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Antibacterial activities of bacterial symbionts of soft corals collected from The water of Wai-Sai Island, Papua

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ABSTRACT

This study evaluated antibacterial activities of bacterial symbionts of soft corals from the water of Wai-Sai Island, Papua. The soft corals collected namely Sarcophyton sp, Sinularia sp and Lobophyton sp associated bacteria were found to have antibacterial activity. Antibacterial activity was determined by disc diffusion method against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis. The results of this study indicated that there were 98 isolates collected from soft corals and two isolates from Sinularia sp associated bacteria RA 4.2 and RA 2.2 were found to inhibit the growth of S. aureus, E. coli, K. pneumonia and B. subtillis.

Keywords: soft corals, antibacterial activities, associated bacteria

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INTRODUCTION

Antibacterial compounds can be found in almost all organisms both from the land and from the sea and from both the land plants and marine plants[(1)]. The main obstacle in finding the anti-bacterial compounds from the marine biota is limitation in the sources of biota that have active compounds in addition to the obstacles in cultivation. Some facts have showed that some of the active compounds isolated from the marine biota are produced by symbiotic microorganisms. Solving the problem is an interesting challenge. The microorganisms isolated from invertebrate or from marine biota can be cultivated based on the desired quantities[(2)].

Soft corals are an important and diverse group of colonial invertebrates belonging to the Phylum Coelenterata (Cnidaria), Class Anthozoa, Subclass Octocoralia[(3)]. The soft coral is a kind of marine biota, which lives around the coral reefs and has high pharmacological significance. According to LaBarre et al. (1986), soft corals contain chemical compounds with anti-depressant activity and space competition. They are potential sources of protein, carbohydrates, as well as fats. Some of them have been studied and found to have toxic substances[(4)].

Soft corals are rich sources of bioactive compounds, such as terpenoid, steroid, and steroid glycoside. A recent study found that about 50% of the extracts of soft corals showed toxicity against fish. In addition, many of secondary metabolite from the soft corals had biological activities, such as anti-fungal, cytotoxic, anti-neoplastic, HIV inhibitor, and anti-inflammatory activities[(5,6)].

The study aims at exploring bioactive compounds of the soft corals from the water of Indonesia.

MATERIALS AND METHODS

1.1. Sampling of soft corals and isolation of bacterial symbionts

Three soft corals were collected by Scuba diving from the water Wai-Sai Island at a depth of 2-14 meters. The tissues were then rinsed with sterile seawater and homogenized. The homogenized tissues were serially diluted, spread on half strength ZoBell marine agar medium and incubated at room temperature for 24 hours. On the basis of morphological features, colonies were randomly picked and purified by making streak plates[7].

1.2. Bacterial Identification

Microscopic observation was preceded with gram staining to figure out the bacteria and group of gram-positive and gram-negative bacteria. On the other hand, biochemical tests included triple agar, sulfide Indol Motility, and citrate use, sugar-to-sugar agar, catalase test, oxydase test, and MacConkey Agar[10].

1.3. Antibacterial test

Antibacterial test of soft corals associated bacteria were performed with the agar diffusion method. Samples were impregnated on to sterile filter paper disc and placed on to the Mueller-Hinton agar which were previously swabbed with *S. aureus, E. coli, K. pneumonia* and *B. subtillis.* Chloramphenicol was used as positive control. All the plates were incubated overnight from inhibition zone formed around the paper disc[7].

1.4. Extraction

Extraction of bioactive components in the soft corals was conducted using the method proposed by Chen (2013). The solvents used in extraction process were *n-hexane*, ethyl acetate, and methanol. The ratio of sample and solvent was 1:3. Samples of soft corals were prepared for 100 grams, then chopped and blended, and added with 300 ml of solvents. The first extraction used methanol solvent, with maceration period of 24 hours to dilute the bioactive components in the soft corals. The macerated samples were filtered using filter paper to obtain the desired residues and filtrates. The residues from methanolic extraction were re-macerated using 300 ml of ethylacetate for 24 hours, while the filtrates were evaporated to separate the solvents from the extracts[8].

1.5. Mass culture and fractionation

A seed culture of the Sinularia sp (RA 4.2 and RA 2.2) was prepared by inoculation of 50 mL Zobell marine broth medium in a 100 mL Erlenmeyer flask in a shaker (30°C/250 rpm) for 48 h. This seed culture (10 mL each) was then transferred to five 2 L Erlenmeyer flask containing 1 L Zobell marine broth and cultured with shaking (250 rpm) for 3 days at 30 °C. Cells were separated by centrifugation (4 °C/7000 rpm/20 min). The supernatant was extracted with ethyl acetate, n-hexane and water. The organic layer was concentrated in vacuo and the concentrate was assayed for antimicrobial activity employing disc diffusion method[9].

RESULTS

The results of inoculation based on serial dilution method, namely series of 10^4 and 10^5, were are adequate to represent all of the bacterial colonies, which grew because the bacteria were not too dense but rather varied. The findings are summarized in Table 1 with 10^5 dilutions. They were chosen because colonies were adequately representative for isolation. The following is the colony of RA bacterium under 10^5 dilutions.

Figure 1. Colony of RA bacterium under 10^5 dilutions

Each colony in the germination of RA, MA, and NA was calculated for the number of bacterial, and the results are presented in Table 1.

Tabel 1. Total number of symbiotic bacterial colony

No	Sponge code	Latin name	Symbiont colony number
1.	RA	*Sinularia sp*	$1,4\ 10^4$
2.	NA	*Sarcophyton sp*	$1,5\ 10^6$
3.	MA	*Labophytum sp*	$3,5\ 10^4$

Table 1 shows that the number of RA symbiont bacteria were fewer than those of NA and MA. Each of the bacterial colonies was isolated based on the shape of colony and representative quantites. The isolated bacteria were grown on a tilt agar medium and incubated for 24 hours.

Table 2. The growth inhibition zone of isolates

No	Isolates	Soft coral	*S.aureus*	*E.coli*	*K.pneumoniae*	*B. subtillis*
1.	RA 2.2	*Sinularia sp*	15±1,2	16±0,98	13±1,32	14±3,23
2.	RA 4.2	*Sinularia sp*	16±0,95	18±2,12	13±1,34	6±2,13

Both of the potential isolates (RA 2.2 and RA 4.2) were extracted in a serial way by using solvents with different polarities, namely *n*-hexane and ethylacetate and water, then evaporated to dessiccation using rotavapor, and finally tested for anti-microbial properties as they were for the crude isolates. The results are presented in Table 3 and 4.

Table 3. Antagonistic effect of cell free supernatants from selected bacterial isolates against the test organisms of RA 2.2

Fraction	*S.aureus*		*E.coli*		*K. pneumonia*		*B. subtilis*	
	24	48	24	48	24	48	24	48
Ethylacetate	8	8	-	-	8	10	8	7
Hexane	6	8	-	-	9	6	9	8
Methanol	10	14	-	6	6	18	17	9

Table 4. Antagonistic effect of cell free supernatants from selected bacterial isolates against the test organisms of RA 4.2

Fraction	*S.aureus*		*E.coli*		*K. pneumonia*		*B. subtilis*	
	24	48	24	48	24	48	24	48
Ethylacetate	4	6	-	6	9	12	8	9
Hexane	8	8	-	9	9	9	8	7
Methanol	10	12	10	11	12	14	6	7

Figure 2. Results of anti-microbial activity test for RA 4.2

The results of RA 2.2 and RA 4.2 isolate identification with gram staining and biochemical assay are presented in Table 5.

Table 5. Morphological and biochemical characterization for the identification of RA 2.2. and RA 4.2

Types of Test	RA 2.2	RA 4.2
Gram staining - Shape of bacteria - Staining	 Rod Gram negative	 Rod Gram negative
Biochemical test -TSI agar - H2S -Catalase -Oxydase	 + - - -	 - - - +
Carbohydrate Fermentation - Glucose - Lactose - Sucrose - Maltose - Manitol	 + - + - -	 - + - - -
Other biochemical tests - Indol - Simmon citrate - Motility - Methyl red - VP - Nitrate Reduction	 - + + - - -	 - + + - - +

Table 5 shows that both of the isolates are gram-negative bacteria. The biochemical tests for both isolates found that RA 4.2 was an *Alteromonas sp* while RA 2.2 was *Pseudomonas sp*. Based on the characteristics and after comparison with the characteristics of bacteria described by Cowan and Steel's (1974), it was concluded that the bacterium was *Pseudomonas sp*[10].

DISCUSSION

Indonesia, the world's largest archipelagic country with 17.508 islands and 81,000 km of coastline, is worldwide recognized as being the richest in the world in term of diversity of marine organisms. Indonesian coral reefs in particular have the highest biodiversity in the world, forming the centre of high diversity of marine organisms[11].

Figure 3. Wai sai Island is an area of Raja Ampat, located near to the bird head of Papua, Indonesia[17]

Raja Ampat, West Papua, Indonesia, is known as one of the world's biodiversity hotspots. It has a unique biogeographical history and is located between the Asia and Australia plates, making it an interesting place to explore biodiversity. Raja Ampat is known as a region with a high diversity of flora and fauna[12].

The soft corals of Sinularia were reported to have five types of diterpene compounds that serve to protect themselves from the predator, namely diterpenes flexibilide, dihydro flexiibilide, sinulariolide, episinulariolide, and episinularil acetate. Of the five compounds, only sinulariolide and flexibilide had inhibitory activity against bacteria. Sinulariolide was found to have minimum inhibitory concentration of 5 ppm, while flexibilide was found to have 10 ppm[13].

Antibacterial activity in this study was determined using disc diffusion method. This method has the ability to rapidly identify active metabolites contained in the extract and can be used as an initial screening of antibacterial activity. However, this method greatly depends on the rate of diffusion of chemical compounds in order to obtain extract that has a small diffusion rate, despite having potentially active compounds, will produce a small test activity.

Screening among 98 marine bacteria associated with three soft corals *Sarcophyton sp, Sinularia sp* and *Lobophyton sp* by disc diffusion method against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* revealed that 2 isolates (RA 2.2 and RA 4.2) from *Sinularia sp* wer more active (inhibitory diameter of 6-18 mm) than any other isolates. Results of the study were presented in Table 3. In another study conducted by Sulistiyani et al (2010), there were 109 isolates collected from soft coral *Sinularia sp*, and 5 isolates were found to inhibit the growth of pathogenic resistant bacteria (*S.aureus, E.coli, Enterobacter sp*[7].

Table 3 and 4 present that *n*-hexane fractions of both RA 2.2 and 4.2 were more active than ethyacetate and methanolic fractions. This shows that the n-hexane soluble compounds were more active. Another study showed that two metabolites featuring norcembranoid diterpenes with antimicrobial properties have been isolated from chloroform extract of soft coral of the genus Sinularia, which was collected from the southern coast of India[14]. Cembranoids constitute a large group of natural products isolated from both marine and terrestrial sources. These macrocyclic diterpenoids have been largely found in gorgonians and alcyonarians (soft corals) of the genera *Lobophytum, Sinularia, Sarcophyton*, and *Clavularia*. They are believed to be an important role within the chemical defence arsenal against other reef organisms[15,16].

The method used for bacterial identification was conventional. In a conventional method, the test may include enrichment phase, selective enrichment, and complete test (biochemical test). Based on the characteristic observation and comparison with the characteristics of bacteria elaborated by Cowan and Steel's (1974), the *Pseudomonas* sp. bacteria of Alteromonas strains was positive for rod morphology, glucose oxidation–fermentation, oxydase, nitrate reduction, growth at 0% and 6% NaCl, and 37 °C growth on GSP agar and acid production from mannitol and negative for Gram reaction, growth at 6% NaCl, swarming motility, and urea hydrolysis[10,15].The motile *Alteromonas and Pseudomonas* are typically water-borne bacterial genera and include opportunistic pathogenic species to crustaceans, fish and other aquatic organisms, as well as to humans.

CONCLUSION

Isolates RA 4.2. and RA 2.2 of Sinularia sp were found to have anti-bacterial activities against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* by using disc diffusion method. The results of *Sinularia sp* associated bacteria revealed RA 2.2 *Pseudomonas sp* and RA 4.2 *Alteromonas sp*.

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