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Original Article

IN VITRO ANTIBACTERIAL ACTIVITIES OF MARINE SPONGE-ASSOCIATED BACTERIA AGAINST PATHOGENIC *VIBRIO* SPP. CAUSES *VIBRIO*SIS IN SHRIMPS

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ABSTRACT

Objective: This study was aimed to isolate and screen marine sponge-associated bacteria producing anti-*Vibrio* compounds and to identify their compounds from the bacterial extract.

Methods: Sponge-associated bacteria were isolated by spread plate method. Their anti-*Vibrio* activity against *Vibrio parahaemolyticus, V. harveyi*, and *V. vulnificus* was determined by dual culture test. Three potential isolates were identified based on 16S-rRNA gene analysis. All isolates producing anti-*Vibrio* compounds was tested for their haemolytic characters in blood agar medium. Anti-*Vibrio* activity of the most potential isolate was also tested by using its supernatant, extract, and concentrated culture. Chemical composition of crude extract derived from that isolate was identified by GC-MS analysis.

Results: 68 bacterial isolates have been isolated from the marine sponge, *Spongia* sp., *Svenzea* sp., *Ircinia* sp., and *Igernella* sp. Of 68 isolates, 15 (22%) isolates had anti-*Vibrio* activities in various spectra against three *Vibrio* species, including *V. harveyi, V. parahaemolyticus*, and V. vulnificus. All isolates producing anti-*Vibrio* compounds were non-haemolytic. Bacterial isolates coded as D6.6, D6.19, and P4.17 have broad spectra. They could inhibit at least two *Vibrio* species as indicated by the clear zone formed around bacterial colonies. Based on 16S-rRNA, these isolates were closely related (similarity ≥ 99%) to *Brevibacterium casei* strain M Sw oHS, *Bacillus altitudinis* strain FJAT 47750, and *Bacillus altitudinis* strain PgBe190, respectively. D6.6 isolate was the most potential isolate, which could inhibit three *Vibrio* species. Consistently, its anti-*Vibrio* activity also confirmed by their supernatant, concentrated culture, and crude extract of that isolate. The crude extract derived from this isolate contained 10 major compounds that are biologically active.

Conclusion: This study suggests that 15 bacteria strains isolated from marine sponges were potentially could inhibit *Vibrio*'s growth *in vitro*. These isolate could be further explored as anti-*Vibrio* agent.

Keywords: Anti-*Vibrio*, Bioactive compounds, GC-MS, Sponge-associated bacteria, 16S-rRNA

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INTRODUCTION

Infectious diseases in shrimp, particularly *Vibrio*sis have become a serious problem in aquaculture. The disease is caused by pathogenic *Vibrio*, *including V. harveyi, V. vulnificus, V. parahaemolyticus, V. alginolyticus, V. anguillarum,* and *V. splendidus* [1]. Even though the use of antibiotics is considered effective for treating *Vibrio*sis, the overuse of those compounds resulted in resistance in some *Vibrio* species. For example, more than 50% of *Vibrio parahaemolyticus* strains isolated from marine and freshwater fish surprisingly presented high resistance to ampicillin (88%), amikacin (64%), and kanamycin (50%) [2]. Consequently, the challenge to find new antibiotics encourages us to look for alternative ways to deal with *Vibrio* infections mainly in shrimp aquaculture.

The oceans are presently being investigated in the search for new active compounds. There is an increased interest in natural compounds produced by organisms living in marine habitats. Based on the database of marine natural products, more than 32,000 compounds have been identified [3]. Interestingly, nearly 75% of Indonesia is ocean. This condition provides an endless source for exploration of marine natural products, particularly those from marine bacteria. The water column of the oceans contains approximately 10^6 bacterial cells per milliliter [4]. In addition, they also have an association with the marine organism, especially sponge. The bacterial density could reach 10^9 cells per cm3 of sponge tissue [5], indicating that the possibility to find diverse potential bacteria isolated from sponge tissue is high. Bioactive compounds extracted from these bacteria have some biological activities, including antibacterial [6-8], antioxidant, antiglycation, antiaging [9], anticancer [10-12], antiviral, and antifungal [13]. Taking into account those potential characters, the investigation of sponge-associated bacteria in producing anti-*Vibrio* compounds needs to be done.

In some previous studies, sponge-associated bacteria isolated from Indonesian Sea showed potent anti-*Vibrio* activities. Nearly 12 (15%) of bacterial strains isolated from sponge markedly exhibited anti-*Vibrio* properties in various spectra [14]. Supporting that studies, marine bacteria isolated from North Java Sea also have antibacterial activity against pathogenic *Escherichia coli* [15]. Based on those reports, marine bacteria isolated from sponge was explored for the discovery of new anti-*Vibrio* compounds. This study was aimed to isolate and screen anti-*Vibrio* activities of sponge-associated bacteria. We also report the identity of the most potential bacterial isolate based on 16S-rRNA analysis.

MATERIALS AND METHODS

Sponge and *Vibrio* spp

Spongia sp., *Svenzea* sp., Ircinia sp., and Igernella sp. were collected from Pramuka Island, Thousand Island, Jakarta. *Vibrio* harveyi P-275 (collection of Research and Development Center of Brackish Water Aquaculture, Maros, Indonesia), *Vibrio* vulnificus 195B, and *Vibrio parahaemolyticus* ATCC 17802 (collection of The Standard of Fish Quarantine, Quality Qontrol and Fishery Product Safety, Jakarta, Indonesia) were used for primary screening targets.

Isolation of sponge-associated bacteria

Nearly 1 g of each sponge biomass was washed by using sterile seawater. It was then macerated and diluted through several dilution serials (from 10^{-1} to 10^{-4}). About 100 µl of each dilution was

plated on seawater complete (SWC) agar medium (5 g peptone, 1 yeast extract, 3 ml glycerol, 750 ml seawater, 250 ml distilled water), Zobel marine agar (ZMA), and nutrient agar medium by using spread plate technique. The inoculated plates were incubated at±28 °C for 24 h. The growing colonies were then characterized and purified on Luria Bertani agar (1 g tryptone, 1 g NaCl, 0.5 g yeast extract, 1.5 agar, 100 ml distilled water).

Screening for anti-*Vibrio* activity from sponge-associated bacteria

Antibacterial activity of sponge-associated bacteria was tested by using the dual culture method. Each *Vibrio* strain was cultured in SWC broth medium for 24h. About 1 % (v/v) was then inoculated to the melted SWC agar medium, and homogenized. The inoculated medium was then poured into the sterilized plate. After the medium was solid, each sponge-associated bacterial isolate was streaked on that medium and incubated at ±28 °C for 24 h. Antibacterial activity was indicated by the formation of a clear zone around the bacterial colonies.

Haemolytic assay

The potential isolates producing anti-*Vibrio* compounds were tested for their haemolysis ability using a blood agar medium. The bacterial isolates were streaked on that medium and incubated for 24 h at ±27 °C. The formation of clear zones around the bacterial colonies indicates that the isolate is haemolytic positive.

Identification of the potential bacteria

The potential bacterial isolates were cultured on SWC medium, incubated at 28 °C and agitated in 120 rpm overnight. About 3 ml of bacterial culture was transferred into a sterile microtube and centrifuged at 10.000 rpm for 10 min. The genomic DNA was extracted using the Genomic DNA Mini Kit (Blood/Cultured Cell, Geneaid, Taiwan). The procedures were carried out according to the manufacturer's instructions. The 16S-rRNA gene was amplified using 1387R primer (5'-GGG CGG WGT GTA CAA GGC-3') and 63F primer (5'-CAG GCC TAA CAC ATG CAA GTC-3') [16] with a targeted fragment of 1300 bp. The PCR reaction was performed under the following conditions: 25 µl of GoTaq Green Mastermix 2x (Promega, Madison, USA), 5 µl of 1387R (10 pmol), 5 µl of 63F primers (10 pmol), 2 µl DNA template (~100 ng/µl), and adjusted with nuclease-free water (NFW) to 50 µl. The cycling conditions (30 cycles) were pre-denaturation 94 °C for 5 min, denaturation 94 °C for 30 s, annealing 55 °C for 45 s, elongation 72 °C for 1 min 30 s, and post-PCR 4 °C for 5 min. The PCR products were sequenced in FirstBase, Malaysia. The sequences were compared to the other 16S-rRNA sequences in GenBank NCBI database (http://ncbi.nlm.nih.gov) using BlastN (Basic Local Alignment Search Tool). The phylogenetic tree was constructed in molecular evolutionary genetics analysis program (MEGA) version 7.0 using the neighbor-joining method.

***In vitro* anti-*Vibrio* assay of the most potential isolate**

The most potential isolate was tested to confirm its anti-*Vibrio* activity. The isolate was cultured in SWC broth medium for 72 h, and incubated at room temperature (±27°C). After incubation, nearly 1.5 ml of that suspension was centrifuged in 10.000 rpm for 5 min. The supernatants and pellets were separated into different eppendorf. The pellets were added with 150 µl of supernatants so that the suspension contained ten times of cell number. About 20 µl of that culture was inoculated onto the SWC agar medium containing the bacterial tests. In addition, nearly 20 µl of supernatants were also inoculated on that medium and the plates were incubated for 24 h at±27°C. About 1 l culture was also used for the extraction of its bioactive compounds. Then, bacterial cultures were added with ethyl acetate solvent in ratio 1:1 (v/v) and shaken continuously for 20 min. The bacterial culture and the ethyl acetate layers were separated. The solvent layer was then evaporated using rotary evaporator at 50oC. The extract was then stored at 4°C. This extract was tested for its anti-*Vibrio* activity in a concentration of 5000 ppm. DMSO and ampicillin (100 ppm) were served as the negative and positive control, respectively.

Chemical identification of the bacterial extract

The extract derived from the most potential bacteria attributed to broad spectrum of anti-*Vibrio* activity was identified by using the GC-MS technique. The GC-MS analysis was carried out in an Agilent Technologies 6890N inert C, USA equipped with a fused capillary column (58 × 0.25 µm ID × 0.25 µm df). For GC-MS identification, an electron ionization system was executed in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and 1µl of suspension was injected (a split ratio of 50:1). The temperature of injector was maintained at 280 °C. The ion-source temperature was 200 °C, and the oven temperature was operated from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 280 °C, then 5 °C/min to 280 °C, ended with a 20 min isothermal at 280 °C. Mass spectra were taken at 70 eV, a scan-interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 47 min. MSD ChemStation Data Analysis software (G1701EA E.02.02.1431) was used for mass spectra and chromatograms analysis.

RESULTS

Bacterial isolates from sponge

From 4 sponge species used, each sponge showed a different number of bacteria. As isolated by using three different medium (SWC, ZMA, and NA), the total number of bacterial isolates from each sponge was found to be diverse. These isolates were selected by their colony morphology (shape, color, texture, optical characters, and size). Seventeen bacterial isolates, 19 isolates, 16 isolates, and 15 isolates were isolated from *Spongia* sp., *Svenzea* sp. *Ircinia* sp., and *Igernella* sp., respectively. In instance, a total of 68 bacterial isolates were obtained from four sponges.

Anti-*Vibrio* activities of sponge-associated bacteria

Of 68 bacterial isolates, 15 isolates (22%) markedly exhibited antibacterial activity against three *Vibrio* species in various spectra (table 1). These isolates were able to inhibit at least one *Vibrio* species. Interestingly, the isolate coded as D6.6 (isolated from Spongia sp.) showed a broad spectrum of anti-*Vibrio* activity. This isolate was able to inhibit all three *Vibrio* species, including *V. harveyi, V. parahaemolyticus,* and *V. vulnificus.* The other bacterial isolates displayed narrow spectra of anti-*Vibrio* activities. They displayed antibacterial activity only in one or two test strains of *Vibrio*.

Haemolytic character of te selected isolates

In the present study, 15 potential isolates showed a negative haemolysis reaction. These isolates were not able to lyse red blood cells in the medium, indicating that the bacteria were suspected not to be pathogenic to human and animal. In this study we used *V. vulnificus* as positive control. There was a lytic zone around the *V. vulnificus's* colony.

The molecular identification bacterial isolates

Three potential isolate coded as D6.6, D6.19, and P4.17 were selected for molecular identification. The 16S-rRNA gene amplification of these isolates showed DNA fragment ~1300 bp in size. Based on BlastN program, both D6.19 and P4.17 isolate were highly homolog (similarity 94% and 99%) with *Bacillus altitudinis* in different strains, and D6.6 was similar to *Brevibacterium casei* (similarity 100%), as shown in table 2. Consistently, D6.19 and P4.17 were located in the *Bacillus* clade, while D6.19 was located in the *Brevibacterium* clade (fig. 1).

The anti-*Vibrio* activity of extract, supernatant, and concentrated culture of the most potential isolate

The supernatant concentrated culture, and extract from D6.6 isolate consistently exhibited clear zone formation (fig. 2). The best inhibition of *Vibrio*'s growth was showed by the concentrated culture against *V. vulnificus*. Ampicillin as a positive control also showed anti-*Vibrio* activity at 100 ppm, while there was no clear zone formation in DMSO treatment.

Table 1: Inhibition of *Vibrio's* growth by sponge-associated bacteria

Sponges	Isolate code	Anti-*Vibrio* activity*		
		V. harfeyi	*V. parahaemolyticus*	*V. vulnificus*
	D6.3	-	+++	-
Spongia sp.	D6.6	+	+	+
	D6.9	+	+	-
	D6.8	+	-	-
	D6.18	+	++	-
	D6.19	++	+++	-
Svenzea sp.	P4.11	+	++	-
	P4.17	-	+++	+
	P4.19	-	-	+
	P4.21	++	-	-
	P5.10	+	-	-
Ircinia sp.	P5.20	+	+	-
Igernella sp.	P6.13	-	++	-
	P6.15	-	++	-

*Clear Zone diameter: 0 mm: -; 0.1-2.5 mm: +;>2.5-5 mm: ++;>5 mm: +++

Table 2: The identity of bacterial isolates based on the 16S-rRNA sequence

Isolates code	Closest relative strain	E-value	Identity	Query cover	Accession number
D6.6	*Brevibacterium casei* strain M Sw Ohs	0.0	94	100	KF777366
D6.19	*Bacillus altitudinis* strain FJAT 47750	0.0	99	100	MG651154
P4.17	*Bacillus altitudinis* strain PgBe190	0.0	100	100	MH211281

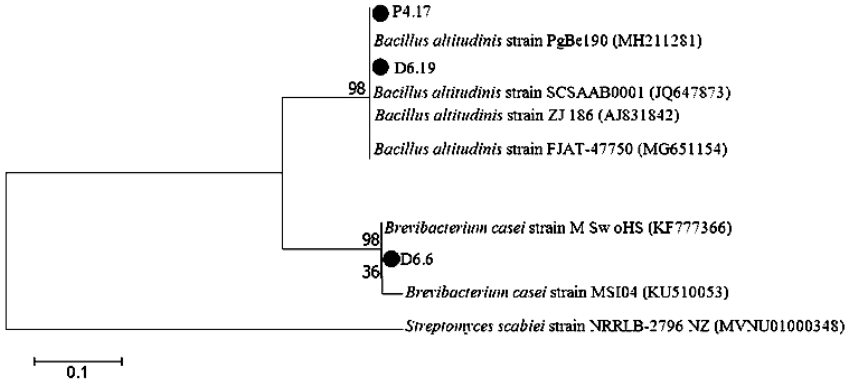

Fig. 1: Genetic relationships of three potential isolates compared to their closest relative strains

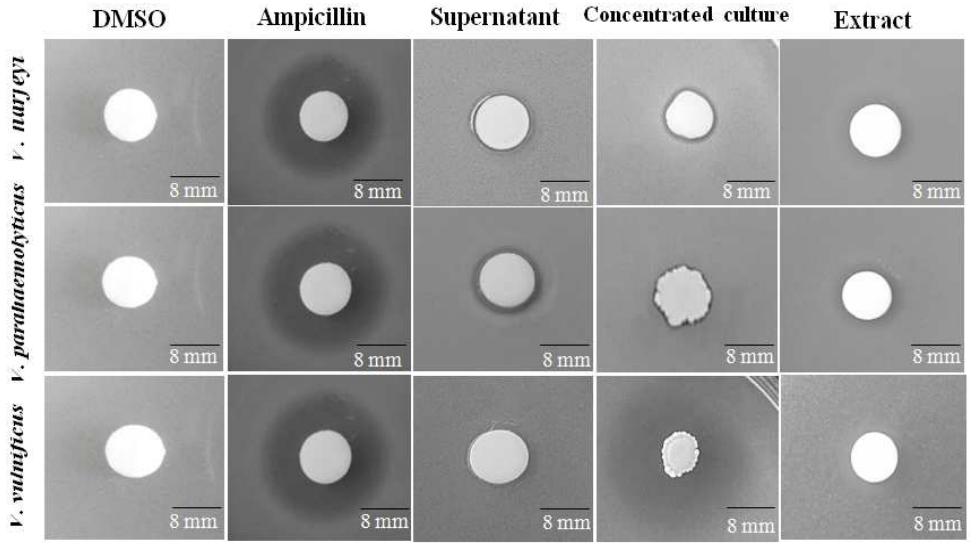

Fig. 2: Anti-*Vibrio* activities of supernatant, concentrated culture, and crude extracts of D6.6 isolate in SWC agar medium after 24 h incubation at±27°C

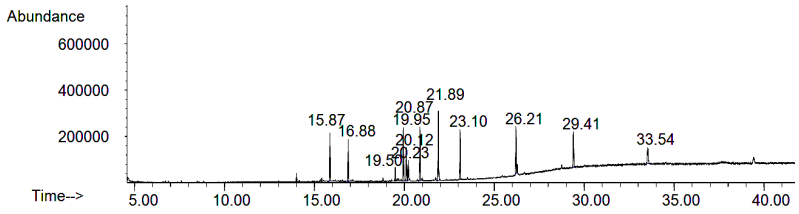

Fig. 3: GC-MS chromatogram of a crude extract derived from D6.6 isolate

Table 3: Ten major compounds in the crude extract derived from D6.6 isolate

No.	Compounds	Formula	Retention time	Peak area (%)	Similarity (%)	Biological activity [references]
1.	Thiophene,2-butyl-	$C_{10}H_{16}S$	15.87	8.18	53	Anticancer, antiinflammation [17]
2.	Octadecane	$C_{18}H_{38}$	16.88	6.90	98	Unknown
3.	Silane, trimethyl-2-propyne-	$C_6H_{12}Si$	19.50	3.20	38	Unknown
4.	Eicosane	$C_{20}H_{42}O$	19.95	9.33	97	Antifungal [18]
5.	2(5H)-furanone, 5-(2-methyl-2-propenyl)-4-methyl-	$C_9H_{12}O_2$	20.12	6.61	97	Unknown
6.	Cyclohexane,1-(cyclohexylmethyl)-2-methyl, cis-	$C_{14}H_{26}$	20.22	4.89	47	Unknown
7.	Heptane,1,7-dibromo-	$C_7H_{14}Br_2$	20.87	10.08	43	Unknown
8.	Fluoranthene	$C_{16}H_{10}$	21.89	15.20	97	Enzyme inhibitor [19]
9.	Docosane	$C_{22}H_{46}$	23.10	9.87	97	Antibacterial [20]
10.	Tetracosane	$C_{24}H_{50}$	26.21	11.91	97	Cytotoxic [21]

Chemical composition of the crude extract from D6.6 isolate

Based on GC-MS analysis, D6.6 derived extract was dominated by ten compounds, including thiophene, 2-butyl-; octadecane; silane, trimethyl-2-propyne-; eicosane; 2(5H)-furanone, 5-(2-methyl-2-propenyl)-4-methyl-; cyclohexane,1-(cyclohexylmethyl)-2-methyl, cis-; heptane,1,7-dibromo-; fluoranthene; docosane; and tetracosane (table 3). These compounds showed different retention time and peak area (fig. 3).

DISCUSSION

In this study, we investigated the potential of sponge-associated bacteria as the candidate of anti*Vibrio*sis on shrimps. The number of bacteria isolated from 4 sponges species was found to be diverse. All sponges, *Spongia* sp., *Svenzea* sp. *Ircinia* sp., and *Igernella* sp. containing at least 15 bacterial isolates proved to be sources of diverse bacteria that produced bioactive compounds. The number of bacteria isolated may be influenced by the isolation technique, nutrient content on medium, and type of sponge used. In this study, a total of 68 isolates were obtained. These culturable bacteria could be the microbiological evidence of symbiotic interaction between the sponge and their bacterial symbiont. Of 68 isolates, 15 isolates (22%) markedly exhibited anti-*Vibrio* activities in various spectra against *V. harveyi, V. parahaemolyticus,* and *V. vulnificus*, as indicated by the clear zone formation around bacterial colonies. The different spectra of anti-*Vibrio* activity indicated the chemical diversity of anti-*Vibrio* bioactive compounds produced by these bacteria. Based on their anti-*Vibrio* activity, it is likely that these isolates have an important role in supporting host defense mechanisms. According to haemolytic assay, these isolates were haemolytic negative, suggesting that these potential isolates were not pathogenic bacteria in human. Thus, these isolates can potentially be explored as the anti*Vibrio*sis agent.

Three isolates coded as D6.6, D6.19, and P4.17, showed great anti-*Vibrio* activity. Two isolates, D6.19 and P4.17 were closely related (similarity ≥ 99%) to *Bacillus altitudinis* strain FJAT 47750 and *Bacillus altitudinis* strain PgBe190, respectively. Surprisingly, D6.6 isolates showed low similarity (94%) to *Brevibacterium casei* strain M Sw oHS suggesting the novelty of this isolate. Both *Bacillus* and Brevibacterium genera were commonly known as the antimicrobial compounds producer. As reported by Gao *et al.* [22], marine *Bacillus* strain has a strong anti-*Vibrio* activity against 29 *Vibrio* strains. Supporting these results, Abubakar *et al.* [23] also demonstrated that *Bacillus* isolates were able to inhibit not only Gram-positive bacteria, but also Gram-negative bacteria, including *V. harveyi, Staphylococcus aureus, E. coli,* and *Pseudomonas aeruginosa*. Other *Bacillus* associated with marine sponge in Thousand Island, Indonesia, also has an excellent anti-*Vibrio* activity against *V. parahaemolyticus, V. vulnificus*, and V. harveyi, as reported by Wahyudi *et al.* [14]. The antibacterial activity of *Bacillus* is likely to be influenced by their capability in synthesizing diketopiperazines, heat resistance compounds in size 1 kDa [24]. Further study needs to be done to identify the anti-*Vibrio* compounds produced by these isolates.

D6.6 isolate was the widest spectrum of anti-*Vibrio* compounds producer, identified as *Brevibacterium casei*. This genus has also well studied as antibacterial compounds producer classified as a broad spectrum of antibacterial against both Gram-positive and Gram-negative bacteria [25]. Kiran *et al.* have successfully investigated the potential of marine *Brevibacterium casei* as *Vibrio* biocontrol. The study suggested that poly-hydroxy butyrate derived from that isolate was able to inhibit pathogenic bacteria on shrimp including *V. alginolyticus* and *V. harfeyi* [26]. Consistently, the anti-*Vibrio* activity of D6.6 isolate has also been confirmed by its supernatant, concentrated culture, and metabolites. The inhibitory effect of the supernatant, culture and extract indicate that the anti-*Vibrio* compound is likely an extracellular molecule.

The capability of supernatant, extract and culture of D6.6 isolate to inhibit *Vibrio* sp. is likely to be caused by activity of docosane as one of the major compounds identified. It has been reported as antibacterial by previous study [20]. The presence of this compound has been identified in the crude extract of that isolate. Wang *et al.* reported that docosane isolated from *Metaplexis japonica* has high and wide antibacterial performance against five Gram-positive and seven Gram-negative bacteria strains [20]. Other compounds were also found as dominant compounds in D6.6-derived extract, including thiophene, 2-butyl-; octadecane; silane, trimethyl-2-propyne-; eicosane; 2(5H)-furanone, 5-(2-methyl-2-propenyl)-4-methyl-; cyclohexane,1-(cyclohexyl methyl)-2-methyl, cis-; heptane, 1,7-dibromo-; fluoranthene; and tetracosane. Some of these compounds have been reported as biologically active compounds. They act as anticancer, antifungal, enzyme inhibitor, and cytotoxic compounds [17-21]. In conclusion, we suggest that D6.6 isolate needs to be further investigated as biocontrol candidate especially for controlling *Vibrio*sis in shrimp caused by *Vibrio* sp. This is the

first report on the anti-*Vibrio* activity of *Brevibacterium casei* isolated from Indonesian marine sponge.

CONCLUSION

Of 68 bacterial isolates, 15 isolates (22%) showed anti-*Vibrio* activities in various spectra against three *Vibrio* species, including V. harveyi, V. parahaemolyticus, and V. vulnificus. Bacterial isolates coded as D6.6, D6.19, and P4.17 have broad spectra. Based on 16S-rRNA, these isolates were closely related to *Brevibacterium casei* strain M Sw oHS, *Bacillus altitudinis* strain FJAT 47750, and *Bacillus altitudinis* strain PgBe190, respectively. The anti-*Vibrio* activity of the most potential isolate (D6.6) are also consistent as showed by its supernatants, concentrated culture, and crude extracts activities. D6.6 derived extract contains 10 major compounds which are biologically active. Based on those potential properties, these sponge-associated bacteria need to be developed as anti-*Vibrio*sis agents.

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AUTHORS CONTRIBUTIONS

Aris Tri Wahyudi has lead this study, took part in experimental design, integrated all experimental data, manuscript writing, and submission. Jepri Agung Priyanto has contributed in laboratory experiments, data analysis, and manuscript writing. Dian Retno Wulandari has contributed in laboratory experiments and data analysis. Rika Indri Astuti has involved in results verification, scientific discussion, and manuscript writing.

CONFLICTS OF INTERESTS

All authors declare that there are no conflict of interest.

REFERENCES

1. Chandrakala N, Priya S. *Vibrio*sis in shrimp aquaculture a review. Int J Scientific Res Sci Eng Tech 2017;2:27-33.
2. Lee LH, Mutalib NSA, Law JWF, Wong SH, Letchumanan V. Discovery on antibiotic resistance patterns of *Vibrio* parahaemolyticus in selangor reveals carbapenemase-producing *Vibrio* parahaemolyticus in marine and freshwater fish. Front Microbiol 2018;9:1-13.
3. Marin Lit; 2018. Available from: http://pubs.rsc.org/marinlit. [Last accessed on 10 Aug 2019].
4. Debbab A, Aly AH, Lin WH, Proksch P. Bioactive compound from marine bacteria and fungi. Microb Biotechnol 2010;3:544-63.
5. Hentschel U, Piel J, Degnan SM, Taylor MW. Genomic insights into the marine sponge microbiome. Nat Rev Microbiol 2012;10:641-5.
6. Thirumalairaj J, Shanmugasundaram T, Sivasankari K, Natarajaseenivasan K, Balagurunathan R. Isolation, screening and characterization of potent marine *Streptomyces* sp. PM105 against antibiotic-resistant pathogens. As J Pharm Clin Res 2015;8:439-43.
7. Murniasih T, Putra MY, Hadi TA. The antibacterial evaluation of *Haliclona* associated bacteria and the relating compounds derived from the host. Asian J Pharm Clin Res 2018;11:412-5.
8. Peela S, Porana S. Isolation and screening of novel streptomyces from sediment of the bay of bengal near srikakulam coast. Int J Curr Pharm Res 2017;9:40-4.
9. Prastya ME, Astuti RI, Batubara I, Wahyudi AT. Antioxidant, antiglycation and *in vivo* antiaging effects of metabolite extracts from marine sponge-associated bacteria. Ind J Pharm Sci 2019;81:344-53.
10. Utami AWA, Wahyudi AT, Batubara I. Toxicity, the anticancer and antioxidant activity of extract from marine bacteria associated with sponge *Japsis sp*. Int J Pharm Bio Sci 2014;5:917-23.
11. Safari WF, Chasanah E, Wahyudi AT. Antibacterial and anticancer activities of marine bacterial extracts and detection of genes for bioactive compound synthesis. Int J Pharm Pharm Sci 2016;8:55-9.
12. Priyanto JA, Astuti RI, Nomura J, Wahyudi AT. Bioactive compounds from sponge-associated bacteria: anticancer activity and NRPS-PKS gene expression in different carbon sources. Am J Biochem Biotechnol 2017;13:148-56.
13. Mayer AMS, Rodriguez AD, Berlinck RGS, Fusetani N. Review marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, anti-tuberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanism of action. Comp Biochem Physiol 2011;153:191-22.
14. Wahyudi AT, Priyanto JA, Maharsiwi W, Astuti RA. Screening and characterization of sponge-associated bacteria producing bioactive compounds anti-*Vibrio* sp. Am J Biochem Biotechnol 2018;14:221-9.
15. Radjasa O. Antibacterial activity of sponge-associated bacteria isolated from North Java Sea. J Coast Dev 2007;10:143-50.
16. Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Iom SJ, *et al*. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 1998;64:795-9.
17. Ahmed MM, Khan MA, Rainsford KD. Synthesis of thiophene and NO-curcuminoids for anti-inflammatory and anti-cancer activities. Molecules 2013;18:1483-501.
18. Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express 2017;7:1-9.
19. Willett KL, Wassenberg D, Lienesch L, Reichert W, Giulio RTD. *In vivo* and *in vitro* inhibition of CYP1A-dependent activity in Fundulus heteroclitus by the polynuclear aromatic hydrocarbon fluoranthene. Toxicol Appl Pharmacol 2001; 177:264-71.
20. Wang DC, Sun SH, Shi LN, Qiu DR, Li X, Wei DS, *et al*. Chemical composition, the antibacterial and antioxidant activity of the essential oils of *Metaplexis japonica* and their antibacterial components. Int J Food Sci Tech 2014;50:449-57.
21. Uddin SJ, Grice D, Tiralongo E. Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureus*. Pharm Bio 2012;50:1276-80.
22. Gao X, Liu Y, Miao L, Li E, Hou T, Liu Z. Mechanism of anti-*Vibrio* activity of marine probiotic strain *Bacillus* pumilus H2, and characterization of the active substance. AMB Express 2017;7:1-10.
23. Abubakar H, Wahyudi AT, Yuhana M. Skrining bakteri yang berasosiasi dengan spons *Japsis* sp. sebagai penghasil senyawa antimikroba. Il Kelautan 2011;16:35–40.
24. Leyton Y, Borquez J, Darias J, Cueto M, Diaz Marrero AR, Riquelme C. Diketopiperazines produces by an *Bacillus* species inhibits *Vibrio* parahaemolyticus. J Aquat Res Dev 2012;3:1-5.
25. Boucabeille C, Mengin Lecreulx D, Henkes G, Simonet J, Heijenoort JV. Antibacterial and hemolytic activities of linenscin OC2, a hydrophobic substance produced by *Brevibacterium linens* OC2. FEMS Microbiol Lett 1997;153: 295-301.
26. Kiran GS, Lipton AN, Priyadharsini S, Anitha K, Suarez LEC, Arasu MV, *et al*. Antiadhesive activity of poly-hydroxy butyrate biopolymer from a marine *Brevibacterium casei* MSI04 against shrimp pathogenic *Vibrio*s. Microbiol Cell Fact 2014;13:1-12.