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Isolation, characterization and identification of sponge-associated bacteria producing antimicrobial compounds

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Abstract. *Wibowo RH, Sipriyadi, Darwis W, Putri DA, Yudha S, Mashudi, Ilsan NA, Renta PP, Masrukhin. 2023. Isolation, characterization and identification of sponge-associated bacteria producing antimicrobial compounds. Biodiversitas 24: 3616-3623.* Sponges are marine biota that is currently in great demand as research object. Sponges also belong to a group of marine life that has symbiotic with bacteria. Bacteria that have formed a symbiotic relationship with a sponge may produce secondary metabolites that protect their host from pathogens. This study aimed to isolate, select, characterize and identify bacteria associated with the sponge *Aplysina* sp. that exhibit antimicrobial activity. The methods used in this study were bacterial isolation, screening, molecular identification, and observing their ability to produce antimicrobial compounds. A total of 16 isolates were isolated on the Sea Water Complete agar medium, and four isolates were able to inhibit the growth of pathogenic microbes including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Four isolates with the most significant inhibitory clear zones were selected for further testing. The isolate APD10 showed the biggest inhibition zone. The molecular identification results showed that the APD3 and APD15 had genetic similarities with *Bacillus subtilis*, isolate APD9 had genetic similarities with *Bacillus paralicheniformis*, and isolate APD10 had genetic similarities with *Bacillus velezensis*. Two isolates (APD3 and APD15) gene sequences for the KS domain in PKS were related to polyketide synthase, while APD10 in KS was related to oxidoreductase. Two isolates (APD3 and APD15) gene sequences encoding domain A on NRPS had a relationship with a bioactive compound in the form of surfactin, APD9 was related to a bioactive compound in the form of bacitracin, and APD10 NRPS was related to adenylation.

Keywords: Antimicrobial, bioactive compounds, PKS-NRPS genes, 16S rRNA sponge-associated bacteria

INTRODUCTION

A marine ecosystem has caught the scientists' attention due to its high natural resources and relationships among all organisms. Marine life forms are widely variable, including sponges, corals, ascidians, gorgonians, sea pens, algae, fungi, and marine associated microbes. They are considered as an important source for the discovery of bioactive secondary metabolites. Sponges, one of the phylum Porifera animals, are one of the oldest Metazoa and are considered important in the evolutionary process. They are also the most abundant source of producing bioactive compounds, so they are of pharmaceutical relevance (Conkling et al. 2019). Sponges have yielded the largest number of secondary metabolites that have been isolated and described by marine natural products chemists (Blunt et al. 2013; Maharsiwi et al. 2020). They are a potential source of bioactive compounds with various biological activities, including antibacterial (Yoghiapiscessa et al. 2016; Sipriyadi et al. 2022; Wibowo et al, 2023, Retnowati and Katili 2023), antifungal (Ozkaya et al. 2014), antiviral (Hassan and Ibrahim 2016), and other bioactivities. The sponge has symbiosis interaction with marine microbes, especially bacteria. To date, 41 prokaryotic phyla have been found in association with sponges (Thomas et al. 2016), and accordingly, this vast genetic potential is hypothesized to be accountable for numerous interactions between sponge symbionts and their hosts (Webster and Thomas 2016; Moitinho-Silva et al. 2017; Chaib De Mares et al. 2018).

Marine organisms, especially sponges, have been reported to synthesize antibacterial bioactive compounds

(Varijakzhan et al. 2021). Many prior studies exploring the antimicrobial activity from sponges associated bacteria have been reported, such as Abubakar et al. (2012), who successfully found 32 (45.71%) and 20 (29.41%) isolates from mesohil and surface of the sponges, respectively. That discovery showed that those obtained bacterial isolates were able to inhibit some pathogenic microbial growth, namely *Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Vibrio harveyii*, *Escherichia coli*, *Candida albicans*, and *C. tropicalis*. According to Orfanoudaki et al. (2021), the *Aplysina aerophoba* contains secondary metabolite compounds, including brominated alkaloids. Vilas et al. (2015) discovered that aeroplysinin, which is found in the sponges *Ianthella ardis* and *Aplysina aerophoba*, has a strong antibiotic and anti-inflammatory effect on gram-positive bacteria. Anteneh et al. (2021) studied and found that sponge *Geodia* sp *Chondrosida* sp, *Ircinia* sp., *Poecilosclerida* sp. and *Crella* sp., *Sarcotragus* sp., *Carteriospongia foliascens*, *Aplysilla sulfurea*, *Dendrilla* sp. and *Tedania tubulifera* have potential as an antimicrobial against 11 human pathogens.

Nowadays, research for new bioactive compounds in bacteria generally focuses on the exploration of the genes encoding bioactive compounds, including nonribosomal peptide synthetase (NRPS) adenylation and polyketide synthase (PKS) that have conserved adenilase (A) domain and ketosynthase (KS) domain, respectively (Rini et al, 2017). The bioactive compounds produced by bacteria are synthesized by multidomain enzymes, namely, polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS). These enzymes are approximately 200–2000 kDa in size. The core catalytic domains of NRPS comprise a thiolation (T), adenylation (A), and condensation (C) domain and usually terminate with a thioesterase (Te) domain (Miller and Gulick 2016; Priyanto et al. 2022). The 16S rRNA gene was used to identify the bacterial isolates. The existence of nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes have to be demonstrated for their potential to synthesize bioactive compounds (Rini et al. 2017).

To date, more than 23,000 natural Poliketides (PKs) and Non Ribosomal Protein (NRP) products have been identified and characterised, including antibiotics (Chen et al. 2022). Although the presence of these genes in the genome does not suggest their expression or function, their presence raises the possibility of identifying organisms capable of producing bioactive compounds (Amoutzias et al. 2016). According to the description above, it is necessary to conduct research on the identification of sponge-associated bacteria and their potency to produce antimicrobial compounds.

MATERIALS AND METHODS

Isolation of sponge-associated bacteria

The sponge used in this study was *Aplysina* sp. collected from Dua Island, Enggano, Bengkulu Province, Indonesia. The identification of sponges was evaluated based on their morphology (Cruz-Barraza et al. 2012). The serial dilution method was applied in this study. One g of sponge biomass was rinsed with sterile seawater and aseptically crushed. These materials were homogenized and serially diluted in 9 mL of 0.85% NaCl solution from 10^{-1} to 10^{-3} dilution. Nearly 100 µL suspension of each serial dilution was plated onto Sea Water Complete (SWC) agar medium (1 g yeast extract, 3 mL glycerol, 5 g peptone, 250 mL distilled water, and 750 mL seawater, 15 g agar) by spread plate technique, then incubated for 24-36 h at ±30°C.

Antagonism assay for pellet and supernatant of the potential bacteria

The activity test of bacteria in producing bioactive compounds was determined using pathogenic bacteria and fungi, specifically *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. First, pathogenic microbes were prepared for antagonistic tests by culturing the pathogenic microbes on tryptic soy broth (TSB) media and potato dextrose broth (PDB) media was used to culture pathogenic fungi for 24 hours. Furthermore, 1 mL of pathogenic microbial culture was transferred into 100 mL of tryptic soy agar (TSA), then poured into a sterile Petri dish. After adding the pathogenic microbial cultures to the tryptic soy agar (TSA) medium, the screened isolates were added to the agar medium. The cells were then incubated for 12-24 h at 30°C in an incubator. Furthermore, the same approach was used to analyze pathogenic yeast using potato dextrose broth (PDB) medium. Chloramphenicol was used as the positive control. A clear zone around the colonies indicates a positive result.

A total of 1.5 mL of the selected isolates suspension was centrifuged at 10.000 rpm for 5 min. The pellets and supernatants were separated into different tubes. The pellets were diluted with 150 µL of supernatants so that the suspension contained ten times of cell number. Subsequently, 20 µL of the concentrated culture was absorbed on a blank paper disc with a diameter of 6 mm, then dropped onto the Sea Water Complete (SWC) agar medium containing the targeted bacteria. Additionally, 20 µL of supernatants were inoculated on the medium for comparison. The plates were then incubated at ±30°C for 24 h.

Antagonism assay of bacterial crude extract

As a starter, 10 mL of 24-hour bacterial suspension was inoculated into 1 L of liquid SWC medium. The culture was incubated and shaken at 120 rpm for 72 h at ±30°C. The bacterial culture was then extracted by adding ethyl acetate solvent 1:1 (v/v) and shaking continuously for 20 min. The solvent layer was separated and evaporated using a rotary evaporator at 42°C. The crude bacterial extracts were diluted in dimethyl sulfoxide (DMSO). In total of 20 µL of 1000 ppm crude extract were injected into the sterile paper disk then placed on to SWC agar plate containing targeted bacteria. dimethyl sulfoxide (DMSO) and 100 ppm of ampicillin were applied as a negative and positive control, respectively. The plates were incubated for 24 hours at ±30°C. This assay was conducted in triplicates, and all antagonism assays were measured with a diameter of the clear zone–diameter of the paper disk in mm.

Molecular characterization of potential bacterial isolates using 16S rRNA gene

The 16S rRNA gene was amplified using a polymerase chain reaction (PCR) machine with a 63F forward primer (5'-CAG GCC TAA CAC ATG CAA GTC-3 ') and 1387 R reverse primers (5'-GGG CGG WGT GTA CAA GGC-3'), Marchesi et al. (1998). The total volume of PCR reaction used was 40 µL consisting of 20 µL of GoTaq Green Master mix 2x, 2 µL of each primer, 4 µL of genomic DNA (~ 100 ng) and 12 µL of nuclease free water (NFW). PCR condition was carried out in 30 cycles with conditions as follows: pre-denaturation (94°C, 5 minutes), denaturation (94°C, 45 seconds), annealing (55°C, 1 minute), elongation (72°C, 1 minute 10 seconds). After amplification, the amplified gene was visualized using the Gel Document System Oxygen. The amplified 16S rRNA gene will be identified by the formation of DNA fragments measuring ±1300 bp.

Molecular identification of the PKS and NRPS genes of potential bacterial isolates

The Ketosynthase (KS) domain in the PKS gene and the Adenylation (A) domain in the NRPS gene from four potential bacterial isolates were amplified using PCR. The total amount of the PCR reaction was 40µL, which included: GoTaq Green Mastermix 2x 20 µL, each primer 2 µL, DNA template (~100 ng) 2 µL and NFW 14 µL. The primer used to amplify domain A (MTF and MTR codes) was forward: 5 '–AAR DSI GGI GSI GSI TAY BICC- 3', reverse: 5 '–CKR WAI CCI CKI AIY TTI AYY TG-3', while the KS domain (KSF and KSR codes) were forward: 5 '–CSA TGG AYC CSC ARC ARC GSVT- 3', reverse: 5 '–GTS CCS GTS CCR TGS SCY TCS AC-3' (Schirmer et al. 2005). The PCR conditions for domain A were modified slightly from Schirmer et al. (2005), specifically pre-denaturation (94°C, 5 minutes), denaturation (94°C, 1 minute), annealing (50°C, 1 minute), elongation (72°C), 1 minute). For 35 cycles, denaturation, annealing, and elongation were performed. In the next stage, post-elongation (72°C, 10 minutes) and cooling (4°C, 5 minutes) were performed. PCR conditions for KS domain with modifications were pre-denaturation (94ºC, 5 minutes), denaturation (94°C, 1 minute), annealing (55°C, 1 minute), and elongation (72°C, 1 minute). Denaturation, annealing, and elongation were repeated for 35 cycles. In the next stage, post-elongation (72°C, 10 minutes) and cooling (4ºC, 5 minutes) were performed. The amplified gene was then visualized using Gel Document System Oxygen. Isolates with the gene coding for domain A showed the formation of DNA fragments measuring 1000 bp, and KS domains showed DNA fragments of 700 bp.

Molecular data analysis

The amplification results were sequenced using First Base services (PT. Genetika Science Indonesia). The 16S rRNA gene sequences were aligned with the Gen Bank using Blast N, while Blast X analyzed the PKS and NRPS gene sequences found in NCBI www.ncbi.nlm.nih.gov/ BLAST/). Phylogenetic tree construction used the Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 with the Neighbor-Joining method 1000 bootstraps (Tamura et al. 2013).

RESULTS AND DISCUSSION

Sponge-associated bacteria and the antimicrobial properties

Isolation of sponge-associated bacteria from *Aplysina* sp. resulted in 263 colonies with 16 selected bacterial isolates morphologically different in the Sea Water Complete (SWC) agar medium for further test. Antimicrobial activities against the Gram-negative bacteria (*E. coli*), the Gram-positive bacteria (*S. aureus and P. aeruginosa*), and pathogen fungi (*C. albicans*) were evaluated for 16 isolates of bacterial sponge-associated *Aplysina* sp. It is confirmed by concentrated pellets, supernatant, and crude extracts using the paper disk assay of 16 isolates. Four isolates (25%) inhibited pathogenic microbes' growth indicated by clear zone formation around the bacterial colonies. The four potential isolates were coded as isolates APD3, APD9, APD10, and APD15 (Table 1).

According to the research finding, the four bacterial isolates indicate bacterial activity against the pathogen microbes tested. Chloramphenicol and nystatin were used as a positive control for bacteria and fungi in this experiment, respectively. Isolate APD10 showed the largest inhibition zone, measuring 26.6 mm and 13.7 mm against bacteria and fungi, respectively. Compared to the inhibition zone produced by the positive control, isolate APD10 produced lower antibacterial activity than C+, but the inhibition zone produced by isolate APD10 was larger than Control positive.

In Table 1, isolate APD10 possessed the broadest spectrum of the antibacterial and antifungal compound against all pathogen microbes tested. Additionally, concentrated culture, supernatants, and metabolite extracts of that bacteria exhibited consistent antibiotic efficacy. The formation of an inhibitory zone around the bacterial colony indicates the diffusion of bioactive compounds synthesized by the bacterium onto the growth medium, inhibiting the growth of targeted bacteria in the diffusion area (Figure 1). According to Esteves et al. (2017), the ability of symbiont bacteria to inhibit target microbial growth is a form of antagonistic activity that is considered to be accomplished by the production of antimicrobial compounds. Endophytic bacteria can produce secondary metabolites that are identical to those produced by their host plants, even in high amounts (Kim and Sudbery 2011).

As illustrated in Figure 1, crude extracts of the bacteria-associated with sponge *Aplysina* sp. are capable of inhibiting the pathogenic bacteria and fungi tested. Antimicrobial compounds may inhibit bacterial growth by causing cell wall damage. Othman et al. (2019) stated that the mechanism of action of alkaloids as antibacterials affects cell division, respiratory inhibition, and enzyme inhibition in bacteria, disrupting bacterial membranes and affecting virulence genes.

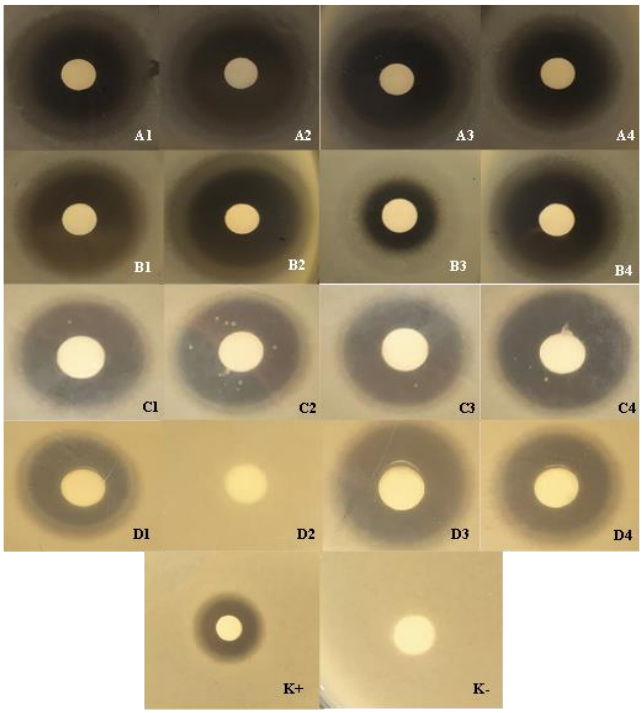

Figure 1. The inhibition zone confirmed from crude extracts of isolates APD3 (1), APD9 (2), APD10 (3), and APD15 (4) against pathogenic bacteria *Escherichia coli* (A), *Pseudomonas aeruginosa* (B), *Staphylococcus aureus* (C), *Candida albicans* (D), chloramphenicol (C+) and DMSO (C-). As a reference, the paper disk in the picture is 6 mm in size

Molecular characterization of 16S rRNA gene for potential bacterial isolates

The 16S rRNA gene can be used to determine the taxonomy, phylogeny (evolutionary relationships), and estimate the range of diversity between species (rates of species divergence) of bacteria. Furthermore, 16S rRNA gene sequencing is used to identify bacteria at the species level and to differentiate closely related bacteria (Wang et al. 2015). For instance, four potential bacterial isolates associated with the sponge *Aplysina* sp. amplified successfully and showed DNA fragment measuring ~1300 bp (Figure 2).

Based on the 16S rRNA gene, isolates APD3 and APD15 had genetic similarities with *Bacillus subtilis* strains YEB L4 and *Bacillus subtilis* strain A2. Isolate APD9 shared genetic similarities with *Bacillus paralicheniformis* strains Sac64 and APD10 as producers of the broadest antimicrobial spectrum, which is very homologous to *Bacillus velezensis* strain LZLJ01 that had a 100% genetically similarity value (Table 2). According to Johnson et al. (2019) which state although convenient and powerful, such usage of 16S has necessitated certain assumptions, e.g., the now historical assumption that sequences of >95% identity represent the same genus, whereas sequences of >97% identity represent the same species. Several prior studies have demonstrated the genus *Bacillus*'s potential to produce antimicrobial compounds, including Wahyudi et al. (2018), who investigated the genus *Bacillus*'s inhibitory activity against pathogenic bacteria that cause vibriosis, *Vibrio* spp.. Then, Sawhney et al. (2018) reported that *B. velezensis* strain CHB2 isolated from the marine sponge *Biemna fortis* on the Andaman coast of South India possessed the ability to inhibit the growth of five pathogens, i.e., *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, and *L. monocytogenes*.

Molecular identification of the PKS-NRPS gene for potential bacterial isolates

Polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) are modular enzymes initially associated with the production of significant compounds in bacteria (Hwang et al. 2020). PKS is responsible for modifying of acyl-coA produced during the synthesis of polyketides. These products have been known as important antibiotics for human beings including erythromycin, rapamycin, and amphotericin B, with function being an antibiotic, immunosuppressant, or antifungal (Staunton and Weissman 2001). While, NRPS is responsible for adding of one amino acid to the polypeptide product. NRPS products include daptomycin, actinomycin D, and cyclosporine A have antibacterial, anticancer, and immunosuppressive properties, respectively (Walsh 2016; Sussmuth and Mainz 2017). Those are only a few examples of the products. Therefore, the evaluation of PKS and NRPS genes in this study was performed as the possible link between the antimicrobial compound production. Bacteria that have the potential to produce bioactive compounds are often characterized by having PKS and NRPS genes. Three potential isolates were successfully amplified for KS domain coding genes using KSF and KSR primers, and four potential isolates with domain A coding genes using MTF and MTR primers (Figure 4A and 4B).

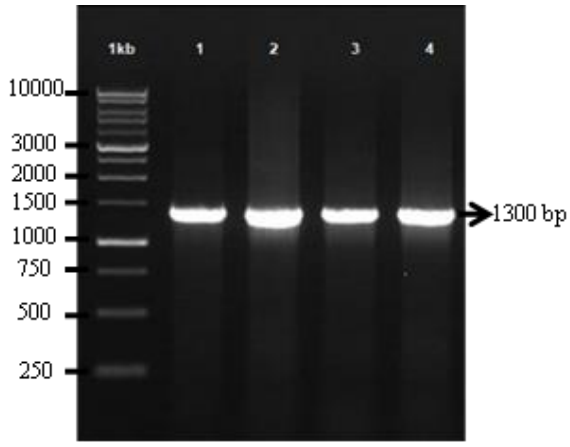

Figure 2. The 16S rRNA gene electrophoresis formed a band measuring ~1300 pb. 1kb: marker 1 kb, 1: APD3, 2: APD9, 3: APD10, 4: APD15

Table 1. Antimicrobial activities of four selected isolates. The number below was evaluated with the diameter of the clear zone minus to diameter of the paper disk in mm

Antagonism assay	Isolate code	Pathogenic microbes (clear zone in mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*C. albicans*
Bacterial pellet	APD3	3.7	0	3.2	7
	APD9	1.7	6	3.5	0
	APD10	6	2.9	0	0
	APD15	4.5	7	4.6	14
Bacterial supernatant	APD3	3	0	3.6	7.2
	APD9	1.7	6.4	3.5	0
	APD10	3.5	6.2	5.2	15.1
	APD15	7.9	4.4	3	13.2
Bacterial crude extract	APD3	20.7	12.3	6.1	13.4
	APD9	22.5	17.9	15.5	0
	APD10	26.6	14.8	10.5	13.7
	APD15	14.3	16	8.7	10.7
	C+ (Chloramphenicol for bacteria and Nystatin for fungi)	25.7	27.2	26.8	13.3
	C- (DMSO)	0	0	0	0

Table 2. Alignment of 16S rRNA gene sequences from 4 potential isolates against data available in NCBI (Blast N)

Isolate code	Homology	Query cover (%)	E-value	Similarity (%)	Accession number
APD3	*Bacillus subtilis* strain YEB L4	90	0.0	97.54	MK258757.1
APD9	*Bacillus paralicheniformis* strain Sac64	99	0.0	98.79	MT479160.1
APD10	*Bacillus velezensis* strain LZLJ01	100	0.0	100	MT645702.1
APD15	*Bacillus subtilis* strain A2	100	0.0	98.39	KC433738.1

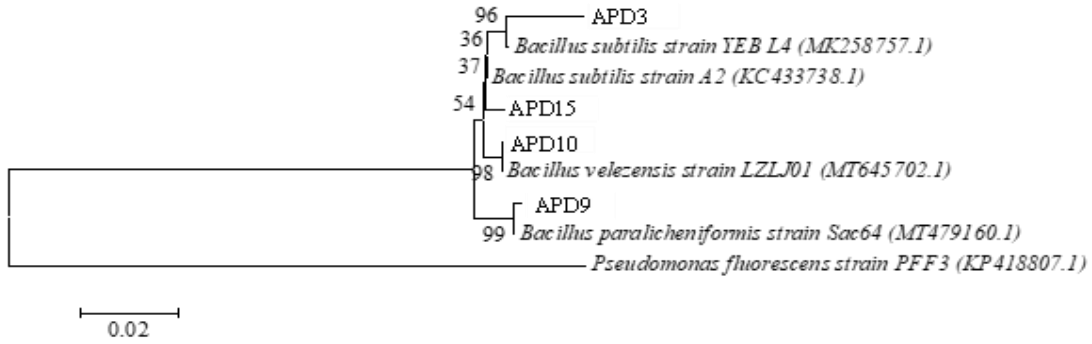

Figure 3. The phylogenetic tree of potential isolates associated with sponge *Aplysina* sp. based on the 16S rRNA gene sequences using the Neighbor Joining Tree method with a bootstrap value of 1000x replicates

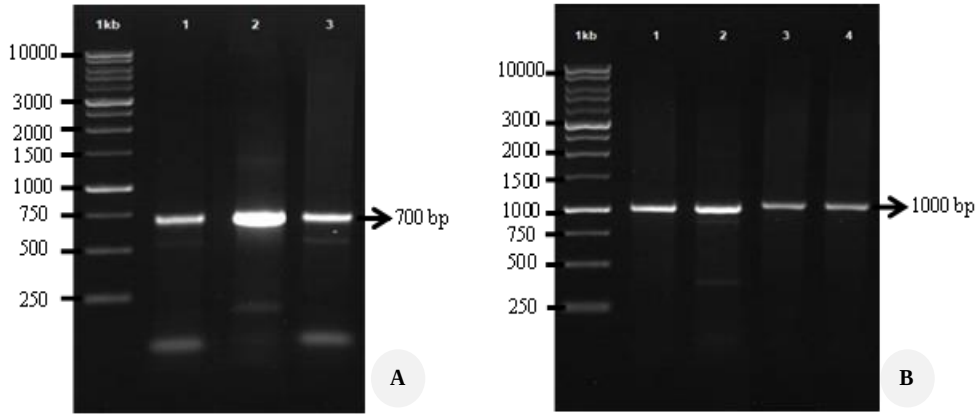

Figure 4. The electrophoresis results. A. The KS domain coding gene (PKS) formed a DNA band measuring 700 bp. 1: APD3, 2: APD10, 3: APD15. B. The A domain coding gene (NRPS) formed a DNA band 1000 bp. 1: APD3, 2: APD9, 3: APD10, 4: APD15, 1kb: marker 1 kb

Based on Tables 3 and 4, the KS domain of isolates APD3 and APD15 have genetic similarity to type 1 PKS gene *Bacillus subtilis* E1 and PKS gene *B. subtilis*. In contrast, domain A for the two isolates has genetic similarities with the bioactive surfactin compound (SrfAC) *B. subtilis*. The APD10 isolate was identical to oxireductase in the KS domain and to *B. velezensis* adenylation in the A domain. Similar to the bioactive compound bacitracin *B. paralicheniformis*, isolate APD9 was only found in the A domain. PKS and NRPS genes were present in the three bacterial isolates designated APD3, APD10, and APD15. NRPS-PKS hybrid may occur with a combination of the NRPS module domain with a PKS module domain in a single open reading frame. Hybrid NRPS-PKS genes may synthesize a novel type of bioactive compound with a hybrid structure (Rini et al. 2017). Amino acid mapping revealed that a fragment of the PKS gene was located in its active site. Isolates APD 3, APD 4, APD 11, and APD 15 were indifferent pattern amino acids compared to APD 10, although these two groups belonged to the same species, including *B. subtilis* (Figure 5).

Phylogenetic tree was constructed to determine the similarities and relationships between nucleotide sequences from potential bacterial isolates associated with the sponge *Aplysina* sp. and comparison strains on Gene Bank data. The phylogenetic tree between the KS domain (Figure 6) and domain A (Figure 7) revealed the formation of two distinct clades. Clade 1 (C1) had isolates of the genus *Bacillus* bacteria with the comparison strain on both phylogenetic trees, whereas clade 2 (C2) included the species *Xanthomonas citri pv. citri* in domain KS and *Alcaligenes faecalis* in domain A was located in outgroups of the phylogenetic tree.

Table 3. Alignment of DNA fragment encoding KS domain of PKS gene from 3 potential isolates against data available in NCBI (Blast X)

Isolate code	Homology	Query cover (%)	E-value	Similarity (%)	Accession number
APD3	Polyketide synthase of type I *Bacillus subtilis* E1	99	5e-88	100	CCU58292.1
APD10	SDR family NAD(P)-dependent oxireductase *Bacillus velezensis*	99	3e-77	97.86	WP_060386947.1
APD15	Polyketide synthase *Bacillus subtilis* subsp. *subtilis* str. 168	99	8e-87	99.24	AAA85145.1

Table 4. Alignment of DNA fragment encoding A domain of NRPS gene from 4 potential isolates against data available in NCBI (BlastX)

Isolate code	Homology	Query cover (%)	E-value	Similarity (%)	Access number
APD3	Surfactin non-ribosomal peptide synthetase SrfAC; *Bacillus subtilis*	89	1e-56	66.85	WP_161621342.1
APD9	Bacitracin non-ribosomal peptide synthetase BaCA; *Bacillus paralicheniformis*	65	4e-40	92.59	WP_1060706871
APD10	amino acid adenylation domain-containing protein *Bacillus velezensis*	100	0.0	99.66	WP_173613238.1
APD15	Surfactin non-ribosomal peptide synthetase SrfAC *Bacillus subtilis*	99	2e-93	96.93	WP_144460361.1

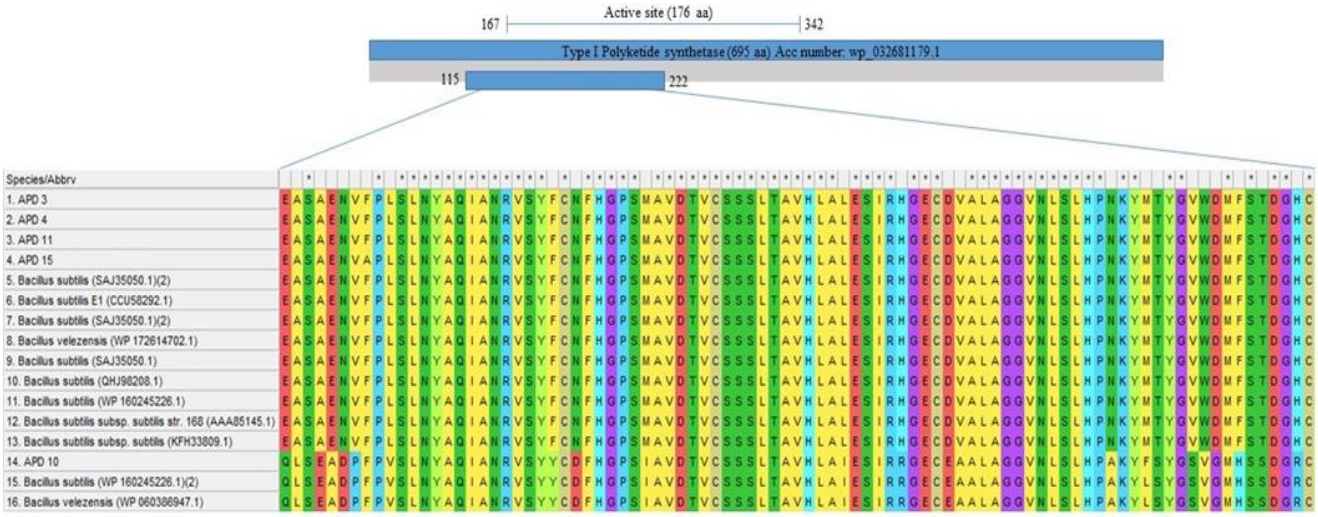

Figure 5. Amino acid mapping of partial PKS gene of isolates (APD 3, APD 4, APD 11, APD 15, and APD 10) with references isolates

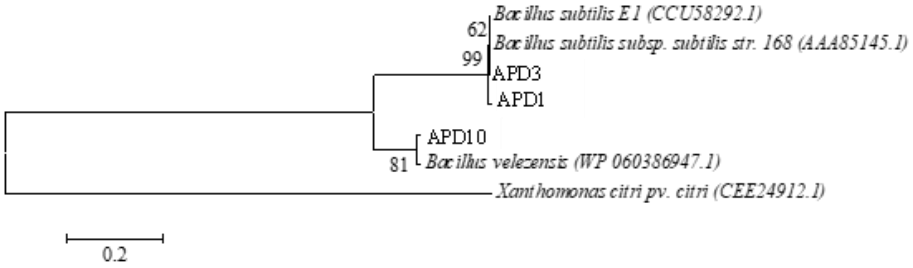

Figure 6. The phylogenetic tree of potential isolates associated with sponge *Aplysina* sp. based on the KS domain coding gene sequences using the Neighbor Joining Tree method with a bootstrap value of 1000x replicates

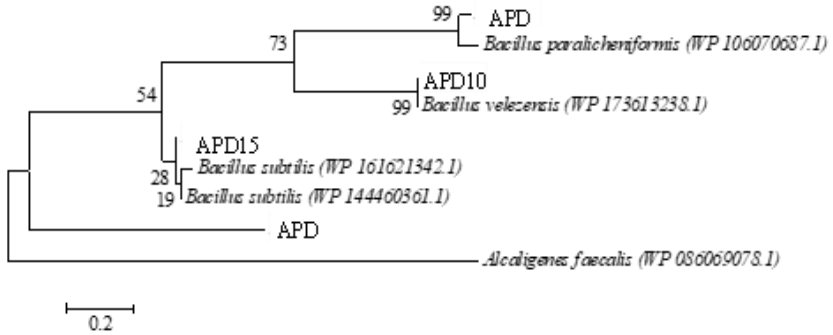

Figure 7. The phylogenetic tree of potential isolates associated with sponge *Aplysina* sp. based on the A domain coding gene sequences using the Neighbor Joining Tree method with a bootstrap value of 1000x replicates

The phylogenetic trees of potential bacterial isolates constructed using the 16S rRNA gene were consistent with those constructed using the KS and A domain coding genes, which indicated that the four isolates were closely related to *Bacillus*. The phylogenetic tree's branches represented the relationships between biological entities. Two highly similar sequences will occur on the same branch and oppositely (Queiroz et al. 2013).

In conclusion, this study confirmed that total of 263 colonies with 16 selected bacterial isolates successfully growth on Sea Water Complete (SWC) agar medium. Isolate APD10 had the highest antimicrobial activity among the four isolates due to the largest inhibition zone against the testing pathogenic microbes. Based on 16S rRNA gene, those 4 isolates were highly homolog to Bacillus genera. This APD10 is important to be further exploited for controlling human pathogenic microbes biologically and essential for the elucidation of bioactive compounds synthesized by this bacterium.

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