Potential of marine sponge-derived fungi in the aquaculture system

MUHAMMAD SYAIFUDIEN BAHRY1,2, OCKY KARNA RADJASA3,4, AGUS TRIANTO1,2*

1Department Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Soedarto, SH, Tembalang, Semarang 50275, Central Java, Indonesia. Tel./fax.: +62-24-7474498, *email: agusstrianto.unsdip@gmail.com.
2Marine Natural Product Laboratory, Centre for Research and Services, Universitas Diponegoro. Jl. Prof. Soedarto, S.H. Tembalang, Semarang 50275, Central Java, Indonesia
3Tropical Marine Biodiversity Laboratory, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Soedarto, SH, Tembalang, Semarang 50275, Central Java, Indonesia
4Research Center for Oceanography, Indonesian Institute of Sciences. Jl. Pasir Puteh I, Ancol Timur, North Jakarta 11048, Jakarta, Indonesia

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 largest commodities in the aquaculture sector in Indonesia and Indonesia is one of the largest exporters of fishery products to Japan, America, and the European Union. 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 causes health 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.g. 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 its 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)](image-url)
The anti-vibrio screening

The anti-vibrio assay was conducted by the agar plug method (Sabdanningsh et al. 2017; Trianto et al. 2020). A total of 3 vibrios 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 27°C 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 30°C (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 27°C 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 35°C 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 (Sabdanningsh et al. 2019). Extracts that have been made with a dilution series (500, 250, 100 μg/disk) are 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 (Sabdanningsh 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-Navarrete 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 TAC GG GGA AAA CTG CCG G-3′) as reverse-primer, 9.5 μL of ddH2O and 1 μL of DNA template. The PCR setting was: denaturation at 95°C for 1 min; 34 cycles of denaturation at 95 °C for 3 min, annealing at 56.1 °C for 1 min, extension at 72 °C for 1 min; final extension at 72 °C 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 megascleres 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 of 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 (Kaewkray et al. 2021), Jamaica (Hua et al. 2007). Monanchora sp. was also found in Indonesia viz; Seribu island (Hadi 2011), North Sulawesi (Calcina 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 Monanchora pulchra has anticancer activity against cervical cancer and monocytic leukemia in human and mouse epidermal cell line in mouse (Kiran et al. 2018). Guanidine, an alkaloid that has cytotoxic properties and prevents EGF-Induced Neoplastic was isolated from Monanchora 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 intracellularare 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](image_url)

**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. Daene spicule, G. Sphaerancora spicule, H. Sigma spicule

| 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 total of 3 fungal isolates were obtained from the Monanchora sponge. The small number of fungi isolated from *Monanchora* sp. 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 SP 3.2 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 Thangaparakasam 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 SP 3.3 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* RH3 strain under the accession number KM246746.1. Primers covered the sequence length of the fungal isolate ITS 1 and ITS 4 with amplification ranged 750bp 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-corncched 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, 250, 100 µg/disk. The highest activity was obtained at a concentration of 500 µg/disk against *V. harveyi* with an inhibition zone of 4.82 ± 0.37mm, while the diameter of the inhibitory zone of positive control was 19.50 ± 1.66mm.

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

| Isolate Code | 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 | +      | +       | +       |

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

**Note:** *bactericidal

**Figure 6.** Phylogenetic tree of *Monanchora* sp. The potential fungus SL-3 SP3.3 is indicated by the red square.
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 enzymic 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 (xylosanase 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 SP 3.3 was EI of 3.75 ± 0.36 which is the lowest enzymic activity compared to amylase and protease. However, the cellulase activity of T. reesei SL3 SP 3.3 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 cellobiohydrolyase group of enzymes, Endo-β-1,4-β-glucanases, and β-1,4-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. reesei SL 3 SP 3.3 has the potential to be applied in marine aquaculture. The antivibrio 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.

**ACKNOWLEDGEMENTS**

The authors would like to thank the Ministry of Research and Technology, Indonesia, which has supported this research through a research grant for the Higher Education Research Consortium (KRU-PT) with contract number 201-01/ UN7.6.1/PP/2020 under the supervision of Dr. Agus Trianto.

**REFERENCES**

Ahmed ME. 2018. Extraction and purification of protease from Aspergillus niger isolation. Pharm Pharmacol Intl J 6: 96-99. DOI: 10.15406/ppij.2018.06.00162.

Alvarez-Navarrete M, Reyina Lopez GE, Flores-Garcia A, Lopez Gomez R, Martinez-Pacheco MM. 2015. Selection and molecular identification of fungal isolates that produce xylanolytic enzymes. Genet Mol Res 14: 8100-8116. DOI: 10.4238/2015.July.17.19.

Areavbin C, Civlentti YD, De Abreu MH, Bitencourt TA, Santos MFC, Berlinck RGS, Hadu E, Beleboni RO, Fachin AL, Marins M. 2014. Antifungal activity of metabolites from the marine sponges Amphimedon sp. and Monanchora arbuscula against Aspergillus flavus strains isolated from peanuts (Araechnis hypogaeus). Nat Prod Commun 9: 33-36. DOI: 10.1177/1934578X1400900111.

Assem H, Khalifa A, EL Salhia M. 2014. Physiological and microbiological indices as indicators of evaluating dietary fungi degraded date pits as a probiotic for cultured Nile tilapia Oreochromis niloticus fingerling and its effect on fish welfare. Egypt J Aquat Res 40: 435-441. DOI: 10.1016/j.ejar.2014.10.004.
Bahry MS, Prangenegers D, Trianto A. 2017. Molecular identification of marine symbiont bacteria of Gastropods from the waters of the Krakal Coast Yogyakarta and its potential as a multi-drug Resistant (MDR) Antifungal Agent. AIP Conf Proc 1803: 02019. DOI: 10.1063/1.4973146.

Chekane B, Pavlik H, Beavestrello G, Bertolino M, Horcadjadas SB, Pansini M, Makapedua DM, Cerrano C. 2017. Demosponge diversity from North Sulawesi, with the description of six new species. Zootaxa 2017: 105-150. DOI: 10.11604/zootax.680.12135.

Carpa R, Cândea A, Remizovschi A, Barbu-Tudoran L, Maor MC. 2018. Cellulase production and morphology of Trichoderma reesei in different experimental conditions. Studia Universitatis Babes-Bolyai Biologia 63: 115-129. DOI: 10.24193/subbiol.2018.2.09.

Colonia BSO, Junior AFC. 2014. Screening and detection of extracellular cellulases (endo- and exo-glucoanases) secreted by filamentous fungi isolated from soils using rapid tests with chromogenic dyes. Afr J Biotechnol 13: 4694-4701. DOI: 10.5897/AJB2014.14221.

Coronado-Ruiz C, Avendaño R, Escudero-Leyva E, Conoto-Baroza G, Chaverrri P, Chavarría M. 2018. Two new cellulolytic fungal species isolated from a 19th-century art collection. Sci Rep 8: 1-9. DOI: 10.1038/s41598-018-24934-7.

Cristianawati O, Sabdannangsh M, Becking LE, Khoei MM, Nuryadi H, Sabdono A, Trianto A, Radaja OK. 2019. Biological activity of sponge-associated fungi from Karimunjawa islands, Indonesia against pathogenic streptococcus pneumoniae. Biodiversitas 20: 2143-2150. DOI: 10.15408/biodivind.v20i00807.

Duranumia K, Yki K, Mato H, Ohtaki Y, Suzuki Y, Shida Y, Ogawasa W. 2019. Proteolytic analysis of Trichoderma reesei in celluase-inducing condition reveals a role for trichodermepapsin (TrAsP) in cellulase production. J Ind Microbiol Biotechnol 46: 831-842. DOI: 10.1007/s10295-019-02155-9.

de Voogd NJ, Cleary DR, Hoeksema BW, Noor A, Van Soest RW. 2006. Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. Mar Ecol Prog Ser 309: 131-142. DOI: 10.3354/meps309131.

de Voogd NJ, Francis D, Cleary R. 2008. Indo-pacific agelas view project Novel cytotoxic marine natural products View project. Mar Ecol 29: 205-215. DOI: 10.1111/j.1439-4856.2008.0238x.

Dermawan AM, Juliante E, Putra MY, Karim F. 2019. Identification and Evaluation of Antibacterial Compounds from the Vibrio sp. associated with the Ascidian Pyocinovella diminuta. Pharrm Sci Res 6: 142-148.

Dienes D, Borjeson J, Häggblund P, Tjerneld F, Lidén G, Réczy K, Stålbrand H. 2007. Identification of a trypsin-like serine protease from Trichoderma reesei QM9414. Enzyme Microb Technol 40: 1087-1094. DOI: 10.1016/j.enmic.2006.08.013.

Drzuhinica IB, Kubick CP. 2017. Genetic engineering of Trichoderma reesei cellulase cascade enzyme production. Microb Biotechnol 10: 1485-1499. DOI: 10.1111/1751-7915.12726.

Dyshlovoy SA, Tabakmakher KM, Haus J, Garcia-Fernandez LF, Kelly M, Hamann MT. 2007. Bazzettalidine alkaloids from the caribbean sponge Monanchora turergiifera and the significant activities against HIV-1 and AIDS opportunistic infectious pathogens. Tetrahedron 63: 11179-11188. DOI: 10.1016/j.tet.2007.08.005.

Duranumia K, Yki K, Mato H, Ohtaki Y, Suzuki Y, Shida Y, Ogawasa W. 2019. Proteolytic analysis of Trichoderma reesei in celluase-inducing condition reveals a role for trichodermepapsin (TrAsP) in cellulase production. J Ind Microbiol Biotechnol 46: 831-842. DOI: 10.1007/s10295-019-02155-9.

de Voogd NJ, Cleary DR, Hoeksema BW, Noor A, Van Soest RW. 2006. Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. Mar Ecol Prog Ser 309: 131-142. DOI: 10.3354/meps309131.

de Voogd NJ, Francis D, Cleary R. 2008. Indo-pacific agelas view project Novel cytotoxic marine natural products View project. Mar Ecol 29: 205-215. DOI: 10.1111/j.1439-4856.2008.0238x.

Dermawan AM, Juliante E, Putra MY, Karim F. 2019. Identification and Evaluation of Antibacterial Compounds from the Vibrio sp. associated with the Ascidian Pyocinovella diminuta. Pharrm Sci Res 6: 142-148.

Dienes D, Borjeson J, Häggblund P, Tjerneld F, Lidén G, Réczy K, Stålbrand H. 2007. Identification of a trypsin-like serine protease from Trichoderma reesei QM9414. Enzyme Microb Technol 40: 1087-1094. DOI: 10.1016/j.enmic.2006.08.013.

Drzuhinica IB, Kubick CP. 2017. Genetic engineering of Trichoderma reesei cellulase cascade enzyme production. Microb Biotechnol 10: 1485-1499. DOI: 10.1111/1751-7915.12726.

Dyshlovoy SA, Tabakmakher KM, Hausch J, Shchekaleva RK, Otte K, Guzi AG, Makarieva TN, Kudryashova FK, Fedorov SN, Shubina LK, Bokemeyer C, Honecker F, Stonik VA, Von Amsberg G. 2019. Guanidine alkaloids from the marine sponge Monanchora pulchra show cytotoxic properties and prevent EGF-induced neoplastic transformation in vitro. Mar Drugs 14: 1-17. DOI: 10.3390/md14070133.

El-Demerdash A, Morieu C, Martin M-T, Rodrigues-stien ADS, Petek S, Demey-stien Mark H, Hall K, Hooper JNA. Al-mourabat A. 2016. Cytotoxic guanidine alkaloids from a French Polynesien Monanchora n. sp. Sponge. J Nat Prod 79: 1929-1937. DOI: 10.1021/acs.jnatprod.6b00168.

Etkin L, Musa SA, Alkassim RS. 2016. Comparison of convenience sampling and purposive sampling. Am J Theor Appl Stat 5: 1-4. DOI: 10.11648/j.atst.201505011.

AOA. 2018. The State of World Fisheries and Aquaculture- Meeting the sustainable development goals. Food and Agriculture Organization of the United Nations, Rome.

Gloeckner V, Wehr M, Motinho-Silva L, Gernert C, Hentschel U, Schupp P, Pawlik JR, Lübeck M, Lübeck PS. 2018. Identification and screening of cellulolytic filamentous fungi. J Appl Sci Environ Manag 15: 203-206. DOI: 10.1007/s10482-018-0328-x.

Gomi H, Honda M, Makihira H. 2007. Classification of Sponges. Kluwer Academic/Plenum Publishers.
oceanica. New Biotechnol 30: 686-694. DOI: 10.1016/j.nbt.2013.01.010.

Pecutily A, Anasontizis GE, Karlström K, Larsson PT, Olsson L. 2014. Morphology and enzyme production of Trichoderma reesei Rut C-30 are affected by the physical and structural characteristics of cellulosic substrates. Fungal Genet Biol 72: 64-72. DOI: 10.1016/j.fgb.2014.07.011.

Proksch P, Ebel R, Dradara RA, Wray V, Steube K. 2003. Bioactive natural products from marine invertebrates and associated fungi. Mar Mol Biotechnol 37: 117-142. DOI: 10.1007/978-3-642-55519-0_5.

Rachmawati D, Hutabarat J, Dewi EN, Windarto S. 2020. Supplementation of papain in feed on growth performance, efficiency of feed utilization, and survival rate of whiteleg shrimp (Litopenaeus vannamei). J Mar Res 9: 215-222.

Radjasa OK, Kencana DS, Sabdono A, Hutagalung RA, Lestari ES. 2009. Antibacterial activity of marine bacteria associated with sponge Aiptos sp. against Multi Drugs Resistant (MDR) strains. Jurnal Matematika dan Sains 12: 147-152.

Radjasa OK, Vaske YM, Navarro G, Vervoort HC, Tenney K, Linnington RG, Crews P. 2011. Highlights of marine invertebrate-derived biosynthetic potential: Their biological potential and possible production by microbial associates. Bioorg Med Chem 19: 6656-6674. DOI: 10.1016/j.bmc.2011.07.017.

Rehman SU, Yang LJ, Zhang YH, Wu JS, Shi T, Haidar W, Shao CL, Wang CY. 2015. Antibacterial activity of the extracts of sponge Monanchora arbuscula against pathogenic bacteria. J Nat Prod 78: 1101-1112. DOI: 10.1021/acs.jnatprod.5b00070.

Sedjati S, Ambariyanto A, Trianto A, Supriyantini E, Ridlo A, Bahry MS, Yusuf S, Beger M, Citra A, Tassakka MAR, Brauwer MDE, Pricella A, Citra A, Trianto A, Radjasa OK, Sibero MT, Sabdono A, Haryati D, Zilullah WOM, Syanindyta AR, Bahry MS, Armondo HD, Supriadi S, Igarashi Y. 2020. The effect of culture media on the number and bioactivity of marine invertebrates associated fungus. Biodiversitas 22: 1717-1724. DOI: 10.13057/biodiv/d220415.

Rodriguez-Iglesias A, Schmoll M. 2019. Protein phosphatases regulate growth, development, cellules and secondary metabolism in Trichoderma reesei. Sci Rep 9: 1-17. DOI: 10.1038/s41598-019-47421-2.

Sabdaningsih A, Cristianawati O, Sibero MT, Aini M, Radjasa OK, Sabdono A, Trianto A. 2019. Anti MDR Acinetobacter baumannii of the sponges-associated fungus from Karimunjawa National Park. AACL Bioflux 12: 1970-1983.

Sabdaningsih A, Cristianawati O, Sibero MT, Nuryadi H, Radjasa OK, Sabdono A, Trianto A. 2017. Screening antibacterial agent from crude extract of marine-derived fungi associated with soft corals against MDR-Staphylococcus haemolyticus. IOP Conf Ser: Earth Environ Sci 55: 012026. DOI: 10.1088/1755-1315/55/1/012026.

Santos MFC, Harper PM, Williams DE, Mesquita JT, Pinto EG, Da Costa-Silva TA, Hajdu E, Ferreira AG, Santos RA, Murphy PJ, Andersen RJ, Tempone AG, Berlinc RGS. 2015. Anti-parasitic Guanidine and Pyrimidine alkaloids from the marine sponge Monanchora arbuscula. J Nat Prod 78: 1101-1112. DOI: 10.1021/acs.jnatprod.5b00070.

Sedjati S, Ambaryanto A, Trianto A, Supriyantini E, Ridlo A, Bahry MS, Wismayantini G, Radjasa OK, Mccaeley E. 2020. Antibacterial activities of the extracts of sponge-associated fungus Trichoderma longibrachiatum against pathogenic bacteria. Squalen Bull Mar Fish Postharvest Biotechnol 15: 81-90. DOI: 10.15578/squalen.v15i2.438.

Sharma AK, Sharma V, Saxena J, Yadav B, Alam A, Prakash A. 2015. Isolation and screening of extracellular protease enzyme from bacterial and fungal isolates of soil. Intl J Sci Res Environ Sci 3: 334-340. DOI: 10.12987/ijres.2015.93340.

Sibero MT, Herdiikawan D, Radjasa OK, Sabdono A, Trianto A, Triningsh D. 2018. Antibacterial activity of sponge associated fungis against vibriosis agents in shrimp and its toxicity to Litopenaeus vannamei. AAOL Bioflux 11: 10-18.

Sila F, Lourenço O, Queiroz JA, Domingues FC. 2011. Bacteriostatic versus bactericidal activity of ciprofloxacin in Escherichia coli assessed by flow cytometry using a novel far-red dye. J Antibiot 64: 321-325. DOI: 10.1038/ja.2011.5.

Suryanarayanan TS. 2012. The diversity and importance of fungi associated with marine sponges. Botanica Marina 55: 553-564. DOI: 10.1515/bot-2011-0086.

Toju H, Tanabe AS, Yamamoto S, Sato H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS ONE 7: e40863. DOI: 10.1371/journal.pone.0040863.

Trianto A, Radjasa OK, Purnaweni H, Bahry MS, Djamaludin R, Tjoa A, Singleton JAN, Dele K, Evan D. 2021. Potential of fungi isolated from a mangrove ecosystem in Northern Sulawesi, Indonesia: Protease, cellulase and anti-microbial capabilities. Biodiversitas 22: 1717-1724. DOI: 10.13057/biodiv/d210147.

Trianto A, Radjasa OK, Sibero MT, Sabdono A, Haryati D, Zilullah WOM, Syanindyta AR, Bahry MS, Armondo HD, Supriadi S, Igarashi Y. 2020. The effect of culture media on the number and bioactivity of marine invertebrates associated fungus. Biodiversitas 21: 407-412. DOI: 10.13057/biodiv/d220415.

Radjasa OK, Saber MT, Sabdono A, Haryati D, Zilullah WOM, Syanindyta AR, Bahry MS, Armondo HD, Supriadi S, Igarashi Y. 2020. The effect of culture media on the number and bioactivity of marine invertebrates associated fungus. Biodiversitas 22: 1717-1724. DOI: 10.13057/biodiv/d220415.