and grey pigment (Figure 2.B); WoHS 3 colonies showed greyish black irregular shape with cottony texture and black pigment (Figure 2.C); while WoHS 4.2 colonies showed circular white with black spots color, cottony texture, and white pigment (Figure 2.D). These visual aids provide a clear understanding of the morphotypes under discussion.

Culturable marine fungi characterization

The growth rate of the isolated marine fungi decreased along with the number of subcultures carried out during isolation. Not all of them can be grown continuously under laboratory conditions, even though all conditions have been adjusted to the initial environmental conditions where the fungi originated. This results in a smaller number of microorganisms, including marine fungi, that have been successfully isolated and cultured continuously in laboratory conditions. Among 47 marine fungi isolates obtained from Jember south coast, there were only 17 culturable ones (ca. 36.17%).

Morphological characterization of culturable marine fungi

The culturable isolates of marine fungi obtained and their morphological characteristics are shown in the Supplementary Data. Some culturable marine fungi show different macroscopic morphological characteristics. Some of them are white, and the other are turning greyish, making them seem like having different morphotypes. As a result, they are coded as different isolates, including WoHS 3, WoHS 3.1, and WoHS 3.2. When the microscopical morphology was performed, they had the same characteristics. It turns out that the difference in macroscopic morphology is caused by color difference because of the culturable marine fungal age. The white color is their color at an early stage of growth, but as they grow older, they are turning grey and a darker grey.

SaH 1 has rhizoid-shaped colony morphology, beige colony and mycelium color, lobate colony edge with raised elevation, powdery colony texture, no exudate drops, and dark brown pigment. The microscopic morphology of SaH 1 shows that this isolate has hyphae that are non-septate, conidiophores that are single-stipitate, vesicles, phialides, and conidia that are ellipses.

WoH 2.1, WoH 3.1, WoH 5, WoH 6, WoH 6.1, WoH 6.2, WoH 4, and WoHS 6 have similar colony morphology, i.e., filamentous colony shape with greenish-brown color, brown mycelium color, raised colony edge with raised elevation, powder colony texture, and no exudate drops. The pigment colors of these isolates are varied, ranging from beige, brown, light brown, to grey. They have similar microscopic morphology. They have conidial hyphae, conidiophores consisting of one stipe, gourd-shaped phialids, and round conidia.

WoHS 2, WoHS 4, and WoHS 4.2 have a circular colony shape with white colony color with black spots, white mycelium color, filiform colony edges with flat elevations, cottony colony texture, no exudate drops, and white pigment color. Microscopically, they have septate hyphae, conidiophores are hyphae-like, simple, and short, and conidia are in chains and in the form of rounded to wide ovals.

WoHS 3, WoHS 3.1, and WoHS 3.2 have irregular colony shapes, with greyish-black color, black mycelium color, undulate colony edge with flat elevation, cottony colony texture, no exudate drops, and black pigment. Microscopically, this species has septate hyphae, conidiophores are septate, geniculate, and conidia are spindle-shaped.

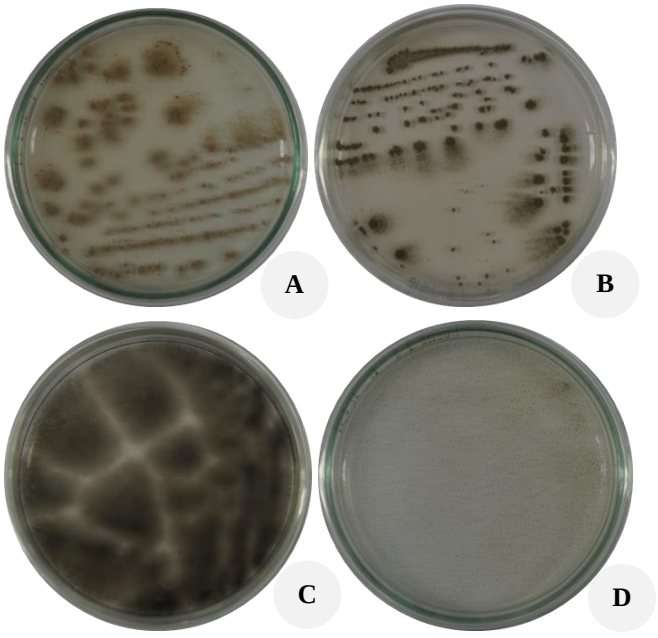

Figure 2. Examples of different morphotypes of successfully isolated marine fungi from Jember south coast, East Java, Indonesia: A. SaH 1, B. WoH 3.1, C. WoHS 3, D. WoHS 4.2

RaP 2 has filamentous colony morphology with green color, white mycelium color, entire colony edge with raised elevation, cottony colony texture, no exudate drops, and yellow pigment. Microscopically, this isolate has septate hyphae, conidiophores are single-type, vesicles, phialides are flask-shaped, and head radiated conidia.

RaP 3 has regular-shaped colonies, grey-olivaceous color, aerial mycelium color, entire colony edge with raised elevation, velvety colony texture, no exudate drops, and olivaceous black pigment. Microscopically, this isolate has septate hyphae, a straight and unbranched conidiophore, and obovoid-shaped conidia.

Molecular characterization of culturable marine fungi

Figure 3 presents the PCR product visualization of culturable marine fungi isolated from Jember south coast. The PCR using an ITS primer was successful, confirmed by the gel electrophoretic pattern showing the complete sequence of the ITS rDNA region at 500-700 bp. The amplicons are shown in a single band, suggesting that the annealing temperature used was appropriate.

The phylogenetic tree of the culturable marine fungi from Jember south coast is illustrated in Figure 4. The result shows that each recognized sample is divided into three clades. *Aspergillus* and *Penicillium* were members of the first clade, with 10 isolates belonging to this clade. Each branch of the second clade included the genus *Cladosporium* and *Curvularia*, with 4 isolates belonging to this clade. There is just one branch of the genus *Scopulariopsis* in the third clade with 3 members of isolates.

Among the 17 culturable marine fungi isolates from the Jember south coast, the dominant genera were *Penicillium* (47.059%), *Aspergillus* (11.765%), *Scopulariopsis* and *Curvularia* (17.647%), and *Cladosporium* (5.882%). The results showed that consecutive dominance were *Penicillium copticola* (35.294%), *Scopulariopsis candida* and *Curvularia lunata* (17.647%), and *Aspergillus stromatoides*, *Penicillium setosum*, *Penicillium javanicum*, *Aspergillus versicolor*, and *Cladosporium cladosporioides* with 5.882%, respectively.

Possible bioactivity for culturable marine fungi

Based on a literature review (Table 1), the culturable marine fungi from Jember south coast exhibit promising bioactivities. Most of these fungi possess cancer chemopreventive-related activities (such as cytotoxic, antitumor, and antileukemia effects), as well as antimicrobial (antibacterial and antifungal), antiviral, and anti-inflammatory properties. These findings may serve as a valuable reference for future studies on the bioactivity of the culturable marine fungi identified in this research.

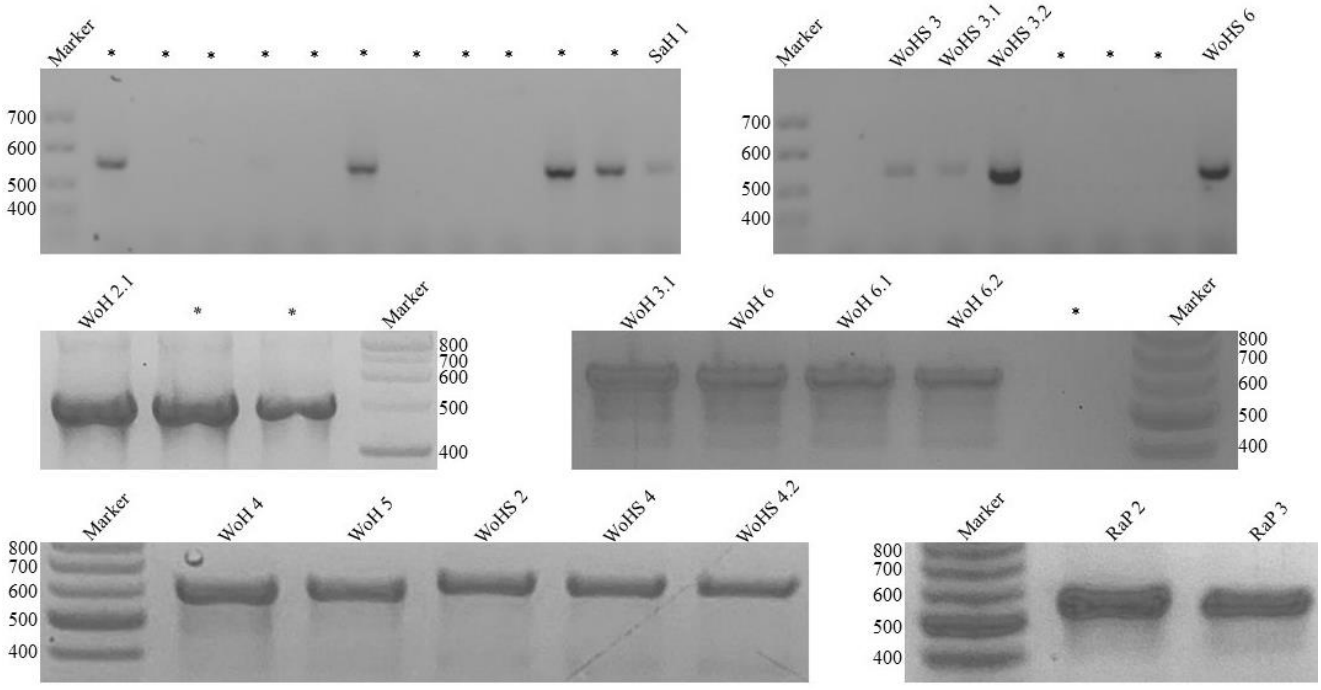

Figure 3. Gel electrophoretic analysis for PCR product visualization using agarose 1.5% and ITS primer of the culturable marine fungi isolated from Jember south coast. The respective sample ID indicated each sample. The asterisk symbol (*) represents unculturable marine fungi isolated from the same location

Discussion

The marine fungi from Jember south coast, East Java, Indonesia were isolated based on their sample and media. Sterilized seawater of origin was used at first as a solvent for the media, ensuring that the salinity of the media matched the salinity of the seawater from which it was sampled. During the isolation process, the solvent was gradually changed from seawater of origin to artificial seawater. It was crucial to adjust the initial salinity value at the time of sampling, as this prevented fluctuations in the condition of the original seawater, which can vary depending on the season. For example, the composition of seawater during the rainy season will differ from that during the dry season, and such fluctuations can affect the fungal growth (Chen et al. 2020). Once they were isolated, continuous culture was done. The wood remains sample had the most fungi, while the least fungi were obtained from the green algae sample. Wood remains are decomposing. On the other hand, the main organisms that decompose organic plant matter are fungi (Fukasawa and Matsukura 2021), making the wood remains sample the best fungi-associated sample.

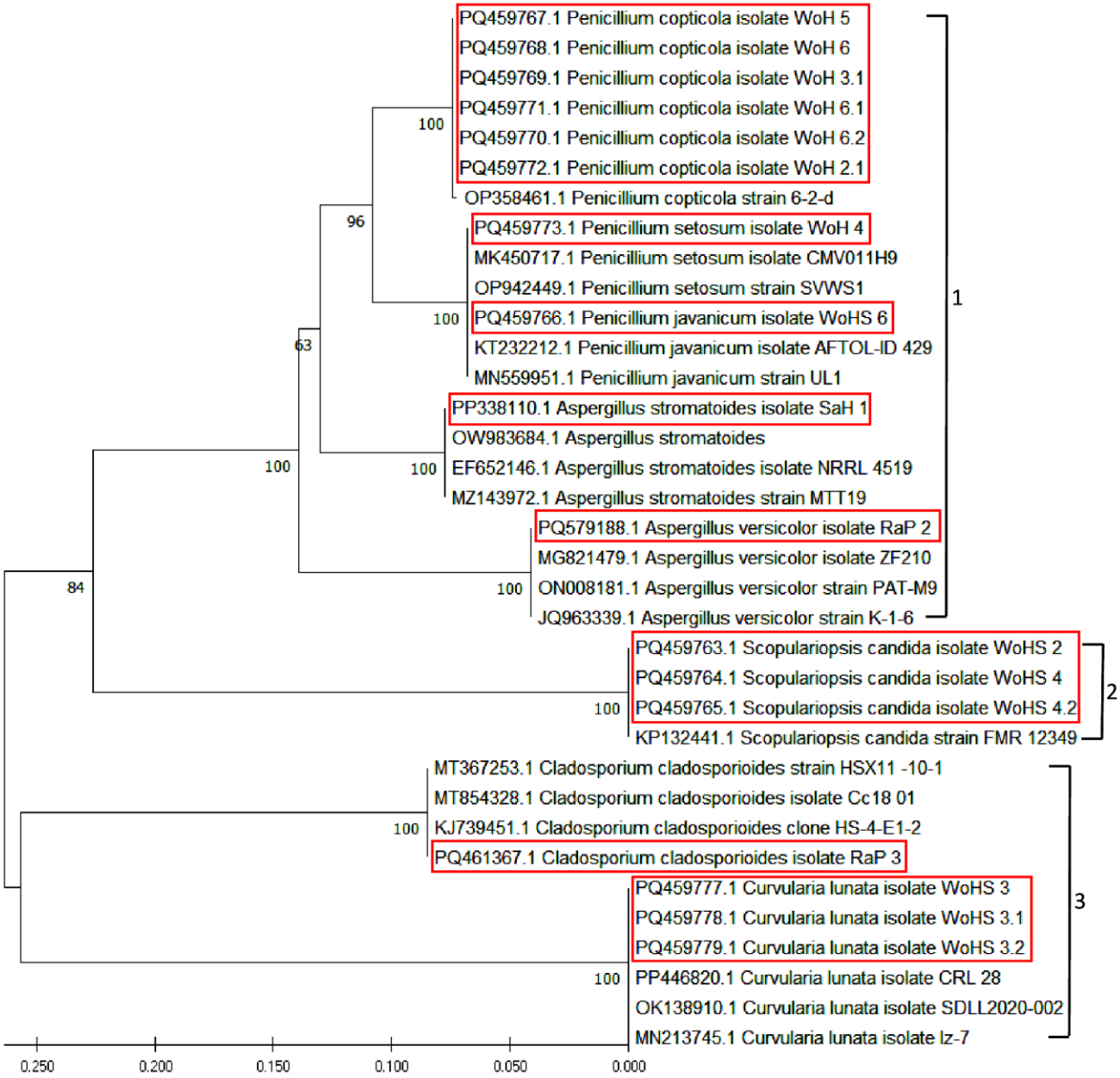

Figure 4. The phylogenetic tree of the culturable marine fungi from Jember south coast. The neighbor-joining was constructed based on the ITS sequence using MEGA11 with the Kimura-2 Parameter model. The data was statistically analyzed by a bootstrapping of 1,000. The culturable marine fungi isolated from Jember south coast were presented in a red bracket. The alpha numerical code in front of the species name of each marine fungal isolate is the accession number of the sequence in GenBank

Table 1. Possible bioactivity for culturable marine fungi from Jember south coast, East Java, Indonesia

Sample ID	Accession Number	Identity	Bioactivity	References
SaH 1	PP338110.1	*Aspergillus stromatoides* EKAP-SaH 1	Cytotoxic activity	Girich et al. (2023); Artasasta et al. (2024)
WoH 2.1	PQ459772.1	*Penicillium copticola* EKAP-WoH 2	Antitumor	Zhang et al. (2022a)
WoH 3.1	PQ459769.1			
WoH 5	PQ459767.1			
WoH 6	PQ459768.1			
WoH 6.1	PQ459771.1			
WoH 6.2	PQ459770.1			
WoH 4	PQ459773.1	*Penicillium setosum* EKAP-WoH 4	Antileukemia and antibacterial activity	de Carvalho et al. (2023); Eshboev et al. (2024); Mamangkey et al. (2024)
WoHS 2	PQ459763.1	*Scopulariopsis candida* EKAP-WoHS 2	Antibacterial activity	Widjajanti et al. (2022)
WoHS 4	PQ459764.1			
WoHS 4.2	PQ459765.1			
WoHS 3	PQ459777.1	*Curvularia lunata* EKAP-WoHS 3	Antimicrobial activity	Nwobodo et al. (2022)
WoHS 3.1	PQ459778.1			
WoHS 3.2	PQ459779.1			
WoHS 6	PQ459766.1	*Penicillium javanicum* EKAP-WoHS 6	Antifungal and antibacterial activity	Shen et al. (2020)
RaP 2	PQ579188.1	*Aspergillus versicolor* EKAP-RaP 2	Antimicrobial, antivirus, and antitumor activity	Lee et al. (2010); Zhang et al. (2022b)
RaP 3	PQ461367.1	*Cladosporium cladosporioides* EKAP-RaP 3	Antifungi and antiinflamation	Han et al. (2021); Li et al. (2024)

Liu et al. (2023) and Zhang et al. (2024) reported that culturing marine microorganisms is a challenge in itself. Given that just 1857 fungal species from 769 genera have been recovered from marine environments, making up less than 1.2% of the over 150,000 species of cultivated fungi found globally, marine fungi are a surprisingly underrepresented group. This is very surprising because fungi are found in every ecosystem, from the intertidal zone and surface water to depths of kilometers, yet the ocean covers 70% of the Earth's surface (Zhang et al. 2024). This study obtained 17 out of 47 marine fungi isolates that are re-culturable (36.17%). Environmental isolates often exhibit instability when adapted to laboratory culture conditions (Guan et al. 2017). This instability may arise due to stress from the artificial environment, selective pressures in the lab setting, or loss of interspecies communication (Puspitasari et al. 2024). Moreover, repeated sub-culturing can further compromise the phenotypic or genotypic stability of these isolates (Cavalcante et al. 2007).

Since some of the marine fungi isolates were unculturable, only the culturable marine fungi isolates were analyzed further for their characteristics. The characterization of the culturable marine fungi morphology was done using GPY agar. The GPY agar is a clear medium, whereas the Hastings media is not crystal clear. The use of GPY agar made the observation of the marine fungal morphology easier; hence, some colonies appear to have different features compared to the reference. *Scopulariopsis candida* colony (WoHS 2, WoHS 4, WoHS 4.2) in GPY agar appears to be white with black spots, and they grow spread out on the surface of the media (Supplementary Data). In contrast, using potato dextrose agar, they are white-yellowish and grow in clusters on the media (Widjajanti et al. 2022). The media used will affect the colony color (Noman et al. 2023). The key identification of fungi based on microscopic morphological characterization is their conidia and conidiophore structure (Hassan and Chang 2022). Yet, the microscopical and molecular characteristics still show the same species identification with the reference (Supplementary Data) (Widjajanti et al. 2022). Thus, the morphological observation is insufficient to determine the marine fungal identity (Hasanah et al. 2022).

The molecular characterization using DNA barcoding was intended to complete the morphological characterization. The visualization of the PCR product of culturable marine fungi isolated from Jember south coast showed a single band (Figure 2) at the expected ITS region at 600 + 113 bp (Porras-Alfaro et al. 2014), indicating that the annealing temperature was suitable. The phylogenetic tree reconstruction findings indicate the taxonomy of the sample species and GenBank genomes (Figure 3). The NJ approach was chosen because it has a lower evolution rate than other methods. This method is more efficient for bootstrapping and processing vast amounts of data (Saitou and Nei 1987). Meanwhile, the K2P model was utilized to estimate each taxon's evolutionary distance better. Furthermore, the K2P model is more accurate and statistically consistent than other models (Patwardhan et al. 2014).

Each sample that has been identified is distributed in 3 clades according to the taxonomy of the species. The first clade contained the genera *Penicillium* and *Aspergillus*, which indicates that these genera have a close relationship. This is because *Aspergillus* and *Penicillium* belong to the same family, i.e., Aspergillaceae (Houbraken et al. 2020). Each species is also distributed on branches with high cluster similarity values. Branch samples WoH 2.1, WoH 6.1, WoH 6.2, WoH 3.1, WoH 5, and WoH 6, which are *P. copticola* species, have 100% cluster similarity, which

indicates high validity. This indicates a high level of confidence (Tamura et al. 2021). Similarly, the branch samples RaP 2, which is *A. versicolor*, and SaH 1, which is *A. stromatoides*. WoHS 6 and WoH 4 samples are on 1 branch with *P. setosum* and *P. javanicum*, with a high cluster similarity value on the branch, which is 100%. The fungi *P. setosum*, which is a new species of fungi, is in the same branch as *P. javanicum*, so it can be concluded that the two species have a very close relationship (Tamura et al. 2021).

The second clade contained the genera *Cladosporium* and *Curvularia* in each branch. These genera are in the same class, i.e., Dothideomycetes, so their relationship is quite close (Salvatore et al. 2021; Ferdinandez et al. 2023). The RaP 3 sample is identified as *C. cladosporioides* and has a very high and valid cluster similarity value, i.e., 100%, as well as samples WoHS 3, WoHS 3.1, and WoHS 3.2, which are *C. lunata*.

The third clade has only 1 branch of the genus *Scopulariopsis*. This genus forms a different clade because it has a different class from the other genus, i.e., *Sordariomycetes* (Skóra et al. 2015). Samples WoHS 2, WoHS 4, and WoHS 4.2, which are *S. candida*, also have 100% cluster similarity.

In some cases, ITS is insufficient to be used for precise molecular identification of some fungi species, including the genus *Penicillium* (Reich and Labes 2017). Nevertheless, samples WoH 2.1, WoH 6.2, WoH 6, WoH 5, WoH 3.1, and WoH 6.1 are highly related to *P. copticola*, with 100% cluster similarity. Therefore, to corroborate the results of molecular characterization, identification analysis was carried out with the support of morphological characterization results (Hasanah et al. 2022). However, since all samples show a close relationship with existing species in the NCBI database with high bootstrap values, there were no significant problems in this study.

The ITS regions are the standard fungal barcode used in fungal diversity studies. Still, they often fall short in providing accurate species-level identification for certain genera like *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, and *Fusarium* (Reich and Labes 2017). For these genera, additional DNA markers, such as translation elongation factor (TEF1-α), calmodulin (CaM), β-tubulin (TUB2), and the RNA polymerase II second-largest subunit (RPB2), are commonly used (Tekpinar and Kalmer 2019). The ITS sequences must be analyzed in conjunction with these protein-coding genes, allowing for differentiation between closely related strains to achieve proper species identification. Multigene phylogenetic analyses, which integrate both ITS and protein-coding genes, are essential in confirming the identity of marine fungi (Chin et al. 2024).

Although it increases accuracy, using several gene markers for species identification has drawbacks. First, since not all genes are equally informative across taxa, it is necessary to choose the right markers carefully. Variability in resolution and possible mistakes in species discrimination may result from this. Furthermore, using additional markers raises the cost and technical complexity because sequencing and analysis require more time and resources. Lastly, the potential for insufficient reference databases and the requirement for sophisticated bioinformatics tools can make data interpretation difficult (Antil et al. 2023; Chac and Thinh 2023).

Among 17 culturable marine fungi isolates obtained in this study, the fungal genera identified were predominantly *Penicillium* (47.059%), followed by *Aspergillus* (11.765%), *Scopulariopsis* and *Curvularia* (each comprising 17.647%), and *Cladosporium* (5.882%). At the species level, the community was primarily composed of *P. copticola* (35.294%), *S. candida* and *C. lunata* (each accounting for 17.647%), along *with A. stromatoides*, *P. setosum*, *P. javanicum*, *A. versicolor*, and *C. cladosporioides*, each representing 5.882% of the total isolates. All of the identified fungi are classified within the phylum Ascomycota (Rudramurthy and Kaur 2024).

Penicillium and *Aspergillus* are usually found abundantly in marine ecosystems (Sen et al. 2022; Behera and Das 2023; Banchi et al. 2024). These genera have adaptive mechanisms that enable tolerance to high hygroscopic pressure, salinity, and temperature variations (Behera and Das 2023), making them culturable (Sen et al. 2022). On the other hand, *C. lunata* can be found not only in marine samples (da Silva et al. 2022) but also in terrestrial environments (Al-Askar et al. 2022). *S. candida* is mostly found in terrestrial environments (Jagielski et al. 2016) or clinical samples (Sandoval-Denis et al. 2013). Even though there is no report on that they can be found in marine ecosystems, their existence there is still possible since they are closely related to *Microascus manginii*, which can be found in the marine ecosystem (Kramer et al. 2016; Sandoval-Denis et al. 2016). On the other hand, *C. cladosporioides* is considered a cosmopolitan fungus; it can be found all over the world in a wide range of environments, including marine ecology. *C. cladosporioides* thrives in various climates and ecosystems, including soil, air, plants, and decaying organic matter. Their ability to grow in diverse habitats, including indoor environments, makes them common and widespread, contributing to their cosmopolitan nature (Lee et al. 2023; Simonetti et al. 2024).

The final identity of the species of the culturable marine fungi from Jember south coast, and their possible bioactivity, is presented in Table 1. The possible bioactivities were collected based on a literature study. Of course, the culturable marine fungi isolated from Jember south coast may exhibit different bioactivities than those from previous studies. Many factors are affecting bioactivity, including the culture conditions. The isolation process of marine fungi itself would delete the communication with other microbes, which in turn will affect the fungi's metabolism, resulting in different natural products (Puspitasari et al. 2024). Different natural products will contribute to different bioactivities (Ahmad et al. 2025). Similar challenges have also been encountered by other researchers, resulting in only 1.2% of marine fungi that are culturable (Zhang et al. 2024). In this case, a researcher needs to decide which aspect would be their focus: the isolation of marine fungi or the targeted bioactivity. One possible solution to get the desired bioactivity while doing the isolation on marine fungi is co-culture (Caudal et al. 2022). Co-culture is a strategy to

increase the molecular diversity of microbial natural products and promote the expression of dormant gene clusters in microorganisms (Peng et al. 2021). Thus, the proposed bioactivity of the culturable marine fungi isolated from Jember south coast, East Java, Indonesia needs to be confirmed further. These data could be used as a basis for drug discovery, with the sources originating from Indonesia to support government goals in self-reliance on drug raw materials (Kartika 2023).

This study reveals the biodiversity that offers a reservoir of unique genetic and metabolic traits, which can be harnessed for various applications, including drug discovery. Leveraging microbial biodiversity data for drug discovery offers immense potential for Indonesia, aligning with the government's goals of self-reliance on drug raw materials. By tapping into the rich microbial resources of the country, Indonesia can not only enhance its pharmaceutical landscape but also contribute to global health solutions. Emphasizing sustainable practices in this endeavor will ensure that biodiversity is preserved while unlocking its therapeutic potential, particularly within the context of Indonesia's unique environment. Hence, further researches need to be done to ensure these possible bioactivities since the site-specific condition of the origin will affect their bioactivity (Puspitasari et al. 2024).

In conclusion, we succeeded in reculturing 17 out of 47 marine fungi isolates from Jember south coast, East Java, Indonesia. Morphological and molecular characterization revealed diversity among the isolates, with both methods yielding consistent taxonomic identification. The genera were dominated by *Penicillium* (47.059%), *Aspergillus* (11.765%), *Scopulariopsis* and *Curvularia* (17.647%), and *Cladosporium* (5.882%). At the species level, they were dominated by *P. copticola* (35.294%), *S. candida*, and *C. lunata* (17.647%). Less dominated species were constituted by 5.882%, consisting of *A. stromatoides*, *P. setosum*, *P. javanicum*, *A. versicolor*, and *C. cladosporioides*. All identified culturable marine fungi from this research belong to the phylum Ascomycota.

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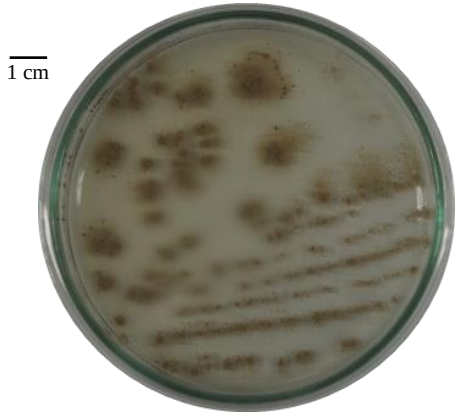
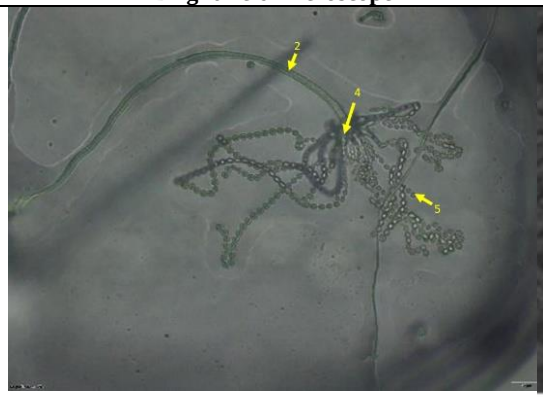
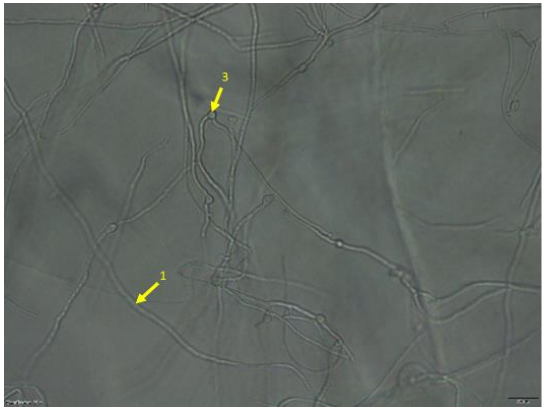
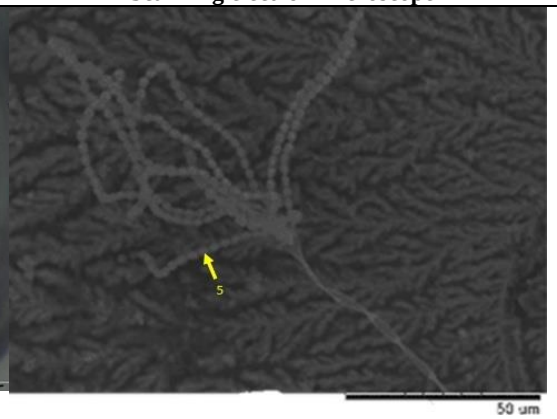
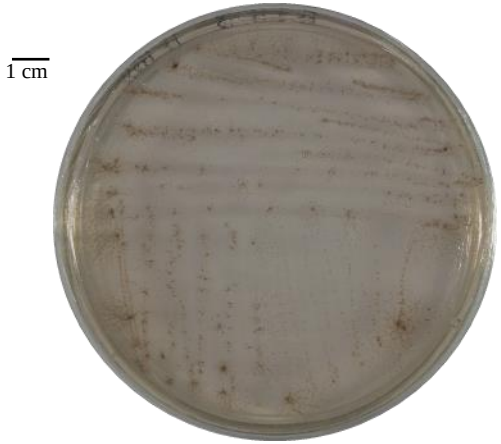
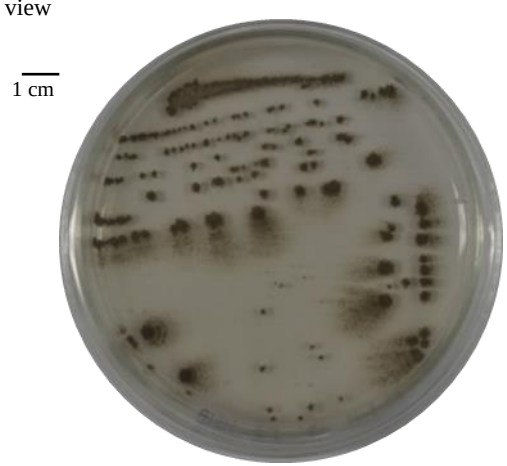
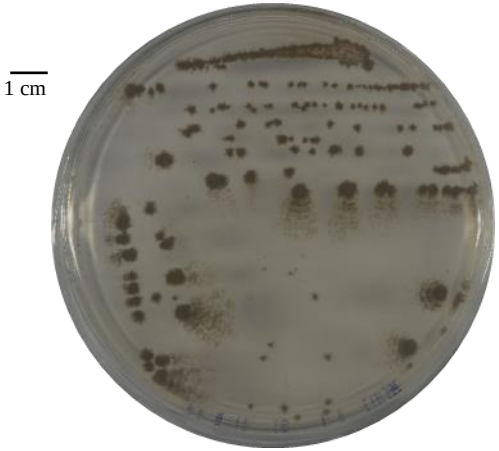
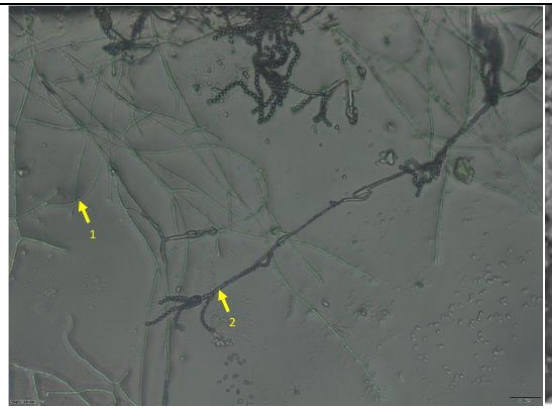
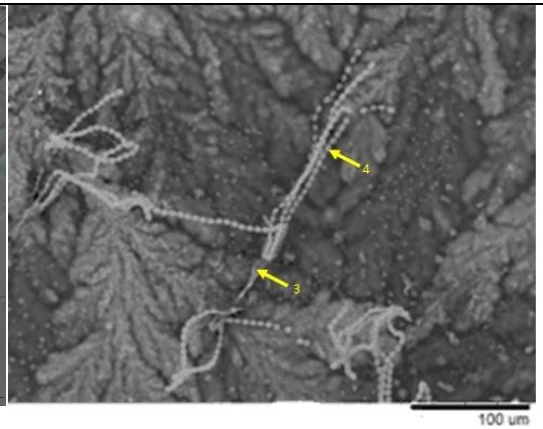
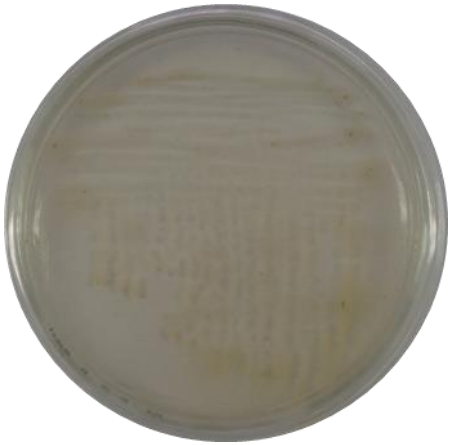
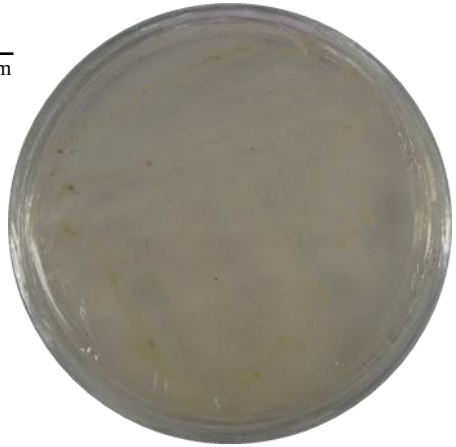
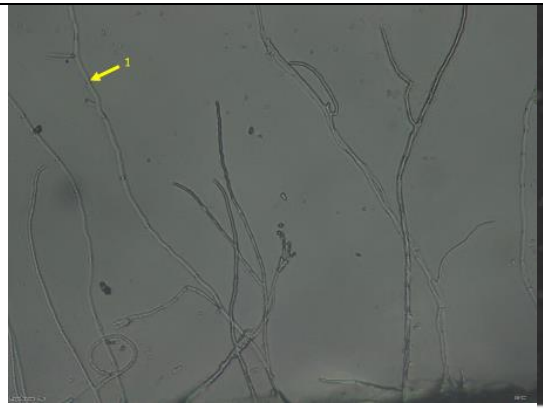
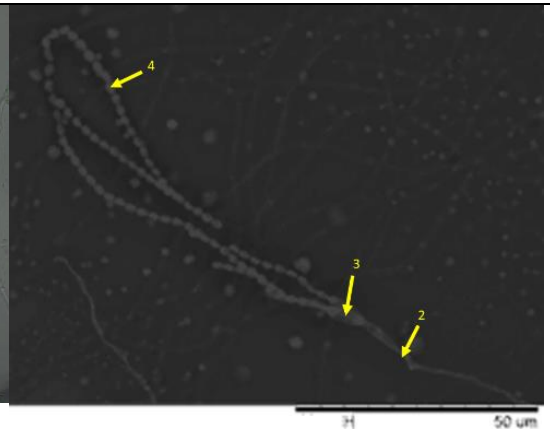
Table S1. Culturable marine fungi isolates from Jember south coast, East Java, Indonesia, with their morphological characteristics

Sample ID	Morphological characteristics			References
	Macroscopic morphology	Microscopic morphology		
		Bright field microscope	Scanning electron microscope	
SaH 1	Top view  Bottom view  Characteristics: 1. Shape: Rhizoid 2. Colony: Cream 3. Mycelium: Cream 4. Edge: Lobate 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Dark Brown 8. No exudate	  Day 4. Mag. 400× Note: 1. Non septate hyphae 2. Conidiophore are single-stipe 3. Vesicles 4. Phialides 5. Conidia are ellipse	 Day 2. Mag. 1.200×	Arifah et al. (2023)

WoH 2.1	Top view  Bottom view  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Cream 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consist single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2 Mag. 1,500×	George et al. (2019); Saif et al. (2020)

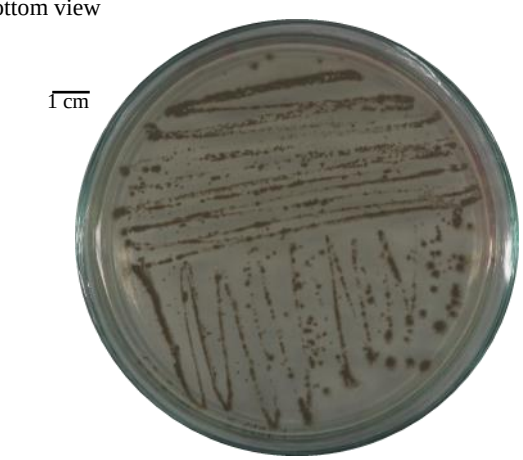
WoH 3.1	Top view  Bottom view  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Grey 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2 Mag. 1, 000×	George et al. (2019); Saif et al. (2020)

WoH 4	Top view 1 cm  Bottom view 1 cm  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Light Brown 8. No exudate	 Day 3. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2 Mag. 1,500×	George et al. (2019); Saif et al. (2020)

WoH 5

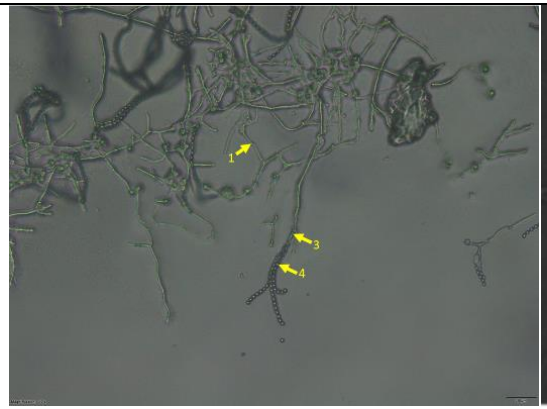
Top view

Bottom view

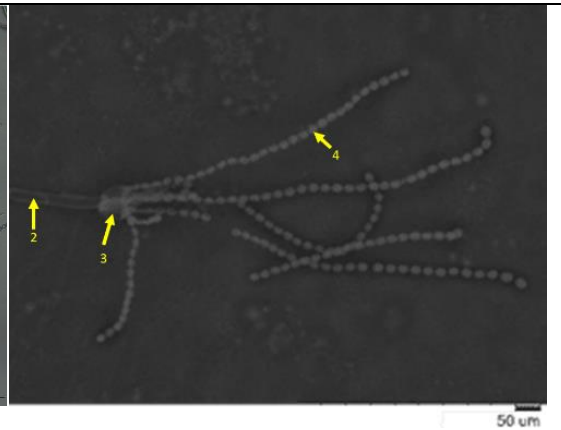


Characteristics:

1. Shape: Filamentous
2. Colony: Greenish-brown
3. Mycelium: Brown
4. Edge: Filiform
5. Elevation: Raised
6. Texture: Powder
7. Pigment: Brown
8. No exudate



Day 5. Mag. 400×

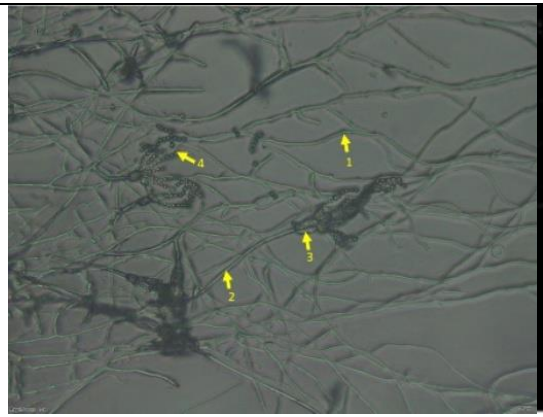
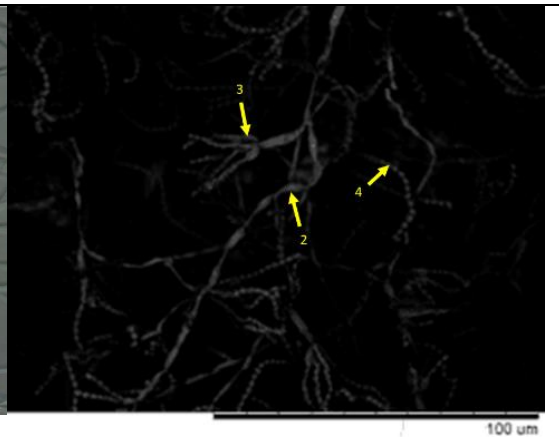


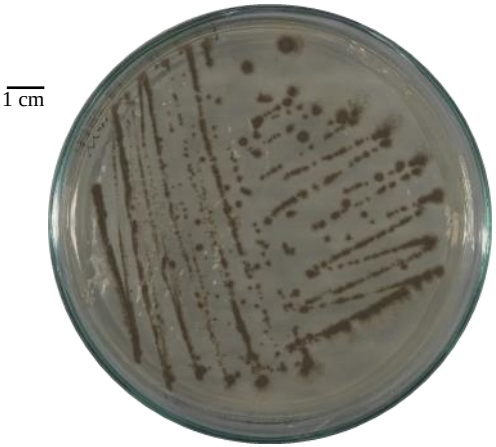
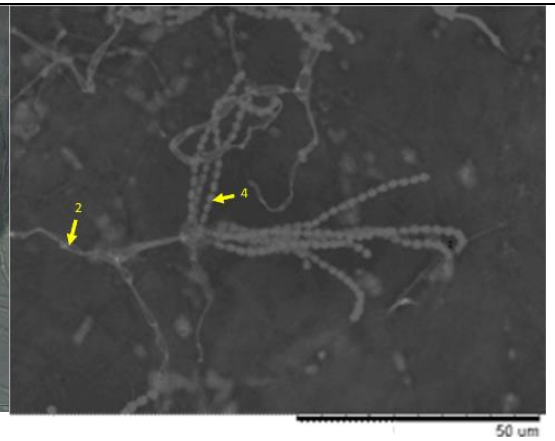
Day 2. Mag. 1,500×

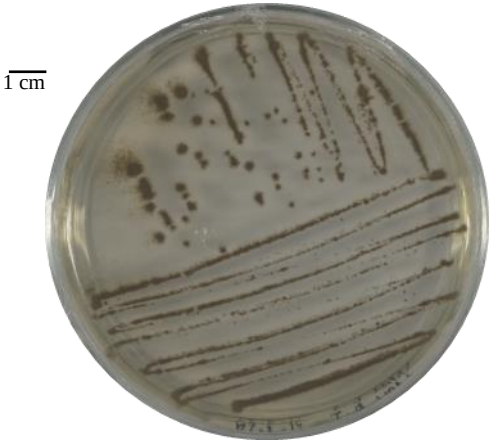
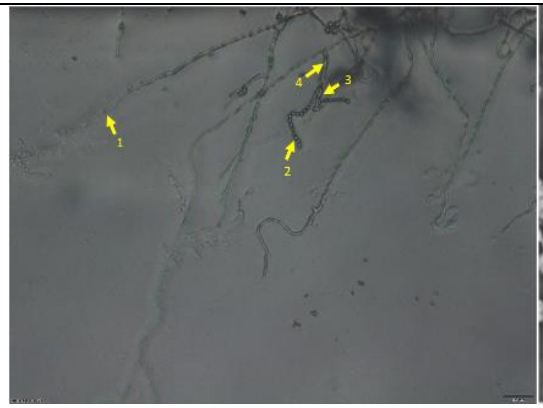
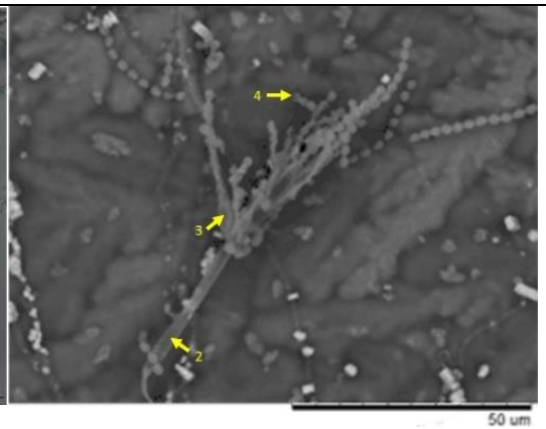
Note:

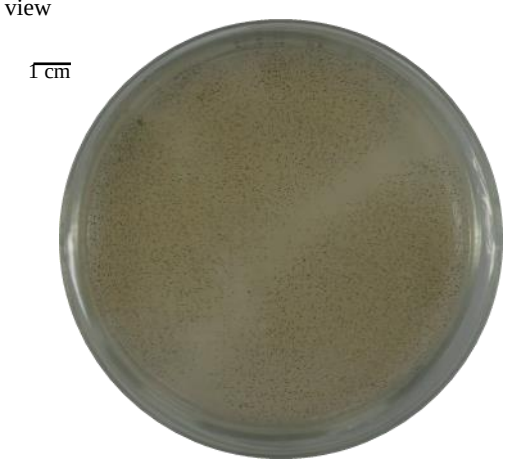
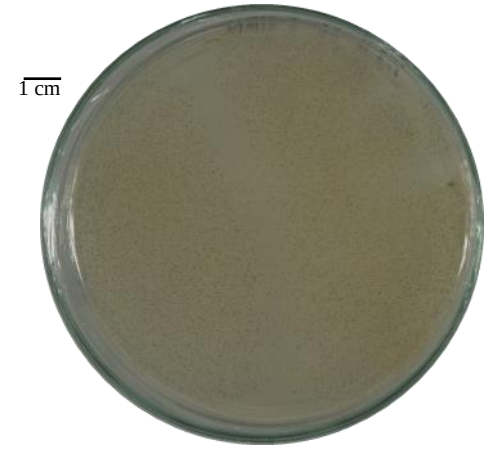
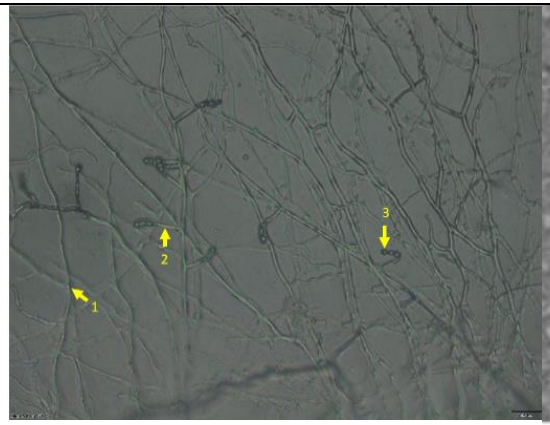
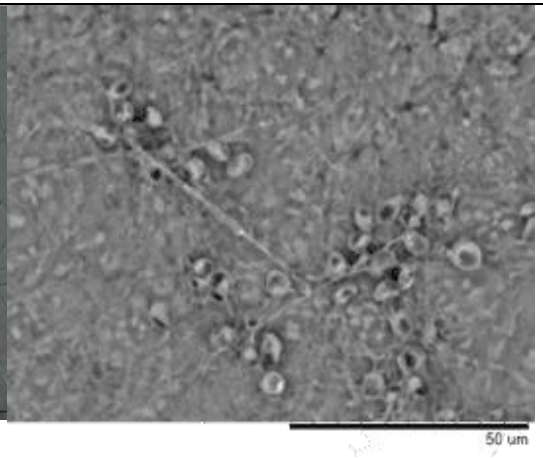
1. Septate hyphae
2. Conidiophore consists of single stipe
3. Phialides are flask shape
4. Conidia are round

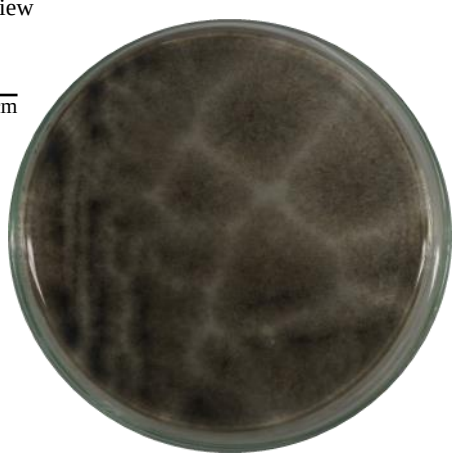
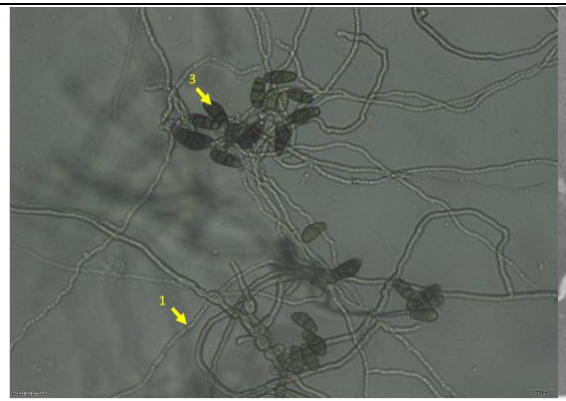
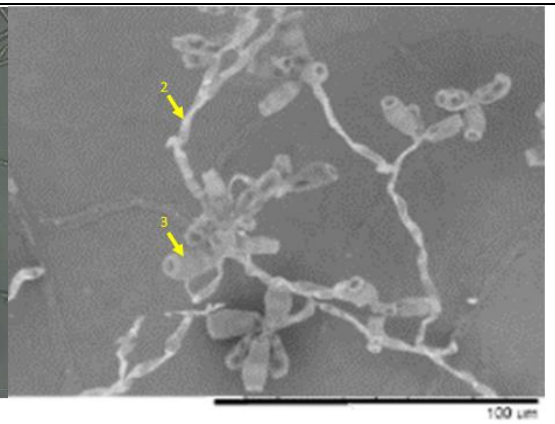
George et al. (2019); Saif et al. (2020)

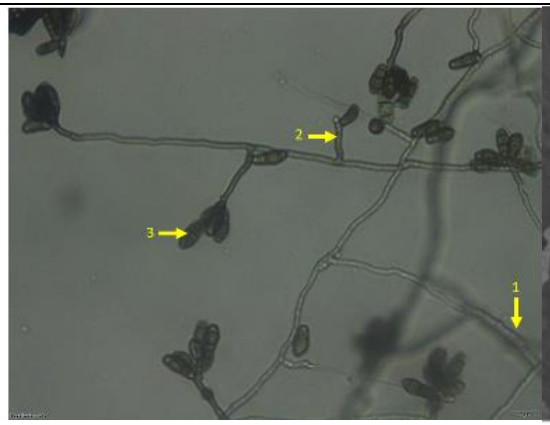
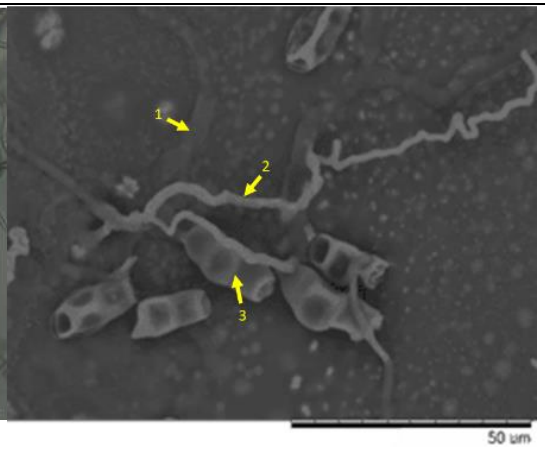
WoH 6	Top view  Bottom view  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Brown 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2. Mag. 1,000×	George et al. (2019); Saif et al. (2020)

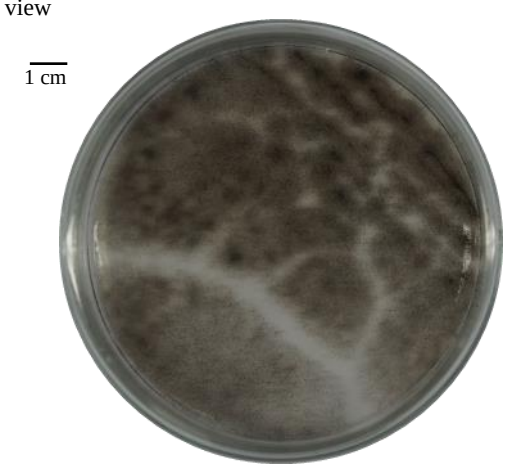
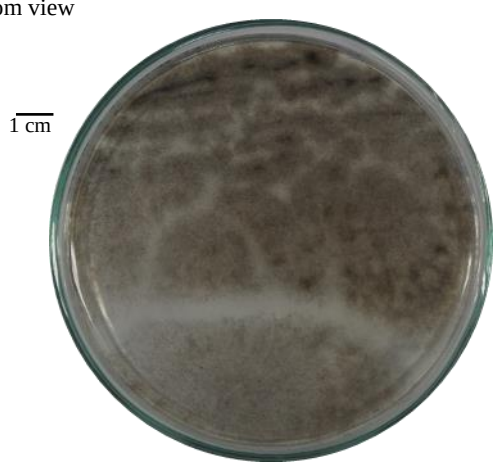
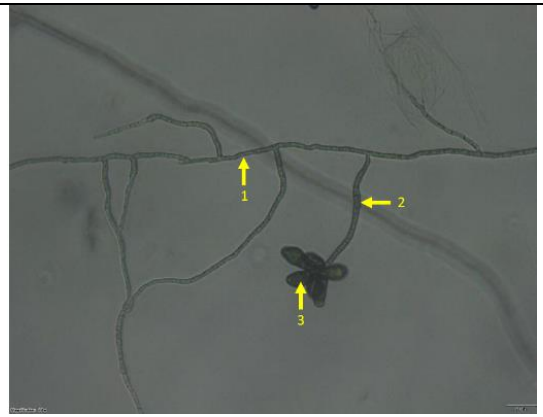
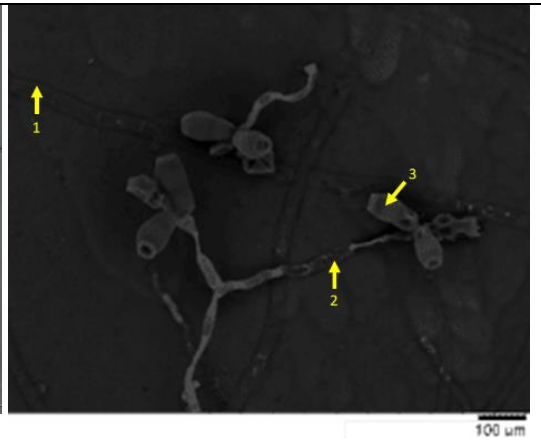
WoH 6.1	Top view  Bottom view  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Brown 8. No Exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2 Mag. 1,500×	George et al. (2019); Saif et al. (2020)

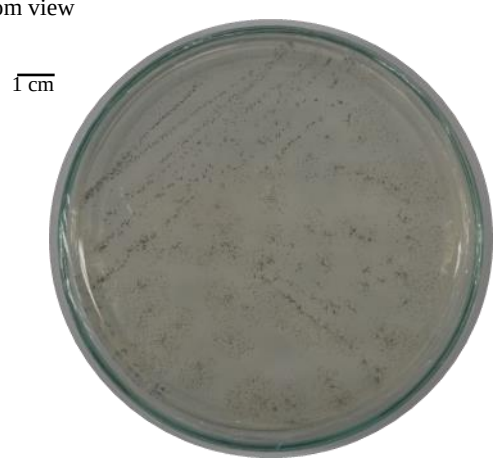
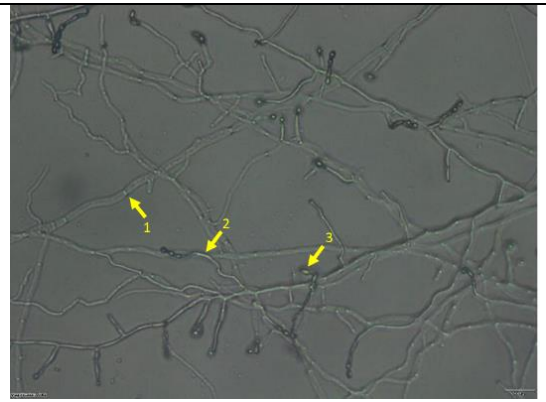
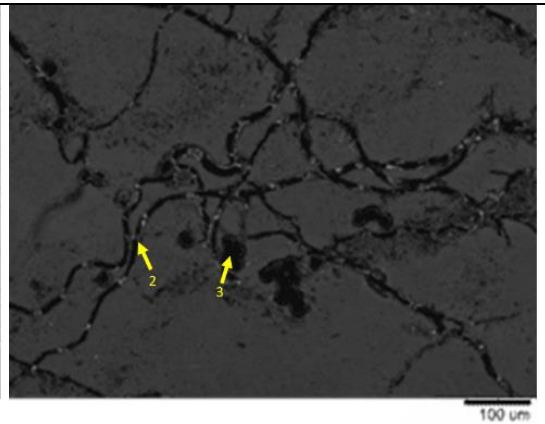
WoH 6.2	Top view  Bottom view  Characteristics: 1. Shape: Filamentous 2. Colony: Greenish-brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Grey 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2 Mag: 1500×	George et al. (2019); Saif et al. (2020)

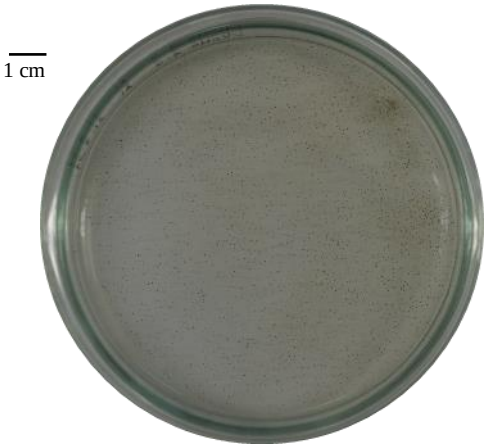
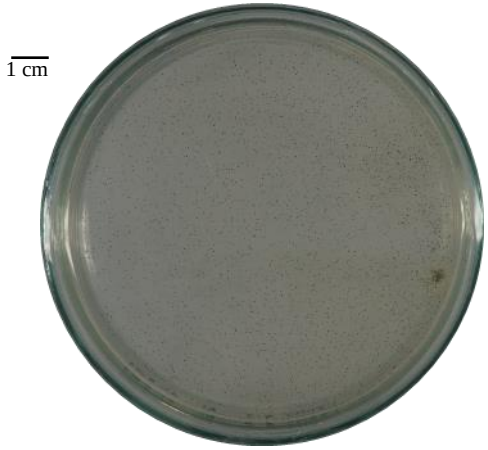
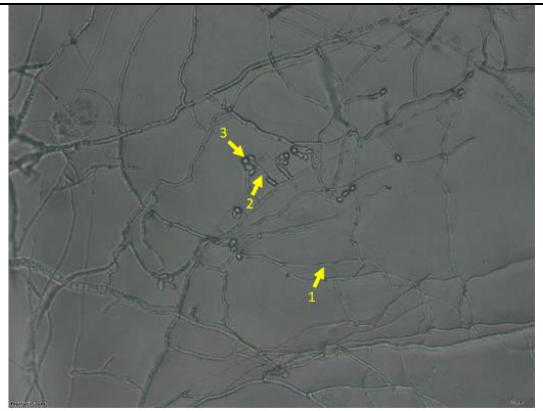
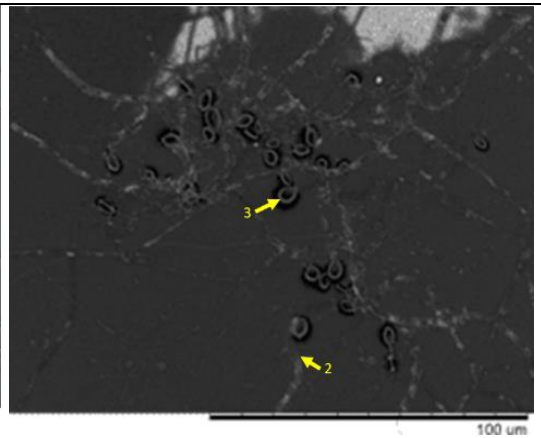
WoHS 2	Top view  Bottom view  Characteristics: 1. Shape: Circular 2. Colony: White with black spots 3. Mycelium: White 4. Edge: Filiform 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: White 8. No exudate	 Day 5. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are hyphae-like, simple, and short 3. Conidia in chains and in the form of rounded to wide ovals	 Day 5 Mag. 1,500×	Widjajanti et al. (2022)

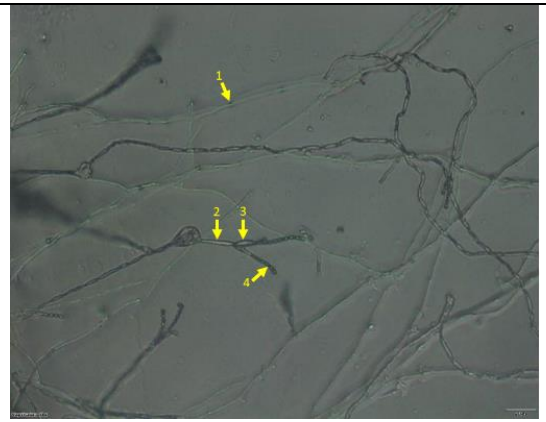
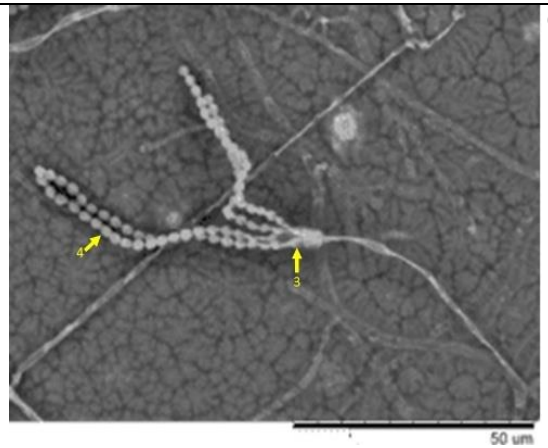
WoHS 3	Top view 1 cm  Bottom view 1 cm  Characteristics: 1. Shape: Irregular 2. Colony: Greyish black 3. Mycelium: Black 4. Edge: Undulate 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: Black 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are septate, geniculate 3. Conidia are spindle shape	 Day 2. Mag. 1000×	Chang et al. (2020); Nwobodo et al. (2022)

WoHS 3.1	Top view  Bottom view  Characteristics: 1. Shape: Irregular 2. Colony: Greyish black 3. Mycelium: Black 4. Edge: Undulate 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: Black 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are septate, geniculate 3. Conidia are spindle shape	 Day 1. Mag. 1,500×	Chang et al. (2020); Nwobodo et al. (2022)

WoHS 3.2	Top view  Bottom view  Characteristics: 1. Shape: Irregular 2. Colony: Greyish black 3. Mycelium: Black 4. Edge: Undulate 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: Black 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are septate, geniculate 3. Conidia are spindle shape	 Day 2. Mag. 1,000×	Chang et al. (2020); Nwobodo et al. (2022)

WoHS 4	Top view  Bottom view  Characteristics: 1. Shape: Circular 2. Colony: White with black spots 3. Mycelium: White 4. Edge: Filiform 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: White 8. No exudate	 Day 4 Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are hyphae-like, simple, and short 3. Conidia in chains and in the form of rounded to wide ovals	 Day 3. Mag. 1,500×	Widjajanti et al. (2022)

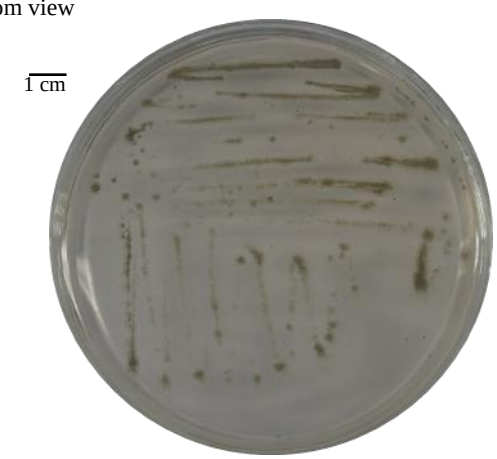
WoHS 4.2	Top view  Bottom view  Characteristics: 1. Shape: Circular 2. Colony: White with black spots 3. Mycelium: White 4. Edge: Filiform 5. Elevation: Flat 6. Texture: Cottony 7. Pigment: White 8. No exudate	 Day 5. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore are hyphae-like, simple, and short 3. Conidia in chains and in the form of rounded to wide ovals	 Day 3. Mag. 1,000×	Widjajanti et al. (2022)

WoHS 6	Top view 1 cm  Bottom view 1 cm  Characteristics: 1. Shape: Filamentous 2. Colony: Brown 3. Mycelium: Brown 4. Edge: Filiform 5. Elevation: Raised 6. Texture: Powder 7. Pigment: Brown 8. No exudate	 Day 5. Mag. 400× Note: 1. Septate hyphae 2. Conidiophore consists of single stipe 3. Phialides are flask shape 4. Conidia are round	 Day 2. Mag. 1,500×	George et al. (2019); Saif et al. (2020)

RaP 2

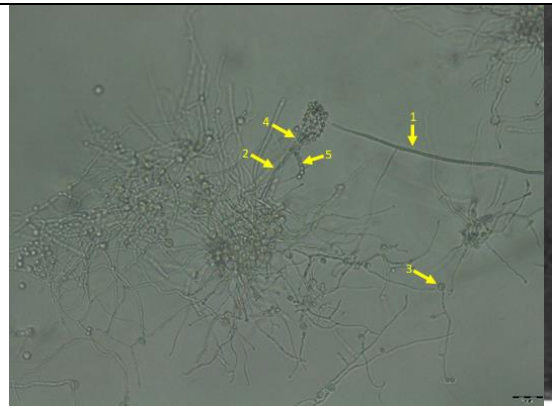
Top view

Bottom view

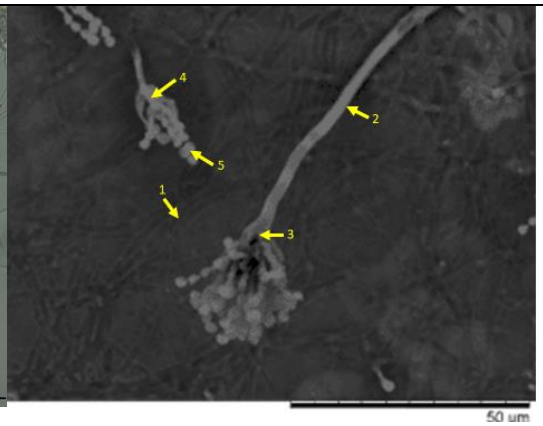


Characteristics:

1. Shape: Filamentous
2. Colony: Green
3. Mycelium: White
4. Edge: Entire
5. Elevation: Raised
6. Texture: Cottony
7. Pigment: Yellow
8. No exudate



Day 4. Mag. 400×

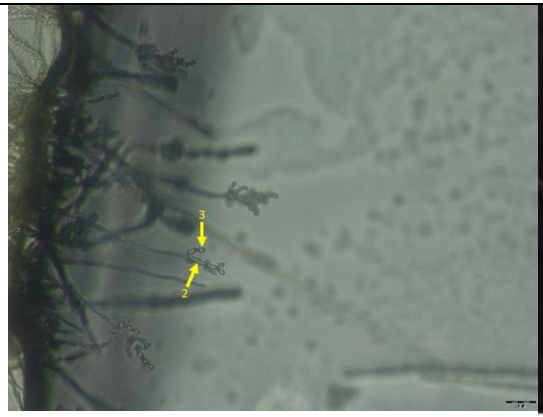
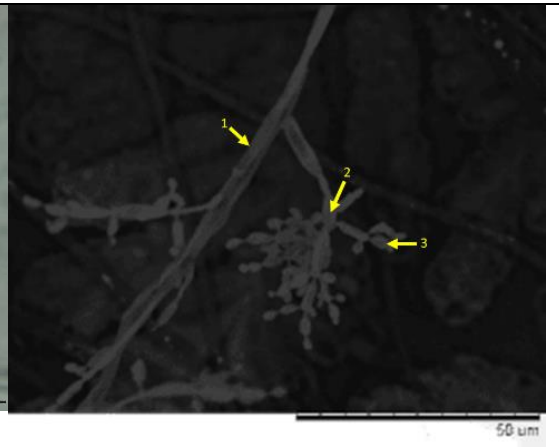


Day 3. Mag. 1,500×

Note:

1. Septate hyphae
2. Conidiophores are single-stipe
3. Vesicle
4. Phialides are flask shape
5. Conidial head radiate

Sklenář et al. (2022)

RaP 3	Top view 1 cm  Bottom view 1 cm  Characteristics: 1. Shape: Regular 2. Colony: Grey-olivaceous 3. Mycelium: White aerial 4. Edge: Entire 5. Elevation: Raised 6. Texture: Velvety 7. Pigment: Olivaceous black 8. No exudate	 Day 4. Mag. 400× Note: 1. Septate hyphae 2. Conidophore is straight and unbranched 3. Conidia shapes were obovoid	 Day 3. Mag. 1,500×	El-Dawy et al. (2021)

Note: the culturable marine fungi were cultured on GPY agar. The colony was documented using Sony camera A6300 with Yongnuo 50mm F1.8 lens. Mag: Magnification