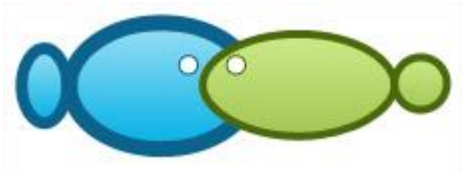

Antibacterial potential of hard coral-associated bacteria against human pathogenic multidrug resistant bacteria

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Abstract. Hard corals are sources of a wide variety of natural compounds with anticancer, anti-inflammatory, antivirus, anti-tumor, and anti-microbial pharmacological properties. This discovery and their consequential extraction to obtain bioactive compounds has led to the overexploitation of reefs, resulting in damaged coral and imbalances in coral reef ecosystems. Hard coral-associated bacteria have been proven to contain compounds similar to their host's. The purpose of this research was to isolate and purify hard coral-associated bacteria, to estimate the potential of their antibacterial activity against human-pathogenic multidrug resistant (MDR) bacteria, and finally to generate a crude extract from the most effective bacterial strains against human-pathogenic MDR bacteria and test it. The sampling of hard corals for this study took place in Panjang Island and Awur Bay, North Java Sea, Indonesia, using purposive sampling methods. Isolation and purification of hard coral-associated bacteria were conducted with serial dilution and streak plate methods on pepton yeast agar media. Screening and confirmation of antibacterial activity were done with the overlay and agar plug method. Furthermore, the most effective bacterial strain was cultivated in liquid medium and extracted using liquid-liquid extraction. Antibacterial assay from crude extract was conducted using the agar disk diffusion method. A total of 32 bacterial-isolates from 7 hard coral genera were obtained: *Acropora, Caulastrea, Favia, Favites, Goniastrea, Isopora,* and *Pachyseris.* 41% of the obtained bacteria were active against human-pathogenic MDR bacteria *Acinetobacter baumannii, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumonia,* methicillin-resistant *Staphylococcus aures* (MRSA)*, Pseudomonas aeruginosa,* and *Staphylococcus haemolythicus*. Most of the active bacterial strains were from hard coral genera *Caulastrea, Favia, Favites,* and *Goniastrea*. Confirmation of the antibacterial activity showed the strongest antibacterial activity against human-pathogenic MDR *E. aerogenes* in two bacterial strains (PHC 43/01 and PHC 49/08) with a zone of inhibition (ZOI) of 25.88±3.23 mm and 21.18±0.17 mm, respectively. The extraction resulted in supernatant and pellet crude extract, but only supernatant crude extract indicated an antibacterial activity against MDR *A. baumannii*, while the pellet did not. The best concentration level for antibacterial activity was 90 µg mL^{-1}, with a ZOI diameter of 11.43±0.85 mm.

Key Words: *Acropora, Acinetobacter baumannii*, crude extract, symbiont, Panjang Island.

Introduction. Infectious diseases are caused by various pathogens, such as viruses, bacteria, and fungi. Community-acquired infections are most frequently contracted by multidrug resistant (MDR) bacteria, namely methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum β-lactamase-producing Enterobacteriaceae (Duin & Paterson 2016). Bacteria from the Enterobacteriaceae family include *Escherichia coli, Enterobacter* sp., and *Klebsiella pneumonia.* The emergence and increase in cases of infections linked to human-pathogenic MDR bacteria has negatively impacted the cost for healthcare and has increased morbidity and mortality rates as well as antibiotic use. Vivas et al (2019) estimate that all currently known antibiotics will no longer be effective at combatting MDR bacteria by 2050. Therefore, the search for new active compounds to

develop the new antibiotics is urgently needed especially against human-pathogenic MDR bacteria.

Since the discovery of penicillin from the fungus *Penicillium notatum*, the exploration of natural products for drug and antibiotic usage has come a long way. Besides land environments, marine environments, which are considered to be unique and more diverse, are host to numerous unexplored compounds with potential. Hard corals are such a source, with compounds that have interesting properties, like antibacterial, antiviral, anti-inflammatory, and cytotoxic activities. *Porites lobata* is a hard coral found in Hawaii that contains compounds that inhibit the growth of bacteria (Gochfeld & Aeby 2008). Other hard coral families showing antibacterial activity through their crude extract are *Acropor*a sp., *Tubastrea* sp., *Tubastrea coccinea*, *Turbinaria* sp. and *Fungia scutaria* (Kelman et al 2006; Meyer et al 2009; Sato et al 2013; Bianco et al 2016). Other compounds with various properties are being extracted from hard coral families like Poritidae, Dendrophylliidae, Milleporidae, Acroporidae, Pocilloporidae, Oculinidae, Helioporidae, Pectiniidae, and Mussidae (Kim 2015). However, the direct extraction of bioactive compounds from hard corals often damages the coral reef ecosystem due to hard coral yielding only small amounts of active compounds (Proksch et al 2002).

Thus, some researchers use the symbiont or associated microorganisms of invertebrates to search for new active compounds. Natural products from marine organisms are mostly sourced from microsymbionts (bacteria and fungi) from invertebrates, i.e. sponges (Sabdaningsih et al 2020), corals (hard corals and soft corals) (Cristianawati et al 2020), tunicates (Ayuningrum et al 2019a), nudibranch (Kristiana et al 2020), bryozoans (Asagabaldan et al 2019), and many more. Bacterial symbionts from marine invertebrates and corals have become a primary target for the exploration of antibacterial compounds for pharmaceutical industry. This study makes an antibacterial evaluation of the potential of hard coral-associated bacteria against human-pathogenic MDR bacteria.

Material and Method

Chemicals and reagents. A variety of chemicals and reagents were used in this study, namely nutrient broth (Hi-media), nutrient agar (Hi-media), Muller Hilton broth (Hi-media), Muller Hilton agar (Hi-media), aquabides (Onemed), ethyl acetate (Merck), methanol (Merck), dimethyl sulfoxide (Merck), paper disc blank (Advantec-Japan), paper disc with antibiotics for positive control using chloramphenicol 30 µg per disc (Oxoid), and finally nitrogen gas (PT. Samator, Semarang).

Sampling and hard coral identification. Hard coral specimens were collected in February, 2016 from Panjang Island and the Awur Bay vicinity in the North Java Sea, Indonesia, by skin diving to a depth of approximately 3 m (Figure 1). Immediately after extraction, the hard corals were placed in sterile ziplock plastic bags and stored inside a cool box with ice gels to keep them refrigerated, and were carefully transported to the Tropical Marine Laboratory, Diponegoro University, Semarang. Identification was subsequently carried out with the samples submerged in water, through morphological observation according to Kelley (2009). Additionally, identification was also done by observing the corallite and matching them with corallite as suggested by Suharsono (2008).

Environmental quality measurements. The measurement of environmental quality included salinity, visibility, temperature and dissolved oxygen (DO) determinations. The parameters were measured using the proper tools including refractometer (Hand Refractometer, Salinity, FG100sa, Hach), Secchi disk, thermometer (PH-689 PH ORP TEMP Meter Digital), and DO meter (WQC HACH SensiON 156-20), respectively. The measurement was conducted 3 times in each sampling point (Figure 1), approximately 300 m from the shore and only at the water surface.

Isolation and purification of microbes. Isolation of hard coral-associated bacteria was done using the serial dilution method (Benson 2002). The hard corals were cut into small

pieces and then crushed with a mortar and pestle. 5 mL of the resulting mush was poured into 4.5 mL of sterile seawater to make a 10^{-1} dilution, and this was continuously diluted until the dilution reached 10^{-4}. Subsequently, 35 µL of this mixture was poured on a sterile peptone yeast agar plate and incubated for 24 h at room temperature (28±2℃). The purification process was carried out using the streak plate method (Willey 2008). Each colony appearing differently in shape, color, elevation, size and margin (Table 2) was separated on a new agar medium. The purified result was then incubated at room temperature for 24 h and observed for growth. The transfer and maintenance of bacterial strains was carried out by transferring pure cultures onto a newly prepared slant.

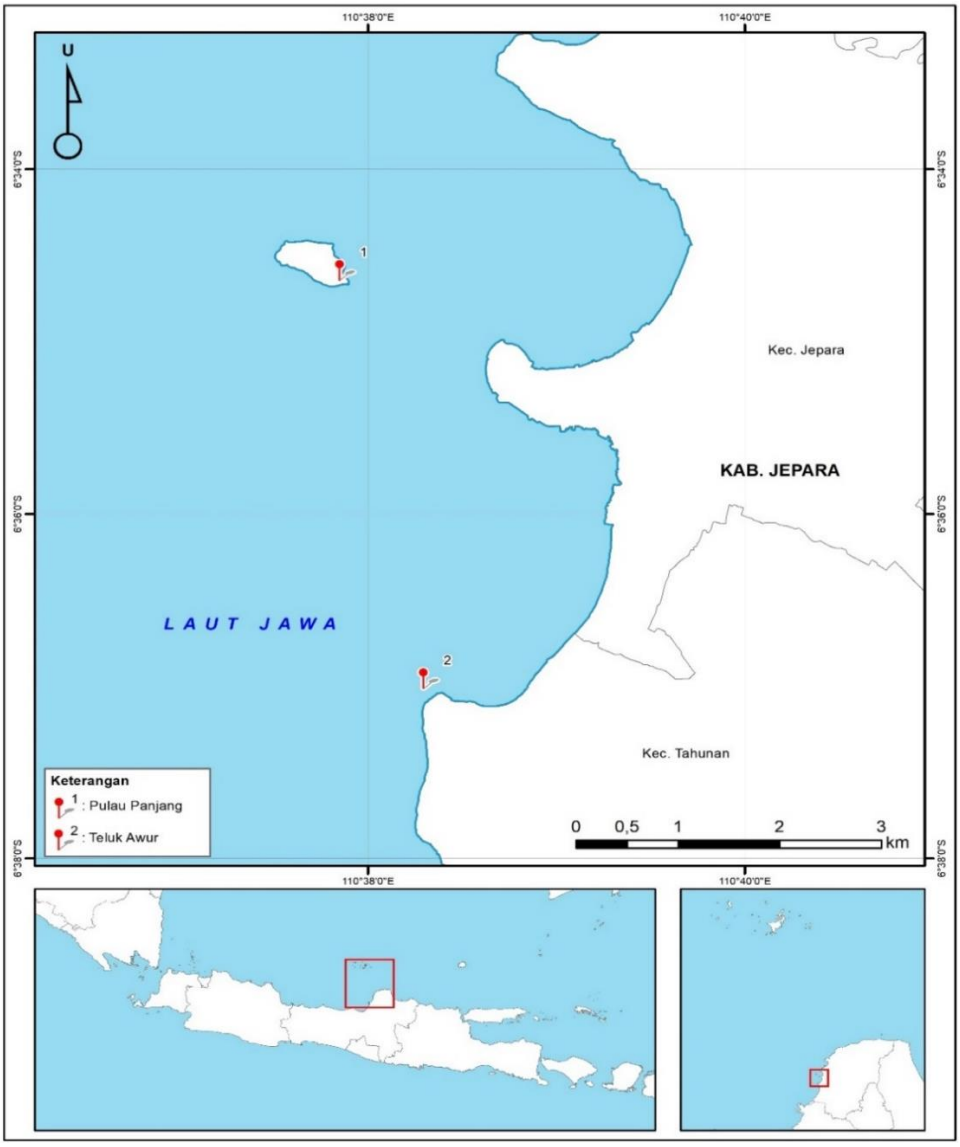

Figure 1. Sites of hard coral sampling. 1 - Panjang Island; 2 - Awur Bay, North Java Sea, Indonesia.

Pathogen preparation. The human-pathogenic MDR bacteria *A. baumannii, E. aerogenes, E. cloacae, E. coli, K. pneumonia, S. aureus,* and *S. haemolyticus* were obtained from Dr. Karyadi Hospital, Semarang, Indonesia. The all human-pathogenic MDR bacteria were cultured a day prior to the antibacterial assay, in a solid medium Muller Hilton agar (MHA). Further preparation included 3-4 colonies of the bacteria being separated and placed in a sterile physiologic salt solution and measured until they reached 0.5 McFarland, or a density of $1x10^7$ CFU mL^{-1}. Bacteria solutions were compared using the McFarland standard (Hi-media).

Screening of antibacterial activity. Screening of antibacterial activity was aimed at determining the biological potential of hard coral-associated microbes against human-pathogenic MDR bacteria. The method used for the antibacterial testing was the overlay method (Radjasa et al 2008). When tested this way, the isolate or hard coral-associated microbes with antimicrobial potency will show a clear zone surrounding it.

Antibacterial assay. Bacterial strains that showed antibacterial activity during the first screening were further tested using the agar block method (Ayuningrum et al 2019b). The bacterial isolates were cultivated 4 days prior to the assay, whereas the human-pathogenic MDR pathogens were cultivated only a single day before. At a density of 10^7 CFU, the pathogens were swapped onto a MHA-medium, and the block from the bacterial strains was subsequently placed onto it. This step was replicated in 3 different MHA plates and the ZOI was measured in mm.

Liquid cultivation and extraction. The extraction of bioactive compounds from *Pseudoalteromonas flavipulchra* was based on a previous study (Ayuningrum et al 2017) and started with the liquid culture of the bacterium. The starter used was 10 mL of *P. flavipulchra* in marine nutrient broth (NB) medium placed on the orbital shaker (Corning) for 1x24 h. Then, the starter was poured in 150 mL of marine NB inside a 250 mL Erlenmeyer and placed on the shaker for 1x24 hour. The culture was done in stages until placed in a 1 L Erlenmeyer containing 500 mL of marine NB. The cultures were incubated and stirred at 100 rpm for 24 h at 25°C. After incubation, the bacteria cultures were centrifuged (Corning) for 10 min at 6000 rpm. Harvesting was done by separating the cells from the medium using a centrifuge. Then, the supernatant was mixed with ethyl acetate and pelleted with methanol solvent 1:1 (v/v) ratio. The mixed suspension was shaken for 15 min, then separated using a separating funnel (Pyrex). The ethyl acetate layer was evaporated using a rotary evaporator (Buchi R-124) at 40°C. Meanwhile, the mixture between pellet and methanol after separation (with filter paper) was collected in vials. The results of both extractions were collected in vial recipients and concentrated using nitrogen gas.

Crude extract antibacterial assay. The evaluation of crude extract antibacterial activity against MDR *A. baumannii* was performed using the disk diffusion method. The first step was making the crude extract concentrations of 15 µg mL^{-1}, 30 µg mL^{-1}, 60 µg mL^{-1}, 90 µg mL^{-1}, 180 µg mL^{-1}, 250 µg mL^{-1}, 350 µg mL^{-1}, and 500 µg mL^{-1}. Then, the MDR 0.5 McFarland was swapped on the Muller Hinton agar medium. The sterile paperdisc (d=8 mm) was placed on the surface, and 30 µL of extract of each concentration was added in each paper disc. The positive control used Chloramphenicol 30 µg per disc, while the negative control used the solvent. Then, the plates were incubated at 37°C overnight and the inhibition zone was measured. All measurement data were determined using a Vernier Caliper (Tricle Brand), in three replicates and expressed as mean±SD (n=3).

Results and Discussion

Environment quality. According to the measurements during the sampling presented in Table 1.

Table 1

Water quality at sampling sites Awur Bay and Panjang Island, Indonesia

No	*Location*	*Water quality*			
		Salinity (‰)	Visibility (m)	Temperature (℃)	Dissolved oxygen (mg L^{-1})
1	Awur Bay	30	0.8	29.3	11.91
2	Panjang Island	30	1.3	29.7	9.9

Identification of hard coral. A total of 7 hard coral specimens were collected from Panjang Island and Awur Bay, Indonesia. They were PP-HC-16-35, PP-HC-16-43, PP-HC-16-45, PP-HC-16-47, PP-HC-16-48, PP-HC-16-49, and PP-HC-16-15. These were successfully identified as belonging to 7 different genera: *Acropora, Caulastrea, Favia, Favites, Goniastrea, Isopora,* and *Pachyseris* (Cristianawati et al 2020).

Isolation and purification of bacteria. From the 7 hard coral genera, a total of 32 axenic bacterial strains were successfully isolated and purified. The highest number of bacterial strains was found on genus *Favia*, followed by genus *Isopora* with a concentration of bacterial strains of 25% and 22%, respectively. *Acropora, Goniastrea*, and *Favites* had the lowest concentrations of bacterial strains, each at 9% (Figure 2).

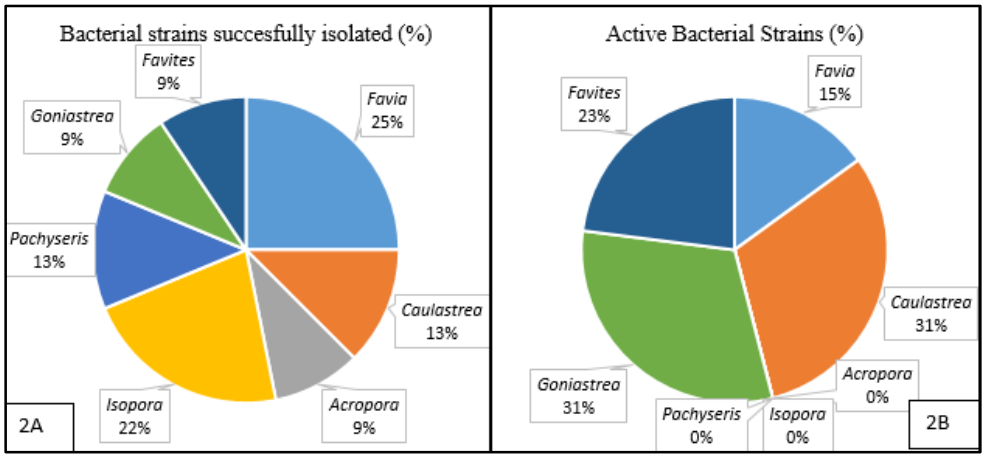

Figure 2. A - the percentage of bacterial isolates successfully isolated from the hard coral specimens. B - the percentage of bacterial isolates from the hard coral specimens that showed antibacterial activity against human-pathogenic multi-drug resistant bacteria.

Morphological characterization. From the 7 hard coral samples, a total of 32 bacterial isolates with unique morphologies were successfully isolated. The results of the morphological characterization are presented in Figure 3 and Table 2.

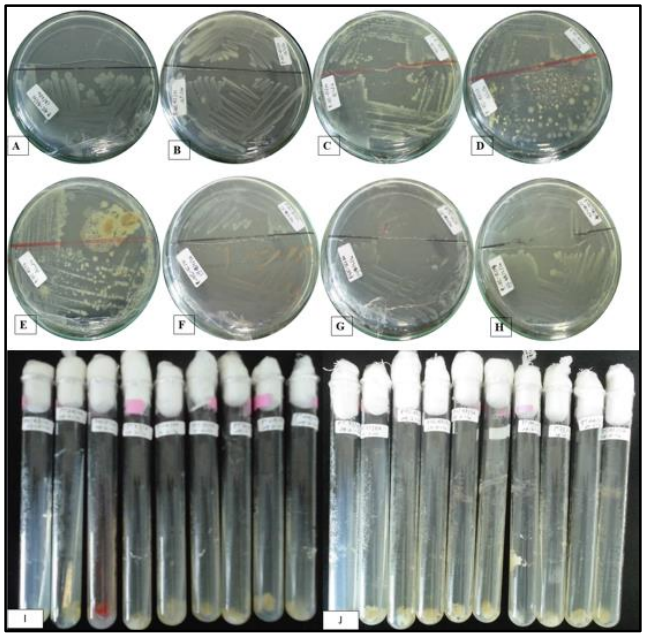

Figure 3. Bacterial isolates from the hard coral specimens showing the colony morphology on the plates (A-H), and in slant media (I-J).

Table 2

The results of the bacterial isolation process from hard coral specimens

No	*Isolate code*	*Morphological characteristics*				
		Color	*Size*	*Shape*	*Margin*	*Elevation*
1	PHC35-01	Red	Small	Round	Entire	Convex
2	PHC35-02	Clear white	Small	Round	Entire	Convex
3	PHC35-03	Turbid white	Medium	Round	Entire	Convex
4	PHC35-04	White	Pin point	Round	Entire	Convex
5	PHC35-05	Yellowish white	Small	Round	Entire	Convex
6	PHC35-06	White	Small	Round	Entire	Convex
7	PHC35-07	Clear white	Big	Filament	Filament	Flat
8	PHC35-08	Turbid white	Small	Round	Entire	Convex
9	PHC43-01	Milky white	Pin point	Round	Entire	Convex
10	PHC43-02	Clear white	Pin point	Round	Entire	Convex
11	PHC43-03	Turbid white	Big	Round	Lobate	Convex
12	PHC43-04	Yellow	Pin point	Round	Lobate	Convex
13	PHC45-01	Turbid white	Medium	Round	Entire	Convex
14	PHC45-02	Translucent white	Pin point	Round	Entire	convex
15	PHC45-03	Turbid white	Small	Round	Entire	Convex
16	PHC49-01	Yellowish white	Small	Round	Entire	Convex
17	PHC49-02	White	Medium	Round	Entire	Convex
18	PHC49-03	Clear yellow	Small	Round	Entire	Convex
19	PHC49-04	Turbid white	Small	Round	Entire	Convex
20	PHC49-05	White	Small	Round	Entire	Convex
21	PHC49-06	White	Pin point	Round	Entire	Convex
22	PHC49-07	White	Big	Round	Entire	Convex
23	PHC47-01	White	Pin point	Round	Entire	Convex
24	PHC47-02	White	Small	Round	Entire	Convex
25	PHC47-03	Clear white	Small	Round	Entire	Convex
26	PHC47-04	Yellowish white	Moderate	Irregular	Lobate	Raised
27	PHC48-01	Yellowish white	Pin point	Round	Entire	Convex
28	PHC48-02	White	Pin point	Round	Entire	Convex
29	PHC48-03	Yellow	Pin point	Round	Entire	Convex
30	PHC15-01	White	Pin pint	Round	Entire	Convex
31	PHC15-02	White	Small	Round	Entire	Convex
32	PHC15-03	Yellow	Small	Round	Entire	Convex

Screening of antibacterial activity against human-pathogenic MDR bacteria. 13 (41% of total strains) bacterial strains presented activity (Table 3 and Figure 4). The antibacterial activity between each hard coral specimen differed. Whereas *Acropora, Isopora,* and *Pachyseris* had no active bacterial isolates (0%), the genera *Goniatrea* and *Caulastrea* had the highest amounts of active bacterial isolates (31%), followed by genera *Favites* (23%), and *Favia* (15%).

Table 3

Screening results

No	*Isolate code*	*Antibacterial activity against human-pathogenic MDR bacteria*							
		AB	*SA*	*PA*	*EC*	*SH*	*EA*	*ECHE*	*KP*
1	PHC43/01	+++	-	+	-	-	-	-	-
2	PHC43/03	-	-	-	+	-	-	-	-
3	PHC43/05	-	+	-	-	-	++	-	-
4	PHC43/08	-	+++	-	-	-	-	-	-
5	PHC49/02	-	-	-	-	+	++	+	-
6	PHC49/04	-	-	-	-	+	-	-	-
7	PHC49/07	-	++	-	-	-	-	-	-
8	PHC49/08	+++					++		-
9	PHC15/02	-	++	-	-	-	-	-	-
10	PHC15/03	-	-	-	+	-	-	-	-
11	PHC15/04	-	+++	-	-	-	-	-	-
12	PHC35/07	-	-	-	-	-	-	-	+
13	PHC35/08	-	-	-	-	-	-	-	+

Note: MDR - multidrug resistant; AB - *Acinetobacter baumannii*; SA - *Staphylococcus aureus*; PA - *Pseudomonas aeruginosa*; EC - *Escherichia coli*; SH - *Staphylococcus haemolyticus*; EA - *Enterobacter aerogenes*; ECHE - *Enterobacter cloacae*; KP - *Klebsiella pneumonia*; (-) - no activity; (+) - zone of inhibition (ZOI) between 7-11 mm; (++) - ZOI between 12-16 mm; (+++) - ZOI between 17-21 mm.

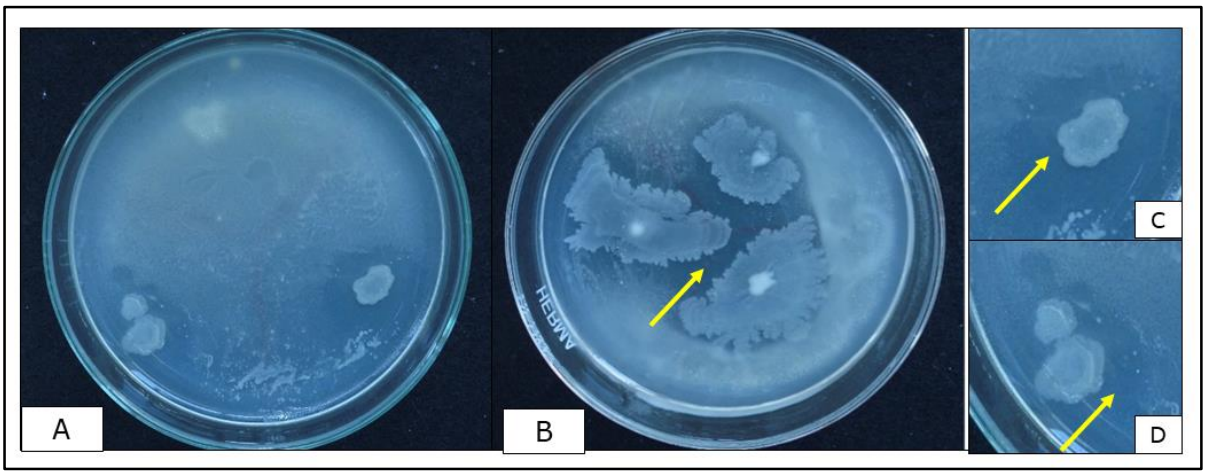

Figure 4. Screening results of bacterial strains from hard coral *Goniastrea* (A) and *Caulastrea* (B). The inhibition zone is indicated by the yellow arrows (B, C, D).

Confirmation of antibacterial activity against human-pathogenic MDR bacteria. Results from confirming the bacterial strains obtained from prior screening showed which isolates retained anti-human-pathogenic MDR activity. Based on these results, the two foremost active bacterial strains were PHC 43/01 and PHC 49/08 with antibacterial activity against *Enterobacter aerogenes* (Table 4).

Table 4

Confirmation results of antibacterial testing of hard coral-associated bacterial strains against human-pathogenic multi-drug resistant bacteria

Bacterial strain	*Repetition 1 (mm)*	*Repetition 2 (mm)*	*Repetition 3 (mm)*	*Average±SD (mm)*
PHC 43/01	22.20	28.25	27.20	25.88±3.23
PHC 49/08	21.00	21.20	21.35	21.18±0.17

Generation of crude extract. One of the best bacterial strains were being cultivated in liquid state and extracted. The result of the extraction process from the medium and pellet is presented in Table 5.

Table 5

The extraction of liquid cultivation from hard coral associated bacteria

	Culture volume (mL)	*Solvent volume (mL)*	*Mass of crude extract (g)*	*Form*	*Color*
Supernatant	700	700	0.1193	Paste	Dark yellow
Pellet	50	50	0.1887	Paste	Dark yellow

The result of yield from supernatant and pellet was different, pellet resulting in more crude extract mass.

Crude extract antibacterial activity. Both crude extracts from previous process were tested for antibacterial activity against MDR bacteria *A. baumannii*. The antibacterial test results are presented in Table 6.

Table 6

The inhibition zone of crud extract from hard coral associated bacteria

		Diameter of inhibition zone (mm)	
		Supernatant	*Pellet*
Concentration (µg mL^{-1})	15	9.97±0.59	8
	30	10.17±0.45	8
	60	10.38±0.19	8
	90	11.43±0.85	8
	150	9.2±0.78	8
	250	9.8±1.13	8
	350	10.46±0.65	8
	500	12.1±1.28	8
Control (+)	Chloramphenicol	24.2±1.3	23.25±0.55
Control (-)	Ethyl acetate	8	-
	Methanol	-	8

Although the pellet gave more crude extract, the antibacterial activity was lower. The best activity was performed by the supernatant crude extract.

Based on Keputusan Menteri Negara Lingkungan Hidup (2004), the environmental parameters such as temperature and dissolved oxygen (DO) were within normal ranges. Salinity and visibility, however, were substandard. Standard salinity for coral is 33-34‰, and visibility is normally more than 5 m (Keputusan Menteri Negara Lingkungan Hidup 2004). As a result, both sampling locations were not within the standard conditions, probably due to the rainy season, which produces run off from main land, decreasing the salinity at the time of sampling. The normal salinity in Panjang Island is 31-33.4‰ (Hartati et al 2019). The visibility in each location was different as well. At Awur Bay, the water was more turbid than at Panjang Island. This is likely because the bay is closer to the mainland and the run off containing sediment particles limiting light penetration through the water. Hard corals are commonly found at Panjang Island rather than Awur Bay due to more favorable visibility (Table 1). Hard corals need high visibility, so that the sunlight penetration could reach the zooxanthellae inside the hard-coral polyp.

The 7 genera of found hard coral commonly live at a depth of 0.5 to 7 m. According to Munasik et al (2012), many more hard coral genera grow in these waters, including 25 genera belonging to 11 different families. Those families are Acroporidae, Agariciidae, Dendrophyllidae, Faviidae, Fungiidae, Merulinidae, Mussidae, Oculinidae, Ectinidae, Pociloporidae, and Poritidae. Hard coral is a holobiont, meaning that there is a symbiosis between metazoan (coral polyps) and microbiomes, as well as with bacteria, archaea, viruses, fungi, and algae. These microbiomes live interlinked with each other to create a suitable environment for coral polyps to flourish. Several studies have been done on this subject. To name a few, a previous study reported successful isolation and purification of bacteria from hard coral specimens of *Pavona* sp. (Ayuningrum et al 2017). Other research studied fungi associated hard coral specimens (Cristianawati et al 2017). Both articles reported that hard coral associated microbiomes showed antibacterial potential against MDR bacteria. Hard corals harbor various microbiomes as a consortium for providing chemicals for growth, development and protection of the host (Kuang et al 2015; Rosenberg & Gophna 2011).

The initiation of hard coral associated bacteria depends on its reproductive strategies; the associated bacteria can move vertically (during reproduction) or horizontally through the water column at each stage of coral life, passively moving through the water column, carried away by the current and suspended sediments from benthos (Rosenberg & Gophna 2011). The microbiomes also make up the different compositions of microbial communities in the mucus, tissue and skeletal parts. According to Bourne et al (2016), microbiomes associated with hard coral were phylogenetically identified as *Actinobacteria* (6%), *Cyanobacteria* (7%), *Firmicutes* (10%), and *Proteobacteria* (68%). In addition, according to Wegley et al (2007), the dominant bacteria living in association with hard

coral are from phylum *Proteobacteria* (mostly *Gammaproteobacteria* and *Alphaproteobacteria*), followed by *Actinobacteria, Bacteriodetes* (mostly *Flavobacteria*) and *Cyanobacteria*. Most symbiont bacteria found in marine invertebrates, including coral, come from the dominant phylum *Proteobacteria*, and are also found in tunicates (Ayuningrum et al 2019b).

Pereira et al (2017) showed that most bacteria associated hard corals have antibacterial activity against the coral pathogen *Serratia marcesent,* which is from the genus *Bacillus*. Another study found that bacterium *Pseudovirio* sp. P12, associated with hard coral, had antibacterial properties against coral pathogenic bacteria (Raina et al 2016). In a study on the antibacterial properties of hard coral associated bacteria against human pathogenic MDR bacteria, bacterial strains that showed potential were identified as *Virgibacilus salarius* and *Pseudoalteromonas flavipulchra* (Ayuningrum et al 2017). Additionally, several compounds with biological activities have already been isolated from hard coral associated bacteria (Radjasa et al 2008). One of these compounds was a sulfur-containing carotenoid from yellowish marine associated hard coral bacterium *Erythrobacter flavus* KJ5. However, the function of the carotenoid remains unclear (Setiyono et al 2019). Another compound successfully isolated from bacteria associated hard coral is tropodithietic acid (TDA). TDA is a sulfur containing compound derived from dimethylsulfoniopropionate (DMSP) catabolism. It showed antibacterial activity against coral pathogen *Vibrio coralliilyticus* and *V. owensii* (Raina et al 2016). In addition to hard corals, tunicates (a coral reef builder) produce the antibacterial compound isatin from its associated bacteria (Ayuningrum et al 2019a). The coral reef builder shares its habitat with many biota and therefore has to protect its home from other species as well as themselves from predators such as the sea urchins and starfish. The coral reef builders and their symbionts collaborate to produce active compounds as a response to chemical ecology. Interestingly, these compounds are also beneficial to humans, as they have antibacterial, antifungal, anti-inflammatory, and other properties.

Conclusions. Hard coral associated bacteria have the potential to be active agents against human pathogenic multidrug resistant bacteria. 13 of the 32 bacterial strains in this study showed antibacterial activity against human pathogenic MDR bacteria *P. aeruginosa*, *K. pneumonia*, *E. aerogenes*, *A. baumannii*, methicillin-resistant *S. aureus*, *E. coli*, *E. cloacae* complex, and *S. hemolyticus*. The crude extract testing from one of the active bacterial strains was able to inhibit the growth of MDR *A. baumannii.* These results should lead to further examination for bioactive compound isolation as antibiotic candidates. In this regard, hard coral associated bacteria are certainly one of the promising sources of bioactive compounds.

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