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Bioactivity of Fungi from the Thousand Islands Against *Staphylococcus aureus* And *Escherichia coli* Using Dilution Method

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Abstract

The rapid advancement of science and technology has brought numerous benefits to human life; however, it is unfortunate that this progress has not extensively tapped into one of Indonesia's abundant biological resources, which is fungi, especially marine fungi. This research was conducted to explore the antimicrobial potential of marine fungi isolated from the Thousand Islands against *Staphylococcus aureus* and *Escherichia coli* using the dilution method. The study aimed to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the obtained isolates against the two test bacteria. The research comprised two phases: the collection of samples in the field, followed by the antimicrobial activity assay using the dilution method in the laboratory. The findings are expected to reveal several types of marine fungi from the Thousand Islands with antimicrobial capabilities that could be developed as alternative antibiotics to replace those that have lost their potency. The results of the study showed that out of the 10 tested marine fungi isolates, all of them exhibited inhibition against the growth of *Staphylococcus aureus* and *Escherichia coli*. The concentrations of 100% and 75% proved to be the most effective in inhibiting bacterial growth. The MIC of the tested marine fungi extracts was determined to be 25%. Moreover, five isolates of marine fungi demonstrated MBC against *S. aureus* and *E. coli*: S.KL5 isolate at concentrations of 75% and 50%, S.AL and S.KL1 isolates at concentrations of 100% and 50%, and S.MA2 and S.KL1 isolates at concentrations of 100% and 75%. The identified best isolates were found to belong to the species *Penicillium* sp. and *Aspergillus niger* .

Keyword: Antibacterial, Marine fungi, , MIC, MBC.

INTRODUCTION

Fungi are eukaryotic microorganisms that exist in filamentous (thread-like) or single-cell forms, typically small in size (microscopic), and obtain their nutrients by absorbing them from their hosts (Ali, 2005). In terrestrial environments, such as forests, fungi commonly grow on decaying organic matter like leaf litter, acting as saprophytes, which play a direct role in maintaining soil fertility and ecosystem balance (Ilyas, 2007). In the air, fungi are found in the form of spores, which can contaminate surrounding objects such as food, walls, and paintings, especially in humid and poorly lit environments. Additionally, in aquatic environments, fungi can be found in freshwater, such as lakes, ponds, and rivers, as well as in saline water, particularly the sea. Typically in the marine environment, fungi thrive on macroalgae and marine corals, or on decaying matter in the sea.

In marine environments, the number of fungi found is relatively fewer compared to terrestrial environments. It is reported that the number of fungal species on the planet ranges from 1.5 to over 5 million, with an estimated 1,100 species described in association with marine environments (Amend et al., 2019). Fungi have been discovered in almost every explored marine habitat, from the sea surface to kilometers beneath the seafloor. Marine fungi contribute to the population cycle of phytoplankton and the biological carbon pump, and they actively participate in marine sediment chemistry. Many fungi have been identified as commensals or pathogens of marine animals (e.g., corals and seagrasses), plants, and algae (Amend et al., 2019).

Fungi have numerous benefits in life, serving as a significant food source used especially in the fermentation industry, and they are highly valuable in the field of health, particularly as producers of antibiotics. The potential of fungi as antibiotic producers has been known for a long time, and ongoing research continues to explore new antibiotics to replace those that have lost their efficacy due to the increasing resistance of microorganisms to certain antibiotics. Therefore, the search for alternative antibiotics is essential.

Despite the remarkable advancements in science and technology that have greatly benefited human life, it is regrettable that the rapidly developing knowledge has not fully utilized one of Indonesia's abundant biological resources—fungi, especially marine fungi. Even in this era of progress, the existence and potential of marine fungi remain relatively unexplored. The antimicrobial potential of marine fungi in Indonesia has not been deeply investigated.

Research results that have been conducted on Air Island, Karang Boko Island, and Kotok Besar Island by Noverita et al. (2016) revealed 18 isolates of marine fungi from three different substrates (seawater, macroalgae, and soft corals). These isolates exhibited the ability to inhibit the growth of S. aureus and E. coli bacteria after undergoing an antibacterial activity test using the dilution method.

The above data only represents a small fraction of the marine fungi present in the Thousand Islands (Kepulauan Seribu). Many other islands in the Thousand Islands are likely to harbor various types of marine fungi that hold significant potential for exploration.

The aim of this research is to obtain potential fungal isolates that can inhibit test microorganisms and may be developed into alternative antibiotics to replace those that have lost their potency.

METHOD

A. Research Location

The research was conducted on four islands in the Thousand Islands (Kepulauan Seribu), North Jakarta, and at the Microbiology Laboratory and Integrated Chemistry Laboratory, Universitas Nasional, JL. Bambu Kuning, Pasar Minggu, South Jakarta.

B. Research Equipment and Materials

The equipment used in this research includes an oven, autoclave, laminar airflow cabinet, rotary shaker, refrigerator, incubator, portable stove, beakers, Erlenmeyer flasks (Pyrex), Petri dishes (Pyrex), test tubes, test tube racks, Bunsen burners, vials, measuring glasses, filter paper, volumetric pipettes, vortex mixer, core borer (cork borer), centrifuge, bulbs, scissors, inoculation needles, scalpels, and rulers.

The materials used in the research are seawater, macroalgae, and coral as samples of microfungi, filter paper, plastic wraps, aluminum foil, cotton, label paper, newspapers, plastic, matches, swabs, *Staphylococcus aureus* and *Escherichia coli* bacteria obtained from the Microbiology Laboratory, Faculty of Biology, Universitas Nasional, 70% ethanol sterilization material, Mueller Hinton Agar (MHA), Nutrient Agar (NA), Potato Dextrose Agar (PDA) media (Scharlau), 30µg chloramphenicol antibiotic, Potato Dextrose Broth (PDB) media, 0.5 McFarland solution, 6mm diameter paper discs (Schleicher), and 0.85% physiological NaCl solution.

C. Procedure

Field Research

Field research was carried out by collecting samples of seawater, coral, and macroalgae at three points in the Thousand Islands area. Seawater samples were taken directly and placed in sterilized bottles. For coral, aseptic techniques were used to collect the samples, which were then cut using sterilized knives and placed in sample bottles filled with sterilized seawater. Macroalgae samples were

aseptically collected and placed in sample bottles filled with sterilized seawater. All collected samples were then placed in a cooler box and transported to the laboratory for further testing.

Laboratory Research

a. Preparation of growth media

Prepared growth media include Nutrient Agar (NA) and Potato Dextrose Agar (PDA) for microbial culture, Mueller Hinton Agar (MHA) and NaCl for antimicrobial activity testing, and Potato Dextrose Broth (PDB).

b. Sterilization of materials and equipment

The materials and equipment used in this research were sterilized. Growth media were sterilized using an autoclave at a temperature of 121°C for approximately 15 minutes, while glassware such as Petri dishes and Erlenmeyer flasks were sterilized using an oven at a temperature of 180°C for 2 hours.

c. Isolation of marine fungi

Marine fungi in the sample bottles were cultured on PDA media, then transferred to slant agar tubes. They were incubated at 37°C for 24-48 hours. After that, the fungi were transferred back to PDA media in solid form using an inoculation needle. They were incubated at 37°C for approximately 1 week. Using a core borer, the isolated fungi were inoculated into PDB media and left for approximately 9 days. Centrifugation was then carried out to obtain the supernatant fluid from the microfungi isolate.

d. Preparation of test microorganisms

Two types of test microorganisms were used for antimicrobial activity testing: S*taphylococcus aureus* and *Escherichia coli*. 1-2 mL solution of the test microorganisms was dissolved in physiological saline (0.85% NaCl). The suspension was standardized using the 0.5 McFarland standard (microbial density of 1.5 x 108) against a black background and bright light.

e. Antimicrobial activity testing using the dilution method

The dilution method involved using a series of test tubes filled with liquid media and a specific number of test microbe cells. Each tube was then tested with serially diluted marine fungi extracts. The tubes were incubated at 36°C for 18-24 hours, and the turbidity was observed. In this study, colony counting was performed for each dilution series using the Total Plate Count (TPC) method. The lowest concentration of marine fungi extract in the tube that showed clear growth (no microbial growth/reduced colony count compared to the negative control) was determined as the Minimum Inhibitory Concentration (MIC). Next, from the tubes showing no growth, the extract was streaked on solid media (Nutrient Agar) without adding the test fungi extract, and incubated for 18-24 hours. The lowest concentration of marine fungi extract in the solid culture, showing no microbial growth, was determined as the Minimum Bactericidal Concentration (MBC).

D. Data Analysis

The data obtained from the research were analyzed descriptively based on their ability to inhibit the test bacteria. Meanwhile, potential fungi that can inhibit the growth of the test microorganisms were identified macroscopically and microscopically based on colony shape, texture, color, hyphae form, and spores.

RESULT AND DISCUSSION

A. Isolated Isolates from the Thousand Islands

A total of 18 marine fungal isolates were successfully isolated from various substrates (seawater, macroalgae, and soft corals) at four locations in the Thousand Islands (Harapan Island, Air Island, Karangbongko Island, and Kotokbesar Island). These 18 isolates are presented in Table 1 and Figure 1.

Table 1. Marine Fungal Isolates Resulting from Isolation on Various Substrates in the Thousand Islands

Location	Substrate	Quantity	Isolate Code
P.Pramuka	Seawater	3	$S_1Al_{1yellow}$, S_1Al_{2White}, $S_1Al_{1Yellow}$
	Macroalgae	1	S_1Ma_{2Putih}
	Soft Corals	4	$S_1Kl_{1\ Balck}$, S_1Kl_{1Blue}, $S_1Kl_{1Yellow}$, S_1Kl_{1White}
P. Air	Seawater	0	0
	Macroalgae	2	$S_2Ma_{1\ White}$, $S_2Ma_{1\ Balck}$
	Soft Corals	1	S_2Kl_{1Green}
P.Karang Bongko	Seawater	0	0
	Macroalgae	2	$S_3Ma_{1\ Green}$, $S_3Ma_{1\ White}$
	Soft Corals	2	$S_3Kl_{1\ White}$, $S_3Kl_{2\ Balck}$
P.Kotok besar	Seawater	1	$S_4Al_{1\ Green}$
	Macroalgae	0	0
	Soft Corals	2	S_4Kl_{1Gray}, $S_4Kl_{1\ Balck}$
Total		18	

Note: S = sample, Al = Seawater, Ma = Macroalgae, Kl = Soft Corals

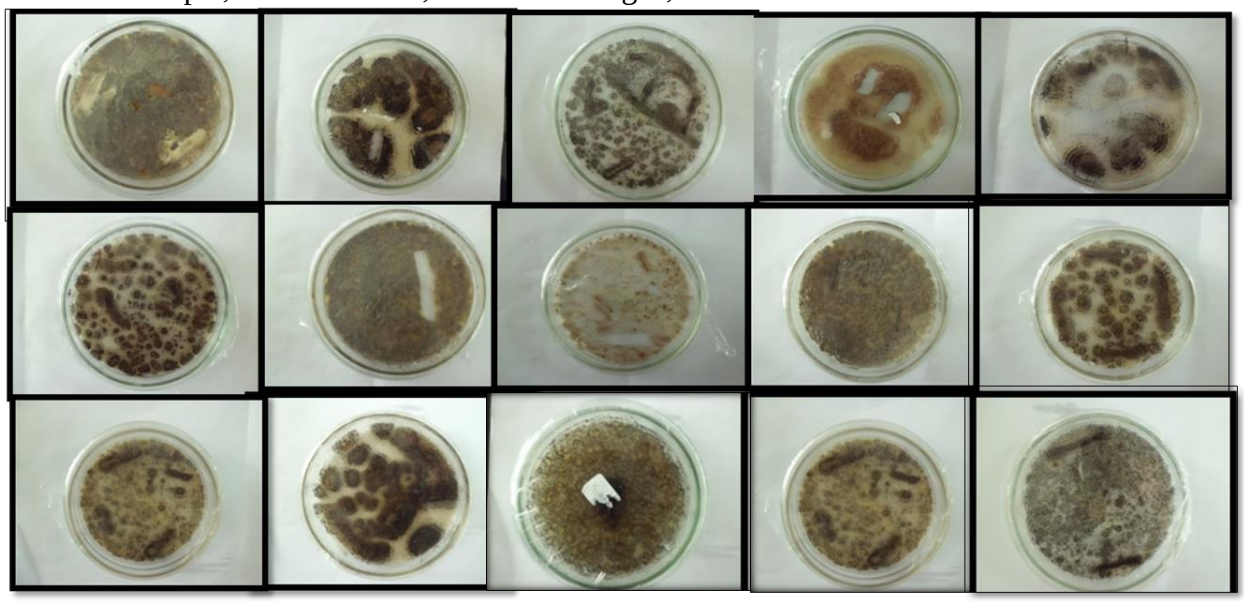

Figure 1. Fungal isolates resulting from isolation from the waters of the Thousand Islands.

B. Antimicrobial Activity of Marine Fungal Isolates against Staphylococcus aureus and Escherichia coli Growth Using the Dilution Method

The inhibitory capability of 10 marine fungal isolates against the test bacteria was observed based on the growth of bacterial colonies after 24 hours of incubation. For further details, refer to Table 2.

Table 2. Average Inhibitory Capability of Marine Fungal Isolates against Test Bacteria *S. aureus* and *E. coli.*

NO	Marine Fungal Isolate Species	Test Bacterial	Concentration				Control	
			25%	50%	75%	100%	Positive	Negative
1	S.AL	*S.aureus*	124	47	4	0	72	805
		E. coli	60	0	0	0	78	822
2	S.MA 1	*S.aureus*	637	440	438	407	75	806
		E. coli	440	476	339	293	72	815
3	S. MA 2	*S.aureus*	573	149	59	0	78	810
		E. coli	522	91	0	0	73	815
4	S. MA 3	*S.aureus*	422	126	59	3	75	810
		E. coli	427	83	7	0	72	821
5	S.MA 4	*S.aureus*	332	137	31	10	76	810
		E. coli	310	69	24	12	73	802
6	S.KL 1	*S.aureus*	611	222	25	0	78	801
		E. coli	484	3	0	0	72	795
7	S.KL 2	*S.aureus*	627	509	417	327	70	800
		E. coli	309	260	297	244	76	800
8	S.KL 3	*S.aureus*	188	51	16	7	77	808
		E. coli	188	54	17	6	82	805
9	S.KL 4	*S.aureus*	112	65	2	0	76	813
		E. coli	340	0	0	0	70	818
10	S.KL 5	*S.aureus*	118	2	0	0	70	820
		E. coli	360	0	0	0	77	815

Table 2 shows that 10 marine fungal isolates have the ability to inhibit both tested bacteria (S. aureus and E. coli), as evidenced by the reduction in the number of bacterial colonies compared to the control (treatment without the addition of marine fungal isolate extracts), and even some treatments showed no growth at all (0 colonies) after 24 hours of incubation. From the data, it can be stated that at the lowest concentration of 25%, there was already a decrease in the growth of the test bacteria, indicating that the Minimum Inhibitory Concentration (MIC) in this study is at a concentration of 25%.

When considering the effectiveness of these marine fungal extracts in inhibiting the growth of test bacteria (Table 1), it is observed that the isolates are more effective in inhibiting *E. coli* compared to *S. aureus*, as seen from the lower number of bacterial colonies that grew in *E. coli* compared to *S. aureus*. This could be due to differences in cell wall structures between Gram-positive and Gram-negative bacteria.

Five marine fungal isolates (S.AL, S.KL 3, S.KL 5, S.KL 4, and S.MA 4) showed the best inhibition of bacterial growth, as indicated by very few or no colonies compared to other marine fungal isolates (Table 1). The results of the One-Way ANOVA test showed that each marine fungal isolate had a highly significant effect on the test bacteria (significance value less than < 0.05). Based on the post-hoc Tukey's Honestly Significant Difference (HSD) test, it was found that five marine fungal isolates, S.AL, S.KL 3, S.KL 5, S.KL 4, and S.MA 4, had equally effective abilities in inhibiting the growth of the test bacteria.

The inhibitory effect of marine fungal isolate extracts increased with increasing concentrations. This can be seen from the reduction in the number of bacterial colonies with increasing concentrations of the tested marine fungal extracts against *S. aureus* and *E. coli*. The 100% concentration showed the best results, with a significant reduction in the number of bacterial colonies,

even to the extent of no growth, followed by the 75% concentration, which was comparable to the negative control (Table 2)

Based on the One-Way ANOVA test of the marine fungal isolates with the test bacteria and treatments, all showed significance values less than 0.05 (Table in Appendix 2). This indicates that the concentration affects the growth of the test bacteria *S. aureus* and *E. coli*. The post-hoc Tukey's Honestly Significant Difference (HSD) test was conducted to determine the most effective concentration among the 10 marine fungal isolates. The results showed that the 100% concentration is the most effective, followed by the 75% concentration, which was comparable to the positive control, indicating that the 100% concentration and the positive control have similar abilities.

C. Minimum Bactericidal Concentration (MBC) of Marine Fungal Isolates against *Staphylococcus aureus* and *Escherichia coli*.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration at which there is no microbial growth after streaking on fresh agar media without the addition of antimicrobial agents and incubated for 24 hours at 37°C (Pratiwi, 2008). The MBC of each marine fungal isolate was determined based on the treatment that showed no bacterial growth (a value of 0) in the previous treatments (Table 1). Subsequently, each tube that showed no growth was streaked on NA medium without the addition of antimicrobial agents and incubated for 24 hours. The results obtained are shown in Table 3.

Table 3. Minimum Bactericidal Concentration (MBC) of Each Marine Fungal Isolate.

NO	Marine Fungal Isolate Species	Test Bacterial	Concentration			
			25%	50%	75%	100%
1	S. AL	*S. aureus*	#	#	#	0
		E. coli	#	0	0	0
2	S.MA 1	*S. aureus*	#	#	#	#
		E. coli	#	#	#	#
3	S.MA 2	*S. aureus*	#	#	#	0
		E. coli	#	#	0	0
4	S. MA 3	*S. aureus*	#	#	#	#
		E. coli	#	#	#	+
5	S.MA 4	*S. aureus*	#	#	#	#
		E. coli	#	#	#	#
6	S.KL 1	*S. aureus*	#	#	#	0
		E. coli	#	#	0	0
7	S.KL 2	*S. aureus*	#	#	#	#
		E. coli	#	#	#	#
8	S.KL 3	*S. aureus*	#	#	#	#
		E. coli	#	#	#	#
9	S.KL 4	*S. aureus*	#	#	#	0
		E. coli	#	0	0	0
10	S.KL 5	*S. aureus*	#	#	0	0
		E. coli	#	0	0	0

Note : (#) No streaking (streak plate) was performed.
(0) No bacterial growth observed.

(+) Bacterial growth observed.

The MBC values resulting from the streak plate (streaking) on agar media are indicated by the absence of bacterial growth (Table 3). There are 5 marine fungal isolates that have MBC values: isolate S.KL 5 against S. aureus at 75% and *E. coli* at 50%, followed by isolates S.AL and S.KL 4 against *S. aureus* at 100% and E. coli at 50%, and then Isolates S.MA 2 and S.KL 1 against S. aureus at 100% and E. coli at 75%. This indicates that these 5 marine fungal isolates have the ability to kill the growth of the test bacteria.

D. Identification

Identification was carried out on the 5 marine fungal isolates that showed inhibitory and bactericidal activity against the test bacteria. Identification was conducted by observing the macroscopic features (colonies) and microscopic features (hyphae and spores) of these five types of fungi. Macroscopic observation was done by examining the color, topography, and the presence of concentric circles on the colonies. Meanwhile, microscopic observation was conducted by examining the structure of hyphae and spores. Referring to the books "Illustrated Genera of Imperfect Fungi" and "A Guide To Tropical Fungi," the five identified marine fungal isolates belong to the species *Penicillium* sp and *Aspergillus niger* (Table 4).

Table 4. Marine Fungal Species After Identification.

No	Marine Fungus	Isolate Code
1	*Pencilium* sp	S.AL
2	*Aspergilus niger*	S.MA2, SKL1, SKL4, S.KL5

The morphology of each isolate is as follows:

Penicillium sp.

Surface colony color: Green fading to yellowish, with a velvety texture (like a carpet), and a globose topography. Reverse side color: Green, with radial lines and concentric circles. Microscopic features: Hyaline, septate. Conidiophores erect, septate, and hyaline to pale-colored. Penicillium sp. usually has penicilli, which are branched, bent-over secondary phialides. Phialides are bottle-shaped with blunt ends and usually clustered in chains at the ends of metulae or directly on conidiophore branches. Conidia are small, elongated or elliptical, unicellular, and hyaline (colorless) or slightly pigmented with somewhat rough walls. The macroscopic and microscopic morphology of the fungus is shown in Figure 2.

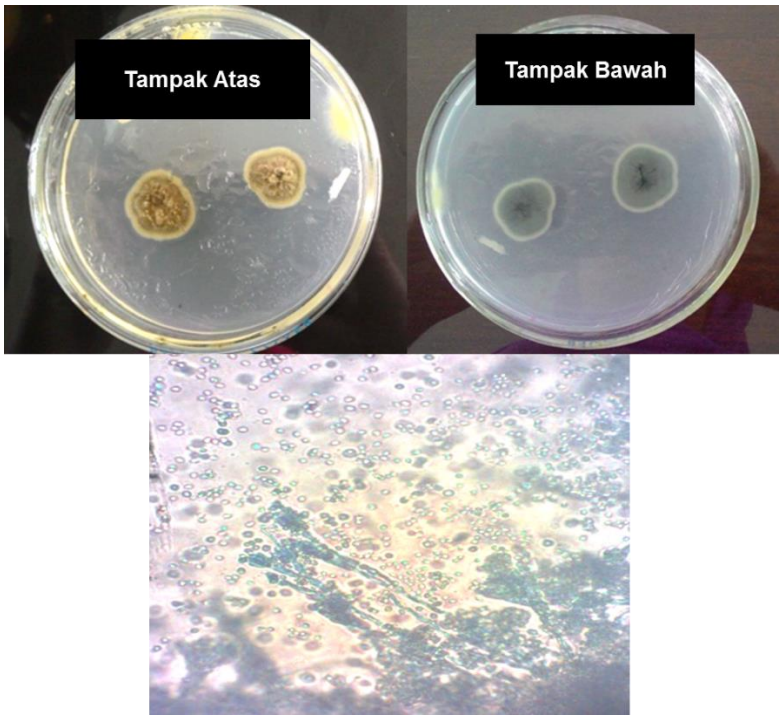

Figure 2. Macroscopic and Microscopic Morphology of *Penicillium* sp.

Aspergillus niger

The colony of *Aspergillus niger* initially has a cottony texture, which changes to fine granular or velvety after conidia formation. It is black in color on the top side and yellowish on the reverse side, with concentric circles and no radial lines. The microscopic morphology of *Aspergillus niger* includes septate hyphae, non-septate conidiophores that emerge from foot cells (swollen and thick-walled mycelial cells). The tip of the conidia is black. Conidia form chains on sterigmata. Macroscopic and microscopic morphology can be observed in Figure 3.

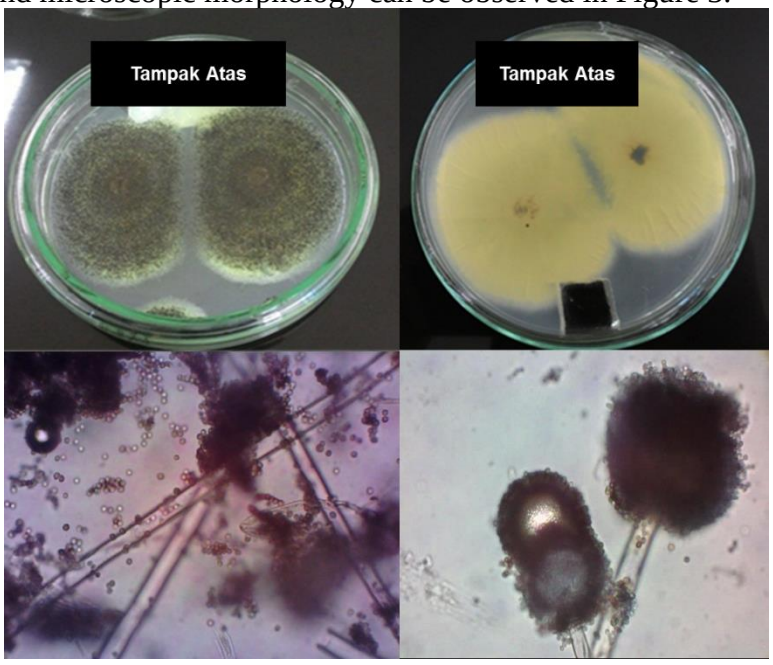

Figure 3. Macroscopic and Microscopic Morphology of *Aspergillus niger*.

DISCUSSION

The ability of the marine fungus extract to inhibit the two types of test bacteria used is due to the presence of antibacterial compounds produced by the 10 isolates of marine fungi. These compounds are capable of damaging the tested bacterial cells, leading to their death. According to Talaro (2008), an antibacterial compound can work by inhibiting the growth of bacteria, either against Gram-positive or Gram-negative bacteria, or it may only work against specific groups of bacteria.

When examining the effectiveness of this marine fungus extract in inhibiting the growth of test bacteria (Table 1), it is evident that the extract from marine fungus isolates is more effective in inhibiting *E. coli* compared to *S. aureus*. This can be observed from the smaller number of bacterial colonies that grew on *E. coli* compared to *S. aureus*. This difference in effectiveness can be attributed, in part, to the structural differences in the cell walls of Gram-positive and Gram-negative bacteria.

Pelczar and Chan (2006) stated that there are differences in the cell wall structures of Gram-positive and Gram-negative bacteria. Gram-positive bacteria consist of a single layer, the relatively thick peptidoglycan layer with a compact wall arrangement. On the other hand, Gram-negative bacterial cell walls have three layers: the outer membrane, middle layer, and inner layer. Gram-negative bacteria tend to have higher lipid content, with peptidoglycan present in the inner layer in smaller amounts compared to Gram-positive bacteria. This condition makes it easier for antibacterial compounds from the marine fungus extract to damage Gram-negative bacterial cells (E. coli), leading to their death.

Apart from the differences in the components of the test bacterial cell walls, the effectiveness of the secondary metabolites produced by the marine fungus extract is also related. According to Losung et al. (2015), marine fungi in symbiosis with marine biota are more effective in inhibiting the growth of Gram-negative bacteria (E. coli) compared to Gram-positive (S. aureus). Furthermore, Renhoran (2012) and Fadilah et al. (2016) found that marine fungi produce antimicrobial compounds that are polar in nature, making them more effective in inhibiting the growth of E. coli.

Riguera (1997) mentioned that the active components that can be extracted from a substance depend on the polarity of the solvent used. Compounds bound to polar solvents include alkaloids, amino acids, polihydroxysteroids, and saponins, while those bound to semi-polar solvents include peptides and depsipeptides, and those bound to non-polar solvents (e.g., hexane) include hydrocarbons, fatty acids, and terpenes. These active components also affect the differences in sensitivity to the test bacteria.

Among the marine fungus isolates, S.AL, S.KL 3, S.KL 5, S.KL 4, S.MA 4 are the most effective in inhibiting bacterial growth, as indicated by the significantly smaller number of colonies or the absence of colonies compared to other marine fungus isolates. However, based on the Tukey's Honestly Significant Difference (HSD) test, it is shown that these five isolates have equal abilities.

The inhibitory effect of the marine fungus extract increases with the increasing concentration applied. This can be observed from the decreasing number of bacterial colonies with the increasing concentration of the marine fungus extract tested against *S. aureus* and *E. coli*. This demonstrates that the concentration has an effect on the inhibition of the test bacteria (*S. aureus* and *E. coli*). At each concentration, the 100% concentration is the most effective, marked by a significant reduction in the number of bacterial colonies, or even the absence of colonies. Subsequently, the reduction in the number of bacterial colonies at a 75% concentration is equally effective as the negative control.

According to Pelczar and Chan (2006), the higher the concentration of antibacterial substances, the higher the inhibitory power. Therefore, if the antibacterial substance used becomes more concentrated, the number of bacterial colonies that grow will decrease. To determine the potential of various concentrations of marine fungus isolates as antibacterial agents, chloramphenicol

is used as the positive control. A concentration that has the same or better ability as the control means it has a very good ability as an antibacterial agent.

Chloramphenicol is used as the positive control because of its ability to inhibit both Gram-positive and Gram-negative bacteria. Chloramphenicol works by inhibiting protein synthesis in bacterial cells. It binds reversibly to the 50S ribosome unit, preventing the binding of amino acids with the ribosome (Setiabudy, 1995). Chloramphenicol is effective in inhibiting the growth of Gram-positive and Gram-negative bacteria because it is a broad-spectrum antibiotic that is active against aerobic and anaerobic Gram-positive and Gram-negative organisms.

The Minimum Bactericidal Concentration (MBC) from the study is obtained from isolate S.KL 5 against *S. aureus* at 75% and *E. coli* at 50%, followed by isolates S.AL, S.KL 1 at 100% and 50%, and then Isolate S.MA 2, S.KL 1 at 100% and 75%. This shows that the five marine fungus isolates have the ability to kill the test bacteria. The Minimum Bactericidal Concentration (MBC) is the smallest concentration at which no bacterial growth is observed after streaking on fresh agar media without the addition of antimicrobial agents and incubation for 24 hours at 37°C (Pratiwi, 2008).

Based on the selective toxicity properties, antimicrobial agents that inhibit bacterial growth are known as bacteriostatic, while those that kill bacteria are known as bactericidal. The minimal concentration required to inhibit growth or kill microorganisms is known as the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), respectively. Antibacterial compounds work by interacting with the bacterial cell wall, leading to inhibited bacterial growth (bacteriostatic) or cell death (bactericidal). Additionally, antibacterial compounds can penetrate the membrane and interact with genetic material, causing bacterial mutations (Amin, 2014).

Bacteriostatic agents work by inhibiting protein synthesis in bacterial ribosomes through passive diffusion through hydrophilic channels and active transportation systems. After the antibacterial agent enters the ribosome, it binds to the ribosome and prevents the entry of the tRNA complex with amino acids in the amino acid reaction, thus preventing bacterial growth (Kimball and John, 2008).

Results of the identification conducted on 5 isolates of marine fungi that exhibited inhibition and killing of test bacteria belong to the species *Penicillium* sp (S.AL) and Aspergillus niger (S.MA2, SKL1, SKL4, S.KL5). Both types of fungi have the ability to inhibit test bacteria due to the production of active compounds. Aspergillus has the capability to inhibit the growth of test bacteria and fungi because it produces secondary metabolites that have the potential as antimicrobials. Generally, the antimicrobial compounds produced by *Aspergillus* are neutral, polar, and possess phenolic groups. Phenol can denature proteins in the cell walls and membranes of bacteria and fungi (Singh et al., 2005).

Four 4-hydroxy-α-pyrones including three newly named nipyrones A–C (1–3) with one germicidin analogue C (4) were discovered from the fungus Aspergillus niger isolated from a sea sponge. These compounds showed good activity against S. aureus and B. subtilis, with minimum inhibitory concentration (MIC) values of 8 μg/mL and 16 μg/mL, respectively, and showed weak antitubercular activity against M. tuberculosis, with MIC values 64μg/mL (Ding *et al.* 2019).

Furthermore, Panda *et al.* (2005) stated that the inhibitory activity against test bacteria and fungi is produced by *Penicillium* due to its ability to produce penicillin, which inhibits the synthesis of peptidoglycan in bacterial cell walls. Penicillin inhibits bacterial cell synthesis by either inhibiting enzyme synthesis or inactivating enzymes that are necessary for peptidoglycan synthesis, an essential component of bacterial cell walls. The inhibition of peptidoglycan synthesis leads to loss of viability and often results in the lysis of bacterial cells. *Penicillium* also produces griseofulvin, a compound

that inhibits the growth of fungi by disrupting the function of spindle fibers and microtubules in the cytoplasm, thus hindering fungal mitosis.

Marine microorganisms have been shown to be a major source of marine natural products (MNP) in recent years, where filamentous fungi are a vital source of bioactive natural products, one of which is *Penicillium* (Ma, H.G et. al. 2016).

CONCLUSION

Based on the research conducted on the antibacterial activity of marine fungi using the dilution method on isolates from seawater, marine coral, and macroalgae, it can be concluded that:

1. Ten isolates of marine fungi tested were capable of inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.
2. Concentration influenced the growth of test bacteria, with concentrations of 100% and 75% showing the most favorable results.
3. The Minimum Inhibitory Concentration (MIC) of the tested marine fungal extracts was 25%.
4. There are five isolates of marine fungi that have Minimum Bactericidal Concentration (MBC) against *S. aureus* and *E. coli*, namely isolates S.KL 5 at 75% and 50% concentration, isolates S.AL and S.KL 1 at 100% and 50% concentration, and isolates S.MA 2, S.KL 1 at 100% and 75% concentration.
5. The identification results indicate that the five best isolates are of the species *Penicillium* sp (Isolate S.AL) and *Aspergillus niger* (Isolates S.MA2, SKL1, SKL4, and S.KL5).

REFERENCES

Ali AJSUoMPM. (2005) Mikrobiologi dasar jilid I.

Amend A, Burgaud G, Cunliffe M, et al. (2019) Fungi in the marine environment: Open questions and unsolved problems. 10: 10.1128/mbio. 01189-01118.

Ali AJSUoMPM. (2005) Mikrobiologi dasar jilid I.

Amend A, Burgaud G, Cunliffe M, et al. (2019) Fungi in the marine environment: Open questions and unsolved problems. 10: 10.1128/mbio. 01189-01118.

Amin LZJM. (2014) Pemilihan antibiotik yang rasional. 27: 40-45.

Ding, L., Ren, L., Li, S., Song, J., Han, Z., He, S. and Xu, S., 2019. Production of new antibacterial 4-hydroxy-α-pyrones by a marine fungus *Aspergillus niger* cultivated in solid medium. *Marine drugs*, *17*(6), p.344.

Ilyas MJB. (2007) Isolasi dan identifikasi mikoflora kapang pada sampel serasah daun tumbuhan di kawasan Gunung Lawu, Surakarta, Jawa Tengah. 8: 105-110.

Kimball and John W. (2008) *Bacteria*, *Kimball's Biology Pages.*

Ma, H.G., Liu, Q., Zhu, G.L., Liu, H.S. and Zhu, W.M., 2016. Marine natural products sourced from marine-derived *Penicillium* fungi. *Journal of asian natural Products research*, *18*(1), pp.92-115.

Panda D, K. Rathinasamy, MK. Santra, et al. (2005) Kinetic Suppression of Microtubule Dynamic Instability by Griseofulvin: Implications for Its Possible Use in The Treatment of Cancer. *Journal of Science and Technology* 102