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Antibacterial Activity of Carotenoid from Bacterial Symbiont Virgibacillussp Strain 19.PP.Sc.1.3 from Sinularia against Streptococcus mutans

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ABSTRACT: Infection disease is a disease can be caused by pathogenic microbes. One of microbes that potentially produced antibacterial compound is bacteria that asosiation with soft corals like Sinularia sp. Bacteria that produced carotenoid pigments isolated from soft coral Sinularia sp. The symbiont bacteria used in this research is Virgibcillus sp strain 19.PP.Sc1.3. Carotenoid pigments of symbionary bacteria were extracted using maceration method with methanol solvent. Identification pigment used spectrofotometer UV-Vis. The antibacterial activity test was used by cup plate technique method with ciprofloxacin as positive control and DMSO as negative control. Carotenoid pigments of symbionary bacteria that potencially as antibacterial againts to Streptococcus mutans with mean of blocked zone in concentration 2% is 0,826 cm, in concentration 3% is 0,972 cm and in concentration 4% is 1,132 cm and blocked zone of positive control is 1,382 cm. There are differences in the antibacterial activity of carotenoid pigment extracts from the Virgibacillussp strain 19.PP.Sc1.3 symbiont of the soft coral Sinularia sp. at concentrations of 2, 3 and 4% against the growth of the pathogenic bacteria Streptococcus mutans.

KEYWORDS: Sinularia sp, bacterial symbiont, Virgibacillus sp. strain 19.PP.Sc1.3, carotenoid, Streptococcus mutans, antibacterial.

I. INTRODUCTION

Infectious diseases are the main cause of high morbidity and mortality rates, especially in developing countries such as Indonesia. Infectious disease is a disease caused by the presence of pathogenic microbes [1]. One of the causes of infectious disease is the bacteria Streptococcus mutans. Streptococcus mutans. Streptococcus mutans is a dental caries bacterium with relatively large numbers, which forms stable extra-cellular polysaccharides, has the ability to colonize at relatively low levels of acidity (pH) on the tooth surface so that it plays an important role in the formation of caries [2]. Pathogenic microbes that cause infections can be treated using antibacterials. Antibacterial compounds are defined as biological or chemical compounds that can kill or inhibit the growth and activity of bacteria [3]. These bioactive compounds can be obtained from several sources, including plants, animals, microbes and marine organisms [4].

According to research conducted by [5], it shows that the soft coral Sinularia sp. produces secondary metabolites that have activity against pathogenic bacteria. One type of compound produced by the soft coral Sinularia sp. is a carotenoid pigment [6]. According to [7] carotenoids are a type of pigment that has the potential to be developed as an antibacterial.

II. MATERIALS AND METHODS

Sample Collection

Soft coral Sinularia sp. taken from Panjang Island, Jepara Indonesia. Retrieval of soft coral Sinularia sp. carried out at a depth of 2 meters. Soft coral Sinularia sp. taken by cutting, then washed with sterile sea water and put in a plastic clip. Furthermore, samples of soft corals Sinularia sp. taken to the laboratory to be isolated.

Isolation of symbiont bacteria

The isolation of microorganisms associated with soft corals was carried out by the distribution method. Sample Sinulariasp. as the results of the sampling were washed with sterile sea water. After that, the sample was cut and crushed. The soft corals that had been crushed were put into petri dishes which partly contain sterile sea water. The sample that has been diluted with sterile water is then carried out a series of dilutions. Dilution was carried out by taking 10 ml of each sample with a sterile pipette, then putting it in an

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erlemeyer flask containing 90 ml of sterile sea water and a sample dilution of 10-1 would be obtained. Samples that had been carried out with 10-1 dilution were taken 1 ml of the sample with a sterile pipette and put into a test tube containing 9 ml of sterile sea water and a 10-2 dilution would be obtained. This method was also used to obtain a sample dilution of 10-3; 10-4; and 10-5. Each dilution series that had been obtained was 1 ml sample and put into a sterile petri dish poured with Zobell 2216E agar media. The petri dishes were then incubated at 30oC for 1-2 days.

Bacterial Culture

Bacterial culture aims to multiply bacteria which would be used for the next process. Pure bacteria were taken with a round loop and then planted in 5 ml of sterile liquid Zobell media in a test tube then shaken for 24 hours. After 24 hours, the bacterial culture was mixed into 45 ml of sterile liquid Zobell media, then shaken again for 2 x 24 hours. Then the second culture was mixed again into 450 ml of new sterile liquid Zobell media and shaken for 3 x 24 hours. The culture was made as many as 8 replications so that the final result was 4 liters. After that the bacteria were separated from the liquid media using a centrifuge. Then the bacterial pellets (sediment) were taken and put into a vial for the extraction process.

Extraction of symbiont bacteria pigments Virgibacillus sp. strain 19.PP.Sc1.3

Extraction was carried out using cold methanol [8]. Pellets were extracted until the bacteria become colorless or pale, which indicates the carotenoid has been completely removed. The extract obtained was then separated from the pellets with a centrifuge at 6500 rpm for 10 minutes, then the extraction was filtered then exposed to nitrogen gas to dry to remove the solvent.

Moleculer identification

Based on previous research, bacteria were moleculer identification using PCR, sequencing, and phylogenetic trees. The results showed that the type of bacteria obtained was Virgibacillus sp. strain 19.PP.Sc1.3 [9].

Extraction of Carotenoid Bacteria Symbionts

Extraction was carried out using cold methanol [8]. Pellets were extracted until the bacteria become colorless or pale, which indicates the carotenoid has been completely removed. The extract obtained was then separated from the pellets with a centrifuge at 6500 rpm for 10 minutes, then the extraction was filtered then exposed to nitrogen gas to dry to remove the solvent.

Carotenoid Identification with a UV-Vis Spectrophotometer

Initial identification of the bacterial symbiont pigment was analyzed using a visible spectrophotometer at a wavelength of 300 - 800 nm. Then observed spectra patterns at a wavelength of 300-600 nm.

Antibacterial Activity Testing

The antibacterial activity test was carried out against the pathogenic bacteria Streptococcus mutansusing the well diffusion method. The dry extract obtained was dissolved in DMSO solvent and made in concentrations of 2, 3, and 4%. The positive control used Ciprofloxacin 0.05%. The media in which the suspension of pathogenic bacteria has been planted and given the carotenoid pigment extract of symbiotic bacteria is incubated at a temperature of 37ºC for 1x24 hours. The clear zone around the well indicates that there is antibacterial activity in the carotenoid pigment extract of symbiotic bacteria.

III RESULT AND DISCUSSION

Soft coral Sinulariasp. taken from Panjangisland, Jepara Regency, Central Java, Indonesia. The samples were isolated from the symbiont bacteria Virgibacillus sp. strain 19.PP.Sc.1.3. The wet cell weight of bacteria Virgibacillus sp. strain 19.PP.Sc.1.3 obtained was 8.7463 grams, which after extraction resulted in an extract of 0.2650 grams. From the results of the wet cell weight of the bacteria and the weight of the pigment extract obtained, the yield can be calculated. The yield obtained was 3.03%. The results of bacterial pigment extraction Virgibacillus sp. strain 19.PP.Sc.1.3 can be seen in Figure 1.

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(a) (b)

Figure 1. Isolate bacteria (a) and Carotenoid Extract (b) from Virgibacillus sp. strain 19.PP.Sc.1.3.

Identification of bacterial carotenoid pigment content from Virgibacillus sp. strain 19 PP.Sc 1.3 was carried out using a spectrophotometer instrument. The spectrophotometer used is a UV-visible spectrophotometer with a wavelength range of 300 – 800 nm. The results obtained from measuring the pigment pattern showed that there were 2 peaks in the range of 400 – 600 nm. The peak is at a wavelength of 478.20 nm with an absorbance of 0.230 and 451.60 nm with an absorbance of 0.266. According to [10] and [11], a spectral pattern with a clear peak in the 400 - 500 nm region is an indication of carotenoid pigments. Based on the results of the spectral pattern obtained, it can be concluded that the pigment from Virgibacillus sp. strain 19 PP.Sc 1.3 is a carotenoid pigment. The results of the carotenoid pigment spectra pattern of symbiont bacteria Virgibacillus sp. strain19 PP.Sc 1.3 are presented in Figure 2.

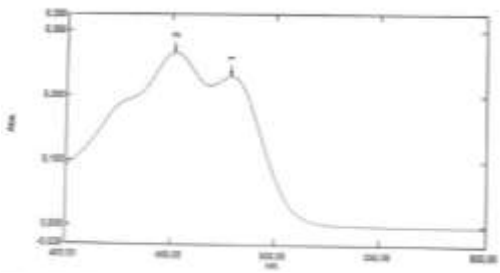

Figure 2. Spectra Pattern of Carotenoid Pigments from Virgibacillus sp. strain19.PP.Sc 1.3

The results of chemical component screening showed that the symbiont bacteria had the same compounds as the soft coral Sinularia sp. namely triterpenoid and carotenoid compounds. According to [7], marine microbes produce the same bioactive compounds as their hosts such as terpenoids, alkaloids, flavonoids and steroids. Based on the screening results obtained, the symbiont bacteria Virgibacillus sp. strain 19 PP.Sc 1.3 produce the same bioactive compounds as their hosts, namely terpenoid compounds. The results of screening for chemical components of symbiont bacteria Virgibacillus sp. strain 19 PP.Sc 1.3 can be seen in table 2.

Table 1. Screening Chemical Compounds from Virgibacillus sp. strain 19 PP.Sc 1.3 extract

Reagent	Literature	Research result	Result
Alkaloid HCl + Dragendroff	Brick red precipitate	No precipitate	-
Flavonoid powder Mg + HCl (p) + amyl alkohol	A red or brown color forms on the amyl alcohol layer	No brown color is formed on the amyl alcohol layer	-
Triterpenoid As.acetat anhidrat + H_2SO_4(p	Purple, red or blue green in color	A purple color is formed	+
Pro-vitamin A $SbCl_3$ 25% in chloroform	A blue solution is formed	No blue solution was formed	-
Tanin $FeCl_3$ 1% Gelatin	The solution is blackish green or dark blue	Doesn't change color	-
Saponin Shaken + HCl 2N	Steady foam	Does not form constant foam	-

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The results of antibacterial activity testing showed that the higher the concentration of carotenoid pigment extract, the greater the antibacterial activity produced table 2 and figure 3.

Table 2. Antibacterial Activity of Carotenoid from Virgibacillus sp strain 19 PP.Sc 1.3

Replikasi	Diameter Zona Hambat (cm)				
	Pigmen Karotenoid 19 PP Sc 1.3			Kontrol	
	2%	3%	4%	Positif	Negatif
1	0,845	0,962	1,104	1,368	0,000
2	0,843	1,002	1,188	1,431	0,000
3	0,774	0,975	1,152	1,384	0,000
4	0,787	0,976	1,120	1,374	0,000
5	0,882	0,946	1,096	1,351	0,000
Rata-rata	0,826	0,972	1,132	1,382	0,000
SD	0,045	0,021	0,038	0,030	-

Figure 3. Inhibition Test Results for Carotenoid Pigments in Symbionts 19.PP.Sc 1.3 against Streptococcus mutans

Based on the data in table 5, it is proven that a 2% concentration has an average inhibition zone of 0.826 cm, while at a 3% concentration the average inhibition zone is 0.972 cm, and at a 4% concentration the average inhibition zone is 1.132 cm. It can be concluded that the increase in concentration of the carotenoid pigment extract of Virgibacillus sp. strain 19.PP.Sc1.3 is directly proportional to the resulting inhibition zone. The larger average zone of inhibition is due to the greater content of active compounds in the extract so that compounds that diffuse into the bacterial suspension planted will further inhibit the growth of Streptococcus mutans bacteria.

The sample's ability to inhibit the growth of Streptococcus mutans bacteria is because the sample contains carotenoid pigments which are a group of terpenoids. According to [12] carotenoid pigments have the potential to be developed as antibacterials. The mechanism of action of carotenoids as antibacterials is to react with porins or transmembrane proteins on the outer membrane of bacterial cell walls, then form strong polymer bonds, resulting in the destruction of porins. Damage to porins is a gateway for the entry and exit of compounds that will reduce the permeability of bacterial cell walls and will result in bacterial cells lacking nutrition, so that bacterial growth becomes stunted or dies [13].

The diameter of the inhibition zone for carotenoid pigments of soft coral symbiont bacteria 19 PP.Sc 1.3 was statistically tested using SPSS (Statistical Product and Service Solutions) 23. Statistical tests were carried out with the aim of determining the differences between concentrations of carotenoid pigment extracts on the growth of Streptococcus mutans bacteria. First, carry out a normality test and homogeneity test. The normality test is carried out with the aim of finding out whether the data is normally distributed or not, while the homogeneity test is carried out with the aim of finding out whether the data is homogeneously distributed or not. Data that is normally distributed is considered to represent the

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sample population. The requirement to be considered normal is homogeneous if the significance value (Sig) is more than 0.05. The SPSS 23 test results show that the data obtained is normally distributed and homogeneous because the significance value is more than 0.05. The SPSS test results are presented in tables 3 and 4.

Table 3. Normality Test Results for Antibacterial Activity of Carotenoid Extracts from Virgibacillus sp. 19.PP.Sc 1.3

Group	Signification	Result
Concentration 2%	0.521	Normally distributed
Concentration 3%	0.870	Normally distributed
Concentration 4%	0.520	Normally distributed
Control (+)	0.395	Normally distributed

Table 4. Homogentiaas Test Results for Antibacterial Activity of Carotenoid Extracts from Virgibacillus sp. strain 19.PP.Sc1.3

Grup	Signification	Result
Based on Mean	0.211	Homogen
Based on Median	0.601	Homogen
Based on Media and with adjusted df	0.603	Homogen
Based on trimmed mean	0.219	Homogen

Normally distributed and homogeneous data were analyzed using parametric tests. Next, a one-way ANOVA test was carried out. The ANOVA results show that there is a significant difference between groups if the significance value is 0.000 ($p < 0.05$). Next, a post hoc test was carried out with the aim of finding out the differences between the groups. Post Hoc test results of the inhibitory power of carotenoid pigments of symbiont bacteria 10 PP.Sc 1.3 against Streptococcus mutans bacteria showed a significance value of less than 0.05, so it can be concluded that there are significant differences between concentration groups and the positive control group. Post Hoc test results are shown in table 5.

Table 5. Post Hoc Test Results of Differences in Carotenoid Pigment Concentrations in Virgibacillus sp. strain 19 PP.Sc 1.3

Concentration	Signification	Result
Concentration 2% vs concentration 3%	0,000	Signification difference
Concentration 2% vs concentration 4%	0,000	Signification difference
Concentration 2% vs positive control	0,000	Signification difference
Concentration 3% vs concentration 4%	0,000	Signification difference
Concentration 3% vs positive control	0,000	Signification difference
Concentration 4% vs positive control	0,000	Signification difference

The results of the antibacterial activity test of the carotenoid pigment extract of the symbiont 19 PP.Sc 1.3 bacteria against Streptococcus mutans bacteria using the Post Hoc test showed significant differences in each group and with the positive control. This shows that each concentration and positive control produces a different inhibitory power in inhibiting the growth of Streptococcus mutans bacteria, so that the inhibitory power produced by the carotenoid pigment solution from 19PP.Sc 1.3 is not able to match the ability of the positive control in inhibiting the growth of Streptococcus mutans.

IV. CONCLUSION

Carotenoid pigment extract from. Virgibacillus sp. strain 19.PP.Sc1.3 has antibacterial activity against the growth of the pathogenic bacteria Streptococcus mutans at concentrations of 2, 3 and 4%. There are differences in the antibacterial activity of carotenoid pigment extracts from the Virgibacillussp strain 19.PP.Sc1.3 symbiont of the soft coral Sinularia sp. at concentrations of 2, 3 and 4% against the growth of the pathogenic bacteria Streptococcus mutans.

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