Jurnal Biodjati 9(1):172-189, May 2024

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Screening Mycelium of Macrofungi Isolates in Tahura Djuanda Bandung As A New Candidate for Biomaterial

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Received: September 30, 2023
Revise from: October 24, 2023
Accepted: January 03, 2024

DOI: 10.15575/biodjati.v9i1.30021

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Abstract. *Grand Forest Park (Tahura) Ir. H. Djuanda, Bandung, West Java, Indonesia, is a secondary nature conservation area rich in biodiversity, including macrofungi, which has not been widely studied. The research aims to isolate, characterize its morphology, and screen its potential as a biomaterial source based on the growth of macrofungal mycelium. The research was carried out using an exploration method at three sampling locations: Maribaya (trail area), Goa Jepang (cave area), and Curug Koleang (waterfall area). The obtained macrofungi were isolated and identified based on their macroscopic and microscopic morphological characteristics and coded based on the location and number of isolates. A comparative evaluation was carried out by one-way analysis of variance (ANOVA) to assess the average mycelial growth of the macrofungal isolates for nine days on PDA. The results showed that there was a total of 62 species of macrofungi from three locations: 22 isolates from the Maribaya (MB) area, 18 isolates from the Goa Jepang (GJP) area, and 22 isolates from the Curug Koleang (CK) area. The isolates that showed the highest mycelium length and represented each research area were MB-07 (63.98 ± 1.21 mm), GJP-01 (81.47 ± 0.41 mm), and CK-13 (72.14 ± 1.20 mm). Isolate GJP-01 from the Goa Jepang area has the potential to become a superior fungus in its ability to expand mycelium and should be developed for mycelium-based material applications.*

Keywords: *bioprospecting, characterization, mycelium-based materials, mycelium growth.*

Citation
Laoli, I. A., Wulandari, A. P., Rachmawati, U., Sukmana, D. R., Dewi, A. H., & Rossiana, N. (2024). Screening Mycelium of Macrofungi Isolates in Tahura Djuanda Bandung As A New Candidate for Biomaterial. *Jurnal Biodjati*, 9(1), 172-189.

Jurnal Biodjati 9(1):172-189, May 2024

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INTRODUCTION

As a mega biodiversity country, Indonesia has a diversity of flora, which can be seen in the reporting of its tropical forests in the lowlands and highlands, which cover 63% of Indonesia's land area. One of the biodiversity found in tropical forests is macrofungi (Djuku et al., 2022). Studies on the diversity of macrofungi have been reported from several forest areas, nature reserves, and protected forests in Indonesia, such as from the Bali region (Proborini, 2012), Sumatra (Wahyudi et al., 2012; Noverita et al., 2019), Kalimantan (Yunida et al., 2014; Sari et al., 2015; Sumarni et al., 2017; Sopialena et al., 2018), Sulawesi (Arini & Christata, 2016). Macrofungi diversity has been found in several areas of Java Island, especially in the DKI Jakarta region (Nur et al., 2021), Central Java (Kramadibrata, 2013; Edyawati et al., 2019), East Java (Witantri et al., 2015; Firdaushi et al., 2018).

Taman Hutan Raya (Tahura) Ir. H. Djuanda is a forest park area used as a tourist park in the Bandung region, West Java. Tahura Ir. H. Djuanda is a secondary natural conservation area located in the Cikapundung and Citarum River sub-regions stretching from Curug Dago to Maribaya which is part of the Mount Pulosari forest group (Rizki & Nurani, 2019). This forest has potential for scientific research and has abiotic conditions that meet the criteria for the growth of wild macrofungi. However, there has been no research regarding the diversity of macrofungi and screening its potential mycelium growth isolated from Tahura Ir. H. Djuanda. Three popular destinations such as the Maribaya (trail area), Goa Jepang (cave area), and Curug Koleang (waterfall area), where macrofungi growth is frequently observed in specific. Its diversity and potential are still up for investigation.

Nowadays, producing commercial products requires a greener method that can also support the ideas of a circular economy, like using macrofungi with mycelium-based materials. A mass of branching, thread-like hyphae called mycelium, a vegetative part of macrofungi, can be utilized as a matrix in applications involving composite materials (Haneef et al., 2017; Jones et al., 2018; Sun et al., 2019; Appels & Wösten, 2021). Mycelium colonizes growth substrates quickly, which makes it an excellent biocomposite material. Its high biodegradability also contributes to its advantages (Irbe et al., 2022). The mycelium of macrofungi has been the subject of numerous investigations; a recent investigation used fungal mycelium with biomass innovation as a biocomposite (Xing et al., 2018; Angelova et al., 2021), fabrication factors that influence the mechanical properties of mycelium-based composites (Haneef et al., 2017; Elsacker et al., 2019), classification of fungal mycelium based on carbon source treatment (Appels & Wösten, 2021), fabrication and characterization of bio-blocks from fungal mycelium for sustainable applications (Joshi et al., 2020). Various applications of fungal mycelium-based biocomposites that have been studied include bioplastic packaging materials (Ziegler et al., 2016; Haneef et al., 2017; Cerimi et al., 2019); tissue engineering replacement materials (Antinori et al., 2020); wound healing (Khamrai et al., 2018); cosmetics sector (Manan et al., 2021); and in construction fields such as bricks, cement, and insulation panels (Jones et al., 2020; Manan et al., 2021).

This research aims to obtain variations of isolates from three areas in Tahura Ir. H. Djuanda and analysis of the macroscopic and microscopic morphological characteristics, as well as screening the potential growth speed of mycelium as a candidate for superior isolates to be applied as mycelium-based material. The results of this study provide the latest information about the potential of macrofungi, particularly those that inhabit Tahura Ir. H.

Djuanda, which has not been explored yet as a new resource for biomaterial applications.

MATERIALS AND METHODS

Sampling Area

Macrofungi samples were collected from April – May 2023 at Forest Park (Tahura), Bandung, West Java, Indonesia (107030' East Longitude and 6052' South Latitude). The environmental temperature and humidity are shown in Table 1. Data collection in this study was carried out by directly observing the presence of the macrofungi in three different representative areas, Maribaya (MB) the trail area, Curug Koleang (CK) the waterfall area, and Goa Jepang (GJP) the cave area (Figure 1).

Table 1. Temperature and humidity in three different areas of Tahura Ir. H. Djuanda Bandung, Indonesia

Environmental factors	Maribaya	Goa Jepang	Curug Koleang
Temperature (°C)	23.4°C	28.25°C	27.4°C
Humidity (%)	73 %	59 %	59 %

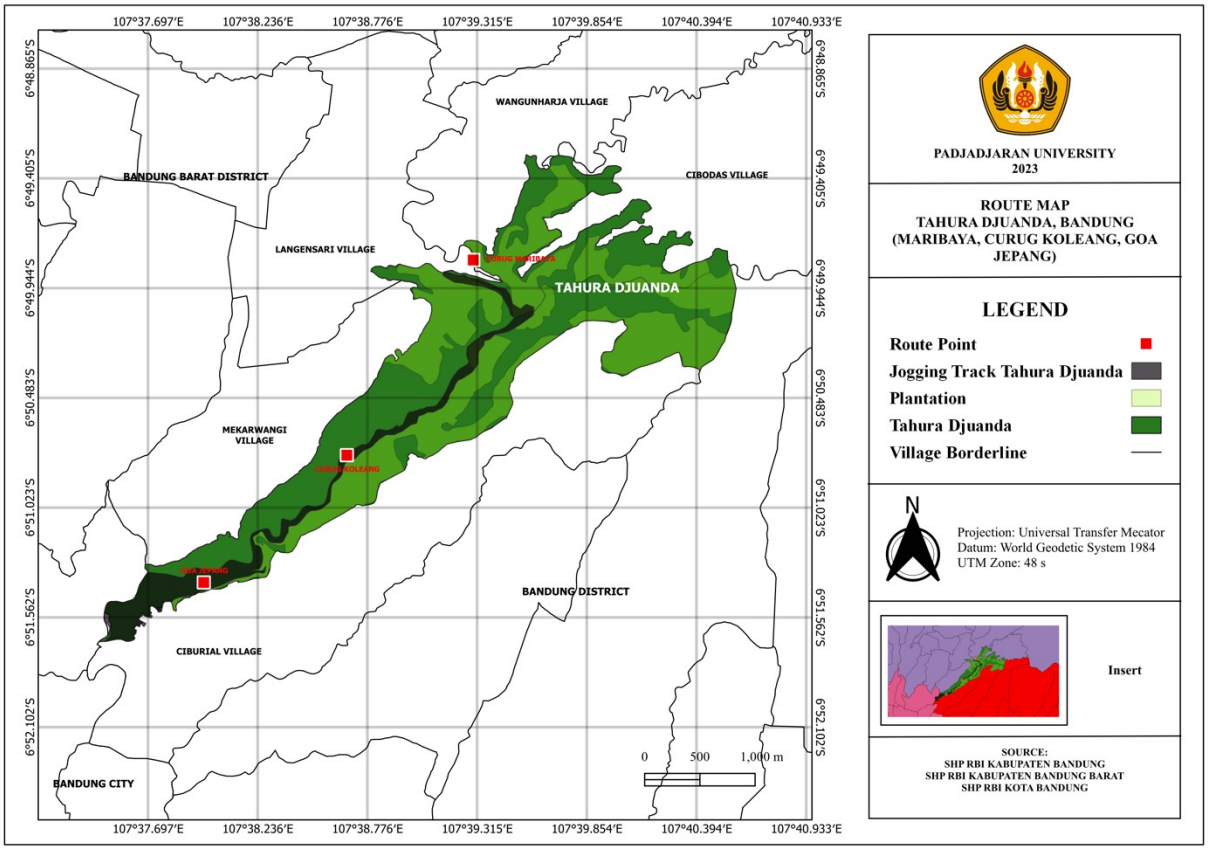

Figure 1. Tahura Bandung route (Maribaya-Curug Koleang-Goa Jepang)

Field Data Collection

Sampling was conducted using the cruise method by exploring the research location and taking every macrofungi sample found in Tahura Ir. H. Djuanda. The macrofungi obtained samples were documented by a Canon 750D DSLR camera (Canon 55 mm Kit Lens, 700 mm Macro Lens) and given a sample number based on the location. The macrofungi specimen was then transferred into a sterile vial using tweezers sprayed with 70% alcohol. All samples were transported to the lab, sterilized with 70% alcohol and stored at -4°C. This method was performed according to Kristin et al. (2020) with several modifications.

Macrofungi Isolation

The isolation of macrofungi was carried out in the Laboratory of the Bioprospecting Study Center for Natural Fibers and Biological Resources, Padjadjaran University, according to the method proposed by Angelova et al. (2021). The macrofungi fruitbodies were cleaned and cut into pieces of 30 mm in size. After that, the surface was sterilized with 70% alcohol (Onemed) for 20 seconds, soaked in 0.1% $Ca(ClO)_2$ for 10 m, and rinsed with distilled water for 1 minute. Thereon, the samples were dried on sterile filter paper. The isolation of macrofungi was carried out using direct planting. Each organ of the plant sample was cut into sizes of 2.5 × 2.5 mm and cultured on Potato Dextrose Agar (PDA) (Merck). Each plate was incubated for ± 5-9 days in dark conditions at temperature of 28°C. The isolates were then stored at 4°C in slant PDA medium.

Characterization of Macrofungi

The obtained macrofungi samples were described for their macroscopic and microscopic morphological characteristics according to Ristiari et al. (2018) with several modifications, including their shape, color, size, and texture. Meanwhile, successfully isolated macrofungi were observed for color, shape, size, margin, opacity, and elevation. Microscopic characterization was carried out using the slide culture method with Lactophenol Cotton Blue (LPCB) (Merck) which was observed after 5-7 days of incubation to see the hyphae structure and the presence of spores. Microscopic fungal hyphae and spores were observed using microscope type of Binocular RCC Multimedia (Digimi 107MT).

Screening Mycelium Growth

Screening macrofungi was measured by calculating mycelium growth based on a modified method of Nguyen et al. (2023). To allow for comparison between fungal isolates, the basal medium PDA was used to observe mycelium growth during 9 days of incubation. Mycelium growth was measured as the radial size of the colony using a digital caliper (SetsuTech). Three repetitions were performed for each measurement, and the average and standard deviation were calculated.

Statistical Analysis

Data were analyzed using GraphPad Prism. All experiments were conducted in triplicate. The obtained data were subjected to one-way analysis of variance (ANOVA) ($P < 0.05$).

RESULTS AND DISCUSSION

Isolation of macrofungi from three areas in Tahura, namely Maribaya (MB), Goa Jepang (GJP), and Curug Koleang (CK) discovered a total of 62 fungal isolates. Each area consisted of MB hosts 22 fungal isolates, followed by CK with 22 fungal isolates, and the least is GJP with 18 fungal isolates. The isolated macrofungi were grouped into five groups based on the variations of the substrate where the fungi grew, consisting of decaying wood, twigs, litter, soil, and tree trunk substrates. In the area of MB, most fungi were found on

twig substrate (8 specimens), while GJP was found in decaying wood (7 specimens) and CK on litter substrate (9 specimens). Macrofungi are primarily found in decaying wood, twigs, and litter substrates (Table 2) because many trees have dense canopies that produce a lot of litter biomass. Putra (2021) states that these substrates are living areas where fungi frequently proliferate. The substrates can provide the nutrient, energy, and carbon source for the macrofungi to grow through decomposition. The ability of macrofungi to colonize a broader set of substrates could be due to their diverse enzyme activity and high carbon and nutrient efficiency. Therefore, substrate availability could be a critical factor in explaining the influence of vegetation on macrofungal diversity, abundance, and distribution (Shi et al., 2014). In secondary forests, significant correlations exist between substrate preference and taxonomic diversity, reflected as higher substrate diversity generally accompanied by higher macrofungal diversity (Ye et al., 2019). Five different macrofungi substrates are found in Tahura. It indicates that Tahura Ir. H. Djuanda has a high macrofungal diversity based on variation of substrates found. Previous studies on macrofungi diversity in Indonesia showed that the average macrofungi found only came from 2-4 different substrates (Dharmawibawa et al., 2015; Christita et al., 2017; Nur et al., 2021; Manalu et al., 2022; Tristina et al., 2022; Afriani & Ratnawati, 2023; Juarsih et al., 2023). This indicates that the macrofungi in Tahura Ir. H. Djuanda have a more diverse substrate compared to macrofungi in other regions of Indonesia. The results of this study can be compared with the research of Armadhan et al. (2023) on the diversity of macrofungi in Karst Forest Kebumen, where the diversity of macrofungi found there also came from 5 different substrates. This result can even be compared to the research of Ye et al. (2019) on macrofungi substrates in the Greater Mekong Sub-region, where the results showed 957 species of macrofungi originating from only 3 different types of substrates, namely root, litter, and soil.

Morphological characterization of macrofungi shows that there are variations in specimens that can be demonstrated based on the shape, color, size, and texture of the fruitbodies. The macrofungi fruitbodies have diverse morphological characteristics, including shapes, colors, sizes, and textures. Characterization of all the macrofungi is based on the morphology of their fruitbodies, which can be seen in Table 2. Based on the shape, the macrofungi found in Maribaya are dominated by the corticoid form. Most corticoid fungi live on woody substrates such as trunks, branches, and twigs; they decompose cellulose, hemicelluloses, and lignin. Thus, in forest ecosystems, they play an essential role not only in nutrient recycling but also in tree growth. Some corticoid species are economically important. Several species are being used in basic and applied research on lignin degradation, making it have a suitable role as a biomaterial (Maekawa et al., 2021). The macrofungi found in Curug Koleang are dominated by the polyporoid form. Polyporoid fungi, which belong to Basidiomycota, are macrofungi with caps or brackets and poroid hymenophores. The polypores can survive for a longer period due to their unique adaptation of producing new layers of spore-producing surfaces, which is ensured by a continuous supply of food material by elevation above ground (Ediriweera et al., 2021). Meanwhile, in Goa Jepang, the macrofungi found are dominated by the pleurotoid form. Fungi with a pleurotoid grow on wood, have gills, and typically form semicircular that are either directly attached to the wood or are attached by means of a rudimentary, lateral stem. The pleurotoid form is polyphyletic (Petersen et al., 2015). The fruitbodies of macrofungi have

various colors, namely brown, yellow, green, orange, black, white, gray, and purple. The size of the macrofungi fruitbodies varies from small and average to large. The macrofungi fruitbodies also have various textures, such as chewy, hard, soft, mushy, dense, and solid.

Isolated fungal specimens that were successfully grown on PDA showed varied characteristics colonies and could be grouped based on color (mostly white, yellow and gray); shape (filamentous, circular, irregular, and rhizoidal); elevation (flat, raised, and umbonate, and crater-shaped); margins (filiform, curled, entire, undulate, and lobate); texture (cottony, smooth, velvety, and powdery); mycelia (aerial and immersed); then the opacity (transparent, translucent and opaque). The visual macroscopic morphology of all macrofungal isolates can be seen in Figure 3. Besides, according to their mycelium, it can be differentiated into fungi with septate and non-septate (coenocytic) hyphae (Figure 4). In the fungal cell wall, septate have valve openings that can be closed. It serves to increase the strength of the mycelium (Lelivelt et al., 2015), by having a strong mycelial structure, it can be developed as biomaterials. In addition, its lightweight and biodegradable structure and its ability to grow from waste sources make mycelium a suitable material for biocomposites.

Furthermore, variations in the presence of spores observed in fungal cultures showed the types of oidium spores, conidiospores, zoosporangia, and sporangiophores (Table 3). Some spore isolates could not be observed for the presence of spores, possibly in the observed incubation period of 5-7 days, and they did not show reproductive activity of the spores. More research on this group of fungi is necessary, with particular attention to variables like light, temperature, nutrition, and gene expression during fruitbodies induction that can also affect the rate at which spores grow (Sakamoto, 2018). By tracking the mycelium's length increase over nine days of incubation, the growth rate of the isolate colony's mycelium was assessed. The variations in mycelial length for every isolate are displayed in Table 4. The mycelial extension representing the three areas has the lowest average of 34 mm, while the highest average is 72.55 mm. The average length of mycelia in each MB, GJP, and CK area is 55.5 mm, 63.9 mm, and 53.96 mm, respectively. The specimen's original growth location was likely warmer (28.25°C) with a humidity of 59.9%, ideal for the fungi's optimal growth, which is likely why the Goa Jepang (GJP) area has the highest mycelium growth rate. All isolates incubated for nine days had their growth rates calculated with three repetitions. The significant p-value was ($p<0.05$), and the average results of the three repetitions (R) were R1 (56.74 mm), R2 (56.15 mm), and R3 (57.29 mm), which shows significant differences (Figure 5.).

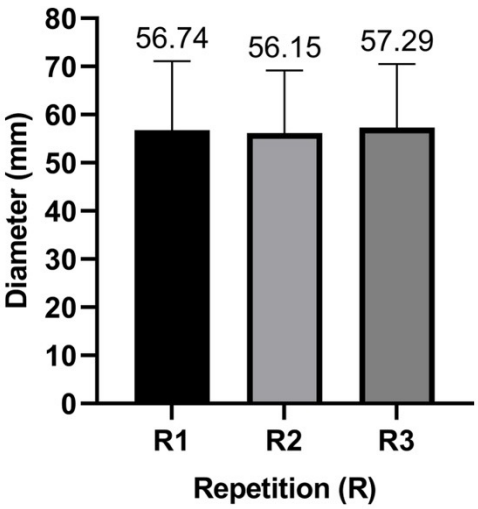

Figure 5. Average growth of all mycelium fungal isolates from three repetitions. Sig. $P<0.05$

In this study, mycelial growth screening was carried out for the first time by comparing 62 species. This has never been reported in previous studies. Some previous studies have

screened mycelial growth but not explored its diversity and only compared at least two fungi species, especially the genera of Ganoderma or Pleurotus (Yan et al., 2014; Nguyen et al., 2023), or even only focusing on just one species (Jayasinghe, 2008; Ma et al., 2014; Luangharn et al., 2014; Bijalwan et al., 2021). All previously reported studies did not focus on screening mycelial growth for potential biomaterial development. Therefore, in this study, many fungi were screened to obtain superior isolates as biomaterial agents. According to Alemu et al. (2022), species selection is one of the most challenging tasks for researchers in effective biomaterial production. Criteria for species selection include mycelium density, growth rate, cost of growth media (substrate), and noxiousness level (Attias et al., 2020). Based on these considerations, screening mycelial growth speed has become very important research to carry out at this time. Isolates MB-07 (63.98 ± 1.21 mm), GJP-01 (81.47 ± 0.41 mm), and CK-13 (72.14 ± 1.20 mm) were the ones that displayed the longest mycelium length and represented each study area. The length of the mycelia is comparable to isolates from Tricholoma caligatuma and Morchella angusticeps which were grown on a glucose carbon source and had mycelium growth of 78 mm and 80 mm, respectively (Kalmis & Kalyoncu, 2008); in contrast, low growth of Agaricus bisporus isolate with the mycelium growth only 35 mm (Yan et al., 2014) or Ganoderma sinense the growth of 43.38 mm (Nguyen et al., 2023). These findings indicate that Goa Jepang originating GJP-01 isolate has the potential to be a superior isolate in terms of mycelial extension ability. In order to investigate the possibility of using the three isolates chosen for further study in the degradation of plant biomass components, their molecular properties must be analyzed down to the species level.

The most effective way to break down lignocellulosic compounds is through wood-rotting fungi, most of which are members of the Basidiomycota family. These fungi are commonly used to produce biomaterials due to their natural ability to stick together and break down lignocellulose (Appels & Wösten, 2021). This group of fungi can degrade cellulose, hemicellulose, and lignin components through enzymatic and non-enzymatic mechanisms whose selectivity is determined by the particular species and environment (Schwarze et al., 2013). The polymerization of complex mycelium components as a component of hyphae to form abundant networks can be facilitated by understanding the role of vegetative mycelium in degrading and colonizing organic substrates (Elsacker et al., 2019). The growth capacity of mycelium is a crucial factor as it affects the properties of the composite material and the duration of the biomaterial manufacturing process. Generally, properties are stiff, elastic, porous, dense, fast-growing, inexpensive, and possess antioxidant and antimicrobial qualities (Alemu et al., 2022). More research is required to enhance the selection criteria for macrofungal species used in biomaterials, focusing on the effectiveness of growth media (substrate), ease of cultivation, and mycelium structure.

Like effective degrading agents, many natural isolates can be combined with substrate material formulations to generate various applications (Irbe et al., 2022). It can be utilized as a biocomposite in industry, including construction, textile materials, medicines, cosmetics, and products like bricks, packaging, and insulating panels. Because the mycelium can colonize growing substrates, it can also be used for various design objects like flower pots and vases, office supplies, and LED lights (Girometta et al., 2019).

Figure 2. Macrofungi fruitbodies isolated from Tahura Bandung, Indonesia Species code: MB (Maribaya); GJP (Goa Jepang); CK (Curug Koleang

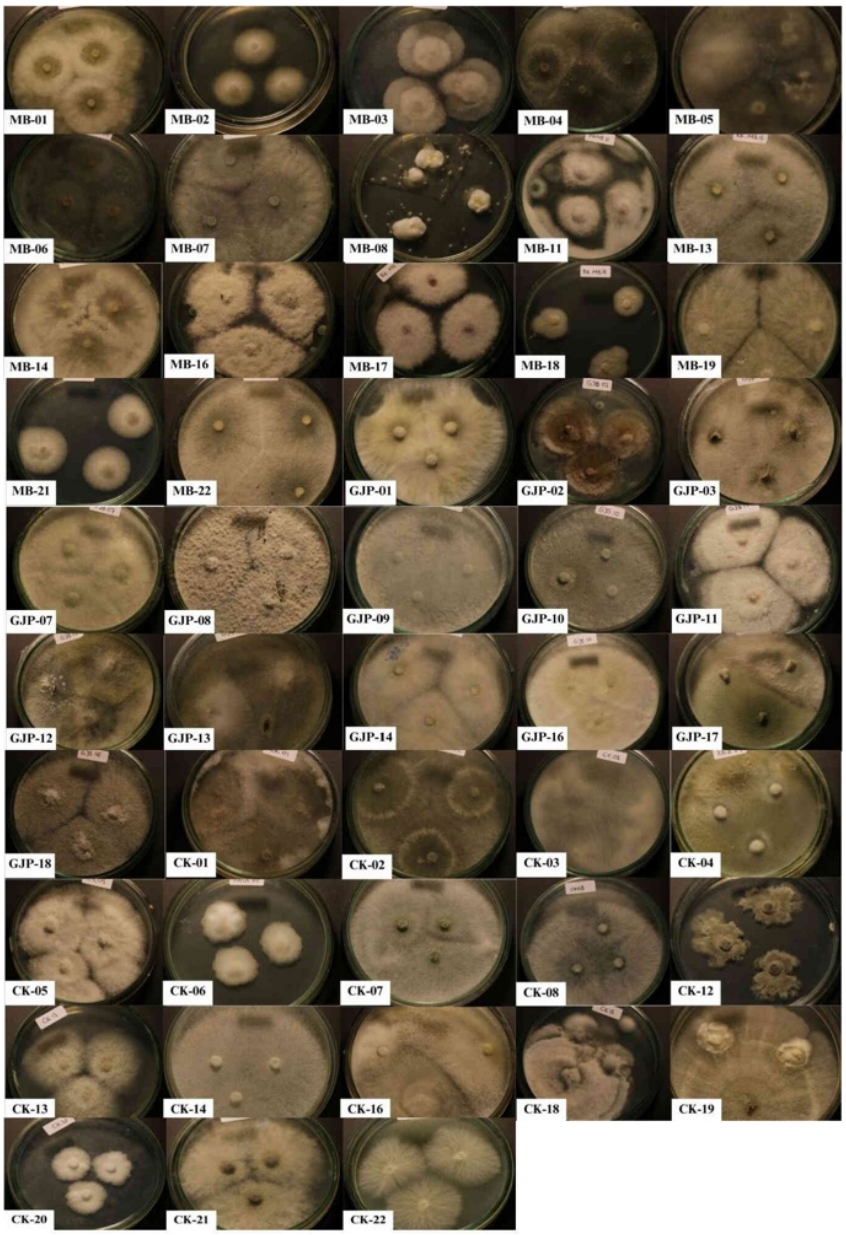

Figure 3. Macroscopic characteristics of mycelium growth from macrofungal cultures growth on PDA medium at 25°C after 9 days of incubation. Species code: MB (Maribaya); GJP (Goa Jepang); CK (Curug Koleang)

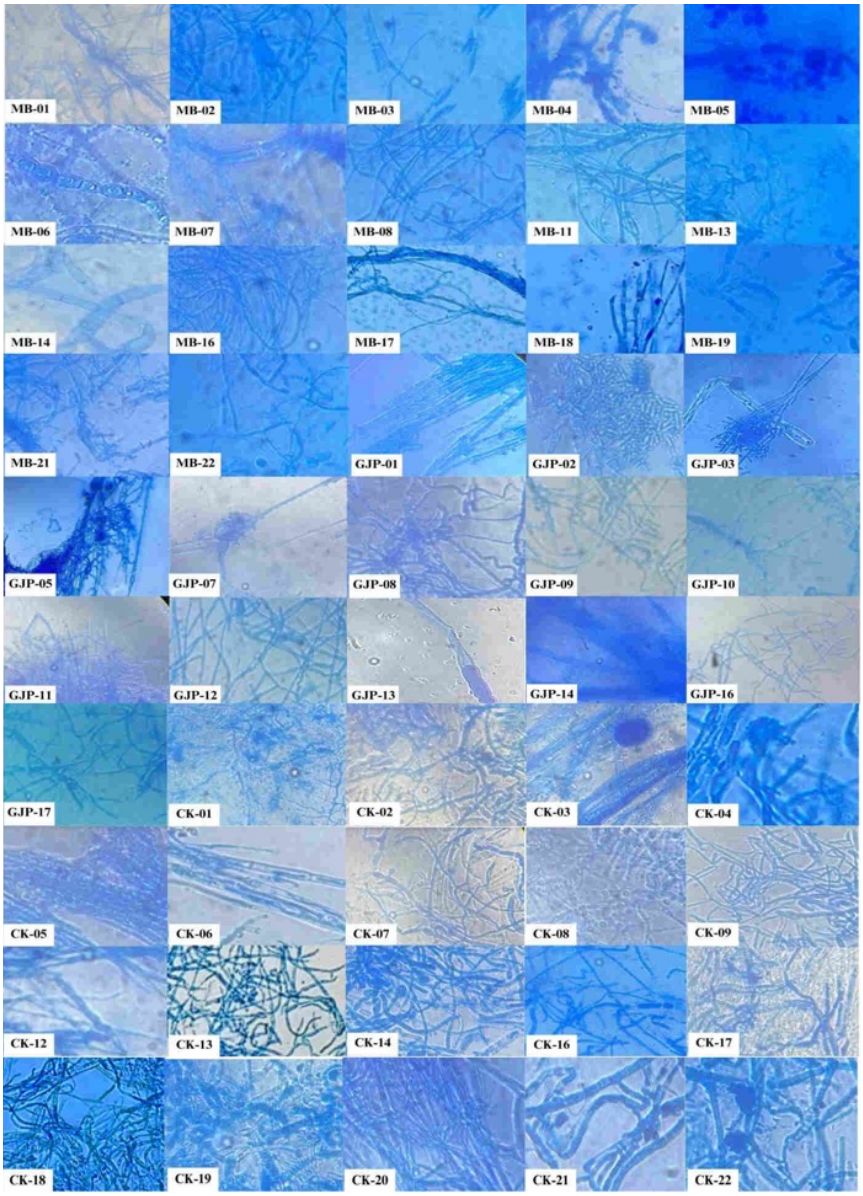

Figure 4. Microscopic characteristics of mycelium hyphae and spore under binocular microscope 400x magnification. Species code: MB (Maribaya); GJP (Goa Jepang); CK (Curug Koleang)

Table 2. Morphological characteristics of macrofungi fruitbodies from Tahura Ir. H. Djuanda Bandung, Indonesia

Region	Substrates	Codes	Morphological characteristics			
			Shapes	Colors	Sizes	Textures
MARIBAYA	Decaying wood	MB-01	Polyporoid	Brown	Large	Chewy
		MB-02	Agaricoid	Yellow	Small	Chewy
		MB-03	Corticoid	Yellowish-white	Average	Chewy
		MB-04	Gomphoid-phalloid	Orange	Small	Hard
		MB-05	Agaricoid	Greenish-brown	Small	Soft
	Twig	MB-07	Pleurotoid	White	Small	Mushy
		MB-17	Pleurotoid	Cream	Average	Hard
		MB-06	Apothecia	Black	Small	Dense
		MB-08	Apothecia	Brown	Small	Chewy
		MB-10	Corticoid	Yellowish-white	Small	Hard
		MB-11	Corticoid	Cream	Small	Mushy
		MB-12	Corticoid	White	Average	Hard
		MB-18	Corticoid	White	Small	Soft
		MB-20	Corticoid	Yellowish-brown	Average	Dense
		MB-21	Corticoid	Brown	Average	Hard
	Litter	MB-09	Agaricoid	Brown	Small	Soft
		MB-16	Agaricoid	Orange	Small	Chewy
	Soil	MB-13	Pleurotoid	Brown	Large	Chewy
		MB-14	Pleurotoid	Yellow	Average	Chewy
		MB-15	Puffball	Brown	Average	Chewy
		MB-19	Pleurotoid	Brown	Large	Dense
		MB-22	Pleurotoid	Brownish white	Average	Soft
GOA JEPANG	Decaying wood	GJP-01	Pleurotoid	Cream	Small	Dense
		GJP-09	Polyporoid	Yellow-patterned	Large	Hard
		GJP-10	Corticoid	Brownish white	Average	Hard
		GJP-14	Pleurotoid	White	Average	Dense
		GJP-15	Pleurotoid	White	Small	Mushy
		GJP-16	Polyporoid	Whitish-brown	Large	Hard
		GJP-17	Polyporoid	Yellowish-white	Large	Hard
	Soil	GJP-02	Agaricoid	Brown	Small	Mushy
		GJP-03	Puffball	Grey	Small	Chewy
		GJP-04	Agaricoid	Cream	Small	Chewy
		GJP-05	Agaricoid	Cream	Small	Hard
	Litter	GJP-06	Pleurotoid	Grey	Average	Mushy
		GJP-08	Agaricoid	Grey	Small	Mushy
		GJP-11	Pleurotoid	Brown	Small	Mushy
		GJP-12	Polyporoid	Brown	Large	Hard
		GJP-13	Corticoid	Brownish-white	Small	Hard
		GJP-18	Agaricoid	Yellow	Average	Mushy
	Tree trunk	GJP-07	Corticoid	White	Large	Dense

Species code: MB (Maribaya); GJP (Goa Jepang); CK (Curug Koleang)

Region	Substrates	Codes	Morphological characteristics			
			Shapes	Colors	Sizes	Textures
CURUG KOLEANG	Litter	CK-01	Pleurotoid	Yellow	Small	Mushy
		CK-02	Agaricoid	Whitish-brown	Small	Mushy
		CK-03	Polyporoid	White	Average	Dense
		CK-04	Puffball	Brown	Average	Hard
		CK-07	Polyporoid	Brown	Large	Hard
		CK-08	Polyporoid	White	Average	Chewy
		CK-09	Agaricoid	Brown	Small	Mushy
		CK-13	Pleurotoid	Brownish-grey	Average	Mushy
		CK-15	Pleurotoid	Creamish-white	Average	Mushy
	Soil	CK-05	Pleurotoid	Purple	Small	Soft
	Twig	CK-06	Corticoid	Orange	Small	Chewy
		CK-16	Pleurotoid	Whitish-cream	Small	Soft
	Decaying wood	CK-10	Corticoid	Blackish-white	Large	Hard
		CK-11	Polyporoid	Blackish-brown	Large	Hard
		CK-12	Polyporoid	Whitish-orange	Large	Hard
		CK-18	Polyporoid	Black and white	Large	Hard
		CK-19	Polyporoid	Beige-orange patterned	Large	Hard
		CK-14	Corticoid	White	Small	Solid
		CK-17	Polyporoid	Brown-patterned	Large	Hard
	Tree trunk	CK-20	Russuloid	Cream	Small	Dense
		CK-21	Agaricoid	White	Small	Chewy
		CK-22	Agaricoid	White	Average	Chewy

Species code: MB (Maribaya); GJP (Goa Jepang); CK (Curug Koleang)

Table 3. Morphology macroscopic and microscopic of macrofungal cultures growth on PDA medium from Tahura Bandung, Indonesia

Isolate Code	Morphological characteristics							Hyphae and Spore Characteristics	
	Color	Form	Elevation	Margin	Texture	Mycelia	Opacity	Type of Hyphae	Type of Spore
MB-01	white	filamentous	flat	filiform	cottony	aerial and immersed	transparent middle	coenocytic	-
MB-02	white	filamentous	flat	filiform	cottony	aerial and immersed	transparent	coenocytic	-
MB-03	white	filamentous	raised	filiform	cottony	aerial	opaque	coenocytic	-
MB-04	white	filamentous	flat	filiform	cottony	immersed	translucent	septate	-
MB-05	white	filamentous	raised	filiform	cottony	aerial and immersed	transparent	coenocytic	conidia
MB-06	white	filamentous	flat	lobate	smooth	immersed	translucent	coenocytic	oidium
MB-07	white	filamentous	flat	filiform	cottony	immersed	opaque	coenocytic	-
MB-08	white	irregular	umbonate	filiform	cottony	aerial and immersed	opaque	septate	-
MB-11	white	filamentous	raised	filiform	cottony	aerial and immersed	opaque	coenocytic	-
MB-13	white	filamentous	flat	filiform	cottony	aerial	opaque	coenocytic	-
MB-14	white	filamentous	raised	filiform	velvety	aerial and immersed	opaque	septate	conidiophore
MB-16	white	filamentous	flat	undulate	velvety	aerial	opaque middle	coenocytic	conidiophore

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Isolate Code	Morphological characteristics							Hyphae and Spore Characteristics	
	Color	Form	Elevation	Margin	Texture	Mycelia	Opacity	Type of Hyphae	Type of Spore
MB-01	white	filamentous	flat	filiform	cottony	aerial and immersed	transparent middle	coenocytic	-
MB-02	white	filamentous	flat	filiform	cottony	aerial and immersed	transparent	coenocytic	-
MB-03	white	filamentous	raised	filiform	cottony	aerial	opaque	coenocytic	-
MB-04	white	filamentous	flat	filiform	cottony	immersed	translucent	septate	-
MB-05	white	filamentous	raised	filiform	cottony	aerial and immersed	transparent	coenocytic	conidia
MB-06	white	filamentous	flat	lobate	smooth	immersed	translucent	coenocytic	oidium
MB-07	white	filamentous	flat	filiform	cottony	immersed	opaque	coenocytic	-
MB-08	white	irregular	umbonate	filiform	cottony	aerial and immersed	opaque	septate	-
MB-11	white	filamentous	raised	filiform	cottony	aerial and immersed	opaque	coenocytic	-
MB-13	white	filamentous	flat	filiform	cottony	aerial	opaque	coenocytic	-
MB-14	white	filamentous	raised	filiform	velvety	aerial and immersed	opaque	septate	conidiophore
MB-16	white	filamentous	flat	undulate	velvety	aerial	opaque middle	coenocytic	conidiophore
GJP-12	white	irregular	flat	filiform	cottony	aerial	opaque	coenocytic	-
GJP-13	white	irregular	flat	filiform	cottony	aerial and immersed	opaque	coenocytic	zoosporangia
GJP-14	white	filamentous	raised	filiform	cottony	aerial	opaque	septate	-
GJP-16	yellow	circular	flat	entire	cottony	aerial	opaque	coenocytic	-
GJP-17	white	irregular	flat	lobate	cottony	aerial and immersed	opaque	coenocytic	-
CK-01	white	irregular	flat	undulate	cottony	aerial and immersed	opaque edge	septate	-
CK-02	white	rhizoid	flat	filiform	cottony	aerial	opaque	septate	-
CK-03	white	filamentous	raised	filiform	cottony	aerial	opaque	septate	-
CK-04	white	circular	raised	entire	cottony	aerial	translucent	septate	sporangia
CK-05	yellow	irregular	crateriform	filiform	velvety	aerial	opaque	septate	-
CK-06	white	circular	raised	entire	velvety	aerial	opaque	septate	-
CK-07	white	circular	flat	entire	smooth	aerial	opaque	septate	-
CK-08	white	filamentous	flat	entire	smooth	aerial and immersed	transparent	coenocytic	-
CK-09	white	circular	flat	entire	velvety	immersed	opaque	septate	-
CK-12	grey	rhizoid	crateriform	undulate	velvety	aerial	opaque	coenocytic	-
CK-13	white	rhizoid	crateriform	filiform	cottony	aerial	opaque edge	septate	-
CK-14	white	circular	flat	filiform	smooth	aerial and immersed	opaque	septate	-
CK-16	white	irregular	crateriform	entire	cottony	aerial	opaque	septate	-
CK-17	white	filamentous	raised	entire	cottony	aerial	opaque	septate	-
CK-18	white	irregular	raised	curled	velvety	aerial	opaque	septate	-
CK-19	white	irregular	flat	undulate	smooth	aerial and immersed	opaque	coenocytic	-
CK-20	white	irregular	raised	undulate	velvety	aerial	opaque	septate	-
CK-21	white	rhizoid	raised	undulate	velvety	aerial	opaque	septate	-
CK -22	white	filamentous	flat	filiform	velvety	aerial	opaque	septate	-

Table 4. Mycelial growth of the fungal isolates from three areas, Maribaya, Goa Jepang, and Curug Koleang, on PDA medium at 25°C after 9 days of incubation

Isolate Code (MB)	Mycelial Growth (mm)	Isolate Code (GJP)	Mycelial Growth (mm)	Isolate Code (CK)	Mycelial Growth (mm)
MB-01	60.48 ± 0.02	**GJP-01**	**81.47 ± 0.41**	CK-01	49.59 ± 1.06
MB-02	63.34 ± 0,82	GJP-02	29.47 ± 1.39	CK-02	46.97 ± 1.55
MB-03	45.19 ± 1.61	GJP-03	74.33 ± 0.07	CK-03	53.02 ± 1.60
MB-04	48.39 ± 0.36	GJP-05	33.23 ± 0.53	CK-04	54.30 ± 3.69
MB-05	60.10 ± 0.25	GJP-07	81.43 ± 3.87	CK-05	58.58 ± 1.91
MB-06	45.07 ± 0.37	GJP-08	63.53 ± 2.02	CK-06	49.94 ± 2.21
MB-07	**63.98 ± 1.21**	GJP-09	73.4 ± 1.79	CK-07	65.27 ± 0.56
MB-08	54.95 ± 0.33	GJP-10	78.47 ± 2.45	CK-08	63.53 ± 3.29
MB-11	61.84 ± 0.38	GJP-11	62.13 ± 2.74	CK-09	27.51 ± 1.11
MB-13	45.70 ± 0.93	GJP-12	72.33 ± 1.69	CK-12	53.61 ± 6.03
MB-14	54.62 ± 0.35	GJP-13	51.27± 0.20	**CK-13**	**72.14 ± 1.20**
MB-16	60.84 ± 0.63	GJP-14	66.8 ± 3.02	CK-14	69.2 ± 4.00
MB-17	60.81 ± 0.24	GJP-16	57.53 ± 1.41	CK-16	51.78 ± 2.12
MB-18	36.89 ± 0.39	GJP-17	69.33 ± 2.49	CK-17	65.09 ± 4.37
MB-19	63.75 ± 0.25			CK-18	54.65 ± 3.25
MB-21	63.20 ± 0.04			CK-19	67.99 ± 0.30
MB-22	54.35 ± 0.12			CK-20	31.86 ± 2.19
				CK-21	46. 45 ± 6.71
				CK-22	43.81 ± 2.86
Average value	55.5 mm		63.9 mm		53.96 mm

CONCLUSION

A total of 62 fungal isolates were discovered through the isolation of fungal cultures from three locations in Tahura Ir. H. Djuanda: Maribaya (MB), Goa Jepang (GJP), and Curug Koleang (CK). There were 22 isolates from MB, 18 from GJP, and 22 from CK in each area. Isolates MB-07 (63.98 mm), GJP-01 (81.47 mm), and CK-13 (72.14 mm) were the ones that displayed the longest mycelium length and represented each study area. The Goa Jepang-isolate GJP-01 has the potential to be a superior isolate in terms of mycelial extension ability. Forthcoming studies must focus on molecular identification in order to identify species that hold promise for utilization as sources of mycelium-based biomaterials

AUTHOR CONTRIBUTION

IAP conducted research, compiled and analyzed data, and wrote the manuscript, UR conducted research and collected data, DRS conducted research and collected data, AHD conducted research and collected data, APW designed research, compiled, wrote and revised the manuscript, NR designed research and revised the manuscript.

ACKNOWLEDGMENTS

The author would like to thank the management of Tahura Bandung for giving permission and support for this research, and to ALG (2203/UN6.3.1/PT.00/2023) for funding this research.

CONFLICT OF INTEREST

There is no conflict of interest during the research work.

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