BIO Web of Conferences **147**, 01007 (2024) https://doi.org/10.1051/bioconf/202414701007
EAFTA 2024

Diversity and antibacterial potential produced by marine endophytic fungi by submerged fermentation from Buton Island, Indonesia

Ismi Isti'anah[1,2,3], *Kustiariyah* Tarman[1,2,*], *Sugeng Heri* Suseno[1], *Roni* Nugraha[1], *Irzal* Effendi[2,4], *Wen-Chien* Lee[5], *Endang* Lukitaningsih[6], *Marianti A.* Manggau[7], and *Rodiah Nurbaya* Sari[8,9]

[1]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, IPB University, Jl. Agatis, 16680, Bogor, Indonesia
[2]Division of Marine Biotechnology, Center for Coastal and Marine Resources Studies (PKSPL), International Research Institute for Maritime, Ocean and Fisheries (i-MAR), IPB University, 16127, Bogor, Indonesia
[3]Department of Marine Biotechnology, Tual State Fisheries Polytechnic, Jl. Raya Langgur, 97611, Maluku, Indonesia
[4]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Faculty of Fisheries and Marine Science, IPB University, Jl. Agatis, 16680, Bogor, Indonesia
[5]College of Engineering, National Chung Cheng University, 62102, Chiayi County, Taiwan
[6]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, 55281, Yogyakarta, Indonesia
[7]Department of Pharmacology, Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
[8]Doctoral Program in Natural Resources and Environmental Management Science (NREMS), Graduate School of IPB University, School Building Floor II IPB Baranangsiang, 16144, Bogor Indonesia
[9]Research Center for Marine and Land Bioindustry, National Research and Innovation Agency, Laterio Building, 14430, Jakarta, Indonesia

Abstract. This study investigated the diversity and antibacterial potential of marine endophytic fungi from Buton Island, Indonesia. This study focused on identifying fungi capable of producing bioactive compounds effective against *Vibrio harveyi.* 32 fungal isolates were obtained from various marine samples. *Aspergillus terreus* (WB 1-2) exhibited the highest antibacterial activity. The growth dynamics of these fungi were analyzed, emphasizing the importance of the log phase for secondary metabolite production. Environmental conditions and mechanical agitation were found to significantly influence growth and metabolite yield. These findings highlight the potential of marine endophytic fungi as sources of novel antimicrobial agents, suggesting promising opportunities for biotechnological and pharmaceutical advancements. This study underscores the untapped potential of marine fungi for the development of new antibiotics.

---

[*] Corresponding author: kustiaz@apps.ipb.ac.id

1 Introduction

The ocean is home to a diverse range of fungi, including Marine Endophytic Fungi that inhabit the roots, rhizomes, and leaves of marine plants. To investigate these fungi, researchers collected samples from these plant structures and employed isolation techniques to obtain fungal colonies. Colonies were subsequently identified using both morphological and molecular methods, with ribosomal RNA (rRNA) sequencing being a common approach for precise identification [1]. Another group of marine fungi, known as Marine Algicolous Fungi (MAF), grows endophytically within macroalgae. These fungi are notable for their ability to produce bioactive compounds as secondary metabolites, and are classified based on their biological activities, including anticancer, antibacterial, antifungal, and antioxidant effects [2].

Indonesia has abundant natural resources, particularly within its marine and fishery ecosystems. There is growing interest in investigating biologically active compounds derived from these sources, especially those that have the potential to significantly influence cellular functions [3]. This study focused on microorganisms, specifically bacteria and fungi, present in diverse marine environments, including seabeds, ocean waters, mangrove forests, fish, and seaweed. Notably, certain microorganisms establish symbiotic relationships with invertebrate species, such as sponges, mollusks, tunicates, coelenterates, and crustaceans, which may lead to the production of unique secondary metabolites [4].

The oceanic ecosystem serves as a rich reservoir of bioactive compounds that provide various advantages for health and well-being. Marine Natural Products (MNPs) are associated with various bioactivities, functioning as antibiotics, antivirals, neuroprotective agents, and anticancer compounds, while also exhibiting anti-inflammatory effects [5]. Southeast Sulawesi, a province in Indonesia, holds significant potential for utilizing mangroves to enhance the fisheries sector and support community welfare. Buton Island, which is located in this province, has abundant fishery resources. The coastal areas of Buton Island are characterised by rich natural resources, including coral reefs, mangrove forests, seagrass beds, and diverse aquatic habitats. Additionally, seaweed is a vital commodity for coastal fisheries of local communities in Southeast Sulawesi [6].

The mangrove forests on Buton Island are coastal forests capable of withstanding high salinity and extreme environmental conditions. These ecosystems provide numerous ecological benefits such as nutrient supply, shoreline erosion protection, nursery habitats, and nutritional sources for fish and crustaceans, while also acting as a buffer against tsunami impacts [7]. Global research has underscored the role of endophytic fungi in mangrove ecosystems. Fungi belonging to the phylum Ascomycota are significant contributors to the production of secondary metabolites, demonstrating antibiotic and cytotoxic activities against pathogenic cells [8].

A notable study identified *Aspergillus terreus* in the root of the mangrove species *Bruguiera gymnorrhiza*, which have been shown to possess antimicrobial, anticancer, anti-filarial, and anti-inflammatory properties [9]. This study aimed to explore the potential of bioactive compounds derived from endophytic marine fungi present in seaweed, seagrass, and mangrove samples collected from Buton Island in Southeast Sulawesi. The primary objective was to explore the diversity of marine endophytic fungi and its antibacterial potential produced by submerged fermentation system. Notably, the antibiotic properties of these bioactive compounds derived from Buton Island have not yet been documented.

2 Materials and Methods

2.1 Isolation and screening of marine endophytic fungi

Seaweed, seagrass, and mangrove leaf samples were collected from Buton Island in southeastern Sulawesi Province. These specimens were identified and air-dried for preservation before being transported to the laboratory for the isolation of endophytic fungi. To ensure sterility, the samples underwent two surface sterilisation treatments: a one-minute soak in distilled water followed by exposure to 5% sodium hypochlorite (NaOCl) to eliminate surface contaminants. The samples were then aseptically placed on Potato Dextrose Agar (PDA) medium, with four to five pieces per Petri dish. These dishes were incubated at room temperature for three to seven days, during which the fungal growths were analyzed for their morphological characteristics. In cases where colonies were not pure, purification procedures were implemented. Well-defined, representative colonies were subsequently transferred to slanted agar media for storage [10].

2.2 Diversity of morphology characterization of marine endophytic fungi

The marine endophytic fungi was initially isolated and subsequently characterized based on their macroscopic morphological attributes, using a Petri dish infused with Potato Dextrose Agar medium. The macroscopic characteristics under examination included the colony's form, colour, and diameter [11].

2.3 Growth curve, antibacterial potential produced by marine endophytic fungi, and its mechanism of action

The stages of fungal cultivation are divided into 2, namely preculture and culture in Potato Dextrose Broth (PDB). Preculture aims to allow marine fungi to adapt to the growth medium. Selected marine fungal isolates are cultivated to determine the growth curve of marine fungi. One ose of prospective isolate samples was taken to be mixed with PDB (PDB) media that had been prepared in Erlenmeyer flasks as much as 100 mL. Then, the isolate that had been mixed in 100 mL PDB was incubated at room temperature for 7x24 hours. After 7x24 hours of incubation, the growing culture media was transferred to new Potato Dextrose Broth (PDB) media as much as 250 mL in a 500 mL Erlenmeyer flask with pH 7 cultivated in static (still) conditions and shaken at a speed of 120 rpm at room temperature for 21 days [12].

The culture media containing mycelia were harvested by filtration using filter paper to separate the mycelia and culture media (culture broth) using filter paper and obtained the extract culture media, then a growth curve was made between the sampling time and the biomass weight. The calculation of the mycelia weight was carried out by taking the fungal biomass that had been filtered using filter paper and dried in an oven for 24 hours at a temperature of 80 °C. The dry weight of the mycelia was determined by calculating the difference in weight between the empty filter paper and the filter paper containing mycelia. Determination of the growth curve of marine fungal isolates was carried out by observing every 3 days to determine the growth of the marine fungal isolates by calculating the dry biomass of mycelia and pH successively from cultivation days 0, 3, 6, 9, 12, 15, 18, 21. Observations of pH values using pH paper on the harvested fungal culture media [13].

3 Results and Discussions

3.1 Selected marine endophytic fungi

Research has been conducted on Buton Island, located in Southeast Sulawesi, which is recognised for its diverse species richness. Mangrove regions, often linked with ecologically important algae, serve as a habitat for a wide array of algae species [14]. The investigation involved the collection of 160 samples, revealing a significant prevalence of endophytic fungi in Chlorophyta, accounting for 60% of the total, thereby making it the most dominant host among all endophytic fungi in the sampled area. Following Chlorophyta, the next four most common hosts for endophytic fungi were identified as Rhodophyta (22%), Seagrass (9%), Mangrove (6%), and Phaeophyta (3%) (see Fig.1).

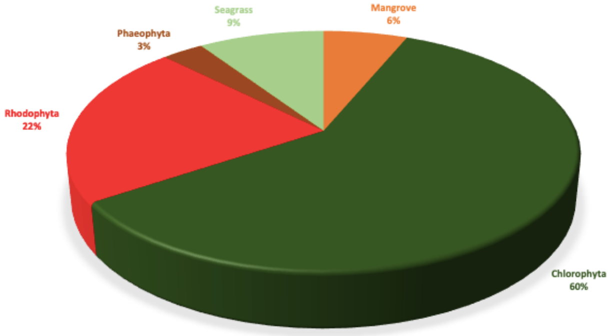

Fig.1. Diversity source of endophytic fungal hosts

A collection of 160 samples was procured and subsequently isolated using Potato Dextrose Agar (PDA) in petri dishes. The samples were then classified into several isolates: seven under the code WB, thirty-two under SM, sixty under LB, fifty-nine under BM, and a pair under KS, as detailed in Table 1. Interestingly, endophytic marine fungi, despite their marine extraction, do not rely on the sea for their life cycle. This characteristic makes them particularly amenable to laboratory cultivation [15]. Furthermore, it was observed that the same algal genus, when sampled from different locations, yielded varying numbers of endophytes.

Table 1. Endophytic marine fungal isolates isolated from Buton Island, Southeast Sulawesi

Isolate Code	Number of Samples
WB	7
SM	32
LB	60
BM	59
KS	2
Total	**160**

The 160 isolates of marine fungi were purified to refine the marine endophytic fungi isolates before conducting a more detailed analysis of their bioactive compounds. Substances with biological activity found in macroalgae, including peptides, polyphenols, fatty acids, and pigments, may play a crucial role in pharmacologically regulating pathogenesis in living organisms [16].

3.2 Diversity of morphology characterization of marine endophytic fungi

Distinct colours and textures were observed in the purified colonies following incubation on PDA plates. By repeatedly cultivating and subculturing the endophytic fungus and comparing attributes such as morphology, growth rate, pigmentation, and colony borders, pure isolates were generated [17]. From a variety of host sources, we identified thirty-two unique strains of endophytic fungi.

The variations in the morphological structure and results of the antagonist test for these marine endophytic fungi are detailed in Table 2. A clear zone indicated strong antibacterial effectiveness, with the size of the zone reflecting the intensity of the effect. Notably, several isolates exhibited strong antibacterial effects against *V. harveyi*, including WB 1-2, WB 6-2, WB 7-1, SM 22-1, SM 27-2, BM-46, and LB 13-A. These isolates produced clear zones with diameters of 13.0 mm, 12.3 mm, 12.3 mm, 11 mm, 11 mm, and 11.3 mm, respectively (Table 2).

Table 2. Purification results of endophytic sample isolates and morphological characteristics

No	Isolate Code	Characterization					Average of diameter (cm)
		Form	Margin	Chromo genesis	Elevation	Texture	
1	WB 1-2	Circular	Entire	Brown	Flat	Smooth	14.0
2	WB 7-2	Irregular	Undulate	White	Raised	Smooth	3.7
3	WB 4-1	Irregular	Entire	Black	Flat	Smooth	1.0
4	WB 6-1	Irregular	Undulate	White	Flat	Smooth	8.0
5	WB 5-1	Circular	Entire	White	Flat	Smooth	7.7
6	WB 3-2	Circular	Filiform	White	Flat	Smooth	2.3
7	WB 6-2	Irregular	Entire	Brown	Raised	Smooth	12.3
8	WB 4-2	Circular	Curled	Black	Umbonate	Concentric rings radial	12.3
9	WB 7-1	Circular	Filiform	White	Flat	Smooth	11.3
10	SM 7-1	Irregular	Undulate	White	Raised	Rough	5.7
11	SM 16-1	Irregular	Entire	White	Flat	Smooth	1.0
12	SM 20-1	Circular	Entire	White	Convex	Smooth	1.7
13	SM 22-1	Circular	Filiform	White	Flat	Smooth	11.0
14	SM 32-1	Circular	Undulate	Black	Flat	Smooth	4.0
15	SM 17-1	Irregular	Undulate	Black	Raised	Rough	11.0
16	SM 5-1	Irregular	Undulate	White	Raised	Smooth	2.7
17	SM 9-1	Circular	Entire	White	Flat	Concentric rings radial	7.0
18	SM 27-2	Circular	Entire	White	Convex	Smooth	3.7

No	Isolate Code	Characterization					Average of diameter (cm)
		Form	Margin	Chromo genesis	Elevation	Texture	
19	SM 26-1	Circular	Undulate	Black	Flat	Concentric rings radial	2.3
20	BM 47 B	Circular	Entire	White	Flat	Smooth	4.3
21	BM 48 A	Irregular	Entire	Green	Raised	Rough	4.7
22	BM 58-B	Circular	Entire	White	Flat	Smooth	2.0
23	BM 32 A	Circular	Entire	Brown	Flat	Smooth	1.0
24	BM 32 B	Circular	Entire	White	Flat	Concentric rings radial	11.0
25	BM 46 A	Irregular	Undulate	Brown	Flat	Smooth	0.0
26	BM 15 B	Irregular	Entire	White	Raised	Smooth	2.0
27	LB 61 B	Circular	Entire	Brown	Flat	Smooth	2.0
28	LB 6-B	Circular	Entire	White	Raised	Smooth	11.3
29	LB-14 A	Irregular	Lobate	Pink	Flat	Smooth	1.0
30	LB 13-A	Circular	Entire	Brown	Flat	Smooth	3.0
31	LB-47 B	Circular	Entire	White	Flat	Concentric rings radial	1.0
32	KS-A	Circular	Entire	White	Umbonate	Concentric rings radial	2.3

Thirty-two fungal endophyte isolates were collected from various sources, including seaweeds (Chlorophyta, Rhodophyta, and Phaeophyta), seagrass, and mangrove leaves. Identification of these fungal isolates was based on colony shape and hyphal structure. Each isolate displays unique traits in terms of shape, margin, colour, elevation, and texture [18]. The colonies primarily exhibited a circular shape with smooth margins. Their colouration varied widely, ranging from white and brown to pink and black shades.

Most colonies were characterised by a flat elevation, with the texture of some isolates being smooth, whereas others displayed a rough surface. Notably, isolates WB 4-2, SM 9-1, SM 26-1, BM 32 B, LB 47 B, and KS-A demonstrated radial concentric rings (Fig. 2). The morphological characteristics of these marine fungi are indicative of their unique biochemical and biological characteristics. Recognising the similarities and differences among these fungal taxa allows for more in-depth analysis [19].

Fig.2. Colony morphology characterization of isolated endophytic fungi and its hosts

3.3 Cultivation of marine endophytic fungi by submerged fermentation, antibacterial potential, and its mechanism of action

Among the various isolates studied, the marine endophytic fungus WB 1-2 demonstrated the most substantial zone of inhibition. This isolate was then subjected to molecular identification, which unequivocally identified it as *Aspergillus terreus*. Subsequently, a growth curve was delineated. The growth trajectory of marine endophytic fungi is typically divided into three primary phases: lag, log (exponential), and stationary. Each phase is characterised by unique physiological and metabolic attributes, which are instrumental in understanding the growth dynamics of fungi in their natural marine habitats [20].

The lag phase, which marks the onset of growth, is a period of adjustment for fungi to new culture conditions [21]. During this phase, cell division is either minimal or non-existent, as fungi acclimate to factors such as nutrient availability, temperature, pH, and salinity. During the initial days of the experiment, specifically from days 1 to 6, the fungi were in the lag phase. During this period, the pH values exhibited minor fluctuations, ranging from 4 to 6, suggesting that the fungi were adapting to their environment (Figure 3).

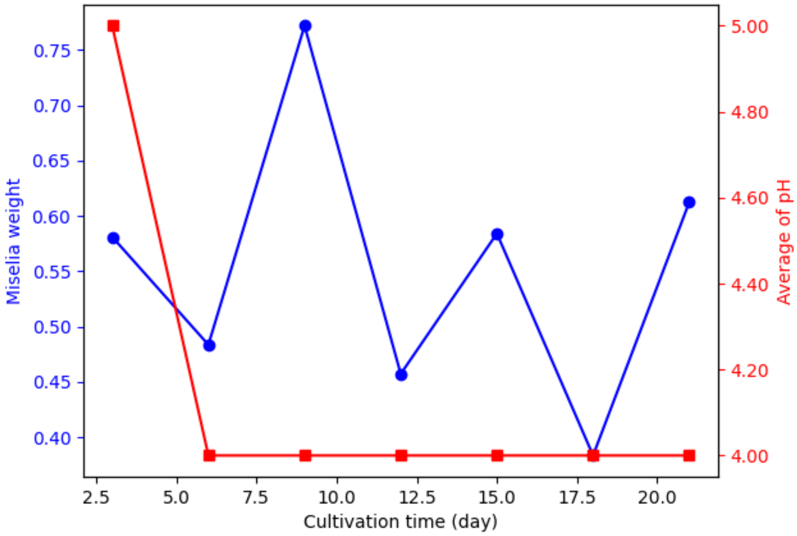

Fig. 3. Growth curve of *Aspergillus terreus* in shaker condition
(Biomass pH)

Biomass measurements during this phase were relatively low, with average values oscillating between 0.79 and 0.995 (as depicted in Figure 3). This implies that the fungi were not actively dividing but were instead focusing on acclimatising to the culture conditions. The lag phase is of paramount importance, as it sets the stage for subsequent growth, enabling the fungi to gear up for more active metabolic processes. The duration of the lag phase can vary significantly among different species and under diverse experimental conditions; however, this period is crucial for preparing cells for future growth [22].

Following successful acclimatisation, marine endophytic fungi enter the log phase, which is characterised by significant cell division and exponential growth. This phase is visually represented by a steep incline on the growth curve, indicating regular doubling of fungal cell numbers. When provided with ample nutrients, these fungi exhibit heightened metabolic activity, effectively harnessing available resources to promote optimal growth. This phase is particularly vital for the synthesis of secondary metabolites, which are of considerable interest in pharmacology owing to their potential bioactive properties [23]. Environmental parameters, including temperature and nutrient availability, are crucial factors that influence growth rates during this stage, with favourable conditions markedly enhancing biomass production [24].

Subsequent to the log phase, fungi progress to the stationary phase, which is characterised by a plateau in the growth curve. During this period, the growth rate declines owing to the depletion of nutrients and accumulation of metabolic by products. The stabilisation of viable cell numbers indicates a shift towards a survival strategy. In this phase, metabolic pathways may undergo modifications and certain species may initiate the production of spores or additional secondary metabolites in response to environmental stressors. Understanding the dynamics of this phase is vital for bioprospecting initiatives, as it can significantly affect both the yield and diversity of bioactive compounds synthesised by marine endophytic fungi [25].

The application of shaking or mechanical agitation during the cultivation of marine endophytic fungi has a pronounced impact on their growth rates and overall biomass yield. Shaking facilitates improved aeration and enhances oxygen availability, which are essential for the growth of aerobic fungi, particularly during the log phase. Increased oxygen levels support elevated metabolic rates and expedite growth, thereby reducing the likelihood of anoxic conditions [26]. Moreover, agitation promotes better homogenisation of the culture medium, ensuring a consistent distribution of nutrients to the fungal cells. This mechanical intervention also mitigates the formation of clumps or aggregates, resulting in a more uniform culture that is capable of efficiently utilising available resources [27].

The growth curve of marine endophytic fungi delineates distinct phases—lag, log, and stationary—each with specific implications for cultivation and biotechnological applications. The lag phase facilitates necessary adaptation, the log phase is critical for rapid growth and secondary metabolite production, and the stationary phase reflects the equilibrium between growth and environmental pressures [28]. The effects of shaking during cultivation are substantial because it enhances aeration, nutrient distribution, and overall growth conditions. A thorough understanding of these growth phases and the influence of agitation is essential to maximise the potential of marine endophytic fungi in diverse scientific and industrial contexts [29].

Recent research has demonstrated that *Aspergillus*, a prominent marine endophytic fungus, is capable of producing approximately 98 unique antimicrobial compounds. This highlights the remarkable chemical diversity and antimicrobial potential of these metabolites, positioning them as promising candidates for the development of novel antimicrobial agents. Many of these compounds exhibit broad-spectrum antimicrobial activity, particularly against pathogens such as *Staphylococcus aureus* and *Escherichia coli* [30]. Another marine endophytic fungus, *L. pseudotheobromae* IBRL OS64, exhibits antibacterial properties against aquaculture-relevant pathogens, with inhibition zones typically measuring less than 10 mm. It is noteworthy that natural compounds sourced from aquatic organisms frequently exhibit significantly higher bioactivity than those derived from terrestrial environments [31].

Endophytic fungi are known for their potential to generate unique bioactive compounds, exhibiting antimicrobial, antioxidant, and anticancer properties, and participating in biotransformation processes [32]. A significant amount of research has been dedicated to the exploration of biologically active substances derived from nature, particularly those capable of effective interaction with a variety of molecular targets. Marine Natural Products (MNPs) are distinguished for their diverse bioactivities, which include antibiotic, antiviral, neuroprotective, anticancer, and anti-inflammatory effects [33]. By studying the mechanisms through which marine endophytic fungi operate as antibacterial agents, specifically as natural antibiotic sources, we can enrich our understanding of their interactions with the immune system (refer to Figure 4).

A diverse range of fungal species from various orders have been discovered to flourish in hypersaline environments. These fungi generate secondary metabolites as a tactic for survival under extreme conditions, which bolsters their resilience against stress [34]. The health advantages linked to bioactive compounds are not just coincidental; they specifically engage with targeted cellular sites, as illustrated in Figure 4. These compounds exhibit strong attraction to receptors, enzymes, and different cellular elements. Once they attach to these sites, they trigger a series of events that modify cellular functions, guiding them toward positive health effects.

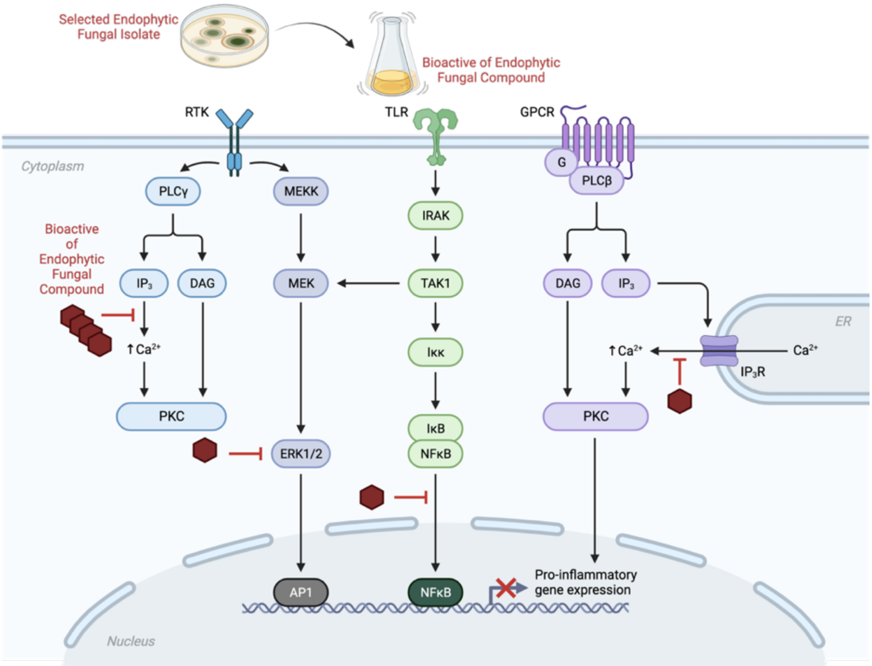

Fig. 4. Possible mechanism of bioactive compound from marine endophytic fungi in immune system (Visualised and generated by BioRender)

Bioactive substances obtained from marine endophytic fungi commence a sequence of reactions starting at the IP3 receptor, resulting in calcium release and the subsequent activation of Protein Kinase C (PKC). A crucial aspect of these compounds is their capacity to affect gene expression. As molecular architects, they can adjust gene activity and orchestrate a complex set of genetic responses that contribute to overall well-being. Furthermore, active compounds derived from endophytic fungi activate NF-ϰB, a vital transcription factor that governs genes involved in both innate and adaptive immunity [35].

These compounds can affect health at the fundamental genetic level by promoting protective genes, suppressing pro-inflammatory signals, and potentially influencing epigenetic changes. The underlying mechanisms of these health-enhancing effects are interconnected and coordinated, leading to a combined impact that exceeds the individual effects of each component [36]. Marine endophytic fungi generate a broad spectrum of natural products, including polysaccharides, enzymes, and bioactive compounds [37]. Marine microorganisms, such as microalgae, bacteria, and fungi, also possess the ability to synthesise amino acids.

Marine fungi show great potential for the development of novel biopharmaceuticals such as immunomodulators and therapies for cancer, inflammation, and infections caused by microbes and fungi. Natural bioactive compounds derived from endophytic fungi have demonstrated increased efficacy, potency, cost-effectiveness, and lower resistance than traditional antimicrobial treatments [38]. These substances are renewable, have distinct properties, and tend to exhibit low toxicity. Consequently, these discoveries offer substantial opportunities for the biotechnological and pharmaceutical industries to reduce strain on public healthcare systems.

4 Conclusions

This study highlights the significant potential of marine endophytic fungi as a source of novel bioactive compounds with notable antibacterial activity. The isolation and identification of 32 fungal isolates from diverse marine environments on Buton Island, Southeast Sulawesi, revealed that *Aspergillus terreus* (isolate WB 1-2) exhibited the most substantial antibacterial effects against *Vibrio harveyi*. The growth dynamics of these fungi, characterized by distinct lag, log, and stationary phases, underscore the importance of environmental factors and mechanical agitation in optimizing biomass production and metabolite yield. The ability of marine endophytic fungi to produce a variety of secondary metabolites during the log phase suggests their potential application in pharmaceutical developments, particularly in combating antibiotic-resistant pathogens. These findings emphasize the need for further research to explore the mechanisms underlying the antibacterial properties of these metabolites, as well as their broader implications for health and disease management. By leveraging the diverse biochemical capabilities of marine endophytic fungi, the biotechnological and pharmaceutical industries can develop innovative solutions to address pressing health challenges. Continued exploration of marine biodiversity will be essential in uncovering new antimicrobial agents and enhancing our understanding of these organisms' ecological roles in marine ecosystems.

The author would like to thank the Directorate Research and Innovation IPB University for providing financial support through the National Research Collaboration/Riset Kolaborasi Nasional (Ri-Na) programme. This funding was awarded to Dr. Kustiariyah, S.Pi, M.Si under Grant Number 509/IT3.D10/PT.01.03/P/B/2023 on 24 November 2023.

REFERENCES

1. L. Torta, S. Burruano, S. Giambra, G. Conigliaro, G. Piazza, G. Mirabile, M. Pirrotta, R. Calvo, G. Bellissimo, S. Calvo, A. Tomasello. Cultivable fungal endophytes in roots, rhizomes and leaves of *Posidonia oceanica* (L.) Delile along the coast of Sicily, Italy. Plants. **11**, 9, 1139 (2022). https://doi.org/10.3390/plants11091139
2. M. Sarasan, J. Puthumana, N. Job, J. Han, J. S. Lee, R. Philip. Marine algicolous endophytic fungi-a promising drug resource of the era. J Microbiol Biotechnol. **27**, 6, 1039-1052 (2017). https://doi.org/10.4014/jmb.1701.01036
3. A. Karthikeyan, A. Joseph A, B. G. Nair. Promising bioactive compounds from the marine environment and their potential effects on various diseases. J. Genet. Engineer. Biotechnol. **20**, 1, 14 (2022). https://doi.org/10.1186/s43141-021-00290-4
4. E.A. Tanur, H. Al Imran, R. Angrianto, N. L. May, A. Anwar. Impact Analysis of Human Activities on Mangrove Conservation in Coastal Sulawesi. West Sci. Nat. Technol. **2**, 01, 1-8 (2024). https://doi.org/10.58812/wsnt.v2i04.742
5. M. J. Struebig, S. G. Aninta, M. Beger, A. Bani, H. Barus, S. Brace, Z. G. Davies, M. D. Brauwer, K. Diele, C. Djakiman, R. Djamaluddin. Safeguarding imperiled biodiversity and evolutionary processes in the Wallacea center of endemism. BioScience. **72**, 11, 1118-1130 (2022).
6. S. Suhardin, A. Bafadal, R. Rosmawaty. Factors influencing seaweed supply in Lakudo District, Central Buton Regency. Int. J. Technol. Edu. Res. **1**, 03, 60-72 (2023).
7. A. P. Cahyaningsih, A. K. Cahyaningsih, A. K. Deanova, C. M. Pristiawati, Y. I. Ulumuddin, L. Kusumaningrum, A. D. Setyawan. Causes and impacts of anthropogenic activities on mangrove deforestation and degradation in Indonesia. Int. J. Bonorowo Wetlands. **12**, 1 (2022). DOI: 10.13057/bonorowo/w120102

8. Z. Q. Bai, X. Lin, Y. Wang, J. Wang, X. Zhou, B. Yang, J. Liu, X. Yang, Y. Wang, Y. Liu. New phenyl derivatives from endophytic fungus *Aspergillus flavipes* AIL8 derived of mangrove plant *Acanthus ilicifolius*. Fitoterapia. **95**, 194-202 (2014). https://doi.org/10.1016/j.fitote.2014.03.021
9. K. M. Ukwatta, J. L. Lawrence, C. D. Wijayarathne. Antimicrobial, anti-cancer, anti-filarial and anti-inflammatory activities of Cowabenzophenone A extracted from the endophytic fungus *Aspergillus terreus* isolated from a mangrove plant *Bruguiera gymnorrhyza*. Mycology. **11**, 4, 297-305 (2020).
10. D. P. Gauchan, P. Kandel, A. Tuladhar, A. Acharya, U. Kadel, A. Baral, A. B. Shahi, M. R. García-Gil. Evaluation of antimicrobial, antioxidant and cytotoxic properties of bioactive compounds produced from endophytic fungi of Himalayan yew (*Taxus wallichiana*) in Nepal. F1000 Res. **9**. (2020) DOI: 10.12688/f1000research.23250.2
11. G. Vinayarani, H. S. Prakash. Fungal endophytes of turmeric (*Curcuma longa* L.) and their biocontrol potential against pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*. World J. Microbiol. Biotechnol. **34**, 1-7 (2018). DOI : 10.1007/s11274-018-2431-x
12. D. Wahjuningrum, S. Hariati, M. Yuhana, I. Effendi, T. Citarasu, D. A. Utami, K. Tarman. Low dose of *Nodulisporium* sp. KT29 metabolite promotes production performance and innate immunity of Pacific white leg shrimp (*Litopenaeus vannamei*) against co-infection of white spot syndrome virus and *Vibrio harveyi*. Aquacult Inter. **30**, 5, 2611-28 (2022). DOI: 10.1007/s10499-022-00924
13. I. Isti'anah, K. Tarman, S. H. Suseno, R. Nugraha, I. Effendi. Penapisan senyawa bioaktif antibakteri fungi laut endofit asal Pulau Buton Sulawesi Tenggara. JPHPI **27**, 7 (2024). DOI: 10.17844/jphpi.v27i7.50489
14. A. Kunzmann, G. Todinanahary, F. E. Msuya, Y. Alfiansah. Comparative environmental impacts and development benefits of coastal aquaculture in three tropical countries: Madagascar, Tanzania and Indonesia. Trop. Life Sci. Res. **34**, 3, 279 (2023). DOI : 10.21315/tlsr2023.34.3.15
15. P. Angelini, A. M. Abdel-Azeem, C. E. Girometta. Bioactive compounds with potential medicinal properties derived from fungi: recent and future developments in microbial biotechnology. Front. Microbiol. **13**, 837586 (2022) https://doi.org/10.3389/fmicb.2022.837586.
16. K. R. Priyanka, R. Rajaram. A critical review on anticandidal properties of marine plants. Reg. Stud. Mar. Sci. **68**, 103258 (2023). https://doi.org/10.1016/j.rsma.2023.103258
17. B. Rehman, S. A. Khan, M. Hamayun, A. Iqbal, I. J. Lee. Potent bioactivity of endophytic fungi isolated from Moringa oleifera leaves. BioMed Res. Int., **1**, 2461021 (2022).
18. S. Hota, K. G. Achary, S. Singh. Identification and Molecular Characterization of *Rhizopus delemar* from Eastern Ghats of State of India and its Biotechnological Applications. Geomicrobiol. J. **41**, 4, 429-439 (2024). https://doi.org/10.1080/01490451.2023.2231925
19. E. G. Jones, K. L. Pang, M. A. Abdel-Wahab, B. Scholz, K. D. Hyde KD, Boekhout T, Ebel R, Rateb ME, Henderson L, Sakayaroj J, Suetrong S. An online resource for marine fungi. Fungal Diversity. **96**, 347-433 (2019).
20. N. Ukhty N, K. Tarman, I. Setyaningsih. 2017. Isolation of endophytic fungi from the coastal plant terong pungo (*Solanum* sp.) and its antibacterial activity against oral pathogenic bacteria. Biotropia. **24**, 1, 9-15. DOI: 10.11598/btb.201.2.741453
21. M. S. Sheteiwy, D. F. Ali, Y. C. Xiong, M. Brestic, M. Skalicky, Y. A. Hamoud, Z. Ulhassan, H. Shaghaleh, H. AbdElgawad, M. Farooq, A. Sharma. Physiological and biochemical responses of soybean plants inoculated with Arbuscular mycorrhizal fungi

and *Bradyrhizobium* under drought stress. BMC Plant Biol. **21**, 1-21 (2021). DOI: 10.1186/s12870-021-02949-z

22. R. Parthasarathy, D. Sruthi, C. Jayabaskaran. Retracted: Isolation and purifications of an ambuic acid derivative compound from marine algal endophytic fungi Talaromyces flavus that induces apoptosis in MDA-MB-231 cancer cells. Chem. Biol. Drug Design. **102**, 5, 1308-1326 (2023). DOI : 10.1111/cbdd.14271
23. M. P. Bhat, M. Rudrappa, A. Hugar, P. V. Gunagambhire, R. S. Kumar, S. Nayaka, A. I. Almansour, K. Perumal. In-vitro investigation on the biological activities of squalene derived from the soil fungus *Talaromyces pinophilus*. Heliyon. **1**, 9, 11 (2023).). DOI : 10.1016/j.heliyon.2023.e21461
24. S. Wang, Y. Bi, W. Quan, P. Christie. Growth and metabolism of dark septate endophytes and their stimulatory effects on plant growth. Fungal Biol. **126**, 10, 674-686 (2022). DOI : 10.1016/j.funbio.2022.08.006
25. P. K. Keshri, N. Rai, A. Verma, S. C. Kamble, S. Barik, P. Mishra, S. K. Singh, P. Salvi, V. Gautam. Biological potential of bioactive metabolites derived from fungal endophytes associated with medicinal plants. Mycol. Prog. **20**, 5, 577-594 (2021). DOI : 10.1007/s11557-021-01695-8
26. R. Geris, V. E. Teles de Jesus, A. Ferreira da Silva, M. Malta. Exploring culture media diversity to produce fungal secondary metabolites and cyborg cells. Chem. Biodiv. **21**, 3, e202302066 (2024). DOI : 10.1002/cbdv.202302066
27. B. Rojas-Sánchez, P. Guzmán-Guzmán, L. R. Morales-Cedeño, M. D. Orozco-Mosqueda, B. C. Saucedo-Martínez, J. M. Sánchez-Yáñez, A. E. Fadiji, O. O. Babalola, B. R. Glick, G. Santoyo. Bioencapsulation of microbial inoculants: mechanisms, formulation types and application techniques. Appl. Biosci. **1**, 2, 198-220 (2022). DOI : 10.3390/applbiosci1020013
28. G. Santamaria, C. Liao, C. Lindberg, Y. Chen, Z. Wang, K. Rhee, F. R. Pinto, J. Yan, J. B. Xavier. Evolution and regulation of microbial secondary metabolism. Elife. **11**, e76119 (2022). https://doi.org/10.7554/eLife.76119
29. B. Fan, D. Parrot, M. Blümel, A. Labes, D. Tasdemir. Influence of OSMAC-based cultivation in metabolome and anticancer activity of fungi associated with the brown alga *Fucus vesiculosus*. Mar. Drugs. 17, 1, 67 (2019). DOI: 10.3390/md17010067
30. Wang B, Cai J, Huang L, Chen Y, Wang R, Luo M, Yang M, Zhang M, Nasihat, Chen G, Huang G. Significance of research on natural products from marine-derived Aspergillus species as a source against pathogenic bacteria. Frontiers in Microbiology. 2024 Sep 19;15:1464135.
31. M. T. M. Jalil, N. A. Zakaria, N. S. M. Suhaimi, D. Ibrahim. Crude extracts of an endophytic fungus attenuate the growth of pathogenic bacteria in aquaculture. Iran J Microbiol. **14**, 3, 383-394 (2022). DOI: 10.18502/ijm.v14i3.9780
32. Abdel-Azeem AM, Salem FM, Abdel-Azeem MA, Nafady NA, Mohesien MT, Soliman EA. *New and future developments in microbial biotechnology and bioengineering* (Elsevier, Amsterdam, 2016). https://doi.org/10.1016/B978-0-444-63505-1.00001-4
33. A. M. Mayer, V. A. Mayer, M. Swanson-Mungerson, M. L. Pierce, A. D. Rodríguez, F. Nakamura, O. Taglialatela-Scafati. Marine pharmacology in 2019–2021: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. Mar. Drugs. **22**, 7, 309 (2024). https://doi.org/10.3390/md22070309
34. S. Sahoo, K. Subban, J. Chelliah. Diversity of marine macro-algicolous endophytic fungi and cytotoxic potential of Biscogniauxia petrensis metabolites against cancer cell lines. Front. Microbiol. **12**, 650177 (2021). https://doi.org/10.3389/fmicb.2021.650177

35. T. Wijesekara, B. Xu. Health-promoting effects of bioactive compounds from plant endophytic fungi. J. Fungi. **9**, 10, 997 (2023). https://doi.org/10.3390/jof9100997
36. E. Wa. Marine Environment, the Secretive World of Endophytic Microorganisms. J. Pharm. Res. **6**, 2 (2022) doi: 10.23880/oajpr-16000269
37. I. Ahmed, M. Asgher, F. Sher, S. M. Hussain, N. Nazish, N. Joshi, A. Sharma, R. Parra-Saldívar, M. Bilal, H. M. Iqbal. Exploring marine as a rich source of bioactive peptides: Challenges and opportunities from marine pharmacology. Mar. Drugs. **20**, 3, 208 (2022). https://doi.org/10.3390/md20030208
38. M. C. Manganyi, C. N. Ateba. Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms. **8**, 12, 1934 (2020). https://doi.org/10.3390/microorganisms8121934