Antibacterial activity of The Soft Coral Symbiont Bacteria *Sarcophyton* sp. Against *Escherichia coli* and *Staphylococcus aureus* Bacteria in The Waters of Lemon Island and Kaki Island, Manokwari

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Abstract. Microorganisms that interact with soft corals contribute to their hosts in the form of protection against marine pathogens. The study aimed to determine the characteristics of symbion bacteria isolated from *Sarcophyton sp soft corals* and their antibacterial activity. Bacterial characteristics were carried out through macroscopic tests by observing the shape of colonies, microscopic tests with Gram staining, biochemical tests including indole tests, motility, MR/VP, Citrate (SCA), temperament, TSIA, catalase, and inhibition tests against pathogenic bacteria *Escherichia* and *Staphylococcus aureus*. The results showed that 13 isolates of soft coral symbiont bacteria could be inhibited. The entire isolate has a round and irregular shape, the edges are flat and notched. Antibacterial isolates have a convex elevation, white and yellow in color. Of the 13 isolates, 3 isolates are gram-positive and 10 isolates are negative. Bacterial isolate is the best antibacterial against *E.coli* with strong inhibitory strength (4 isolation) and very strong (3 isolation). For bacterial isolates, it is the best antibacterial against *S. aureus* with strong inhibitory strength (4 insulation) and very strong (1 isolation). From the results of the inhibition test, all isolates can produce antibacterial properties that inhibit the growth of *E.coli* and *S. aureus bacteria*..

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1 Introduction

Coral reefs are ecosystems that have biodiversity and are highly biologically productive. Prokaryotic groups in corals are very diverse and abundant but information related to microbial diversity is still limited [1]. The influence of microbes on the ecological sustainability of coral reefs is largely not considered the most important factor. Bacteria associated with corals have various functional roles in relation to coral hosts. The health and survival of coral reefs is significantly influenced by the composition of associating microbial communities. Microbial communities have an important role in ecological resilience [2].

Microorganisms associated with soft corals contribute to their hosts in the form of protection against marine pathogens [1]. Marine bacteria have an abundance of antimicrobial molecules that can be utilized [3]. Bacteria that interact with their hosts produce secondary metabolites for defense against the surrounding aquatic environment [4-5]. The genera *Bacillus* and *Pseudomonas* have the highest proportion of antimicrobial activity as protection against the soft coral host *Sarcophyton glaucum* [6].

Damage to coral reef ecosystems, especially in the use of soft coral bioactive compounds, can be reduced by separating associated bacteria. Bacterial filtration against soft corals *Sarcophyton* sp. has been carried out by Huda et al, and obtained bacterial isolates capable of inhibiting *Escherichia coli* and *Staphylococcus aureus* bacteria [7]. This proves that soft coral association bacteria are one of the promising sources as an alternative source of bioactive compounds. Excavation of bioactive compounds by utilizing soft coral biomass *Sarcophyton* sp. can have an impact on the overexploitation of soft corals, so it is necessary to find natural antibacterial sources that are environmentally friendly and conservative.

Papuan waters hold high soft coral biodiversity [8, 9]. This underlies research related to bacteria that symbiote with soft corals is important for further study. The purpose of this study is to obtain bacterial isolates from soft corals *Sarcophyton* sp. which have antibacterial activity that can kill or inhibit pathogenic bacteria *E.coli* and *S.aureus* as well as to know the biochemical characteristics of bacteria with antimicrobial activity.

2 Methods

2.1 Research location and time

This research was carried out from May to June 2021. Sampling of soft corals *of Sarcophyton* sp. carried out in the waters of Lemon Island and Kaki Island, Manokwari Regency, West Papua Province, Indonesia (Figure 1). Samples were collected by SCUBA divers from a depth of 10 to 15 m in the waters of Lemon Island (00° 53'11.99" S and 134° 05'00.14" E) and Kaki Island (00° 44'34.80" S and 133° 59'36.98" E).

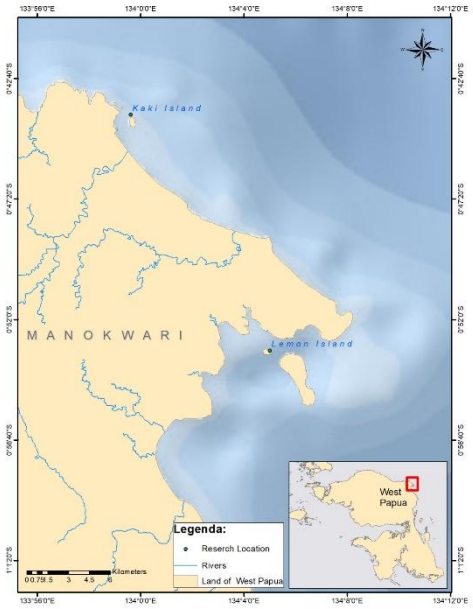

Fig. 1. Research locations in the Waters of Lemon Island and Kaki Island, Manokwari Regency, West Papua Province, Indonesia .

2.2 Data collection

The sampling method used is the purposive sampling method, which focuses on the target by considering certain criteria for the object in accordance with the research objectives [10]. Soft coral samples were taken as much as ±50 g, put into a vial containing seawater, and stored in a cool box filled with ice cubes to be taken to the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, University of Papua. Identification of soft corals *Sarcophyton* sp. was carried out by observation of spicules at the Aquatic Resources Laboratory, Faculty of Fisheries and Marine Sciences, University of Papua.

2.3 Data Analysis

The colony morphological data, Gram staining is a technique used to identify bacteria and check for bacterial pathogens, and biochemical tests were analyzed descriptively with the help of tables and figures. The data on the diameter of the zone of inhibition surrounding an antimicrobial agent-impregnated disc represents the area devoid of bacterial growth. This region, characterized by its lack of microbial colonies, is commonly referred to as the clear zone. Its diameter provides a quantitative measure of the antimicrobial agent's effectiveness against the tested bacterial strain. Measurements are expressed in millimeters (mm). The measurement results are the average of the three measurements [11].

2.4 Isolation of soft coral symbiont bacteria *Sarcophyton* sp

Soft coral samples (Figure 2) were washed using aquades three times (trituation). The sample is fined, and diluted in NaCl to a dilution of 10-4. The test tube was incubated for 2 x 24

hours at 37°C [12,13]. The dilution process is carried out using a multi-level dilution technique [14].

Samples were taken as much as 1 ml to be inoculated in nutrient agar in a petri dish ± 15-20 ml aseptically. The samples were then incubated at 37°C in an incubator for 2 x 24 hours. Dominant bacterial colonies are taken and used in testing. The purification method used is a scratch cup [15]. Colonies that have the same morphology because they come from the division of one cell are pure colonies [16]. The method used for purification is a scratch cup [10]. A gram staining test was then carried out on the bacterial isolate that was successfully purified.

The method used for antibacterial activity tests is the agar diffusion method [17]. The activity test was carried out by inoculating 200 µl of bacterial suspense (*S.aureus* and *E.Coli*) into 20 ml of NA medium that was still warm at 36-37°C (liquid) and then flattened. The bacteria are then left to freeze so that the bacterial suspension freezes and then a paper disk that has been soaked in bacterial isolate is inserted into each dish that has been inoculated with *S. aureus* and *E. Coli* bacteria. Bacterial isolates that have antibacterial activity are characterized through two stages, namely morphological observation (colony and gram) and biochemical tests which include: the indole test, MR/VP, motility, citrate, Triple Sugar Iron Agar (TSIA), and catalase test.

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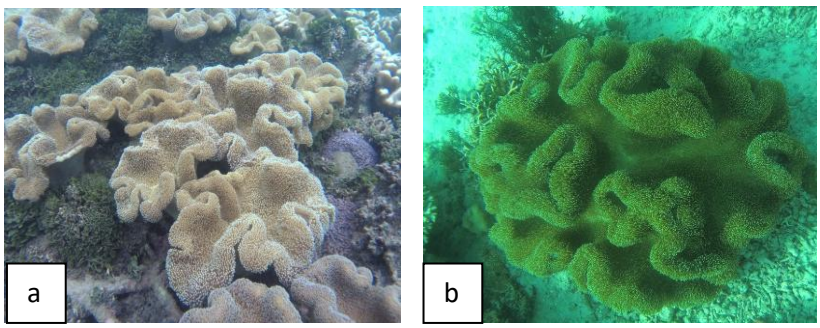

Fig. 2. Types of *Sarcophyton* sp found in the waters of Kaki Island and Lemon Island, Manokwari

Morphological observations are carried out by observing various isolates that have antibacterial abilities. These observations include shape, elevation, edges, and color. Pure cultures are characterized by the same shape, elevation, and edges, so a gram staining test is carried out [18]. Gram-positive bacterial cells are purple to blue while gram-negative bacteria are red.

The biochemical test in the form of an indole test is carried out by taking bacterial isolate as much as one ose, inoculated in indole medium (tryptone broth), incubated for 24 hours, adding a few drops of *Kovac's reagent* to the broth culture. Positive results indicate a pink or color change in the media. The MR (Methyl Red) test was carried out by isolating bacteria as much as one ose inoculated in liquid MR-VP medium in a test tube and incubated for 3x24

hours at a temperature of 37°C. Methyl-red is added as many as 5 drops on top of the bacterial isolate preparation. Positive results when pink to red complexes are formed, which indicates that the microbe produces acid. The VP (Voges Prokauer) test was carried out by taking bacterial isolate as much as one ose, inoculated in liquid MR-VP medium in a test tube, and incubated for 3x24 hours at a temperature of 37ºC. The medium was then added 0.2 mL of 40% KOH and 0.5 mL of alfanaphthol and then beaten for 30". Positive results if the medium changes color. The motility test was carried out by isolating from the culture stock as much as one ose and then inoculated by piercing it in upright SIM medium, incubating at 37°C for 1 x 24 hours. The SIM medium indicates a positive result (motile) if there is a propagation around the needle prick while a negative result (non-motile) if it is the other way around.

The Simmons Citrate Agar (SCA) test was carried out by implanting bacterial colonies in zig-zag scratches on the simmons citrate agar seeding medium. The next stage is the medium is incubated at 37°C for 18-24 hours. Blue colonies show positive results while green colonies show negative reactions. TSIA (Triple Sugar Iron Agar) test, bacterial isolate is taken as much as one ose, inoculated by being inserted into TSIA medium. The bacterial isolate was then taken one more from each culture stock and scratched on the surface of the medium and then incubated for 1x24 hours at a temperature of 37°C. The changes observed after incubation are that the color of the medium becomes yellow indicating acid, the color of the medium becomes redder indicating that the medium becomes alkaline, the color becomes black indicating the formation of $H_{2S\ and}$ when the medium is lifted indicates that the microbes are able to produce gas. The catalase test was carried out by taking 1 ose of bacteria isolated from each culture stock and then dipping it into the H_2O_2 reagent that had been filled into a test tube. The results of the catalase test will show a positive result when gas bubbles are formed at the ose, and negative results if no gas bubbles are formed.

The antibacterial activity test in the form of extraction of secondary metabolite compounds was carried out by culturing the bacterial isolate of the soft coral *association Sarcophyton* sp. taken 1 ose, planted in a sterile Nutrient Broth (NB) liquid medium, and then incubated for 24 hours at a temperature of 37°C. The medium containing bacterial isolate that has been purely cultured is put into an incubator shaker for 3 days, and bacterial cells and supernatants are separated with a centrifuge speed of 9000 rpm for 15 minutes [18, 19].

Testing of antibacterial activity was carried out by the agar diffusion method [19]. The test pathogenic microbes used are pathogenic microbes against humans, namely *S. aureus* and *E.coli* bacteria. The rejuvenated test pathogenic microbes were taken as much as 0.1 ml and put into a petri dish containing 20 ml of NA medium and then spread until evenly using a spreader. The supernatant obtained is dripped on a disc paper as much as 10 ml and then left to dry. The disc paper after drying is placed on the agar medium aseptically. Microbes that are able to produce antibacterial substances will inhibit pathogenic bacteria which are characterized by the presence of clear zones around the disc paper.

The provisions of an antibacterial substance can be said to be very strong if the resistance area has a value of 20 mm or more, it is said to be strong if the resistance area is 10-20 mm, it is said to be moderate if the resistance area is 5-10 mm and if the resistance area is 5 mm or less, it means that the antibacterial power is weak [14].

2.5 Data analysis

The colony morphological data, gram properties, and biochemical tests were analyzed descriptively with the help of tables and figures. The data on the diameter of the inhibition zone was collected by measuring the vertical, horizontal, and diagonal diameters in the clear zone around the disc using a ruler with an accuracy of 0.01 mm 3 times to avoid measurement

errors. Measurements are expressed in millimeters (mm). The measurement results are the average of the three measurements [11].

3 Results and discussion

The results of filtering 30 bacterial isolates from the waters of Lemon Island obtained 6 isolates that have the ability to antibacterial activity that inhibits *E.coli* and *S.aureus* bacteria, while from Kaki Island from 15 isolates, 7 isolates were obtained. This is shown by the formation of a clear zone on the *Nutrient Agar* (NA) medium. The clear zone produced by the potential isolate indicates that the bacterial isolate secretes chemical substances thereby inhibiting the colonization of other microbes. The results of the study were similar to the study [7] which filtered the soft coral association bacteria *Sarcophyton* sp and found that two bacterial isolates were able to inhibit *E.coli* and *S.aureus bacteria*.

3.1 Characterization of the best isolate producing antibacterial activity

The best isolate results that can inhibit two test bacteria *S. aureus* and *E.coli* from Kaki Island are given the isolate codes K1, K2, K4, K6, K7, K9, and K11, while the isolates from Lemon Island Waters are given the codes L1, L2, L3, L4, L5, L6, and L7. All of the isolates were characterized by morphological observations (colonies and gram properties) and biochemical tests which included the indole test, MR/VP catalase test, motility test, SCA test, and TSIA test.

The results of the observation of the colony's morphology were circular and irregular shapes. The edges of the colony are flat (entire) and notched (lobate). All bacterial isolates have a convex elevation, white and yellow (Table 1), this is supported by [20] which states that bacterial colonies can be white, yellowish-white, to golden yellow. The morphology of bacterial isolate colonies found is generally circular, irregular, filamentous, and rhizoid in the form of bacterial colonies. The elevation is raised, convex, flat, umbonate, and crateriform. The edges are in the form of entire, undulate, filiform, curled, and lobate [21].

The results of cell morphological observation by gram staining method showed that of the 13 isolates obtained, generally, colonies were irregular, lobate margins, yellow, gram-negative, and bacillus cell shape [19, 21].

Silica-shaped isolates are indicated by gram staining of purple and round bacteria because they are able to retain crystalline violet dyes even though they are given alcohol-bleaching solutions [19, 21]. The L2, L3, L4, and L6 isolates are red, which means that the bacteria are gram-negative because the bacterial cells do not absorb the main paint (iodine) strongly, so they are rinsed with alcohol and stained with safranin anti-paint (Figure 3). The results obtained are in line with the opinion [22, 23], which states that almost all marine bacteria are gram-negative.

The results of the observation of colony morphology and gram staining of the isolate of *Sarcophyton* sp soft coral symbiont bacteria originating from the waters of Lemon Island, Manokwari can be seen in Table 1. The shape of the colony, the shape of the cell, and the determination of the gram found are in line with [21].

Table 1. Morphology of soft coral symbiont bacteria *Sarcophyton* sp of waters of lemon island and Kaki Island, Manokwari by colony and gram staining.

Isolation Code Bacteria	Shape Colony	Elevation	Banks	Color	Grams	Cell Shape
L1	Circular	Convex	Lobate	White	Positive	Basil
L2	Irregulars	Convex	Lobate	Yellow	Negative	Cocus
L3	Circular	Convex	Entire	Yellow	Negative	Cocus
L4	Irregulars	Convex	Lobate	Yellow	Negative	Cocus
L5	Circular	Convex	Entire	Yellow	Positive	Basil
L6	Irregulars	Convex	Lobate	White	Negative	Cocus
K1	Irregulars	Convex	Entire	Yellow	Positive	Basil
K2	Circular	Convex	Lobate	White	Negative	Basil
K4	Circular	Convex	Entire	White	Negative	Cocus
K6	Irregulars	Convex	Lobate	White	Negative	Basil
K7	Irregulars	Convex	Entire	White	Negative	Basil
K9	Irregulars	Convex	Entire	White	Negative	Basil
K11	Circular	Convex	Lobate	White	Negative	Basil

Caption: L = Lemon; K= Kaki

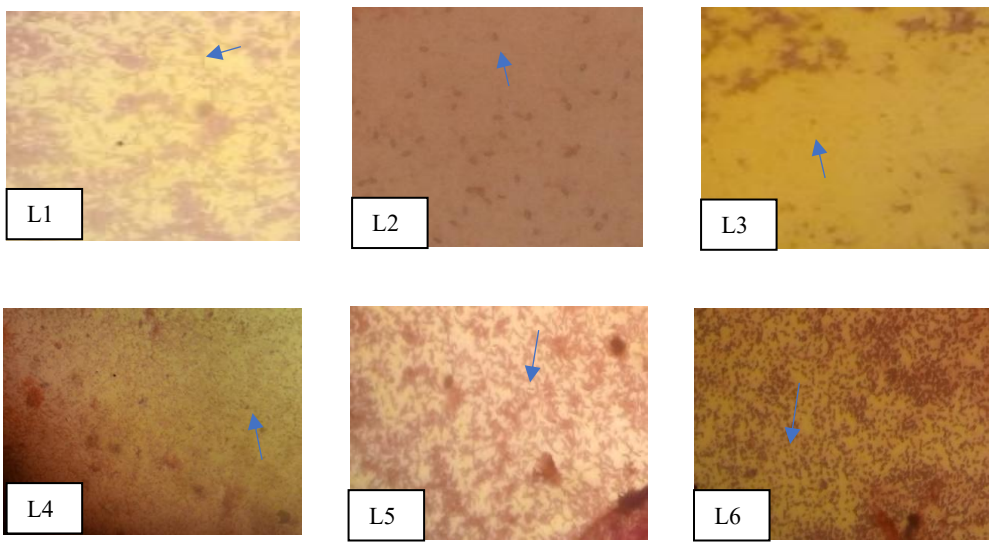

Fig. 3. Example of a sample of the gram staining test of symbiont bacteria soft corals *Sarcophyton* sp from the waters of Lemon Island, Manokwari. L = Lemon

3.2 Biochemical Tests

Biochemical tests were carried out using reagent tests [24]. The results of the biochemical test can be seen in Tables 2 and 3. The indole test is a test to determine the ability of

microorganisms from a sample to produce indole from tryptophan. The results of the indole test showed positive in L4 and L6 bacterial isolates from the waters of Lemon Island, Manokwari. It is known that a red color is formed on the top layer of the liquid after the administration of the Kovac reagent. Thus the bacteria are able to decompose tryptophan. The production of indole in bacteria aims to examine the ability of bacteria to degrade the essential amino acid tryptophan [18]. The amino acid tryptophan is a component of amino acids commonly found in proteins, so amino acids can easily be used by microorganisms due to protein breakdown [25].

Table 2. Observation of biochemical tests on coral symbiont bacterial isolates soft coral *Sarcophyton* sp from the Waters of Lemon Island, Manokwari

No	Test type	Isolation code					
		L1	L2	L3	L4	L5	L6
1	Indol Test	-	-	-	+	-	+
2	MR Test	+	-	-	-	+	-
3	VP Test	-	-	+	+	+	+
4	Motility Test	-	-	-	-	-	-
5	SCA Test	-	-	-	+	-	+
6	TSIA Test	+	-	-	-	+	-
7	Catalase Test	-	-	-	-	-	-

(+) = Positive; MR: Methy-red; SCA: Simmons Citrate Agar
(-) = Negative; VP: Voges Prokauer; TSIA: Triple Sugar Iron Agar

The Methyl Red test aims to determine the ability of a bacterium to produce mixed acids. The results of *the Methyl Red test* were known to be two isolates (L1 and L5) from the waters of Lemon Island and all isolates from Peraiaran Pulau Kaki that had been tested showed positive results.

This indicates that 9 bacterial isolates in fermenting glucose can produce high concentrations of acid. This is because in this test a red color is formed on the medium. The addition of a methyl red pH indicator can indicate a change in pH to acid. Methyl red is red in an environment with a pH of 4.4 and yellow in an environment with a pH of 6.2. Some bacteria ferment glucose and produce a variety of acidic products in large concentrations that will lower the pH of the growth medium to 5.0 or lower [26].

The Voges-Proskauer *test* aims to detect the presence of 2,3-butanediol compounds. The tests carried out gave positive results in L3, L4, L5, and L6 isolates from the waters of Lemon Island. This is shown by the occurrence of pink color change in the solution which indicates the presence of a 2,3-butanediol compound. The pink discoloration of the solution after the addition of Barrite reagent (40% KOH and 5% alfa-naftol solution) indicates the presence of 2,3-butanediol compound. The compound 2,3-butanediol functions as an intermediate to produce acetoin, namely (acetyl methyl carbinol or 3-hydroxbutanone) [27].

Table 3. Observation of biochemical tests on coral symbiont bacterial isolates soft coral *Sarcophyton* sp from the waters of Kaki Island, Manokwari.

No	Test type	Isolation Code						
		K1	K2	K4	K6	K7	K9	K11
1	Indole	-	-	-	-	-	-	-
2	MR	+	+	+	+	+	+	+
3	VP	-	-	-	-	-	-	-
4	Motility	+	+	+	+	+	+	+
5	SCA	+	+	+	+	+	+	+
6	TSIA	-	-	+	+	-	-	-
7	Catalase	-	-	-	-	-	-	-

(+) = Positive; MR: Methy-red; SCA: Simmons Citrate Agar
(-) = Negative; VP: Voges Prokauer; TSIA: Triple Sugar Iron Agar

The motility test is one of the aspects that is looked at to prove the presence or absence of movement of bacterial isolates inoculated on Nutrient Agar media. Based on the tests that have been carried out on six bacterial isolates, the results are negative. This is because there is no spread of isolated colonies around inoculents. This is consistent with the statement [25] that the negative result is that the bacterial growth does not spread and only grows straight at the puncture site.

The citrate test is used to see the ability of microorganisms to use citrate as an energy source. The results of the citrate test found that from the waters of Lemon Island, only two isolates (L4 and L6) were found to be positive, while from the waters of Kaki Island, all isolates showed a positive reaction. This is shown by the color change from green to blue. This color change occurs because in Simon's Citrat media, there is a pH indicator bromine thymol blue. Microorganisms that are able to decompose citrate, the acid is removed from the culture medium, thus changing the color of the medium from green to blue [26].

The Triple Sugar Iron Agar test aims to determine the ability of a bacterium to ferment sugar to produce acids or gases. TSIA medium contains 3 types of sugars, namely glucose, lactose, and sucrose. There are also red phenol and FeSO4 indicators to show the formation of H2S which is indicated by the presence of black deposits. The red color on the agar indicates an alkaline reaction, while the yellow color indicates an acid reaction [28]. The results of the TSIA test (Tables 2 and 3) showed that from both locations, only 4 bacterial isolates showed positive results.

The catalase test serves to determine the ability of bacteria to produce the catalase enzyme. The positive catalase test results were shown by the presence of air bubbles after the bacteria were dripped with a 3% H2O2 solution. Negative results are shown by the absence of air bubbles. Based on the results of the catalase test, it was found that all bacterial isolates reacted negatively after being dripped with 3% H2O2, this was characterized by the absence of the formation of air bubbles in all isolates. This suggests that the entire isolate lacks the catalase enzyme that can degrade hydrogen peroxide (H2O2) into water and O2 [26].

3.3 Antibacterial activity test

The results of the antibacterial activity test showed the formation of an inhibitory zone which was characterized by the appearance of a clear zone around the disc paper. Bacteria that exhibit antimicrobial activity are characterized by the formation of clear zones around bacterial colonies [1, 3, 7, 11, 14]. This indicates the sensitivity of *E.coli* and *S.aureus*

bacteria to the centrifugated supernatant solution of each symbion bacterial isolate isolated from *Sarcophyton* sp. The symbiont bacteria supernatant *Sarcophyton* sp has the ability to inhibit the growth of *E.coli* and *S.aureus* bacteria as shown by the formation of clear zones.

The antibacterial test was carried out using two test bacteria, namely *E. coli* and *S. aureus* against 13 extracts of secondary metabolites (supernatants) of bacteria that symbiosis with soft corals *Sarcophyton* sp. Antibacterial test of soft coral symbiont *bacteria Sarcophyton* sp from the waters of Lemon Island and Kaki Island, Manokwari against *Escherichia coli* bacteria is seen in Table 4 while against *Staphylococcus aureus* bacteria is seen in Table 5.

This study obtained 13 isolates that have potential antibacterial production. Of the 13 isolates, 3 isolates were positive gram isolates, and 10 negative gram isolates, the shape of which was in the form of bacilli and cocus. Thirteen bacterial isolates were obtained from the soft coral symbion bacteria *Sarcophyton* sp, all of which were shown to have antibacterial activity. Where bacterial isolate is the best antibacterial against *Escherichia coli* with strong inhibitory strength (4 isolation) and very strong (3 isolation) while against *S. Aureus*, strong inhibition strength (4 isolation) and very strong (1 isolation).

The difference in the diameter of the inhibitory zone formed between *E.coli* bacteria as gram-negative and *S. aureus* as gram-positive is due to the ability of the soft coral symbion bacteria to extract compound *Sarcophyton* sp. The mechanism of action of antibacterial can occur through several ways namely damage to the cell wall, changes in cell permeability, and inhibition of protein and nucleic acid synthesis. Many factors and circumstances can affect the work of antibacterial, including the number of bacteria, bacterial species, and the presence of organic matter [1, 3, 7].

The causative factor for the difference in the diameter of the inhibition zone between *E.coli* and *S. aureus* bacteria is the difference in cell wall components between gram-positive bacteria and gram-negative bacteria, thus affecting the work of *Sarcophyton* sp soft coral symbiont bacterial extract as an antibacterial. The difference in the sensitivity of gram-positive and gram-negative bacteria to antibacterial substances is caused by differences in wall structure [21].

Table 4. Antibacterial test of *Sarcophyton* sp soft coral symbiont bacteria from the waters of Lemon Island and Kaki Island, Manokwari against *Escherichia coli* bacteria.

Isolation Code Bacteria	Average Diameter of Inhibition Zone (mm)	
	24 hours	48 hours
L1	7,3	7,6
L2	6	8,66
L3	8,66	12
L4	4,33	8,33
L5	7,33	11
L6	3,66	6,66
K1	14,10	21,22
K2	17,94	20,65
K4	6,77	6,77
K6	4,78	21,32
K7	10,79	11,16
K9	12,04	18,06
K11	9,29	8,55

L = Lemon; K= Kaki. The data is the average of 3 repetitions

The cell wall structure of *E. coli* bacteria as gram-negative bacteria has a more complex arrangement of the cell wall. This bacterium has a cell wall with a high lipid content and a multilayer cell wall structure, namely lipoproteins, phospholipid outer membrane, and lipopolysaccharides. The outer membrane of phospholipids can reduce the entry of antibacterial substances into the cell so that the cell wall of *E.coli* bacteria will be more difficult for antibacterial substances to penetrate [1,6,7]. Bacterial isolates were performed from Sarcophyton sp in Tegal Island, Lampung in 10 isolates [7]. The results obtained [7], only 2 bacterial isolates were proven to have antibacterial activity against E. coli and S. aureus. In this study, the 13 isolates obtained all had antibacterial activity where 3 gram isolates (+) and 10 gram isolates (-).

Table 5. Antibacterial test of *Sarcophyton* sp soft coral symbiont bacteria from the waters of Lemon Island and Kaki Island, Manokwari against *Stapylococus aureus* bacteria.

Isolation code bacteria	**Average diameter of inhibition zone (mm)**	
	24 hours	48 hours
L1	16	19,66
L2	5,33	6
L3	14,66	16
L4	4	4,66
L5	4	4,33
L6	6	6,66
K1	11,61	10
K2	13,64	13,56
K4	8,50	8
K6	10,17	10,60
K7	18,85	23,09
K9	12,60	12
K11	12,26	7,55

L = Lemon; K= Kaki. The data is the average of 3 repetitions

The cell wall structure of S. *aureus* bacteria as gram-positive bacteria [29], has a simple cell wall structure with more peptidoglycans, and few lipids, and the cell wall contains polysaccharides (teikoic acid). This makes it easier for antibacterial compounds to enter the cell so that generally the average bacterial growth increases from the beginning of the test to its peak at 48 hours.

Different antibacterial activity occurred due to the difference in the diffusion rate of antibacterial compounds in the agar medium, the type and concentration of the compound gave different inhibitory zone diameters at the length of incubation time of 48 hours. The inhibitory zone formed is due to the presence of bacterial secondary metabolite compounds that have antibacterial activity [1,3,6,7]. Secondary metabolite compounds produced from bacteria that symbiosis with soft corals *Sarcophyton* sp are thought to function in the defense system and show antibacterial activity against test bacteria, namely *E.coli* and *S.aureus*.

Marine bacteria associated with invertebrates, including soft corals, have higher antibacterial activity than other bacteria [30]. Therefore, bacteria that are in symbiosis with *Sarcophyton sp*. can contribute as a new alternative source, particularly as an antibacterial. Like bacteria *Paenibacillus campinasensis* isolated from soft corals *Lobophytum sp.* which has antibacterial activity *E. coli* and *S. aureus* [31]. Communities of bacteria associated with

soft corals *Sarcophyton glaucum* in the Red Sea [1], where the species of bacteria found are Gammaproteobacteria, Actinobacteria, and Firmicutes.

Generally, bacteria that are symbiotic with soft corals have the same bioactive compounds as their hosts. Bacteria that are symbionts with soft corals *Sinularia* sp. have been tested for activity against *E.coli*, *Proteus* sp., *Enterobacter* sp., and *S. aureus* by the agar diffusion method. One in 10 symbiont bacteria has antibacterial activity, with an inhibitory zone size of 9.067 ± 0.305 mm against *E coli* [32].

Diverse microbial communities hold a high diversity of potential proteins. Organisms that share some specific functions can coexist when they differ in other ecological requirements. The diversity of marine microbes reflects the incredible diversity of microbial metabolism and highlights the undiscovered genetic potential in the ocean of microbes [33].

Marine microorganisms with their enormous chemical diversity, provide a rich source of bioactive compounds. Increased research on marine microorganisms as producers of promising new compounds with potential medical applications has led to an increased exploration of marine microbial resources for the discovery of new antibiotics [34].

4 Conclusion

Based on the research that has been conducted, it can be concluded that 13 isolates were obtained that have the potential to produce antibacterial. The entire isolate has a round and irregular shape, the edges are flat and notched. Antibacterial isolates have a convex elevation, white and yellow in color. Of the 13 isolates, 3 gram-positive isolates and 10 negative isolates were obtained, in the form of bacilli and cocci. Thirteen bacterial isolates were obtained from the soft coral symbion bacterium *Sarcophyton* sp, all of which were shown to have antibacterial activity. Where bacterial isolate is the best antibacterial against *Escherichia* with strong inhibition (4 isolation) and very strong (3 isolation) while against *S. Aureus*, strong inhibition strength (4 isolation) and very strong (1 isolation). From the results of the inhibition test, it was found that all isolates have the ability to produce antibacterial properties that inhibit the growth of *Escherichia* and *S. aureus* bacteria.

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