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Potential of marine sponge-derived fungi in the aquaculture system

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Abstract. *Bahry MS, Radjasa OK, Trianto A. 2021. Potential of marine sponge-derived fungi in the aquaculture system. Biodiversitas 22: 2883-2892.* Organic waste from aquaculture is one of the triggers of disease outbreaks and a decrease in water quality that urgently needs to be resolved. Indonesia has a high diversity of sponges including their associated microorganisms that potential in the field of biotechnology. This study aimed to determine the enzymatic and anti-vibrio activity of fungi associated with marine sponges and identify potential fungi. The specimen of sponges was collected from Samalona Island, South Sulawesi, Indonesia. The enzymatic and anti-vibrio assay was conducted by using the plug method and the activity was determined by a clear zone around the fungal isolates. Fungal identification was carried out molecularly using universal primers ITS1 and ITS4 and phylogenetic tree analysis. The fungal isolates were screened for the extracellular enzyme activity (amylase, cellulase, protease) and anti-vibrio activity against *Vibrio parahaemolyticus*, *V. harveyi*, and *V. vulnificus*). A total of three fungal isolates have been isolated from the sponge *Monanchora* sp. Isolate SL 3 SP 3.3 had potential enzymatic activities with Enzymatic Indeks (EI) 3.95±0.17 on amylase, 3.75±0.36 on cellulase, 5.38±0.30 on protease. The highest anti-vibrio activity was obtained against *V. harveyi* with an inhibition zone diameter of 4.82 ±0.37 mm. The results of fungal identification showed that isolate SL3SP3.3 had a sequence length of 638 bp and was closely related to *Trichoderma reesei* a.k.a *Hypocrea jecorina* with a similarity value of 99.69%.

Keywords: Amylase, anti-vibrio, associated fungi, cellulase, protease, sponge

INTRODUCTION

Aquaculture is an important aspect of the security of Indonesia's food resources. The development of marine aquaculture is increasing along with the high demand of the international market. Fish and shrimp are the leading commodities in the aquaculture sector and Indonesia is one of the largest exporters of fishery products to Japan, America, and the European Union (Wati 2018). However, the disease outbreaks in marine aquaculture, including vibriosis, are serious problems in the Indonesian mariculture industry. The Food and Agriculture Organization of the United Nations (FAO 2018) reports that these infections cause international losses of nearly US $ 3 billion per year.

The biggest problem in aquaculture is that 40-60% of the total production cost is allocated to feed, while the efficiency of feed absorption is not optimal (Olmos et al. 2011). This is due to aquaculture fish are carnivores that do not easily digest vegetable protein, while the carbohydrates in the feed are only absorbed by 20% because they are not the main energy source (Kurniawan et al. 2019). Excess nutrition cause problems because it requires more energy and prolongs the digestion period to hydrolyze protein, fat, and carbohydrate bonds (Rachmawati et al. 2020). On the other hand, improper pond management causes poor water quality that leads to vibriosis disease which can cause mass mortality in cultured shrimp and environmental pollution (Kusumaningrum and Zainuri 2015). The marine sponge is a marine organism that has high bioactivity. The genus Monanchora is rich in sources of novel secondary metabolites exhibiting diverse biological activities. The major group of metabolites of the genus Monanchora is guanidine-derived alkaloids (Dyshlovoy et al. 2016), which were isolated from different Monanchora species (Wang et al. 2013), and steroid (Wang et al. 2013). Guanidine-derived alkaloids (Dyshlovoy et al. 2016), showing the wide scope of biological activities, e.i. anti-parasitic (Santos et al. 2015), anticancer and antibacterial (Gogineni et al. 2020), antiviral (Hua et al. 2007), antifungal (Arevabini et al. 2014) and cytotoxic (El-Demerdash et al. 2016). The potential sources of natural products in Indonesia so far have not been well explored. The development of new drugs derived from marine biota is currently a concern of researchers because of its excellent potential and the unique structure of their secondary metabolites. Bioactive compounds derived from the sea can be an alternative in the development of new antibacterial drugs and biotechnological sources (Radjasa et al. 2009, 2011).

Suryanarayanan (2012) stated that marine bioactive substances are produced by sponges and produced by microbes living in or around the hosts called holobiont, including marine fungi. Fungi are categorized as "marine fungi" if they are obligate and sporulate independently in seawater (Proksch et al. 2003). The microbes associated

with sponges provide excellent bioprospects, such as antiviral, broad-spectrum antibacterial, antifungal, and antiprotozoal. Broad-spectrum antibacterial means that act against Gram-positive and Gram-negative pathogenic bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Clostridium* spp., *Escherichia* spp., and *Pseudomonas* spp. (Indraningrat et al. 2016). Some marine fungi *Trichoderma* sp. and *Penicillium* sp. isolated from sponges have activity against bacteria that cause vibriosis (Sibero et al. 2018). Fungi also produce hydrolytic and/or oxidative enzymes to play an important role in the ecological environment as decomposers (Panno et al. 2013) and has industrial application for biotechnological enzyme viz. alginate lyase, amylase, cellulase, chitinase, glucosidase, inulinase, keratinase, ligninase, lipase, nuclease, agarase, phytase, protease, and xylanase, cellulase, amylase, lipase, and pectinase.

MATERIALS AND METHODS

Sample collection

The sponge samples were collected from Samalona Island, Makassar, South Sulawesi, Indonesia: 5° 07' 37,410" SL 119° 20' 24,010" EL (Figure 1) at 5-10 m depth. The purposive random sampling method was used for the sampling method (Etikan et al. 2016). Sponge samples were documented under and above the water using underwater labels for identification purposes. Samples were transferred into the sterile ziplock and stored in the coolbox to avoid contamination.

Isolation and purification of the fungi

Fungal isolation was performed by tapping method according to Trianto et al. (2020). Sponge samples were cleaned using sterile marine water to remove microbial contaminant on the surface of the sponge then cut into approximately 1x1cm and tapped into the sterile PDA plate (Merck, Germany) with three repetitions. After 7 days of incubation, the emerging fungi were purified into the new sterile PDA plate using the plug method and incubated for 3-5 days at room temperature until the fungus grew (Wittriansyah et al. 2016). PDA was supplemented with chloramphenicol (2%) to avoid bacterial contamination.

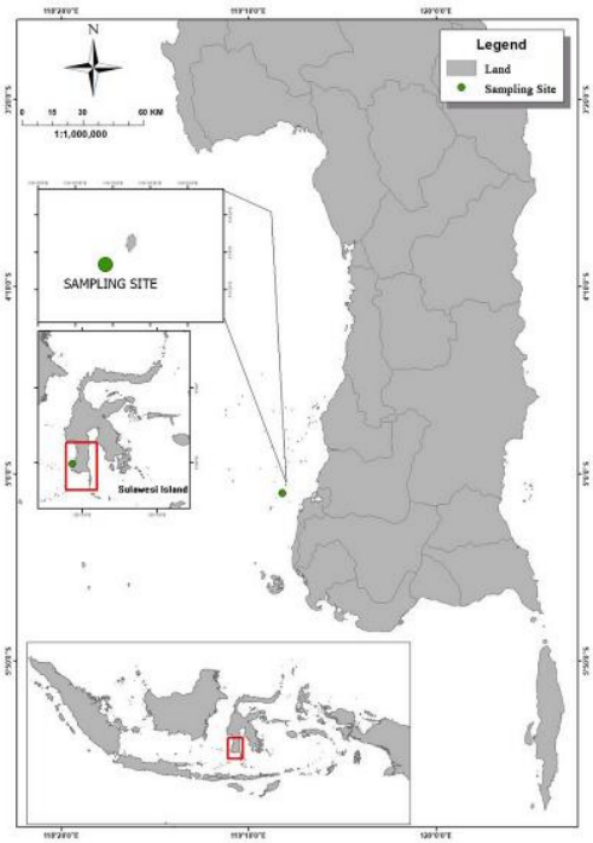


Figure 1. Sampling site of *Monanchora* sp. in Samalona Island, South Sulawesi, Indonesia (5° 07' 37.410" SL 119° 20' 24.010" EL)

The anti-vibrio screening

The anti-vibrio assay was conducted by the agar plug method (Sabdaningsih et al. 2017; Trianto et al. 2020). A total of 3 vibriosis causative (*Vibrio harveyi, V. parahaemolyticus,* and *V. vulnificus*), collection of Tropical Marine Biotechnology Undip Laboratory, Semarang were used for anti-vibrio screening. The Vibrio bacteria were grown on nutrient broth to a concentration of 0.5 McFarland and then inoculate on a trypticase soy agar (TSA) plate (Merck, Germany) using sterile cotton swabs (ONEMED, Indonesia). Seven days old of the fungal disk was plugged on TSA and incubated at 27ºC for 24h (Sibero et al. 2018; Cristianawati et al. 2019).

The enzymatic activity assay

Cellulase-producing fungi were screened on a CMC agar plate. A circle shape fungi (8 mm) from PDA medium was inoculated on a CMC agar plate (CMC 1%, Agar 2%) and incubated for 7 days at 30ºC (Coronado-Ruiz et al. 2018). Amylase activity was carried out using a soluble starch agar plate (2% soluble starch, and 2% agar). The fungal disk was placed on the soluble starch agar plate and incubated for 7 days (Khokhar et al. 2012; Ogbonna et al. 2014). Gram's iodine stain (2.0 g KI and 1.0 g iodine in 300 mL distilled water) was used as a hydrolysis indicator. On the last day of the incubation, CMC plates and soluble agar were flooded with a 10 mL Gram's iodine stain for 10 min (Colonia and Junior 2014). The amylase activity was determined by the starch hydrolysis, which can be seen in the presence of hydrolysis zone around the fungal plate colony (Lübeck and Lübeck 2018). The skimmed milk agar (SMA) plate was used to determine the extracellular protease production (Sharma et al. 2015). The SMA plate was made by mixing the suspension of agar and marine water (2,5%) then sterilized at 121°C for 15 min. The mixture was poured into a solution of 10% (w/v) of skimmed milk powder (Merck, Germany) that heated in a water bath at 50°C. The screening was done by inoculating the fungal disk onto the SMA plate and incubated at 27ºC for 96 h. The hydrolysis zone around the colony indicates protease activity due to the casein hydrolysis process (Kamath et al. 2010; Maitig et al. 2018). The enzymatic activity was determined by clear zone formation around the fungal disk (Lusi et al. 2017). The enzymatic index (EI) was measured as a semi-quantitative estimate of the enzyme activities, according to the formula below (Coronado-Ruiz et al. 2018; Maitig et al. 2018).

$$EI = \frac{Diameter\ of\ clear\ zone}{Diameter\ of\ colony}$$

Extraction and evaporation

After 7-day of incubation, the medium and mycelia of fungi were extracted using ethyl acetate as a solvent by maceration (Handayani et al. 2016) for 72 hours with solvent replacement every 24 hours (Sedjati et al. 2020). The filtrate was evaporated using a rotary vacuum evaporator (Eyela® N101, Tokyo, Japan) at 35ºC to get the concentrated extract (Bahry et al. 2017).

The anti-vibrio assay

The bioassay for anti-vibrio was carried out using the agar disk diffusion method (Sabdaningsih et al. 2019). Extracts that have been made with a dilution series (500, 250, 100 μg/disk) and diffused on a paper disk (6mm, Oxoid. ltd) The disk was placed on the surface of the plate that had been inoculated with vibriosis vector and incubated for 2x24 hours. Observations were carried out every 24 hours. Antibiotic chloramphenicol 30 μg was used as a positive control and solvent (DMSO 10%) was used as a negative control. (Dermawan et al. 2019).

Identification for potential sponge

Identification of sponges was performed by observing the shape of the spicules under a microscope (Sabdaningsih et al. 2019). The distribution and taxonomy of sponges were confirmed by using the online World Porifera Database, while the book Systema Porifera: A Guide to the Classification of Sponges was used as a reference for the identification of morphology and spicules. (Hooper and Van Soest 2002; De Voogd et al. 2008; van Soest et al. 2012).

Molecular identification for potential fungal

The DNA extraction of potential fungus was carried by DNA MiniPrep (ZYMO Research, USA). DNA amplification was performed using a polymerase chain reaction (PCR) thermal cycler (Biorad T100™, USA) and *internal transcribed spacer* (ITS) as the region of fungal DNA (Alvarez-Narrete et al. 2015). The reaction was performed using a total volume of 25 µL PCR mix which contain 12.5 µL of GoTaq Green Master Mix (Promega, USA), 1 µL of ITS1 (5′- TCC GTA GGT GAA CCT GCG G-3′) as forward-primer, 1 µL of ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) as a reverse-primer, 9.5 µL of ddH2O and 1 µL of DNA template. The PCR setting was: denaturation at 95ºC for 1 min; 34 cycles of denaturation at 95 ºC for 3 min, annealing at 56.1 ºC for 1 min, extension at 72 ºC for 1 min; final extension at 72 ºC for 7 min and cooling at 4°C until the reaction over (Trianto et al. 2021). The quality of the PCR products was assessed using electrophoresis at 1% agarose. The visualized PCR results were analyzed at 1st Base Laboratories, Malaysia through PT. Genetics Science Jakarta for sequencing. DNA sequences were analyzed for homology using the Basic Local Alignment Search (BLAS) (www.ncbi.nlm.nih.gov). Phylogenetic trees were reconstructed and analyzed using MEGA 7.0 software while the neighbor-joining method with 1000 bootstrap replication was chosen for statistical analysis. (Kumar et al. 2016; Trianto et al. 2021).

RESULTS AND DISCUSSION

The Spermonde archipelago of Makassar water was chosen as the sampling site because of the biodiversity of the marine invertebrate especially the marine sponge (De Voogd et al. 2006). Samalona Island is the middle inner zone of the Spermonde archipelago which is dominated by

a healthy coral reef ecosystem (Muller et al. 2014; Yusuf et al. 2021). The sample of SL.3-SP3 is an encrusting sponge that covers a dead gorgonian.

The photograph of sponge SL.3-SP3 and the spicule were presented in Figure 2. The SL.3-SP3 sponge has four different megasleres i.e.; style (Figure 2C. D), oxea (Figure 2E), diaene (Figure 2F), sphaerancora (Figure 2G), sigma c (Figure 2H). Based on its megascleres, the sample SL.3-SP3 sponge is identified as *Monanchora* sp. (Van Soest et al. 1996) reported that *Monanchora* sp. contains all spicules including styles and sigma.

The characteristics *Monanchora* sp. are Crambeidae without pseudoastrose, encrusting to a lobate or ramose life form with smooth or extended into corrugated or spined projections surface (Hooper and Van Soest 2002). This sponge is commonly found around the world viz; Brazil (Santos et al. 2015), Thailand (Kaewkrajay et al. 2021), Jamaica (Hua et al. 2007). *Monanchora* sp. was also found in Indonesia viz; Seribu island (Hadi 2011), North Sulawesi (Calcinai et al. 2017).

The bioactive compounds in *Monanchora* sp. are mostly found as alkaloids, i.e. batzelladine isolated from the Caribbean sponge *Monanchora* sp. has activity against human cancer cell lines, protozoa, HIV-1, and AIDS opportunistic infectious pathogens (Hua et al. 2007). Monanchocidin from the *Monachora pulchra* has anticancer activity against cervical cancer and monocytic leukemia in human and mouse epidermal cell line in mouse (Ibran et al. 2018). Guanidine, an alkaloid that has cytotoxic properties and prevents EGF-Induced Neoplastic was isolated from *Monachora pulchra* (Dyshlovoy et al. 2016). Gogineni et al. (2020) reported that Monanchocidin A has the terrific activity against pathogenic microorganisms including bacteria (*Staphylococcus aureus* ATCC 29213, Methicillin-resistant *S. aureus* (MRSA) ATCC 33591, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068), and fungi (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Aspergillus fumigatus* ATCC 204305, and *Cryptococcus neoformans* ATCC 90113). It is proven that activity is bigger than the antibiotic control (Ciprofloxacin and amphotericin B).

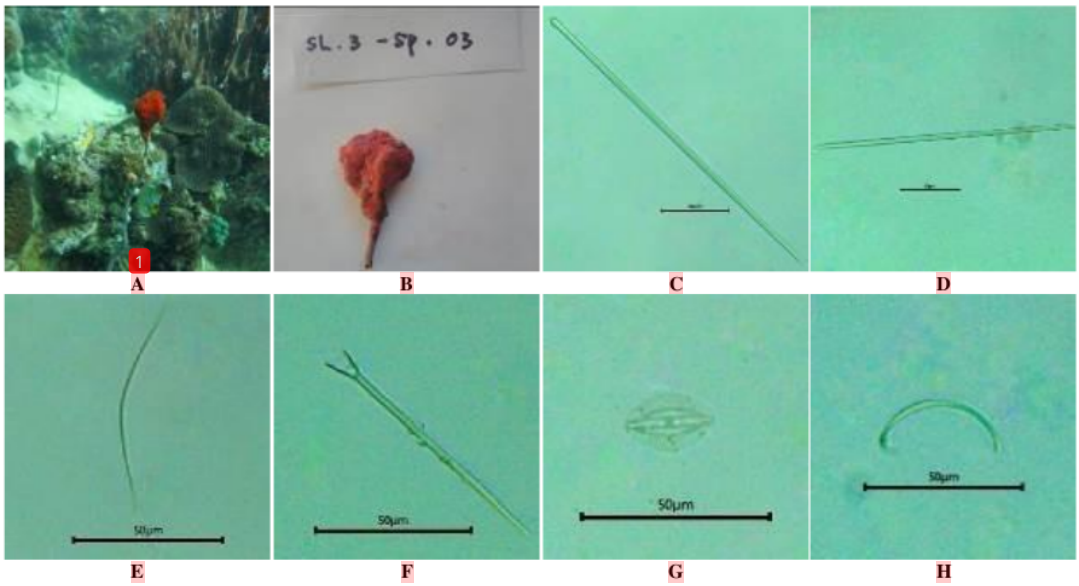
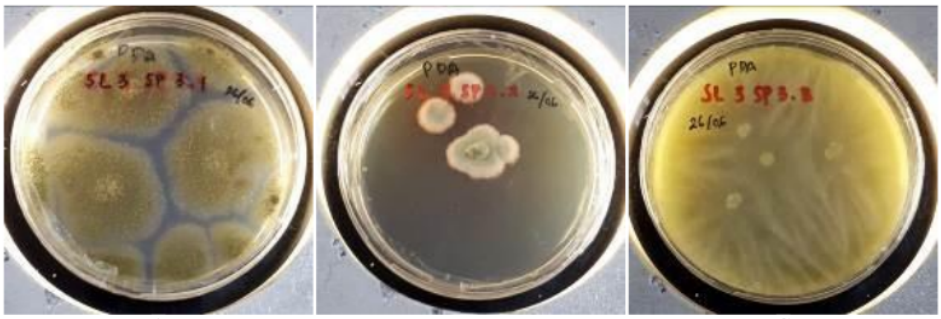


Figure 2. The picture of the SL 3 SP 3 sponge and its spicules. A. The under the water picture, B. The above the water picture, C. Style spicule, D. Style spicule, E. Oxea spicule, F. Diaene spicule, G. Sphaerancora spiclue, H. Sigma spicule

Table 1. The morphology of 3 fungal isolates from sponge SL.3-SP.03

Isolate code	Colour	Filament	Spora	Note
SL 3 SP 3.1	Green	Nonfilamentous	Spore	-
SL 3 SP 3.2	Grey	Nonfilamentous	Spore	-
SL 3 SP 3.3	White-green	Filamentous	Non-sporous	Produce yellow pigment



A B C

Figure 3. The morphology of fungal colony of: A. SL.3-SP.03.1, B. SL.3-SP.03.2, C. SL.3-SP.03.3

A total of 3 fungal isolates were obtained from the Monanchora sponge. The small number of fungi isolated from *Monanchora* was due to the Monanchora sponges were categorized as low microbial abundance (LMA) sponges (Gloeckner et al. 2014). A previous study by Kaewkrajay et al. 2021 showed that there was no culturable microbial found from 6 samples of *M. unguiculata* sponge taken from the Gulf of Thailand, South China Sea. All three fungal isolates have different characteristics, as shown in Figure 3, and their morphological characteristics were presented in Table 1. The isolate SL 3 SP 3.3 has a unique characteristic by producing yellow pigment which was shown in the color change of the medium.

The results showed that the SL3 SP3.2 isolate had neither enzymatic activity nor antibacterial against three Vibrio species. Isolate SL 3 SP 3.3 had antibacterial activity against three Vibrio species and three enzymatic activities, while the SL 3 SP 3.1 isolate only inhibits the growth of *V. vulnificus* (Table 2). Antibacterial activity is categorized as bacteriostatic and bactericidal. According to Silva et al. (2011) bactericidal activity was indicated by the absence the bacterial colony growth or reduction of 99.9% inoculum bacterial and bacteriostatic activity was indicated as maintenance of the original inoculum or a reduction of less than 99.9% the inoculum bacterial.

The enzymatic activity of fungal isolates is shown in Figure 4.A (amylase), 4.B (cellulase), and 4.C. (protease). A clear area or hydrolysis zone around the fungal colony indicated hydrolysis of the test media due to the activity of the enzyme (amylase, cellulase, protease) (Rengasamy and Thangaprakasam 2018). Cellulase-producing microorganisms were screened on agar plates enriched with CMC as a carbon source and using Gram iodine as an indicator. Qualitative determination is based on the presence of cellulose hydrolysis which is characterized by a clear zone around the fungal colony. This is due to the interaction of iodine with cellulose and its degraded components so that the integral biopolymer retains Gram iodine dye (Coronado-Ruiz et al. 2018). For protease production, the nitrogen source of natural protease production was determined by using different sources (peptone, tryptone, casein, and yeast extract) (Ahmed 2018).

Molecular identification of SL3 SP3.3 fungal isolate

The molecular identification process was initiated by extracting DNA of potential isolates SL 3 SP 3.3 using a DNA extractor. The results of electrophoresis visualization are used to determine the success of DNA extraction as indicated by the appearance of bands (white lines) from the PCR product samples (Figure 5) Figure 5. shows that DNA samples of fungal isolate SL 3 SP 3.3 have been successfully extracted with a length of ± 500 base pairs. This stage determines the feasibility of the sample for sequencing.

BLAST analysis on NCBI was used to determine the level of similarity of the isolates compared to the isolates in GenBank data. The sample was analyzed based on the similarity of the nucleotide acid composition with a certain basepair length. Table 3 shows the result of homology analysis of the isolate sequence of SL 3 SP 3.3 which has a sequence length of 638 bp and has a 99.69% similarity with the *Trichoderma reesei* RHa strain under the accession number KM246746.1. Primers covered the sequence length of the fungal isolate ITS 1 and ITS 4 with amplification ranged 750 and 500bp (Yan et al. 2011). ITS primer is a primer that matched 99% of ascomycete and basidiomycete taxa (species, subspecies, or varieties) based on public sequence databases named in silico analysis (Toju et al. 2012).

The phylogenetic tree with the maximum likelihood method is shown in Figure 6 and was made based on the Internal Transcribed Spacer (ITS) region, with 1000 bootstrap replications. The number of each node presents bootstrap values from Neighbor-Joining (NJ). The sample has a branch trust value of 1000 (100%) with the *Trichoderma reesei* acc number KM246746.1. which is shown by its position to form the same clade.

T. reesei has specific hyphae characteristics and has blue color with methylene blue dye under the microscope. The culture medium affects the morphological shape of

fungus. Carpa et al. (2018) reported that *T. reesei* formed "bundle" granules which were difficult to distinguish between off-cornched mycelia and growth hyphae and off-cornched conidiophores with sporangial heads on solid media observed by SEM. *T. reesei* lengthens the hyphae and increased hyphal branching to increase interaction with the substrate thereby increasing the production of enzymes. *T. reesei* is commonly found in asexual form (teleomorph *Hypocrea jecorina*) (Zhang et al. 2019).

Bioassay was performed to evaluate the activity of SL 3 SP 3.3. (*T. reesei*) crude extract against three species of Vibrio (*V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*) with the concentrations of 500, 256, 100 μg/disk. The highest activity was obtained at a concentration of 500 μg/disk against *V. harveiy* with an inhibition zone of 4.82 ± 0.37mm, while the diameter of the inhibitory zone of positive control was 19.50 ± 1.66mm.

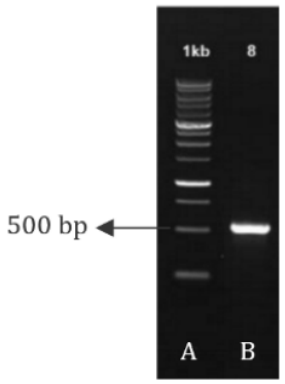


Figure 5. A. DNA ladder, B. DNA template of isolate SL 3 SP 3.3

Tabel 2. Screening of anti-vibrio activity and enzyme activity of fungi isolated from sponge SL.3 SP03

Isolate Code	Anti-vibrio activity						Enzyme activity		
	V. harveyi		*V. vulnificus*		*V. parahaemolyticus*		Amilase	Selulase	Protease
SL 3 SP 3.1	-	-	+	Static	-	-	-	-	-
SL 3 SP 3.2	-	-	-	-	-	-	-	-	-
SL 3 SP 3.3	+	bactericidal	+	bactericidal	+	bacteriostatic	+	+	+

Tabel 3. Identification of potential fungal isolated from *Monanchora* sp. based on BLAST analysis using the ITS region

Sponge	Isolate	Sequence length (bp)	Acc. no.	Next relative by GenBank alignment (AN, an organism)	Similarity (%)	Family
Monanchora sp.	SL 3 SP 3.3	638	MW555831	KM246746, *Trichoderma reesei* RHa	99.69%	Hypocreaceae

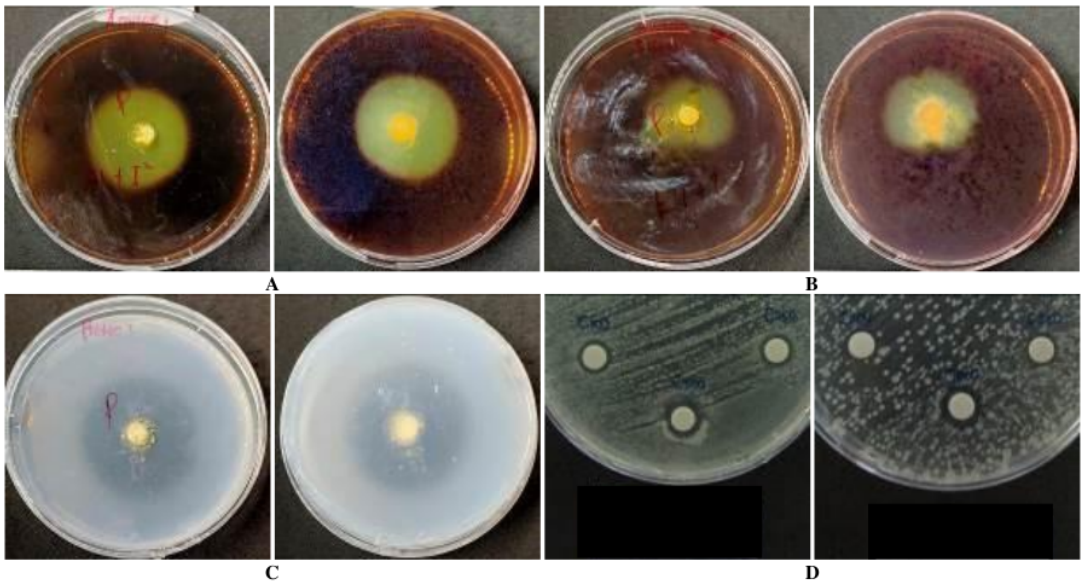


Figure 4. Screening of enzymatic activity of SL3 SP 3.3 isolate: A. Amilase, B. Selulase, C. Protease, and D. Anti-vibrio Please indicate in Figure 4.D with an arrow which one is bacteriostatic and which one is bactericidal

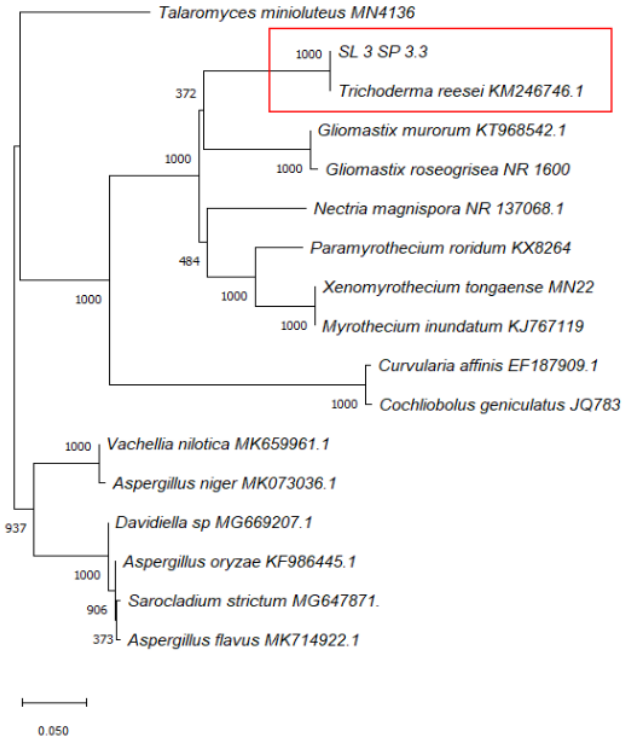


Figure 6. Phylogenetic tree of *Monanchora* sp. The potential fungus SL-3 SP3.3 is indicated by the red square

Enzymes produced by *T. reesei* play an important role in the synthesis of antibiotics through the mechanism of myco-parasitism in bacteria and antibiosis against bacteria. Exocellular enzymes such as cellulolytic, hemicellulolytic, pectolytic, and proteolytic enzymes can damage the main polymer component that makes up the microbial cell walls so that they can function as biocontrols against pathogens. The study results showed that the SL3 SP3.3 extract was able to inhibit the growth of 3 Vibrio species that are Gram-negative bacteria. The anti-vibrio activity of SL3 SP3.3 due to *T. reesei* to release exocellular enzymes to attack the vibrio bacterial cell walls in the form of lipopolysaccharides peptidoglycans which are polysaccharides and also proteins that can be hydrolyzed by cellulase and protease enzymes. Sorbicillinoid compound is an example of a secondary metabolite produced by the sponge-derived fungus *T. reesei* (HN-2016-018) with potent antibacterial activity, especially against Gram-negative bacteria (Rehman et al. 2020). In the future, *T. reesei* has the potential to be applied in marine culture as a biocontrol agent against pathogenic diseases. A study by Assem et al. (2014) showed that fungi *T. reesei*-degraded date pits (FDDP) have the potential to reduce the density of bacterial population in the intestines of *Oreochromis niloticus* fish without impacting the fish weight or health welfare condition. A previous study by Liu et al. (2016) reported that the *Trichoderma* population plays a role in suppressing the disease caused by *Saprolegnia* in aquaculture.

Tabel 4. Diameter of inhibition zone of *Trichoderma reesei* SL 3 SP 3.3 extract against three Vibrio species

Isolate code	Concentration (µg/disk)	Inhibition zone (mm)		
		V. harveyi	*V. parahaemolyticus*	*V. vulnificus*
SL.3-SP.03.3	500	4.82 ±0.37*	3.83±0.2*	4.14±0.32*
	250	3.47±0.18*	1.67±0.15	1.57±0.1
	100	2.32±0.06*	1.51±0.15	1.1±0.02
	-	-	-	-
	+	19.50±1.66*	15.76±1.48*	17.45±1.23

Note : * bactericidal

Tabel 5. Enzymatic Index (EI) of *Trichoderma reesei* isolate SL 3 SP 3.3

Enzymatic activity	Diameter of colony (mm)				Diameter of clear zone (mm)				Enzymatic Index (EI)				
	R1	R2	R3	A	R1	R2	R3	A	R1	R2	R3	A	stdev
Amylase	10.28	9.31	9.76	9.78	38.82	36.79	40.11	38.57	3.78	3.95	4.11	3.95	0.17
Selulase	9.41	8.26	8.04	8.57	32.80	29.76	33.43	31.99	3.48	3.60	4.16	3.75	0.36
Protease	8.44	9.42	8.78	8.88	46.71	47.43	48.94	47.69	5.53	5.04	5.57	5.38	0.30

Note: R1: first repetition, R2: second repetition, R3: thirth repetition, A: Average, Stdev: Standard Deviation

Enzymatic index (EI) is the semi-quantitative approach to measure enzyme activities. The results showed that *T. reesei* has higher protease activity than other enzyme activity. The enzymatic index of protease was EI of 5.38 ± 0.30. The mechanism of the proteolytic activity is due to the hydrolysis of protein bonds originating from skim milk agar (SMA) media into simpler amino acids. Dienes et al. (2007) found that proteolytic activity in the fungus *T. reesei* which was later identified as a serine protease from fungus (a trypsin-like), has similarities protease P27 enzyme from *Trichoderma harzianum*. The proteolytic activity of *T. reesei* was originated from protein kinase, casein kinase II and protein kinase C10 which were synthesized by several gene transcription factors in the form of XYR1 (xylanase regulator 1), ACE1 (activator of cellulases 1), ACE2, HAP2/3/5 (HAT associated proteins), and CRE1 (Rodriguez-Iglesias and Schmoll 2019).

Trichoderma reesei is widely known as a cellulase-producing microbe that has been applied in various fields of biotechnology. In this research, the cellulase activity of *T.reesei* SL3 SP3.3 was EI of 3.75 ± 0.36 which is the lowest enzymatic activity compared to amylase and protease. However, the cellulase activity of *T.reesei* SL3 SP3.3 was higher than the cellulase-control organism *P. ostreatus* EI of 1.8 ± 0.1 and largest cellulase produce-fungi (*Penicillium chrysogenum*) (EI of 3.3 ± 0.2) of Coronado-Ruiz et al. (2018). There are three types of cellulase enzymes in *T. reesei*: the cellobiohydrolase group of enzymes, Endo-β-1,4-D-glucanases, and β-D-glucosidases (Druzhinina and Kubicek 2017). At least three genes are responsible for regulating the cellulase and hemicellulase genes, namely ACE3, XYR1, and Crt1. Gene yellow pigment regulator 1 (ypr1) also has the responsibility to produce yellow pigment in *T. reesei* as shown in Figure 3.C. The genes are regulated by the finetuned cooperation between several transcriptional factors in *T. reesei*. (Zhang et al. 2019). For industrial applications, several optimizations are used to maximize cellulase production such as; protein induction (Daranagama et al. 2019), modification of growth substrate (Peciulyte et al. 2014), transcriptomic engineering (Pakula et al. 2016). The correlation between protease activity and cellulase activity in *T. reesei* is still unclear or even non-existent (Rodriguez-Iglesias and Schmoll 2019).

Filamentous fungi can degrade several types of polysaccharides that are naturally abundant in nature. Starch is one of the polysaccharides are composed of glucose. Based on enzymatic assay (Tabel 5), SL 3 SP 3.3 isolate has an amylolytic activity (EI of 3.95 ± 0.17). Fungi synthesize large amounts of starch-hydrolytic enzymes, such as α-amylase, glucoamylase, and α-glucosidase. These enzymes play an important role in the induction of starch, dextrin, or maltose. Amylolytic cleaves the 1,4-glycosidic bonds in starch (polysaccharides) into glucose, maltose, and other oligosaccharides. The enzyme is encoded by the gene encoding-amylase (amyA/B/C) (Wang et al. 2020). Therefore, *T. reesei* has the potential as a probiotic added to the aquaculture fish feed (Assem et al. 2014).

The biotechnological potential of fungi isolated from marine sponge *Monanchora* sp. is quite promising. Considering that one of the problems in the aquaculture system is the poor regulation of water quality which effect the remaining feed containing protein, cellulose, and starch that are not completely degraded so that the enzymatic ability of the isolate *T. ressei* SL 3 SP 3.3 has the potential to be applied in marine aquaculture. The anti-vibrio ability of *T. reesei* SL 3 SP 3.3 has the potential as a biocontrol to overcome the diseases in marine aquaculture, which are dominated by vibriosis disease due to its anti-vibrio activity. Bioremediation and probiotics are the most potential mechanisms for resolving these problems.

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Potential of marine sponge-derived fungi in the aquaculture system

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