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ORIGINAL RESEARCH

Sponge-associated fungi from a mangrove habitat in Indonesia: species composition, antimicrobial activity, enzyme screening and bioactive profiling

Mada Triandala Sibero · Yasuhiro Igarashi · Ocky Karna Radjasa · Agus Sabdono · Agus Trianto · Dewi Seswita Zilda · Yusuf Jati Wijaya

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Abstract There is no report of diversity, biological properties and bioactive compounds of sponge-associated fungi from Indonesia's mangrove ecosystem to date. This study was designed to isolate sponge-associated fungi from a mangrove ecosystem in Mangkang, to screen the antimicrobial and extracellular enzyme properties of the isolates, characterize the biologically promising isolates using molecular approaches, and profile the secondary metabolites using phytochemical and thin-layer chromatography (TLC) analyses. An unidentified sponge that lived in association with mangrove roots was collected from Mangkang. Total of eight associated fungi were isolated from the sponge. Among all isolates, only two fungi SPMKF 1 and SPMKF 6 produced extracellular amylase, another two fungi SPMKF 4 and 5 showed antibacterial activity against MRSA, and only one fungus SPMKF 8 was able to produce extracellular amylase and show antimicrobial activity against ESBL *E. coli*, *Salmonella enterica* ser. Typhi strain MDR and *C. albicans*, while SPMKF 2, SPMKF 3 and SPMKF 9 did not show any biological properties. The result of genetic

M. T. Sibero (✉) · O. K. Radjasa · A. Sabdono · A. Trianto
Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Campus, St. Prof. Soedarto SH., Semarang 50275, Central Java, Indonesia
e-mail: madatriandalasibero@lecturer.undip.ac.id

M. T. Sibero
Marine Science Techno Park (MSTP), Teluk Awur Campus, St. UNDIP, Jepara District 59427, Central Java, Indonesia

Y. Igarashi
Biotechnology Research Center, Department of Biotechnology, Toyama Prefectural University, Imizu, Toyama, Japan

O. K. Radjasa · A. Sabdono
Tropical Marine Biotechnology Laboratory, Building of Marine and Oceanography Laboratory Lv. 2, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Campus, St. Prof. Soedarto SH., Semarang 50275, Central Java, Indonesia

A. Trianto
Marine Natural Products Laboratory, Building of Central Laboratory Lv. 2, Diponegoro University, Tembalang Campus, St. Prof. Soedarto SH., Semarang 50275, Central Java, Indonesia

D. S. Zilda
Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, St. KS. Tubun Petamburan VI, Jakarta 10260, Indonesia

Y. J. Wijaya
Department of Oceanography, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Campus, St. Prof. Soedarto SH., Semarang 50275, Central Java, Indonesia

characterization proved that SPMKF 1 was *Cladosporium tenuissimum*, SPMKF 4 was *Eutypella* sp., SPMKF 5 was *Lasiodiplodia theobromae*, SPMKF 6 was *Fusarium keratoplasticum* and SPMKF 8 was *F. solani*. Furthermore, an amylase gene was detected in fungi SPMKF 1, 6 and 8 while among the BGC, only NRPS genes were detected in SPMKF 4, 5 and 8. Interestingly, several same metabolites indicating the same retention factor (R_f) values in TLC were detected in the fungal crude extracts by TLC.

Keywords Fungi · NRPS · Sponge · Mangrove ecosystem

Introduction

Since the discovery of penicillin from fungi, substantial amounts of studies were devoted to the fungal bioactive compounds. Exploration of fungal metabolites is significantly increased after the scientists figured out that marine fungi, especially sponge-associated fungi, produce structurally unique bioactive compounds (Hasan et al. 2015; Imhoff 2016). This fungal group produced certain potential bioactive compounds for pharmaceutical application such as citreorosein (anti-obesity activity, IC_{50} = 0.17 μM), truncateol M (antiviral activity, IC_{50} = 8.8 μM), misszrtine A (cytotoxic, IC_{50} = 3.1 μM), and aspergillusene A (antibacterial activity, MIC = 4.3 μM) (Wang et al. 2014; Zhao et al. 2015; Noinart et al. 2017; Zhou et al. 2018). Nevertheless, it is noted that most of the bioactive compounds and their producer were isolated from sponges collected from seagrass or coral reef ecosystems. On the other hand, among the three marine ecosystems, the study of sponge-associated fungi from a mangrove ecosystem is rarely reported.

The Mangkang mangrove forest is located close to a residential area that gives direct anthropogenic effects. It is believed that the anthropogenic effect impacts the ecosystem conditions and has a role on shifting of microbial diversity and ecology (Atkinson et al. 2016; Labbate et al. 2016; Basyuni et al. 2018). In addition, Trianto et al. (2017) stated that anthropogenic activity gave influence to the biological activity of the sponge-associated fungi. The latest study on sponge-associated fungi from mangrove ecosystem was performed by Calabon et al. (2018). They mentioned that from 22 genera of ascomycetes from mangrove-originated sponge in Aklan, Philippines, *Aspergillus* was the most dominant genus with 23 isolates followed by *Mycelia* (n = 21 isolates) and *Penicillium* (n = 14 isolates). This study reports only the biodiversity analysis but not the information on the biological activity of this fungal group. In addition, sponge-associated fungi were rarely reported as the producer of the extracellular enzymes. Therefore, it would be interesting to study such enzyme activity.

Indonesia as a tropical country also has mangrove ecosystem that is neglected as a source of sponge-associated fungi and various biological activities. The purposes of this study were to isolate sponge-associated fungi from the mangrove forest in Mangkang, to screen the antimicrobial and extracellular enzyme activities of the isolates, to characterize the biologically promising isolates using molecular approaches, and to profile the secondary metabolites using phytochemical and thin-layer chromatography (TLC) techniques.

Methodology

Sampling

A mangrove-associated sponge was collected from a mangrove forest in Mangkang district, Central Java in August 2017 (Fig. 1). The sponge grew on the mangroves roots which was submerged in water. The sample was put in a sterilized zip-lock plastic and then transferred to Laboratory of Tropical Marine Biotechnology, Diponegoro University for fungal isolation. Several environment parameters such as substrate, salinity, pH, water temperature, and total dissolved solid (TDS) were measured in situ. Salinity was measured using refractometer; pH, water temperature and TDS were measured using Water Tester Multi-Parameter (Yieryi-pH986); while turbidity was measured using Secchi disk.

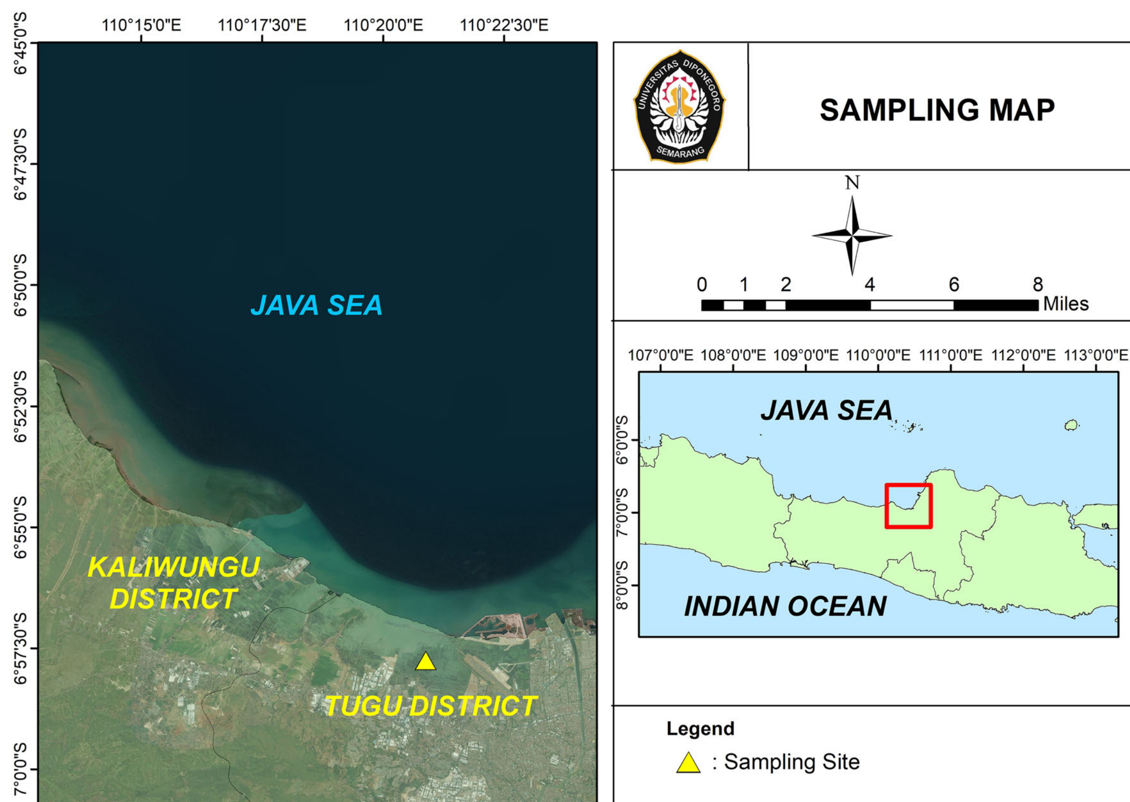

Fig. 1 Sampling site in Mangkang mangrove forest, Central Java, Indonesia

Fungal isolation

Potato dextrose agar (PDA) (HiMedia) with chloramphenicol (2%) was used as isolation media. Tapping method was carried out for fungal isolation (Kjer et al. 2010). We prepared three plates for environmental control. The sample was cleaned and washed with sterilized fresh water followed by 70% alcohol to eliminate the non-target microorganisms and then the alcohol's residues were eliminated by a second washing with sterilized fresh water. Three pieces of mesohyl were resized into 2×2 cm^2 and then transferred onto PDA for incubation (27 °C) until the fungal mycelium grew on the tissue or agar. During the isolation, two Petri dishes with PDA were prepared for environmental control. Each mycelium that grew on mesohyl was compared to the fungi that grew on environmental control. Isolates with different morphological characteristic with the control were transferred into a new medium as a single colony.

Screening of antimicrobial activity

Several clinical pathogenic dermatophytes such as *Candida albicans*, *Malassezia furfur*, and *Trichophyton rubrum* as well as clinical multi-drug-resistant bacteria extended spectrum β-lactamase *Escherichia coli* (ESBL), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella enterica* ser. Typhi were obtained from General Hospital Dr. Kariadi, Semarang. Dermatophytes were revived and recultured on PDA (30 °C, 24 h) while MacConkey agar was used for the Gram-negative pathogen and Mueller–Hinton agar (MHA) (HiMedia) for MRSA (32 °C, 24 h). Agar plug method was applied in this step (Balouiri et al. 2016). All active fungi were cultivated in Malt Extract Broth (HiMedia) for 7 days (shaking culture, 30 °C) and then extracted using 1-butanol.

Profiling of bioactive compounds

Qualitative detection of several groups of bioactive compounds such as alkaloids, flavonoids, phenol hydroquinone, saponins, steroids/terpenoids, tannins, with additional of glycosides, free amino acids, and peptides was carried out according to the phytochemical analysis protocol (Nurjanah et al. 2016; Cita et al. 2017; Gul et al. 2017). The samples were diluted in dimethyl sulfoxide (DMSO) at a concentration of 200 mg/mL.

Alkaloids

Sample (2 mg) was dissolved in 1 mL of 2 N H_2SO_4 (Merck) and then filtered. The filtrate was separated into three test tubes (200 µL/test tube) and then a few drops of Dragendorff (Wako, Japan) reagent were added into the first test tube, Mayer reagent to the second test tube and Wagner reagent to the third test tube. Positive result of Dragendorff was indicated by a yellowish to orangish precipitate, a white-creamy to yellowish precipitate indicated the positive result of Mayer analysis, while reddish to brown precipitate indicated the positive result of Wagner analysis.

Flavonoids

Magnesium powder (Mg, 0.1 mg), amyl alcohol (0.4 mL), and alcohol (1 mL) were added to sample solution (200 µL). The presence of orange to yellow line color in amyl alcohol layer indicated the positive result for this analysis.

Phenol hydroquinone

Sample solution (200 µL) was added into 70% ethanol (1 mL). Then a few drops of 5% $FeCl_3$ were added. The formation of green to green-blue color indicated the presence of phenol hydroquinone.

Saponins

Sample (2 mg) was diluted in water (5 mL), heated, shaken vertically until foam was formed, and kept for 15 min. One drop of 2 N HCl was added to check the foam stability. The presence of stable foam indicated a positive result for this step.

Steroids/Terpenoids

Liebermann–Burchard method was applied to detect the presence of steroids/terpenoids in the crude extract. The sample (2 mg) was mixed with a few drops of acetic anhydride, boiled and cooled. A few drops of concentrated H_2SO_4 were added from the sides of the test tube until brown ring at the junction of two layers appeared. The green coloration of the upper layer and the formation of deep red color in the lower layer indicated a positive result.

Tannin

Detection of tannin was done by the addition of gelatin solution to the sample. Positive result was indicated by the whitish-yellow precipitate.

Glycosides

Keller–Kiliani method was applied to detect the presence of glycosides. The sample solution (200 µL) was added with glacial acetic acid with one drop of 2% $FeCl_3$. Then concentrated H_2SO_4 (500 µL) was added from the tube sides. A brown ring between the layers indicated a positive result.

TLC profiling

Thin-layer chromatography (TLC) was carried out for the further bioactive compounds profiling with silica gel 60 (Merk, F254) as the stationary phase while *n*-hexane, ethyl acetate and methanol as mobile phase with ratio 14:5:1.

Screening of extracellular enzymes

Screening medium for amylase contained 1% of peptone (HiMedia), 0.05% of yeast extract (HiMedia), 1% of soluble starch (Merck) and 3% of agar bacteriological (Oxoid). For cellulose screening, the media contained 1% of peptone (HiMedia), 0.05% of yeast extract (HiMedia), 1% of CMC and 3% of agar bacteriological (Oxoid). Gelatinase screening was performed in media that contained 3% of gelatin and 1% of dextrose. These media were sterilized at 121 °C for 20 min by autoclaving. Skim milk was used for protease screening then sterilized at 121 °C for 5 min by autoclaving. Fungi were inoculated into the media and then incubated for 4–7 days at 27 °C for screening of amylase, cellulase and protease. Screening of gelatinase was done by inoculating the fungi into the media and then incubated for 4–7 days at 27 °C, after that the media were transferred into a refrigerator (4 °C) for overnight. Amylase activity was detected by the presence of clear zone around the fungal colony after the addition of iodine while cellulase activity was detected by the same protocol after the addition of phenol red. Activity of gelatinase was indicated by the presence of liquefaction after preservation at 4 °C for overnight. The presence of clear zone in skim milk media indicated the protease activity (Balan et al. 2012; Bonugli-Santos et al. 2015).

Molecular characterization

DNA isolation

Isolation of fungal DNA was carried out according to Sibero et al. (2018). Fungi were recultured for 7 days at 27 °C and then the mycelia were transferred into a sterilized microtube. One milliliter of Bidest water ($_{dd}H_2O$) and 100 µL of phosphate buffer saline (PBS) were added and kept overnight at 4 °C as a mixture. The mixture was separated using centrifugation (12,000 r.p.m., 10 min, 4 °C) and the supernatant was discarded. One milliliter of sterilized $_{dd}H_2O$ was added into the pellet and mixed using vortex. The mixture was separated again using the same protocol; this step was repeated three times. The pellet was mixed with 50 µL of 20% Chelex 100 and heated in a heating block at 85 °C for 5 min. The mixture of pellet and 20% Chelex 100 was mixed using vortex and reheated at 85 °C for another 5 min. The mixture was separated again using centrifugation (12,000 r.p.m., 10 min, 4 °C), then the supernatant was transferred into a new microtube as DNA template.

DNA barcoding

DNA barcoding in *internal transcribed spencer* (ITS) region was carried out for fungal identification. Amplification for ITS region was performed using ITS1 (forward) and ITS4 (reverse) primers (Table 1). PCR mix for genes amplification consisted of 1 µL of forward primer, 1 µL of reverse primer, 0.5–1.0 µL of DNA template, 12.5 µL of GoTaq Green Master mix from Promega and $_{dd}H_2O$ until the final volume was 25 µL/tube. PCR condition followed the protocol of Sibero et al. (2018) was used: 30 cycles of denaturation at 95 °C for 1 min, annealing at 50–55 °C for 1 min and extension at 72 °C for 1 min while the post-cycling extension was done at 72 °C for 7 min. PCR products were sequenced by 1st Base Laboratories Sdn Bhd, Malaysia. Basic Local Alignment Search Tool (BLAST) was carried out for isolates identification. The phylogenetic tree was reconstructed in MEGA X software package with bootstrap number 1000.

Screening of PKS-I, PKS-II, NRPS and amylase genes

Screening of PKS-I, PKS-II, NRPS and amylase genes was carried out using specific primers mentioned in Table 1. The PCR condition was set to follow the condition in the barcoding stage.

Table 1 Primers were used in this study

Primer's name	Oligonucleotides	Target	References
ITS 1	5′-TCC GTA GGT GAA CCT GCG G-3′	Fungal barcoding	Sibero et al. (2018)
ITS 4	5′-TCC TCC GCT TAT TGA TAT GC-3′		
A2gamForward	5′-AAG GCN GGC GSB GCS TAY STG CC-3′	NRPS gene detection	Radjasa et al. (2005)
A3gamReverse	5′-TTG GGB IKB CCG GTS GIN CCS GAG GTG-3′		
KAF1	5′-GAR KSI CAY GGI ACI GGI AC-3′	PKS-I gene detection	Amnuaykanjanasin et al. (2005)
KAR1	5′-CCA YTG IGC ICC RTG ICC IGA RAA-3′		
IIPF6	5′-TSG CST GCT TCG AYG CSA TC-3′	PKS-II gene detection	Sun et al. (2012)
IIPR6	5′-TGG AAN CCG CCG AAB CCG CT-3′		
AmyF	5′-AGG CGC GCC AGT GCT GAA ACG GCG AAC AAA TCG AA-3′	Amylase gene detection	Afzal-javan and Mobini-dehkordi (2013)
AmyR	5′-TTG CGG CCG CTC AAT GGG GAA GAG AAC CGC TTA AG-3′		

Results

The mangrove forest in Mangkang district, Central Java, is located close to the residential area (Fig. 1). Moreover, the local communities utilize it for a shrimp farming that has impacted the biochemical composition in the water. The water quality of the sampling site had a salinity value of 30.7 ‰, temperature of 31 °C, pH 8.7, TDS value of > 2000 ppm, and visibility of 0.2 m. The sampling site had sandy–mud sediment with depth range 0.1–1.0 m. The high TDS content in the water, low visibility, and sandy–mud sediment were expected to give low diversity of sponge from this area. In this location, one sponge species was collected as a mangrove associate. Figure 2 shows the characteristics of the sponge. It had yellow color covered by the sediment, grew on the *Rhizophora mucronata*'s roots and spread on the bamboo bridge near the roots. It had a thickly encrusting form, had branching mass of cylinders to volcano-shape tubes (ramose) between 0.5 and 2.5 cm with oscula at the top, surface rugose, firm and compressible texture (Fig. 2a). This sponge had oxea-shape spicules (Fig. 2b, c). From this sponge, eight fungi were isolated on PDA after 10 days of cultivation. Figure 3 shows the diversity of the fungi isolated from this sponge. All isolates had different morphological characteristics with fungi that grew on the control medium. This step is very important to confirm the fungi originated from the sponge tissues. Biological properties such as antimicrobial activity and ability to produce extracellular enzymes were examined by in vitro method (Table 2). It shows that among eight isolates, there were five prospective fungi that showed antimicrobial and enzyme activities. Fungi SPMKF 1 and 6 only produced amylase, SPMKF 4 and 5 only showed inhibition zone against MRSA, while SPMKF 8 formed inhibition zones against ESBL *E. coli*, *S. enterica* ser. Typhi, *C. albicans* and indicated the presence of the amylase. This result indicated that fungus SPMKF 8 was suggested as the most prospective isolate for microbial bioprospecting. All isolates which showed antimicrobial and enzyme activities were identified using molecular approaches.

Fungus SPMKF 1 was identified as *Cladosporium tenuissimum* (99% similarity, accession number MK881131), SPMKF 4 as *Eutypella* sp. (99% similarity, accession number MK881164), SPMKF 5 as *Lasiodiplodia theobromae* (99% similarity, accession number MK881166), SPMKF 6 as *Fusarium keratoplasticum* (98% similarity, accession number MK881303) and SPMKF 8 as *F. solani* (100% similarity, accession number MK881752). Due to genetic distance, three clades were reconstructed in the phylogenetic three (Fig. 4). The Dothideomycetidae clade contained SPMKF 1 and 5, Hypocreomycetidae clade included SPMKF 6 and 8, while SPMKF 4 was separated out in a group of Xylariomycetidae clade. The presence of biological gene cluster (BGC) such as NRPS, PKS-I, and PKS-II as well as genes encoding amylase was detected in this study. The result of the screening of BGC and amylase genes is shown in Fig. 5.

Among all the BGCs that were screened, only NRPS genes showed positive result. It was proved by the presence of the clear bands in the gel electrophoresis under UV illumination. Figure 5 shows that the isolates SPMKF 4, 5 and 8 had NRPS genes around 200 bp. The figure also shows the presence of the genes that

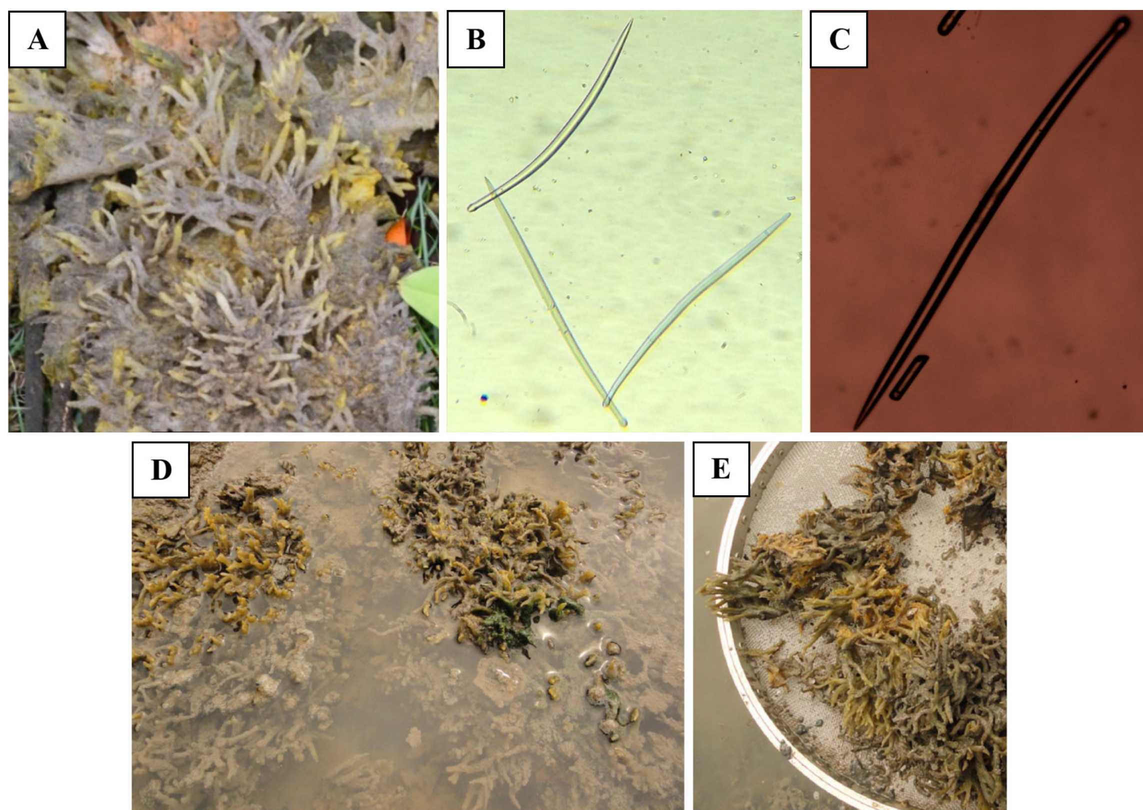

Fig. 2 Sponge from mangrove forest in Mangkang district (**a** Sponge specimen, **b**, **c** oxea-shape spicules, **d**, **e*** *Amorphinopsis atlantica*). (*Source*: Santos et al. 2018)

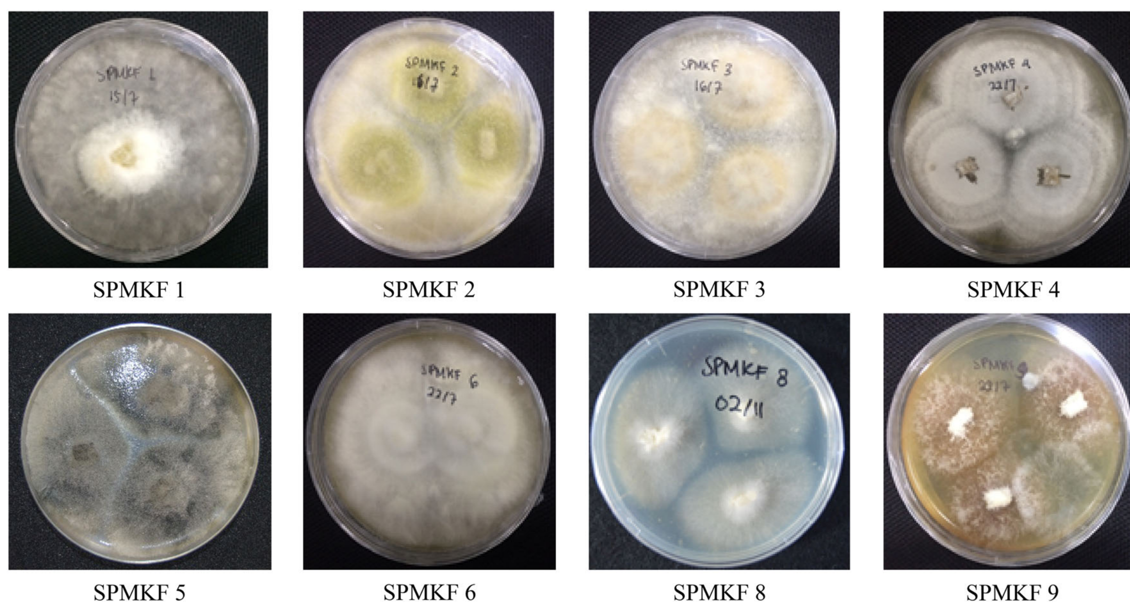

Fig. 3 The diversity of associated fungi isolated from an unidentified sponge collected at the mangrove forest in Mangkang, Central Java

Table 2 Biological properties of sponge-associated fungi

Isolates	Pathogen						Enzyme			
	MDR bacteria			Dermatophyte						
	ESBL	MRSA	SET	CA	TR	MF	A	C	G	P
SPMKF 1	–	–	–	–	–	–	√	–	–	–
SPMKF 2	–	–	–	–	–	–	–	–	–	–
SPMKF 3	–	–	–	–	–	–	–	–	–	–
SPMKF 4	–	√	–	–	–	–	–	–	–	–
SPMKF 5	–	√	–	–	–	–	–	–	–	–
SPMKF 6	–	–	–	–	–	–	√	–	–	–
SPMKF 8	√	–	√	√	–	–	√	–	–	–
SPMKF 9	–	–	–	–	–	–	–	–	–	–

ESBL extended spectrum β-lactamase *E. coli*, *MRSA* methicillin-resistant *S. aureus*, *SET S. enterica* ser. Typhi, *CA C. albicans*, *TR T. rubrum*, *MF M. furfur*, *A* amylase, *C* cellulose, *G* gelatinase, *P* Protease

√: presence of clear zone; –: absence of clear zone

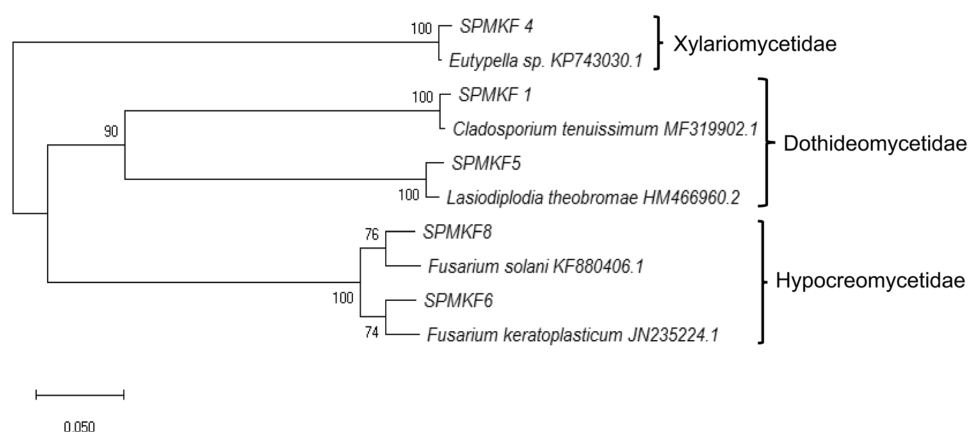

Fig. 4 Phylogenetic tree of prospective sponge-associated fungi that exhibited biological properties

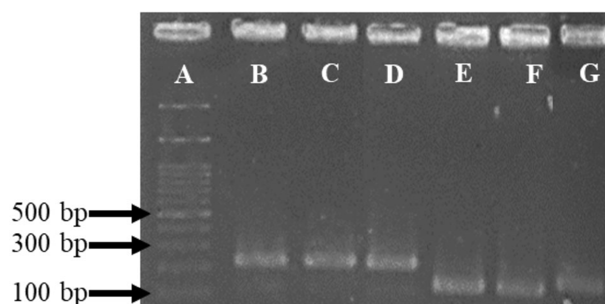

Fig. 5 The presence of BGC and amylase genes in prospective fungi (a: Marker, b: NRPS in SPMKF 4, c: NRPS in SPMKF 5, d: NRPS in SPMKF 8, e: amylase genes in SPMKF 1, f: amylase genes in SPMKF 6, and g: amylase genes in SPMKF 8)

encoded amylase in SPMKF 1, 6 and 8 around 100 bp. These results supported the previous data that obtained from the screening of antimicrobial and enzyme activities by in vitro method.

Bioactive compounds in SPMKF 4, 5 and 8 were profiled by phytochemical analysis and thin-layer chromatography (TLC). The result of phytochemical analysis shows that all isolates produced alkaloids, and phenol hydroquinone, while tannins were produced by *Eutypella* sp. SPMKF 4 and *Fusarium solani* SPMKF 8

Table 3 Group of bioactive compounds in prospective isolates

No.	Analysis	Isolates		
		SPMKF 4	SPMKF 5	SPMKF 8
1.	Alkaloids			
	a. Dragendorff	+	+	+
	b. Mayer	+	+	+
	c. Wagner	+	+	+
2.	Flavonoids	+	−	+
3.	Tannin	+	−	+
4.	Phenol Hydroquinone	+	+	+
5.	Steroids/terpenoids	−	−	−
6.	Saponin	−	−	+
7.	Glycosides	−	−	−

(+: positive result, −: negative result)

(Table 3). In addition, saponins were only produced by *F. solani* SPMKF 8. For further metabolite profiling, TLC analysis had been performed in this study. Figure 6 and Table 4 show the result of this analysis.

All prospective fungi were suggested to produce alkaloids and phenol hydroquinone derivatives by the positive result of the qualitative analysis. Moreover, fungi *Eutypella* sp. SPMKF 4 and *F. solani* SPMKF 8 showed positive result for flavonoids and tannins. In addition, only a fungus *F. solani* SPMKF 8 was suggested to produce saponin derivatives (Table 3). According to the result of TLC, the metabolites in the crude extract from *Eutypella* sp. SPMKF 4 were fractionated to be five fractions, *L. theobromae* SPMKF 5 had three fractions while *F. solani* SPMKF 8 had six fractions (Fig. 6). We noted that several fractions from different isolates had the same retention factors (R_f) on TLC plate (Fig. 6 and Table 4). Bands with R_f values of 0.05 and 0.12 appeared in crude extract of *Eutypella* sp. SPMKF 4 and *F. solani* SPMKF 8; a band with R_f value of 0.40 was detected in the crude extracts of *L. theobromae* SPMKF 5 and *F. solani* SPMKF 8; a band with R_f value of 0.62 was detected in all crude extracts; while a band with R_f value of 0.68 was detected in crude extracts of *Eutypella* sp. SPMKF 4 and *L. theobromae* SPMKF 5.

Discussion

In comparison to the study of sponge diversity from coral reef and sea grass ecosystems, the sponge diversity from mangrove ecosystem in Indonesia could be assessed as a neglected resource due to barely any report on this interest. The only report of this topic was done by Becking et al. (2013) that examined the diversity of

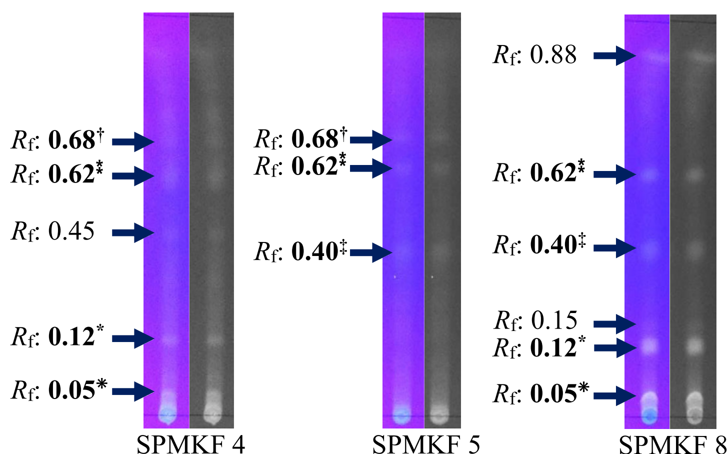

Fig. 6 TLC profile of the fungal metabolites under UV light illumination at 356 nm (same notations indicated the same R_f value)

Table 4 Retention factors (R_f) of the fungal metabolites in the TLC plates

No.	Isolate	Retention factor (R_f)							
1.	SPMKF 4	0.05*	0.12*			0.45	0.62[a]	0.68[†]	
2.	SPMKF 5				0.40[‡]		0.62[a]	0.68[†]	
3	SPMKF 8	0.05*	0.12*	0.15	0.40[‡]		0.62[a]		0.88

Same notations indicated the same R_f value in TLC plates

mangrove-associated sponge in Maratua coastal mangrove and Kakaban marine lakes. These locations had crystal-clear water with diverse genera and species of marine sponges such as *Stelletta*, *Geodia*, *Chondrosia*, *Clathrina*, *Dendrilla*, *Spongionella*, *Dysidea*, *Spongia*, *Cacospongia*, *Semitaspongia*, *Cliona*, *Spirastrella*, *Stylissa*, *Amorphinopsis*, *Axinyssa*, *Halichondria*, *Haliclona*, *Neopetrosia*, *Oceanapia*, *Acarnus*, *Raspailia*, *Tedania*, *Cinachyrella*, *Paratetilla* and *Hexadella*. It was reported that Maratua had higher salinity, temperature and pH than the Kakaban marine lake. Interestingly, the diversity of mangrove-associated sponge was inversely proportional. The sponge diversity in Maratua coastal mangrove was lower than in Kakaban marine lake. It proved that the abiotic parameters had important role in the diversity of the sponge in mangrove ecosystem. Furthermore, the sampling site had a relatively high value of salinity, temperature, pH and turbidity, and the sandy–mud sediment allegedly became a limiting factor of the sponge composition.

According to morphological characteristics in Fig. 2, the sample was suggested as *Amorphinopsis* sp. The morphological characteristics of the sample such as the thick encrust, ramose form, and yellow color were similar to *Amorphinopsis* (Fig. 2d, e) (Santos et al. 2018). However, molecular approach is strongly suggested to confirm the species. The production of ramose form in a sponge was suggested as the morphological adaptation mechanism to prevent the detriment from sedimentation (Ávila and Ortega-Bastida 2015). Furthermore, Hunting et al. (2013) stated that the composition of dissolved organic matters (DOM) and the symbiotic microorganisms had an important role in structuring a sponge community and diversity in coral reef and mangrove ecosystem. Even though further chemical and physical parameters were not investigated in this study, we speculated that the low visibility, high total suspended matters and sedimentation in this site kill the larvae or prevent its settlement and inhibit the growth of other non-branching species in Mangkang mangrove forest.

A total of eight fungal strains were isolated from this sponge and characterized as filamentous fungi (Fig. 3). This finding is very important because this study was the first report of sponge-associated fungi that originated from Indonesia's mangrove ecosystem. Furthermore, among all isolates, five fungi showed potential to produce amylase and bioactive compounds. According to Fig. 4, all isolates are the member of ascomycetes that are grouped into three clades based on the phylogenetic distance. Calabon et al. (2018) reported that ascomycetes are also the dominant fungal group in the mangrove-associated sponge in New Washington, Philippines. They also reported that *Aspergillus* spp. dominates the fungal diversity, while in this study, *Aspergillus* was not isolated. It was noted that several fungi that suspected as *Aspergillus* and *Penicillium* grew on the environmental control (Fig. 7); however, those fungi were not found on the mesohyl part.

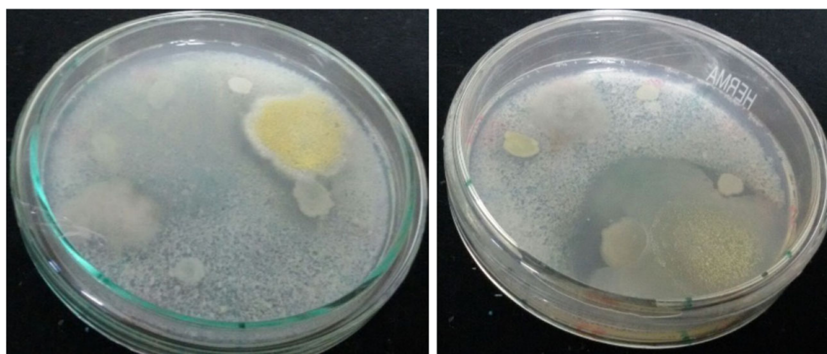

Fig. 7 Fungi that grew on environmental control

Fig. 8 Clear zone indicated the amylase activity of three sponge-associated fungi after the addition of iodine to the soluble-starch media

It is suggested that the ecological condition that the sponge thrives played a big role in the diversity. Nonetheless, the role of ascomycetes in sponge is still understudied.

This study proved that three isolates, *Eutypella* sp. SPMKF 4, *Lasiodiplodia theobromae* SPMKF 5, and *Fusarium solani* SPMKF 8, produce antimicrobial against the pathogenic microorganisms. These fungi are known as potential producers of pharmaceutically prospective compounds. Recently discovered antimicrobial compounds from *Eutypella* sp., *L. theobromae* and *F. solani* are eutypellenoids B, eut-Guainae sesquiterpene, libertellenone A, (+)-(R)-de-O-methyl-lasiodiplodin, fusariumester B, and 7–desmethyl fusarin C (Kyekyeku et al. 2017; Wang et al. 2018; Zhou et al. 2017; Liu et al. 2018; Umeokoli et al. 2018; Yu et al. 2018). The ability of these isolates to produce antimicrobial and other bioactive compounds shown in Table 3 may suggest that these fungi are potentially contributing to the chemical defense of the sponge host. Furthermore, *C. tenuissimum* SPMKF 1, *F. keratoplasticum* SPMKF 6, and *F. solani* SPMKF 8 showed amylase productions (Fig. 8). We propose that these fungi have an important role to breakdown the complex nutrient from the environment. This idea is supported by Nguyen and Thomas (2018) who suggested that *Fusarium* and *Cladosporium* as a saprophyte could contribute to nutrient uptake by breakdown of the plant-derived detritus or other plankton from water. In addition, regarding the symbiotic interaction between the microbes and the host sponge, it can be also suspected that the prospective fungi produce extracellular enzymes to help the host sponge to breakdown the roots so that the sponge is able to spread and expand the colony. Nevertheless, further study is needed to explain the role of the fungal enzymes for its host.

The presence of biosynthetic gene clusters (BGC) was screened to understand the ability of the prospective fungi to produce bioactive polyketides and non-ribosomal peptides. Some studies demonstrated that the presence of biosynthetic gene cluster (BGC) such as polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) genes in microorganisms is correlated to their capability to inhibit the growth of other microorganisms (Passari et al. 2016; Böhringer et al. 2017; Samak et al. 2018). We detected the existence of PKS-I, PKS-II and NRPS genes in *Eutypella* sp. SPMKF 4, *L. theobromae* SPMKF 5, and *F. solani* SPMKF 8. According to Fig. 4, it is noted that all isolates showed a clear band of NRPS genes. Although *Fusarium* is known to bring NRPS and PKS genes in its genome naturally (Hansen et al. 2012; Romans-Fuertes et al. 2016), this is the first report of the discovery of NRPS genes in *Eutypella* and *Lasiodiplodia*. It should also be noted that only a few peptides are reported from these genera (Wei et al. 2014). Therefore, we suspect that NRPS genes in *Eutypella* and *Lasiodiplodia* are silent. The presence of NRPS genes in *Eutypella* sp. SPMKF 4, *L. theobromae* SPMKF 5, and *F. solani* SPMKF 8 raises a presumption that the isolates produce non-ribosomal peptides compounds which contribute to antimicrobial property (Agrawal et al. 2017). To prove this hypothesis, isolation of antimicrobial compounds through bio-guided isolation protocol is essential to be done. The result of metabolite profiling using TLC plates showed that several fractions of the crude extracts contained spots of the same R_f. This finding strongly indicated the fungi produce similar or even the same bioactive compounds. We suggested that it was caused by the gene transfer that might occur when the fungi lived together in the host (Nakashima et al. 2016). In addition, the presence of alkaloids, flavonoids, tannin, saponin (Table 3), non-ribosomal peptides indicated a possibility to find novel bioactive compounds from

sponge-associated fungi in mangrove ecosystem, while the amylase of these fungi could be applied in various industries.

Conclusion

A mangrove-associated sponge collected from Mangkang mangrove forest gave a total of eight associated fungi. Among all isolates, only two fungi SPMKF 1 and SPMKF 6 produced extracellular amylase, another two fungi SPMKF 4 and 5 showed antibacterial activity against MRSA, and only one fungus SPMKF 8 was able to produce extracellular amylase and show antimicrobial activity against ESBL *E. coli*, *Salmonella enterica* ser. Typhi strain MDR and *C. albicans*. The result of genetic characterization proved that SPMKF 1 was *Cladosporium tenuissimum*, SPMKF 4 was *Eutypella* sp., SPMKF 5 was *Lasiodiplodia theobromae*, SPMKF 6 was *Fusarium keratoplasticum* and SPMKF 8 was *F. solani*. Furthermore, an amylase gene was detected in fungi SPMKF 1, 6 and 8 while among the BGC, only NRPS genes were detected in SPMKF 4, 5 and 8. Phytochemical analysis indicated the presence of alkaloids and phenol hydroquinone in SPMKF 4, 5 and 8; flavonoids and tannins in SPMKF 4 and 8, while saponin was only detected in SPMKF 8. Interestingly, several same metabolites indicating the same retention factor (R_f) values in TLC were detected in the fungal crude extracts by TLC.

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Compliance with ethical standards

Conflict of interest None.

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