

Bioprospecting Marine Endophytic Fungi from Buton Island: Antibacterial Activity and Cellulase Production for Sustainable Blue Economy



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Received : 01 June 2025

Accepted : 31 December 2025

Published : 31 December 2025

Academic Editor: Ulfah Amalia, S.Pi, M.Si, Ph.D

©Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 2021. Accreditation Number: 169/E/KPT/2024. ISSN: 2089-5690, e-ISSN: 2406-9272. <https://doi.org/10.15578/squalen.1024>

Introduction

Marine ecosystems represent a significant reservoir of biological diversity and functional novelty, yet many of their microbial components remain insufficiently explored. Among these, marine fungi, particularly marine endophytic fungi (MEF), have gained increasing attention due to their ecological roles and biotechnological potential (Nikijuluw, 2017; Abdel-Razek et al., 2020). MEF reside asymptotically within marine plants and macroalgae, where they contribute to nutrient cycling, host resilience, and metabolic exchanges. Their ability to produce enzymes and secondary metabolites positions them as promising candidates for sustainable biotechnological applications (El-Bondkly et al., 2021).

Recent studies have demonstrated that MEF can synthesize a diverse array of bioactive compounds,

Abstract

Marine-derived endophytic fungi represent an underexplored reservoir of biologically active metabolites and enzymes with industrial relevance. This study investigated the fungal diversity associated with seaweed, seagrass, and mangrove leaf tissues collected from Buton Island, Southeast Sulawesi, with particular emphasis on antibacterial properties and cellulolytic activity. Thirty-two fungal isolates were successfully recovered and characterized based on morphological features. Antibacterial assays against *Vibrio harveyi* showed that several isolates, notably WB 1-2, WB 6-2, and SM 27-2, produced clear inhibition zones ranging from 11 to 13 mm, demonstrating notable antibacterial efficacy. Among all isolates, *Aspergillus terreus* (WB 1-2) exhibited the highest cellulase activity and was therefore selected for further enzymatic evaluation. The crude cellulase displayed maximum activity at pH 4 and 70°C. Hydrolysis products were qualitatively analyzed using HPLC, confirming the presence of glucose. These results highlight the dual potential of marine endophytic fungi as sources of antibacterial agents and thermostable cellulases. This work lays the groundwork for future development of eco-friendly enzyme production and marine-based antimicrobial resources, supporting sustainable biotechnological innovation within the blue economy framework.

Keywords: biotechnology, blue economy, cellulase, mangrove, sustainable

including antibacterial, antifungal, antioxidant, and anticancer agents (Giddings & Newman, 2019; Khattab & Farag, 2022). These properties are particularly relevant in the context of increasing antibiotic overuse and the emergence of resistant pathogens, which demand alternative, environmentally compatible solutions. In addition to pharmaceutical relevance, MEFs are recognized as potential sources of industrial enzymes such as cellulase, which plays a crucial role in biomass degradation, waste management, and bioenergy-related processes (Carroll et al., 2022). However, despite growing global interest, research on MEF-derived enzymes and antibacterial activity remains geographically uneven and taxonomically limited.

Buton Island, located in Southeast Sulawesi, Indonesia, hosts a mosaic of marine habitats, including mangrove forests, coral reefs, seagrass meadows, and

extensive seaweed beds (Melansari, 2022). These ecosystems support high biodiversity and underpin local fisheries and coastal livelihoods (Basyuni et al., 2022). Mangroves and macroalgae in particular are recognized as important reservoirs of endophytic fungi, which may differ in metabolic capacity depending on host species and environmental conditions (Isti'anah et al., 2024a). Previous investigations have reported that endophytic fungi isolated from mangroves, such as *Aspergillus terreus* associated with *Bruguiera gymnorrhiza*, can produce biologically active secondary metabolites with antimicrobial and anti-inflammatory properties (Chen et al., 2022; Jeewon et al., 2019). These findings suggest that the unique environmental conditions of Buton Island may support MEF with distinct functional traits.

Despite this potential, systematic studies focusing on MEF from Buton Island—particularly those integrating isolation, antibacterial screening, and enzymatic activity assessment—remain scarce. Most existing studies emphasize secondary metabolites without concurrently evaluating enzymatic functions such as cellulase production, which is directly relevant to sustainable industrial applications within the blue economy framework (Pang et al., 2023; Vega-Portalatino et al., 2023). This lack of integrated assessment represents a clear research gap, limiting the understanding of how local marine fungal diversity may contribute to both environmental sustainability and applied biotechnology.

Within the context of the blue economy, there is a growing need to identify marine-derived biological resources that support economic development while minimizing ecological impact (Kalogerakis et al., 2015). Marine endophytic fungi, particularly those associated with mangroves and seaweeds, represent an underexplored group with potential applications in enzyme production and antimicrobial development for aquaculture and environmental management. However, baseline data on their diversity, functional properties, and application-oriented potential from Buton Island are still limited.

Therefore, this study addresses this gap by investigating marine endophytic fungi isolated from seaweed and mangrove samples collected on Buton Island. The specific objectives of this research are: (i) to isolate and characterize marine endophytic fungi from selected marine hosts; (ii) to screen the isolates for antibacterial activity against relevant marine pathogens, including *Vibrio harveyi*; and (iii) to evaluate their cellulase-producing capability under controlled fermentation conditions. By focusing on both antibacterial and enzymatic activities, this study aims to provide foundational data supporting the sustainable utilization of marine fungal resources within the blue economy, without overstating their immediate industrial applicability.

Materials and Methods

Isolation and screening of marine endophytic fungi samples

The research was carried out on Buton Island, Southeast Sulawesi Province, where samples of seaweed, seagrass, and mangrove leaves were collected for the isolation of endophytic fungi. Collected plant materials were carefully labeled and air-dried to preserve their condition during transport to the laboratory. Upon arrival, the samples underwent a two-step surface sterilization process to eliminate potential contaminants: first, they were briefly rinsed with distilled water, then immersed in 5% sodium hypochlorite (NaOCl). Sterilized plant tissues were then placed on Potato Dextrose Agar (PDA) supplemented with 2% NaCl to mimic the natural salinity of the marine environment, with four to five pieces of tissue per Petri dish. The plates were incubated at approximately 25 °C for 3–7 days. Emerging fungal colonies were monitored for morphological characteristics to ensure purity, and any mixed or impure colonies were re-isolated. Pure, morphologically distinct isolates were subsequently transferred to slanted agar media for long-term preservation, thereby maintaining the integrity of the cultures for downstream analyses (Al-Rajhi et al., 2022).

Diversity of morphology characterization of marine endophytic fungi

To capture a broad spectrum of potential fungal hosts, samples were collected from the roots, rhizomes, and leaves of marine plants. Fungal isolation was performed using standard microbiological procedures on Potato Dextrose Agar (PDA), a medium that provides a conducive environment for fungal growth. The emerging colonies were characterized based on their macroscopic morphology, including colony shape, color, diameter, surface texture, elevation, and the presence of aerial or submerged mycelia. These detailed observations enabled a comprehensive evaluation of morphological diversity among isolated fungi, thereby enhancing understanding of marine fungal biodiversity in these ecosystems (Taritla et al., 2021).

Antagonistic test of marine endophytic fungi for selection isolates

The antibacterial activity of marine endophytic fungi against *Vibrio harveyi* was evaluated using a dual-culture agar diffusion method. A suspension of *V. harveyi* was mixed with sterile Nutrient Agar (NA) medium and poured into Petri dishes. After solidification, a 1 cm diameter agar plug containing actively growing fungal mycelium was placed on the surface of the inoculated medium. Plates were incubated at 37 °C for 24 h.

Antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding the fungal disc using calipers. A commercial chloramphenicol disc (30 µg/disc) was used as a positive control, while plates inoculated with *V. harveyi* without fungal discs served as negative controls. All assays were conducted in triplicate (Isti'anah et al., 2024b).

Cellulase Enzyme Production

The fungal isolate selected for cellulase production was identified as *Aspergillus terreus* based on macroscopic colony characteristics and microscopic morphological features. This isolate was selected for further analysis due to its superior cellulolytic activity observed during preliminary screening on CMC agar. To induce extracellular cellulase production, *A. terreus* was cultured in a carboxymethyl cellulose (CMC) medium under submerged fermentation conditions.

Following fermentation, the culture was centrifuged at 5000 rpm for 15 min at 25 °C using a Universal 32 R Hettich centrifuge (Germany). The resulting supernatant was collected as the crude enzyme extract and stored at -20 °C until analysis. This crude enzyme preparation was used to assess cellulase components, including endoglucanase (CMCase), exoglucanase (filter paperase, FPase), and β-glucosidase activities, according to El-Baroty et al., (2019).

Endoglucanase activity was determined by incubating 0.5 mL of crude enzyme with 0.5 mL of 2% (w/v) CMC prepared in 0.05 M sodium acetate buffer (pH 5) at 30 °C for 30 min. Reducing sugars released during the reaction were quantified using the dinitrosalicylic acid (DNS) method, following IUPAC recommendations (Silveira et al., 2014). After the addition of the DNS reagent, the reaction mixture was heated to 100 °C for 30 min, and the absorbance was measured at 540 nm using a spectrophotometer. Enzyme activity was defined as the amount of enzyme releasing 1 µmol of glucose per minute under the assay conditions and expressed as units per gram dry substrate (U/gds). All assays were conducted in triplicate in accordance with standardized IUPAC procedures (Poszytek et al., 2016).

HPLC analysis of cellulase enzyme

Monosaccharides in the hydrolysates, including glucose, xylose, and arabinose, were quantified using high-performance liquid chromatography (HPLC) on an Agilent 1100 system equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector. The separation and detection conditions were carried out according to established protocols (Luo et al., 2017).

Authentic monosaccharide standards, including glucose, xylose, and arabinose, were analyzed under identical chromatographic conditions to identify retention times and construct calibration curves. Additional standards, namely succinic acid and acetate, were included to account for other soluble metabolites detected in the hydrolysates. Calibration curves generated from these standards were used to determine compound concentrations in the samples quantitatively.

Results and Discussion

Isolation and screening of endophytic fungi samples

The study on Buton Island, Southeast Sulawesi, aimed to explore the diversity of marine endophytic fungi associated with different marine plants, including mangroves, seagrasses, and algae. Among 160 samples collected across various marine environments, Chlorophyta (green algae) harbored the greatest number of endophytic fungi, accounting for 60% of the total isolates (Table 1).

This dominance suggests that Chlorophyta may provide an ideal environment or possess specific traits that favor the colonization of endophytic fungi.

Among the isolated marine endophytic fungi, the highest proportion originated from Chlorophyta, followed by Rhodophyta (22%), seagrasses (9%), mangroves (6%), and Phaeophyta (3%). This distribution indicates that different marine plants vary in their capacity to host endophytic fungi, with the LB (Labeka) and BM (Bungi Matangia) sampling sites contributing approximately 75% of the total isolates (Figure 1).

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

Isolat Code	Amount of Samples
WB	7
SM	32
LB	60
BM	59
KS	2
Total	160

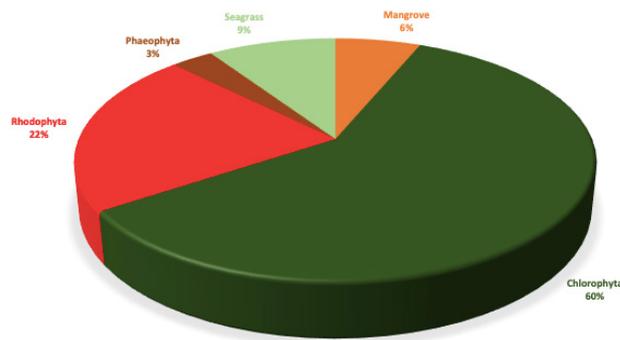


Figure 1. Diversity Source of Endophytic Fungal Hosts

Following isolation, a careful selection and purification process was conducted to retain only the most viable and bioactive fungi, minimizing contamination and emphasizing strains with the greatest potential for cellulase production (Teixeira et al., 2019). Although originating from marine habitats, these fungi were successfully cultured in the laboratory without seawater supplementation, allowing a detailed study (Overy et al., 2019).

From a total of 160 isolates, those showing promising characteristics underwent bioactivity screening, focused on cellulase enzyme production. These findings highlight the biotechnological potential of marine-derived fungi, particularly for enzyme-based applications, and emphasize the importance of methodical isolation and selection to identify strains with high industrial relevance (Sopalun et al., 2021).

Diversity of Morphology Characterization of Marine Endophytic Fungi

In this study, marine endophytic fungi isolated from various marine plant species in Buton Island were subjected to detailed morphological characterization. The isolates exhibited a broad spectrum of colony colors, ranging from pale to vibrant hues, and varied textures when incubated on Potato Dextrose Agar (PDA) plates. For instance, certain isolates formed velvety colonies with a distinct radial pattern, while others displayed a smooth, glossy appearance, indicating significant diversity in their growth forms (Table 2). To ensure pure strains, the fungi were subjected to repeated cultivation and subculturing.

The process involved transferring individual colonies to fresh PDA plates and monitoring for uniformity in growth characteristics over successive generations. The morphological traits, including form, margin, chromogenesis, elevation, and texture, were meticulously documented, providing insights into the phenotypic variability among the isolated fungi (Teresia et al., 2020). This diversity in morphology indicates the fungi's adaptation to their specific marine environments and suggests potential variability in their metabolic profiles,

which is crucial for subsequent bioactive compound screening (Hutapea et al., 2021).

During the study, the marine endophytic fungi displayed a broad range of morphological characteristics, including variations in colony form, color, elevation, and texture. The isolates exhibited circular and irregular forms, with margins ranging from entire to undulate and filiform. This morphological diversity indicates the presence of multiple fungal species or strains, each potentially adapted to specific environmental conditions. For instance, some isolates displayed radial concentric rings, suggesting a unique growth pattern that could be linked to their ecological niche or metabolic activity. Such detailed morphological characterization is crucial for the preliminary identification of fungal species and lays the groundwork for further taxonomic studies (Pham et al., 2021).

The study also highlighted the significance of chromogenesis, or color production, in differentiating between fungal isolates. The isolates varied widely in color, ranging from white to black, brown, pink, and even green (Figure 2). This color variation may reflect the pigments the fungi produce, which could be linked to their ability to produce bioactive compounds. Additionally, the colonies' elevations and textures differed significantly, with some colonies flat while others were raised, convex, or umbonate. The texture ranged from smooth to rough, with some isolates showing concentric rings indicative of radial growth patterns. These morphological traits aid in species identification and provide insights into the ecological roles and potential applications of these fungi (Zanne et al., 2020).

The identification process relied heavily on examining colony form and hyphal morphology. Thirty-two endophytic fungi isolates from marine sources, including seaweeds, seagrasses, and mangrove leaves, were meticulously analyzed. The study emphasized the importance of these morphological characteristics in the preliminary classification of the fungi. Differences in form, margin, and chromogenesis among the isolates suggest that these fungi may have evolved to occupy

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

No	Isolat Code	Characterization					Taxonomic Estimates
		Form	Margin	Chromogenesis	Elevation	Texture	
1	WB 1-2	Circular	Entire	Brown	Flat	Smooth	<i>Aspergillus</i> sp.
2	WB 7-2	Irregular	Undulate	White	Raised	Smooth	<i>Penicillium</i> sp.
3	WB 4-1	Irregular	Entire	Black	Flat	Smooth	<i>Cladosporium</i> sp.
4	WB 6-1	Irregular	Undulate	White	Flat	Smooth	<i>Penicillium</i> sp.
5	WB 5-1	Circular	Entire	White	Flat	Smooth	<i>Aspergillus</i> sp.
6	WB 3-2	Circular	Filiform	White	Flat	Smooth	<i>Aspergillus</i> sp.
7	WB 6-2	Irregular	Entire	Brown	Raised	Smooth	<i>Aspergillus</i> sp.
8	WB 4-2	Circular	Curled	Black	Umbonate	Concentric rings radial	<i>Cladosporium</i> sp.
9	WB 7-1	Circular	Filiform	White	Flat	Smooth	<i>Aspergillus</i> sp.
10	SM 7-1	Irregular	Undulate	White	Raised	Rough	<i>Penicillium</i> sp.
11	SM 16-1	Irregular	Entire	White	Flat	Smooth	<i>Aspergillus</i> sp.
12	SM 20-1	Circular	Entire	White	Convex	Smooth	<i>Aspergillus</i> sp.
13	SM 22-1	Circular	Filiform	White	Flat	Smooth	<i>Aspergillus</i> sp.
14	SM 32-1	Circular	Undulate	Black	Flat	Smooth	<i>Cladosporium</i> sp.
15	SM 17-1	Irregular	Undulate	Black	Raised	Rough	<i>Cladosporium</i> sp.
16	SM 5-1	Irregular	Undulate	White	Raised	Smooth	<i>Penicillium</i> sp.
17	SM 9-1	Circular	Entire	White	Flat	Concentric rings radial	<i>Aspergillus</i> sp.
18	SM 27-2	Circular	Entire	White	Convex	Smooth	<i>Aspergillus</i> sp.
19	SM 26-1	Circular	Undulate	Black	Flat	Concentric rings radial	<i>Cladosporium</i> sp.
20	BM 47 B	Circular	Entire	White	Flat	Smooth	<i>Aspergillus</i> sp.
21	BM 48 A	Irregular	Entire	Green	Raised	Rough	<i>Penicillium</i> sp.
22	BM 58-B	Circular	Entire	White	Flat	Smooth	<i>Aspergillus</i> sp.
23	BM 32 A	Circular	Entire	Brown	Flat	Smooth	<i>Aspergillus</i> sp.
24	BM 32 B	Circular	Entire	White	Flat	Concentric rings radial	<i>Aspergillus</i> sp.
25	BM 46 A	Irregular	Undulate	Brown	Flat	Smooth	<i>Aspergillus</i> sp.
26	BM 15 B	Irregular	Entire	White	Raised	Smooth	<i>Aspergillus</i> sp.
27	LB 61 B	Circular	Entire	Brown	Flat	Smooth	<i>Aspergillus</i> sp.
28	LB 6-B	Circular	Entire	White	Raised	Smooth	<i>Aspergillus</i> sp.
29	LB-14 A	Irregular	Lobate	Pink	Flat	Smooth	<i>Penicillium</i> sp.
30	LB 13-A	Circular	Entire	Brown	Flat	Smooth	<i>Aspergillus</i> sp.
31	LB-47 B	Circular	Entire	White	Flat	Concentric rings radial	<i>Aspergillus</i> sp.
32	KS-A	Circular	Entire	White	Umbonate	Concentric rings radial	<i>Aspergillus</i> sp.



Figure 2. Colony morphology characterization of isolated endophytic fungi and their hosts a) Isolant code WB and SM, and b) Isolant code BM, LB, and KS

distinct ecological niches within the marine environment. Such adaptations are likely linked to their biochemical and biological traits, making these fungi promising candidates for biotechnological applications. The detailed morphological analysis conducted in this study provides a foundation for understanding the diversity and potential of marine endophytic fungi from Buton Island (Shaumi et al., 2021).

Antagonistic test of marine endophytic fungi with the pathogenic bacteria

The antibacterial activity of 32 marine endophytic fungal isolates against *Vibrio harveyi* was systematically evaluated. All isolates exhibited some degree of inhibition, with clear or hazy zones observed around the fungal colonies. Notably, isolates WB 1-2, WB 6-2, WB 7-1, SM 22-1, SM 27-2, BM 46, and LB 13-A demonstrated the highest activity, producing inhibition zones of 11–13 mm (Fanele & Ndlovu, 2023; Table 2). These results indicate that certain marine endophytic fungi can produce bioactive compounds that suppress pathogenic bacterial

growth, supporting previous reports on marine fungi as sources of natural antibacterial agents (Mohamed et al., 2021).

The isolate WB 1-2 showed particularly strong activity (Figure 3), consistent with earlier studies showing that marine fungi, such as *Nodulisporium* sp. KT29 exhibited inhibition zones of 9.3–14 mm against various bacteria (Wahjuningrum et al., 2022). Similarly, *Aspergillus terreus* SHE05 displayed activity against *Aeromonas hydrophila* with zones of ~14 mm (Karwehl & Stadler, 2016). These comparisons suggest that WB 1-2 and related isolates may harbor broad-spectrum antibacterial metabolites, although this study was limited to a single Gram-negative bacterium. Testing against Gram-positive pathogens would be necessary to evaluate the spectrum of activity fully.

The observed antibacterial effects are likely due to secondary metabolites produced by the fungus during growth. In marine endophytes, these compounds often include polyketides, alkaloids, and peptides that disrupt bacterial cell walls or metabolic pathways (Martyniuk

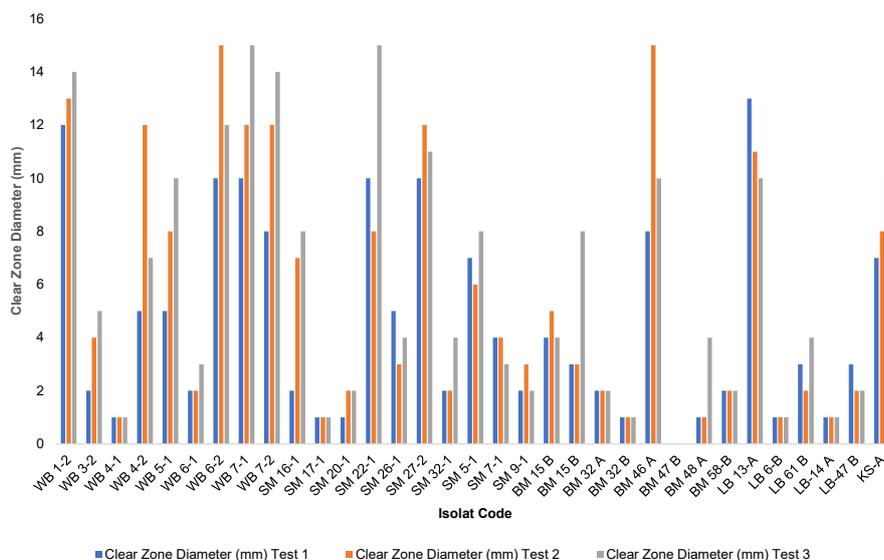


Figure 3. Results of Antagonist Test for Marine Endophytic Fungi with *V. harveyi*

et al., 2020). The variation in inhibition zones among isolates suggests that metabolite production is strain-dependent and influenced by the specific marine host and environmental conditions (El-Latif et al., 2021).

Beyond their antibacterial potential, these marine fungi may possess other biotechnologically valuable traits, such as enzyme production. The ability of isolates such as WB 1-2 to produce cellulase underscores the dual potential of marine endophytes for both antimicrobial and industrial applications (Corbu et al., 2023; Zhu et al., 2023). Such properties are particularly relevant to sustainable blue economy initiatives, where microbial products can support eco-friendly aquaculture and bioindustrial processes (Bouley et al., 2023).

In conclusion, the results demonstrate that marine endophytic fungi from Buton Island are a promising source of antibacterial compounds against *V. harveyi*, with WB 1-2 identified as the most potent isolate.

While the current study focused on a single Gram-negative bacterium, further testing against Gram-positive pathogens and detailed chemical characterization of the metabolites would strengthen the understanding of their antimicrobial potential. These findings provide a targeted framework for future exploration of marine fungi as natural antibacterial agents and as contributors to sustainable marine biotechnology (Sandrawati et al., 2020).

Cellulase Enzyme Production

Cellulase enzyme activity is defined as 1 μmol of reducing sugar produced by the enzyme per minute during the hydrolysis of cellulose substrates. Enzyme activity can describe the purity of an enzyme (Grata, 2020). Enzyme crude extract activity from the marine fungus *Aspergillus terreus*, fractionation results, can be seen in Figure 4.

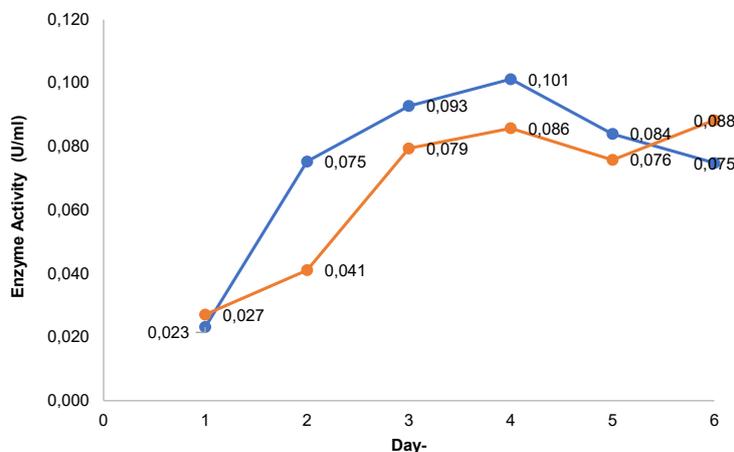


Figure 4. Enzyme Activity Curve of Marine Fungi *Aspergillus terreus* (Sample A. Sample B)

The cellulase enzyme produced by *Aspergillus terreus* exhibited optimal activity at pH 4 (Figure 4), indicating that acidic conditions favor its catalytic efficiency. These results suggest that the enzyme could be suitable for biotechnological applications requiring acidic environments, such as biomass hydrolysis or certain industrial fermentation processes. (Kuhad et al., 2016).

The cellulase enzyme used in this study was obtained as a crude extract from *Aspergillus terreus*; no fractionation was performed before the activity assays. The crude enzyme preparation was considered representative for measuring the combined activity of endoglucanase (CMCase), exoglucanase (FPase), and β -glucosidase, providing an overall assessment of cellulolytic potential (El-Baroty et al., 2019). Among the total 160 fungal isolates screened, *A. terreus* was selected for further study due to its superior cellulolytic activity observed during preliminary screening on CMC agar, as well as its ability to produce clear zones of cellulose degradation. This selection criterion ensured that the isolate with the highest enzymatic potential was investigated in detail.

Enzyme activity was quantified by measuring the amount of reducing sugar released during cellulose hydrolysis, a key indicator of cellulase efficiency. Optimal activity was observed at pH 4, emphasizing the

importance of maintaining specific pH conditions for maximal enzyme performance, a critical consideration for industrial applications (Yadav et al., 2022). Temperature also influenced activity, with cellulase activity increasing up to 70 °C before slightly declining at higher temperatures (Figure 6). This behavior highlights the thermal tolerance of *A. terreus* cellulase and informs potential applications under varying industrial conditions, while the decrease above 70 °C suggests a thermal limit for process design (Gasparotto et al., 2015).

Comparing enzyme activity across different pH and temperature conditions provides valuable data for selecting the optimal conditions for cellulase production in specific applications. For instance, in biofuel production, where lignocellulosic biomass breakdown is necessary, the ability to produce cellulase at optimal pH and temperature can significantly enhance the efficiency of the saccharification process. The results suggest that *A. terreus* could be a valuable source of cellulase for such applications, provided that the operating conditions are carefully controlled to maintain enzyme stability and activity (Nargotra et al., 2022).

Overall, the findings of this study enhance the understanding of cellulase production by marine fungi and its potential industrial applications. The crude cellulase

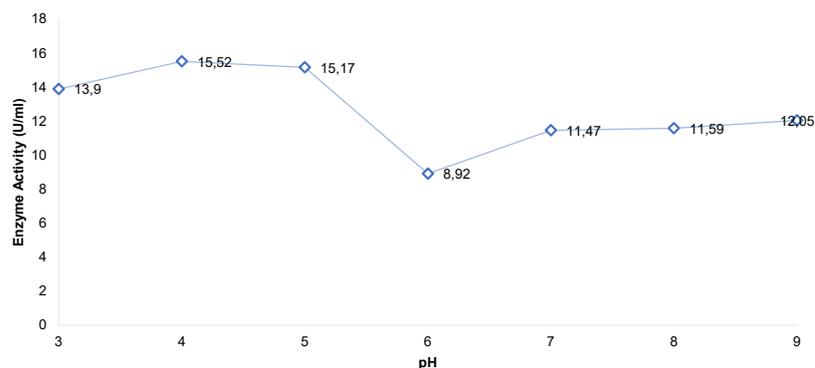


Figure 5. Enzyme Activity (U/ml) Curve on Different pH of Marine Fungi *Aspergillus terreus*

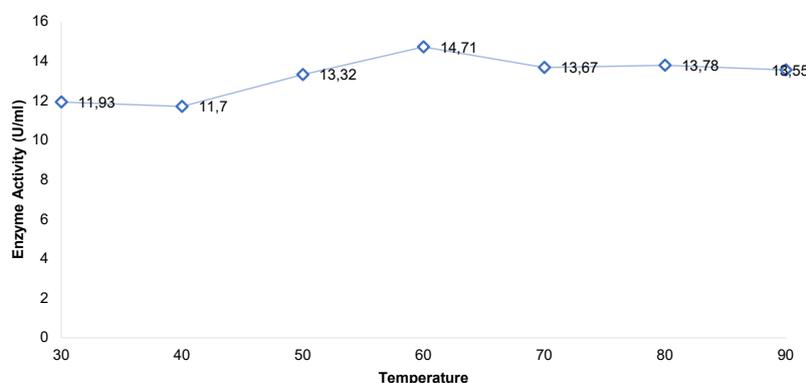


Figure 6. Enzyme Activity Curve on Different Temperatures of Marine Fungi *Aspergillus terreus*

from *Aspergillus terreus* exhibited optimal activity at pH 4 and 70 °C, demonstrating both acid and moderate heat tolerance. Compared to cellulases from other fungi, such as *Trichoderma reesei* and *Penicillium oxalicum*, which typically show optimal activity at pH 4–5 and 50–60 °C (Kuhad et al., 2016; Silveira et al., 2014), the marine *A. terreus* enzyme shows slightly higher thermal stability. In comparison with bacterial cellulases, for example from *Bacillus* spp., which often display higher thermal tolerance but lower activity under acidic conditions, *A. terreus* provides a balanced profile suitable for processes requiring acidic pH and moderate to high temperatures (Gasparotto et al., 2015).

By identifying the optimal conditions for *A. terreus* cellulase activity, this study provides a practical framework for its potential exploitation in biotechnological processes, including biomass hydrolysis, bioenergy production, and industrial enzyme applications. These results also reinforce the value of marine endophytic fungi as a sustainable enzyme source, aligning with the goals of the blue economy, which utilizes marine resources for both economic growth and environmental conservation (Cisneros-Montemayor et al., 2021).

HPLC analysis of cellulase enzyme

The HPLC chromatogram of hydrolysates produced by *Aspergillus terreus* cellulase revealed several distinct peaks, indicating the presence of multiple products generated during cellulose hydrolysis. A prominent peak appeared at approximately 5.928 minutes, likely corresponding to cellooligosaccharides (short-chain oligosaccharides) such as cellobiose or cellotriose, which are intermediate products of cellulose breakdown. Glucose, the expected monomeric product of cellulase activity, was detected at around 8.675 minutes, confirming that the enzyme effectively hydrolyzed cellulose into simple sugars.

It should be noted that no glucose standards were run in this analysis, and therefore, the exact concentration of glucose or other monosaccharides could not be quantified. The chromatogram provides a qualitative indication of cellulase activity and the distribution of hydrolysis products rather than precise concentrations. The peak pattern, however, aligns with typical cellulase hydrolysis profiles reported in previous studies, where initial cleavage produces oligosaccharides that are subsequently converted to glucose (Kumar et al., 2018).

Figure 7. HPLC chromatogram of hydrolysates from *Aspergillus terreus* cellulase. The peak at 5.928 min likely represents oligosaccharide intermediates, while the peak at 8.675 min corresponds to glucose produced during cellulose hydrolysis. Quantitative concentrations were not determined due to the absence of monosaccharide standards.

Marine endophytic fungi, such as *Aspergillus terreus*, are known to produce a wide array of natural products, including polysaccharides, enzymes, and bioactive compounds, with significant promise for various biotechnological applications (Ameen et al., 2021). Compared to conventional antimicrobial treatments, the high efficiency and potency of these natural products suggest their potential for developing new biopharmaceuticals, such as immunomodulators and treatments for cancer, microbial, and fungal diseases (Cutolo et al., 2024). This is particularly relevant in the context of the blue economy, where sustainable and renewable resources from marine environments are increasingly sought after to alleviate the burden on public healthcare systems (Ekasari et al., 2023). These findings highlight the potential of marine fungi to contribute to the pharmaceutical industry, providing new avenues for developing effective, low-toxicity treatments. Integrating such natural products into pharmaceutical pipelines could revolutionize the treatment of various diseases, offering

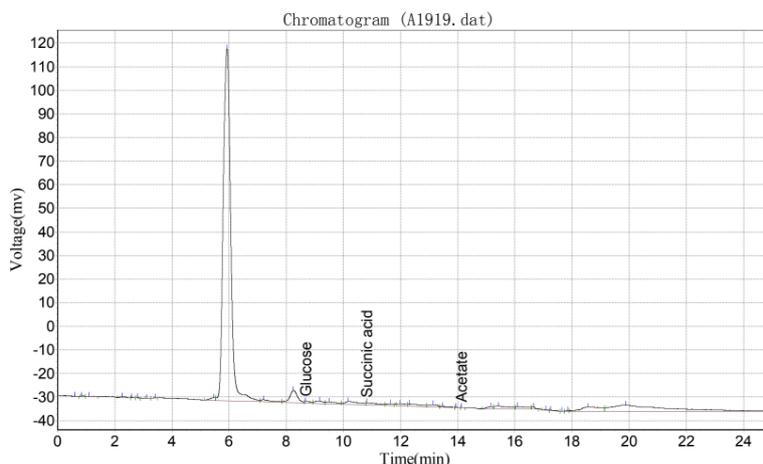


Figure 7. Chromatogram of *A. terreus* cellulase enzyme

cost-effective and less resistance-prone alternatives to traditional therapies (Rotter et al., 2023).

Conclusions

This study investigated the diversity, antibacterial activity, and cellulase production of marine endophytic fungi isolated from Buton Island, Southeast Sulawesi. A total of 32 fungal isolates were obtained from various hosts, including seaweed, seagrass, and mangrove leaves, and were characterized morphologically and taxonomically. Antibacterial screening against *Vibrio harveyi* revealed that several isolates, particularly WB 1-2, WB 6-2, and SM 27-2, exhibited clear inhibition zones of 11–13 mm, indicating notable antibacterial potential. Among the isolates, *Aspergillus terreus* (WB 1-2) was selected for cellulase production due to its superior activity on CMC agar. The crude enzyme demonstrated optimal activity at pH 4 and 70 °C, producing hydrolysates that included glucose, detected qualitatively by HPLC at ~8.675 minutes. Although exact glucose concentrations were not determined, the presence of glucose confirmed effective cellulose hydrolysis. These results highlight that marine endophytic fungi from Buton Island can serve as a source of bioactive compounds and industrially relevant enzymes. While further quantitative analysis and broader antibacterial testing are needed, this study provides a foundation for future research on the biotechnological applications of marine fungi in sustainable enzyme production and in the management of aquaculture pathogens.

Acknowledgements

The author would like to express their gratitude to the Ministry of Education, Culture, Research, and Technology for their generous support through the funding provided by the Scientific Research Program for the Doctoral Dissertation Research Scheme. The contract number for this grant is 3797/IT3.L1/PT.01.03/P/B/2022, dated May 11, 2022. This grant was awarded in the name of Dr. Kustiariyah, S.Pi, M.Si. This research received technical support from the National Research and Innovation Agency, Indonesia, and the College of Engineering at National Chung Cheng University, Taiwan (R.O.C.).

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