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

ARIS TRI WAHYUDI*, JEPRI AGUNG PRIYANTO, DIAN RETNO WULANDARI, RIKA INDRI ASTUTI
Division of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia
Email: aristri2011@gmail.com

Received: 07 Jul 2019, Revised and Accepted: 30 Sep 2019

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 Igermella 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

INTRODUCTION

Infectious diseases in shrimp, particularly Vibriosis have become a serious problem in aquaculture. The disease is caused by pathogenic Vibrio, including V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, and V. splendidus [1]. Even though the use of antibiotics is considered effective for treating Vibriosis, 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 10^4 bacterial cells per milliliter [4]. In addition, they also have an association with the marine organism, especially sponge. The bacterial density could reach 10^6 cells per cm^3 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, antitycation, antitaging [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 Igermella 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 parahaemolyticus ATCC 17802 (collection of The Standard of Fish Quarantine, Quality Control 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^-9). 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), Zobell 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, 400 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 24 h. 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-rDNA 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 at 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 50°C. 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 G, 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 1 μl 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., Sventzea sp. Ircinia sp., and I. gernello sp., respectively. In total, a list of 68 bacterial isolates was 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 (isolated from S. dessipatum) 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 the 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 of bacterial isolates

Three potential isolate coded as D6.6, D6.19, and P4.17 were selected for molecular identification. The 16S-rDNA 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 canseii (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 shown 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. harveyi | V. parahaemolyticus | V. vulnificus |
|-------------|--------------|-----------------------|------------|---------------------|--------------|
| Spongia sp. | D6.3         | -                     | ++         | +                   | -            |
|             | D6.6         | +                     | +          | +                   | -            |
|             | D6.9         | +                     | +          | +                   | -            |
|             | D6.8         | +                     | -          | +                   | -            |
|             | D6.18        | +                     | ++         | +                   | -            |
|             | D6.19        | ++                    | ++         | +                   | -            |
| Svenzea sp. | P4.11        | +                     | ++         | +                   | -            |
|             | P4.17        | -                     | +++        | +                   | -            |
|             | P4.19        | -                     | -          | +                   | -            |
|             | P4.21        | +                     | ++         | -                   | -            |
|             | P5.10        | +                     | -          | -                   | -            |
|             | P5.20        | +                     | +          | +                   | -            |
|              | 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
Supporting these results, Abubakar reported that the producer strain has a strong anti-Vibrio activity. According to Gao 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. paraohaemolyticus, 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, high 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-(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 antiVibrios 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 Igerella 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 microbial 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. paraohaemolyticus, 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 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).

**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. Some of these compounds have been reported as biologically active compounds [17-21]. In conclusion, we suggest that D6.6 isolate has high 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-(cyclohexylmethyl)-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 Vibrios in shrimp caused by Vibrio sp. This is the

---

**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-                      | C₅H₅S           | 15.87          | 8.18          | 53             | Anticancer, antinflammation [17]  |
| 2   | Octadecane                             | C₁₉H₃₈          | 16.68          | 6.90          | 98             | Unknown                          |
| 3   | Silane, trimethyl-2-propyne-           | C₆H₁₂Si         | 19.50          | 3.20          | 38             | Unknown                          |
| 4   | Eicosane                               | C₂₀H₄₀          | 19.95          | 9.33          | 97             | Antifungal [18]                  |
| 5   | 2(5H)-furanone, 5-(2-methyl-2-propenyl)-4-methyl- | C₁₉H₂₈O₂        | 20.12          | 6.11          | 97             | Unknown                          |
| 6   | Cyclohexane,1-(cyclohexylmethyl)-2-methyl, cis- | C₂₁H₂₆        | 20.22          | 4.89          | 47             | Unknown                          |
| 7   | Heptane,1,7-dibromo-                   | C₂₆H₅₈Br₂       | 20.87          | 10.08         | 43             | Unknown                          |
| 8   | Fluoranthene                           | C₁₉H₁₄Br       | 21.89          | 15.20         | 97             | Enzyme inhibitor [19]            |
| 9   | Docosane                               | C₂₈H₅₈         | 23.10          | 9.87          | 97             | Antibacterial [20]               |
| 10  | Tetracosane                            | C₃₀H₆₀         | 26.21          | 11.91         | 97             | Cytotoxic [21]                   |

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

ACKNOWLEDGMENT
This work was supported by Competence-Based Research/Basic Research from The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia 2018 [Contract No.: 129/SP2H/PTNBH/DRPM/2018] and 2019 [Contract No: 3/E1/KP. PTNBH/2019] to Aris Tri Wahyudi. Therefore, the authors thank and appreciate for all the supports given to carry out this research.

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. Chandrakalana N, Priya S. Vibrosis in shrimp aquaculture: a review. Int J Scientific Res Sci Eng Tech 2017;2:27-33.
2. Lee LH, Matalib NSA, Law JWF, Wong SH, Letchumanan V. Discovery of 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. Debba A, Aly AH, Lin WH, Proksch P. Bioactive compound from marine bacteria and fungi. Microm Biotechnol 2010;3:44-63.
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. Murinasi S, Putra MY, Hadi TA. The antibacterial evaluation of Haliclonia 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 streptomycoses from sediment of the bay of bengal near srirakulam coast. Int J Curr Pharm Res 2017;9:40-4.
9. Prastya ME, Astuti RI, Batubara I, Wahyudi AT. Antioxidant, antiutergenic and in vitro antitumor activities 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 Japisa 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 Sci 2016;8:855-9.
12. Priyanto JA, Astuti RI, Nomura J, Wahyudi AT. Antibioic compounds from sponge-associated bacteria: anticancer activity and NRPS-PKS gene expression in different carbon sources. Am J Biochem Biotechnol 2017;13:149-56.
13. Mayer AMS, Rodriguez AD, Berlinck RGS, Fusetani N. Review marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimarial, antiprotozoal, antitubercolsis, 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 S, 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, Rainford KD. Synthesis of thiophene and NO-curcuminoinds for anti-inflammatory and anti-cancer activities. Molecules 2013;18:483-501.
18. Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Splenomyces strain KX052460 for the biological control of Rhizoctonia solani AG-3 strain KX052461 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 compounds. Int J Food Sci Technol 2014;50:449-57.
21. Uddin SJ, Grice D, Tiralonge A. Evaluation of cytotoxicity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant Acrostichum aureus. Pharm Biol 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 benasiosis dengan spore Japisa sp. sebagai penghasil senyawa antimikroba. Il Kulbarub 2011;1:35-40.
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. Boucabelle C, Mengin Lecreuls D, Henkes K, Simonet J, Heijenoort JV. Antibacterial and hemolytic activities of Lentinus 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 butrate biopolymer from a marine Brevibacterium casei MS104 against shrimp pathogenic Vibrios. Microbiol Cell Fact 2014;13:1-12.