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NOVEL RAJA AMPAT MARINE BACTERIA PRODUCING ANTIBACTERIAL AGAINTS *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS*

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Abstract

Numerous new chemicals with potential biological activity have been shown to be abundantly produced by marine microorganisms. Antibacterials contained in secondary metabolites of marine bacteria can be a discovery for treating diseases caused by bacteria. The use of antibacterials from secondary metabolites of marine bacteria can reduce cases of resistance caused by bacteria. This research aims to explore marine bacterial isolates from Raja Ampat Island, Indonesia, which have the ability to produce antibacterial compounds. Isolation of bacteria used the spread plate method, screening of antibacterial activity of secondary metabolites of marine bacteria used well diffusion, determination of Minimum Inhibitory Concentration used the dilution method, and determination of the Minimum Bactericidal Concentration used the spread plate method. Seven strains of Raja Ampat marine bacterial isolates were found to be bacilli and gram-negative. Secondary metabolites of marine bacteria have antibacterial activity against *Salmonella typhi* with an inhibition zone of 8.50 mm and *Staphylococcus aureus* with an inhibition zone of 8.46 mm and have a MIC at a concentration of µl/ml against *Salmonella typhi* and *Staphylococcus aureus*. Secondary metabolites of marine bacteria do not have MBC against *Salmonella typhi* and *Staphylococcus aureus*.

Index Terms: Raja Ampat, Marine Bacteria, Antimicrobs, *Salmonella Typhi, Staphylococcus Aureus.*

1. INTRODUCTION

Concerns with bacterial pathogens as infection agents are concerning in public health [1]; according to data from the CDC's (Central for Disease Control and Prevention) antibiotic resistance threats report, at least 2.8 million people are infected with antibiotic-resistant germs yearly, and at least 35,000 people die in the USA [2]. Antibacterials contained in secondary metabolites of marine bacteria can be a discovery for treating diseases caused by bacteria [3]. The use of antibacterials (i.e., protease inhibitors) from secondary metabolites of marine bacteria can reduce cases of resistance caused by bacteria [4].

The Indonesian marine area is 2.5 times wider than the land area [5]. There are many different kinds of habitats in the maritime environment that may be used to find new sources of natural bioactive resources [6]. On the other hand, bioprospecting of marine metabolites and microorganisms with high biotechnological potential has gained widespread interest due to the variability and richness of the marine environment [7]. One

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of the seas in Indonesia that has the potential to have many natural resources is the marine of Raja Ampat Islands, Papua.

The Raja Ampat Islands are located in the world's coral triangle, making the Raja Ampat Islands the richest in biodiversity as bioactive compounds, including marine bacteria [8], [9]. Therefore, exploring marine bacteria in the Raja Ampat Islands is very interesting. Numerous new chemicals with potential biological activity have been shown to be abundantly produced by marine microorganisms [10]. Secondary metabolites produced by marine bacteria have the potential to develop and have a distinctive structure due to complex environmental conditions and potent bioactivity [11]. A promising strategy for drug development is the ongoing search for secondary metabolites in microorganisms isolated from unexplored habitats [12]. According to research by Mahdiyah [4], 136 isolates were filtered in Raja Ampat marine and associated with the *Japis* sp. Isolates showed protease inhibitor activity, which reached 90% in three isolates. This research aims to explore marine bacterial isolates from Raja Ampat Island, Indonesia, which have the ability to produce antibacterial compounds.

2. METHODS

2.1 Isolation of marine bacterial

The Raja Ampat Island seawater is diluted into 10^{-1} – 10^{-5} and then poured into NA (nutrient agar) media using the dispersion method. 20 ml of sample poured into NA medium and spread using L rod, then incubated at 37^{o}C for 24 – 48 hours. The growing bacterial colonies were seen and differentiated based on their characteristics [13].

2.2 Morphological identification

Identification of bacterial morphology aims to see the physical characteristics of bacteria. The results of bacterial morphological characteristics are described in the table based on the bacterial colony's colour, shape, elevation, and edge. Bacterial morphology is determined based on the bacterial colony [14].

2.3 Screening antibacterial activity

The well dilution method is carried out by 20 µl suspension of *Salmonella typhi* and Staphylococcus *aureus* bacteria, equivalent to a standard solution of Mc. Farland 0.5 was put into the NA medium and spread with the L rod. Then 3 holes of 6 mm diameter were made with a cock borer, and the supernatant of marine bacteria was added, then incubated at 37^{o}C for 18 – 24 hours. The inhibition created by a zone around the holes is observed, documented, and measured with calipers [4].

2.4 Determination of minimum inhibitor concentration (MIC)

The nutrient broth (NB) was put into all test tubes, and the supernatant from marine bacteria was added to each test tube with a concentration (v/v) of 5 µl/ml, 7 µl/ml, 10 µl/ml, and 15 µl/ml. Each test tube was added with a suspension of *Salmonella typhi* and *Staphylococcus aureus* bacteria, which had been adjusted to the Mc. Farland 0.5, and

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then incubated at 37^{o}C for 24 hours. The lowest concentration that shows no cloudiness in the tube is the MIC [15], [16].

2.5 Determination of minimum bactericidal concentration (MBC)

20 µL of MIC was put into solid media and spread using the spread technique using L rods, then incubated at 37^{o}C for 24 hours. Observe whether bacteria grow and then count the bacterial colonies with a colony counter. The lowest concentration where there is no bacterial growth is the MBC [4].

3. RESULT AND DISCUSSION

3.1 Isolates marine bacteria

The serial dilutions carried out succeeded in obtaining 7 isolates from Raja Ampat seawater with different characteristics. Environmental structure influences how communities assemble and what metabolic pathways aid in that assembly [17]. The growing bacterial colonies were very dense at a dilution of 10^{-1}. Furthermore, the bacterial density decreases the higher the dilution series. This is because the dissolved bacterial cell content is diluting or decreasing. The serial dilution technique is a simple method for estimating the number of microorganisms in an environment without replicating [18].

3.2 Characteristics of marine bacteria

Observe the characteristics of marine bacteria that have the potential to be antibacterial by looking at the shape, colour, elevation, and edges of the colony, according to Jackman [19] and Zhou [20]. It can be seen in Table 1 that all colonies have a rod shape (bacillus). Isolates 1, 4, and 5 were cream-colored, isolates 2, 3, and 6 were clear white, and 7 were milky white. All isolates had flat elevations and even edges.

Table 1: Characteristics of Raja Ampat marine bacteria

Isolate	Characteristics				
	Shape	Color	Elevation	Edge	Gram Staining
BAL1	Rod	Cream	Flat	Even	Negatif
BAL2	Rod	Clear White	Flat	Even	Negatif
BAL3	Rod	Clear white	Flat	Even	Negatif
BAL4	Rod	Cream	Flat	Even	Negatif
BAL5	Rod	Cream	Flat	Even	Negatif
BAL6	Rod	Clear white	Flat	Even	Negatif
BAL7	Rod	Milky white	Flat	Even	Negatif

3.3 Antibacterial activity

Antibacterial activity screening in this study used the diffusion method, and the results showed the presence of an inhibitory zone around the wellbore derived from BAL 7 marine bacteria in the Raja Ampat Islands, West Papua, Indonesia (Table 2). The widest diameter of the inhibition zones from BAL 7 was 8.50 mm against *S. typhi* and 8.46 mm against *S. aureus*. The current methodology for assessing antibiotic susceptibility testing

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(AST) involves determining an antibiotic's capacity to suppress bacterial growth in vitro under controlled experimental settings [21]. Compared with the AST of the Clinical and Laboratory Standards Institute (CLSI) using methicillin breakpoints, *S. aureus* can be classified as resistant to 20 ml of BAL 7 suspension. The antibiotic activity of BAL 7 (compared to quinolones) against *S. typhi* is also in the resistant category because the inhibition zone was less than 23 mm [22]. However, the CSLI standard uses pure compound antibiotics (drugs) [23]. Furthermore, this research used crude suspension from marine bacteria. A molecule's chemical structure and affinity for a certain biological target determine its antibiotic action [24]. According to Mardiana [25], marine bacteria in symbiosis with living things such as sponges can produce 5-10 more secondary metabolites than free marine bacteria. This is following this study, which uses free marine bacteria without symbiosis, so the antibacterial activity produced is not much, so the results of the antibacterial activity are only moderate.

Table 2: Antibacterial activity of Raja Ampat marine bacteria

Pathogens	Isolates	Clear zone diameter (mm)
Salmonella typhi	BAL 1	8,13
	BAL 2	8,16
	BAL 3	8,20
	BAL 4	8,20
	BAL 5	8,23
	BAL 6	8,36
	BAL 7	8,50
Staphylococcus aureus	BAL 1	8,13
	BAL 2	8,16
	BAL 3	8,20
	BAL 4	8,20
	BAL 5	8,23
	BAL 6	8,36
	BAL 7	8,46

3.4 Minimum inhibitory concentration of marine bacteria

The lowest antibiotic concentration at which bacterial growth is totally inhibited is known as the minimum inhibitory concentration or MIC value. A bacterial strain's in vitro sensitivity or resistance to an antibiotic is determined by its MIC [26]. Three critical uses of MIC values are a foundation for classifying a pathogen's susceptibility to a specific antibiotic, for organisms presenting equivocal results, and particularly in the absence of a clinical breakpoint [27]. Based on Table 3, the MIC value of BAL 7 against *Salmonella typhi* and *Staphylococcus aureus* was obtained at a concentration of 15 µl/ml. According to the Clinical and Laboratory Standards Institute [28], the antibiotic susceptibility testing (AST) of BAL 7 antibiotics against *S. aureus* can be categorized as resistant when compared to various antibiotics (such as vancomycin, erythromycin, and others) which have sensitive breakpoints less than 8 µl/ml. MIC on *S. typhi* also showed resistance to crude extracts of antibiotics from BAL 7 when compared with antibiotics ciprofloxacin and levofloxacin, where the susceptibility is classified as resistant if the concentration is more

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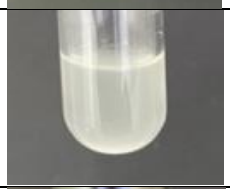
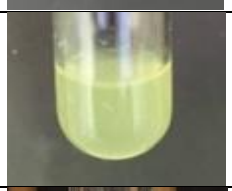
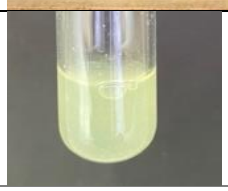
than 2 µl/ml [29]. From the two bacteria, the clarity of the test tube was seen more clearly in the test tube with *Salmonella typhi* bacteria. Antimicrobial drugs are considered therapeutically effective when their toxicity selectivity allows them to eliminate invasive bacteria without causing harm to host cells [30].

Table 3: MIC Raja Ampat marine bacteria

Pathogens	Concentrations	View	Pictures
Salmonella typhi	5 µl/ml	Cloudy	
	7 µl/ml	Cloudy	
	10 µl/ml	Cloudy	
	15 µl/ml	Slightly Cloudy	
	Negative control	Cloudy	
	Positive control	Clear	
Staphylococcus aureus	5 µl/ml	Cloudy	

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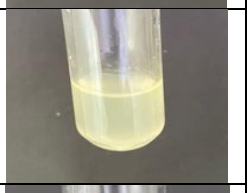
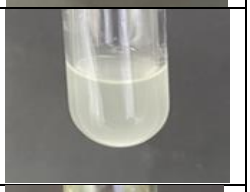
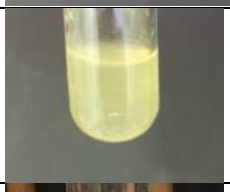
	7 µl/ml	Cloudy	
	10 µl/ml	Cloudy	
	15 µl/ml	Slightly Cloudy	
	Negative control	Cloudy	
	Positive control	Clear	

Notes: a) the significance value of the Kruskal-Wallis Test; b) the significance value of the Mann-Whitney Test. The negative control used bacteria suspension, and the positive control used chloramphenicol.

3.5 Minimum Bactericidal Concentration of marine bacteria

Antimicrobial susceptibility testing (AST) of a particular antibiotic is measured by the MIC, and there are times when this information is not enough to treat people with a weakened immune system or other situations where bacterial death is critical. Therefore, the minimum bactericidal concentration (MBC) is a more accurate indicator of antibiotic action in certain situations [31]. The antibacterial activity test in this study was continued to determine the killing power or MBC of BAL7 against *Salmonella typhi* and *Staphylococcus aureus*, in accordance with Magréault [21] and Khairiah[32]. The MIC obtained from the previous test, where all microbial growth could be inhibited (15 µl/ml) and characterized by the appearance of a clear broth culture, was used for MBC testing. The determination of the value of the MBC was carried out by pouring the MIC test solution on solid NA media. After incubation for 24 hours, the culture was then observed, and the results can be seen in Table 4. The observations showed that MIC of BAL 7 did not show any killing power against *Salmonella typhi* and *Staphylococcus aureus*. This can be seen from the

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growth of the test bacteria on NA media, and the same appearance is also shown in the negative control experiment where only absolute bacteria are grown. A different thing was shown in the positive control culture (Chloramphenicol), which showed no growth of the test bacteria on NA media, which was an indicator of the antibiotic's ability to kill bacteria.

Table 4: MBC of marine bacteria

Isolate	Concentration	Replication		
		I	II	III
Salmonella typhi	15 µl/ml	Grow Colony	Grow Colony	Grow Colony
	Negative control	Grow Colony	Grow Colony	Grow Colony
	Positive control	Clear	Clear	Clear
Staphylococcus aureus	15 µl/ml	Grow Colony	Grow Colony	Grow Colony
	Negative control	Grow Colony	Grow Colony	Grow Colony
	Positive control	Clear	Clear	Clear

Notes: A concentration of 15 µl/ml was used for the concentration of secondary metabolites with MIC; the negative control used bacteria suspension, and the positive control used chloramphenicol.

4. CONCLUSION

Based on the results of research on the Isolation and Identification of Marine Bacteria Producing Antibacterial Against Salmonella typhi and Staphylococcus aureus, it can be concluded that 7 isolates were found in the sea water of the Raja Ampat Islands, West Papua, all of which were rod-shaped; cream, clear, and milky white; flat colonies; and even edges. The results of screening for the antibacterial activity of secondary metabolites which had the highest antibacterial activity were found in BAL 7 with antibacterial ability against Salmonella typhi forming an inhibition zone of 8.50 mm and against Staphylococcus aureus forming an inhibition zone of 8.46 mm. However, in antibiotic susceptibility testing, both test bacteria were classified as resistant at a suspension concentration of BAL 7. The results of the antibacterial activity test showed a Minimum Inhibitory Concentration (MIC) of 15 µl/ml for both test bacteria; however, in the antibiotic susceptibility test, both test bacteria were also classified as resistant at the suspension concentration of BAL 7. The secondary metabolite BAL 7 does not have a Minimum Bactericidal Concentration (MBC). According to the researchers, the resistance observed was because the suspension used was not a pure concentration of LAB 7 secondary metabolite compounds. This is one of the weaknesses of this research. However, there is an opportunity to prove the antimicrobial potential of BAL 7 after it is purified.

Conflict of Interest

All authors declare no conflict of interest in this article.

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