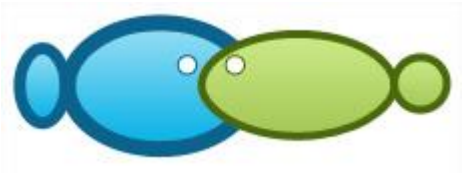

Bioactive antibacterial compounds against bacterial pathogens and potential production of extra cellular enzymes from sponge associations in Karimunjawa Island, Indonesia

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Abstract. Several studies have found that active compounds from the sea are antibacterial and have extracellular enzyme activity. Sponge is one of the marine animals that has the potential to be used in the pharmaceutical field. Microbes associated with sponges are believed to have the same secondary metabolite compounds as their sponge hosts. The purpose of this study was to obtain isolates of sponge symbiont bacteria that have potential as antimicrobial effect against Vibrio species and against bacteria which developed a multiple drug resistance (MDR) and at the same time have lipolytic, proteolytic, amylolytic and cellulolytic activities. The research method used in this study was descriptive exploratory and experimental, concerning the diversity of association microbes, extract cytotoxicity, antimicrobial effect against Vibrio species and against bacteria which developed MDR, extracellular enzyme activity and molecular identification of selected bacteria as potential materials for antibiotics. The results showed that 209 isolates were found from the sponge association and 10 selected isolates had an antimicrobial effect against Vibrio species and against bacteria which developed a MDR, as well as lipolytic, proteolytic, amylolytic and cellulolytic activities. Of the 10 selected isolates, 9 isolates of different types were identified which included six types of bacteria from the genus Bacillus, namely: *Bacillus firmus* strain Xmb067, *Bacillus oceanisediminis* strain Xmb065, *Bacillus flexus* strain RP-UL, *Salibacillus* sp. NT N53, *Bacillus* sp. M-237-24 and *Bacillus pumilus* strain Lmb061. Two bioactive bacteria were identified in the genus Halomonas, namely *Halomonas venusta* partial and *Halomonas* sp. MD5, and one was identified in the genus Brevundimonas, namely *Brevundimonas* sp. N5. These types of bacteria are promising for the bioindustry field.

Key Words: antibacterial, enzyme activity, Bacillus, Halomonas, MDR, Vibriosis, sponge.

Introduction. Karimunjawa Island has a marine ecosystem comprising mangroves, seagrass and coral reefs. Marine ecosystems have many functions for the marine organisms as esential habitat for mollusca and fish (Maheswari et al 2011; Ariyanto et al 2020; Staveley et al 2020) and as a source of bioactive compounds such as polyketides and non-ribosomal peptides (Radjasa 2004), steroids, triterpenes, saponins, flavonoids, alkaloids, and tannins (Abdel-Aziz et al 2016; Ariyanto et al 2019a), antimicrobial and fermentative properties (Pringgenies et al 2020), amino acid (Ningsih et al 2020), element content (Ariyanto et al 2019b), cytotoxicity (Kim et al 2021). Commercial antibiotics have been proven to be beneficial for the treatment of infectious diseases (Nigam et al 2014), but these antibiotics have weaknesses: they are specific to certain pathogenic bacteria and their residues accumulate in the tissue. Currently, several groups of pathogenic bacteria have developed resistance to antibiotics derived from terrestrial microorganisms such as ampicillin, penicillin, ciprofloxacin, erythromycin, gentamicin, imipenem and vancomycin (Dwiprahasto 2005), a condition known as multiple drug resistance (MDR). Cases due to infection with pathogens and resistance require a solution in order to get a cure from infection. However, the discovery of new natural active compounds, with broad-spectrum bactericidal capabilities, facilitates the disease control.

Marine microorganisms have contributed to most of these bioactive compounds. They can produce the same metabolites as their host. A part of the search for bioactive compounds is directed towards the microorganism community living in sponge tissue, as symbionts (Proksch et al 2002). The development of bioactive compounds from sponges is facing obstacles, due to their rarity. Also, it takes about 13,000 kg of *Bryozoans neritina* biomass to obtain 18 g of bryostatin (Taylor et al 2007), which is not feasible without endangering the species. Marine microorganisms contribute to the synthesis of most of the bioactive compounds. producing the same metabolites as their host (Proksch et al 2002). In particular, sessile marine organisms such as sponges are thought to rely heavily on the defense mechanism of their symbionts, which produce bioactive compounds (Varijakzhan et al 2021; Bibi et al 2021).

Sponges are marine biota that produce secondary metabolites with various bioactive groups and have prospects in the field of pharmacology, including antibacterials. Of the 169 tested bacteria species, 70 were found to be active against at least one of the tested bacterial or fungal pathogen, while 37% of the tested bacteria showed activity against *Staphylococcus aureus*, including methicillin-resistant strains (Brown 2021). This study aimed to obtain isolates of sponge symbiont bacteria that have an antimicrobial effect against Vibrio species and against bacteria which developed a multiple drug resistance (MDR) and at the same time have lipolytic, proteolytic, amylolytic and cellulolytic activities.

Material and Method

Research material. The materials used in this study were sponges and sponge-associated microbes obtained from the coast of the Karimunjawa, Jepara. The isolates used for the antibacterial activity test were isolates of Vibrosis bacteria (*Vibrio harveyi*, *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*), collected from the Center for Brackish Water Cultivation of Jepara and bacteria with multiple drug resistance (MDR): *Staphylococcus aureus*, *Escherichia coli* and *Staphylococcus epidermidis*, obtained from the Faculty of Medicine, Diponegoro University, Semarang.

Sponge sample collection. Sponge sampling was carried out at the location of coral reef ecosystems, seagrass ecosystems and mangrove ecosystems, in the waters of Karimunjawa island, Central Java, Indonesia. Stations were chosen purposively, where the survey results indicated the presence of good coral reefs.

Sponge identification. The identification used the Hooper & Soest (2002) method, by observing the external shape, color, oscula, spicules and surface. The morphological form was verified on the sponge identification portals, available at the links: http://spongeguide.org and http://www.marinespecies.org/porifera/porifera.php?p= specimens.

Testing the antibacterial potential of sponge tissue. The Betancourt method (Betancourt-Lozano et al 1998) was used. Pathogenic bacterial cultures were grown on agar media for 24 h. Fresh sponge samples of 1 cm^2 wide and 0.5 cm thickness were placed on agar media and incubated for 24 h before observing the inhibition zone.

Sponge association microbial isolation. The isolation of sponge symbiont bacteria was using the method of Radjasa et al (2007). Sponge samples were washed with sterile seawater. 1 cm^2 of sponge was crushed and immersed in 100 mL of Zobell 2216E (1/2 strength) media, then incubated for 24 h. A graded dilution was performed 10 times. 0.1 mL of isolate were inoculated on a Zobell 2216E solid marine medium, and then incubated for 3-4 days, at room temperature. Purification was performed, based on the colony morphology.

Potential symbiont bacteria grouping. The Repetitive-Polymerase Chain Reaction (rep-PCR) method (Radjasa et al 2007) was used, with a primer BOX A1R (base sequence 59-CTACGGCA AGGCGACGCTGACG-39). The steps consisted of DNA

preparation, amplification, electrophoresis, UV observation and cluster analysis, using the PRIMER 5 program.

Proteolytic, amylolytic and cellulotic activity testing. The enzymatic activities included testing the production of protease, amylase and cellulase enzymes. The enzymatic activity test referred to the journal by Setyati et al (2016), with slight modifications. The proteolytic activity was tested by growing the test bacteria on Zobell agar media enriched with skim milk (1%), then by incubating them for 2x24 hours. Bacteria were grown by doting technique. The proteolytic activity identification was carried out by looking at the clear zone formed.

The amylase enzyme activity was carried out by growing the test bacteria using the doting technique on Zobell media enriched with 1% soluble starch. Bacterial growth was observed after the incubation process for 2x24 hours. After that, the media was dripped with iodine to see the amylolytic activity, based on the inhibition zone formed. Cellulase enzyme activity was tested by growing the test bacteria on Zobell agar media enriched with 1% CMC. Bacteria were grown by doting technique. Furthermore, the media that already contained bacteria was incubated within 2x24 hours. After the incubation, the iodine was dripped onto the media to see cellulosic activity.

Molecular identification of isolates from each genomic cluster. For the molecular identification of isolates, the Radjasa method (Radjasa et al 2007) was used with the primers 18F (5'-ATC TGG TTG ATC CTG CCA GT-3') and 18R (5'-GAT CCT TCC GCA GGT TCA CC-3'). The steps consisted of DNA preparation using a Kappa ready mix master kit for PCR, with amplification and evaluation by UV observation electrophoresis. The purification and the sequencing of PCR products were carried out by PT, using the same primer as Genetic Science Indonesia. The determination of the species or of the genus and of the nucleotide sequences of each sample were analyzed using the BioEdit Sequence Alignment Editor, version 7.0.9.1. Sequences were aligned using the ClustalW Multiple Alignment program and compared with MycoBank DNA data (http://www.mycobank.org) and BLAST data (http://www.blast.ncbi.nlm.nih.gov/blast). The aligned data was then reconstructed using the maximum parsimony analysis to obtain a phylogenetic tree, using the Mega5 program with bootstrap values calculated from 1,000 replications (Tamura et al 2007).

Results and Discussion. The results of sampling at the coral reef ecosystem station have obtained 8 types of sponges, which were *Callyspongia aerizusa*, *Clathria reinwardti*, *Tectitethya* sp., *Halisarca* sp., *Dercitus* sp., *Clathria* sp., *Speciospongia inconstans* and *Haliclona cymaeformis*.

Sponge samples were obtained from sampling at the research station, namely coral reef, seagrass, and mangrove ecosystems, which were then tested for their bioactivity against the vibrosis pathogenic bacteria (*Vibrio harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*) and MDR pathogenic bacteria (*Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *Pseudomonas aeruginosa*). The results of the sponge tissue test showed that the sponge tissue had antibacterial activity against 5 sponge samples from coral reef ecosystems, 5 sponge samples from seagrass ecosystems and 5 sponge samples from mangrove ecosystems (Figure 1).

The sponge isolation samples that were successfully obtained were 28 samples with 209 isolates, which included 8 samples from sponges in coral reef ecosystems (71 isolates), 11 samples from seagrass ecosystem sponges (70 isolates) and 9 isolates from mangrove ecosystem sponges (68 isolates), as shown in Table 1. All isolates of the sponge samples had various morphological characteristics of bacterial colonies, which were: color, shape, edge and elevation of the colony.

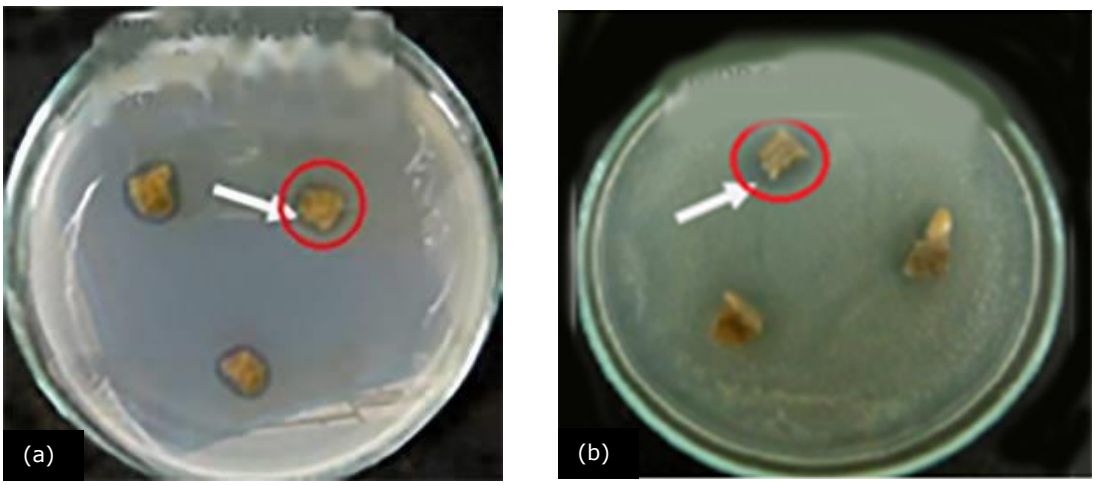

Figure 1. Qualitative test of sponge antibacterial activity against (a) *Escherichia coli* and (b) *Staphylococcus aureus*.

Table 1

Samples of sponge isolation from coral, seagrass and mangrove ecosystems

Sample	*Isolate*										
Isolate Coral	K.S1 8	K.S2 12	K.S3 6	K.S4 9	K.S5 11	K.S6 8	K.S7 8	K.S8 9			
Isolate Seagrass	L.S1 8	L.S2 7	L.S3 8	L.S4 12	L.S5 4	L.S6 4	L.S7 3	L.S8 13	L.S9 3	L.S10 6	L.S11 2
Isolate Mangrove	M.S1 9	M.S2 3	M.S3 5	M.S4 5	M.S5 10	M.S6 6	M.S7 15	M.S8 4	M.S9 11		

Based on results of the study, it was shown that sponge samples from coral ecosystems had 4 colors for each frequency of isolates, namely yellow (11), pink (1), milky white (37) and transparent (22). The colony morphology contained 5 characteristic shapes for each isolate's frequency of occurrence, which were rooted (9), circular (15), oval (12), irregular (24) and dot (11). There were 4 types of morphology characteristics of the edge of the colony for each isolate's frequency of occurrence, namely: wavy (23), stringy (9), curly (6) and flat (33). In colony morphology, there were 5 types of elevation with each isolate's frequency of occurrence, namely: hilly (8), concave (11), convex (28), flat (22) and thick flat (2), as shown in Figure 2a.

Sponge samples from the seagrass ecosystem station had 9 colors with each isolate frequency of occurrence, namely orange (1 isolate), reddish orange (1), light orange (3), dark orange (1), cream (53), light yellow (4), dark yellow (1), pink (2) and white (4). In the characteristic morphology of the colony, there were 2 color forms for each isolate's frequency of appearance, which were round (46) and irregular (24). In the characteristic morphology of the edge of the colony, there were 2 types of color edges for each isolate's frequency of appearance, namely grooved (21) and smooth (49). Meanwhile, in the morphology of the colony elevation characteristics, there were 2 types of color for each isolate's frequency of appearance, namely convex (32) and flat (38) as shown in Figure 2b.

Sponge samples from the mangrove ecosystem showed colony morphology with color characteristics; there were 4 colors for each isolate frequency of occurrence, namely yellow (10), pink (1), milky white (37) and transparent (20). In the characteristic morphology of the colony, there were 5 forms for each isolate's frequency of appearance, namely rooted (9), round (14), oval (14), irregular (21) and point (10). In the characteristic morphology of the edge of the colony, there were 4 types of edges for each isolate's frequency of occurrence, namely wavy (22), stringy (10), curly (4) and flat (32). While in the morphology characteristic of the elevation of the colony, there were 5 types

of elevation for each isolate's frequency of occurrence, namely hilly (8), concave (10), convex (28), flat (19), and thick flat (3) (Figure 2c).

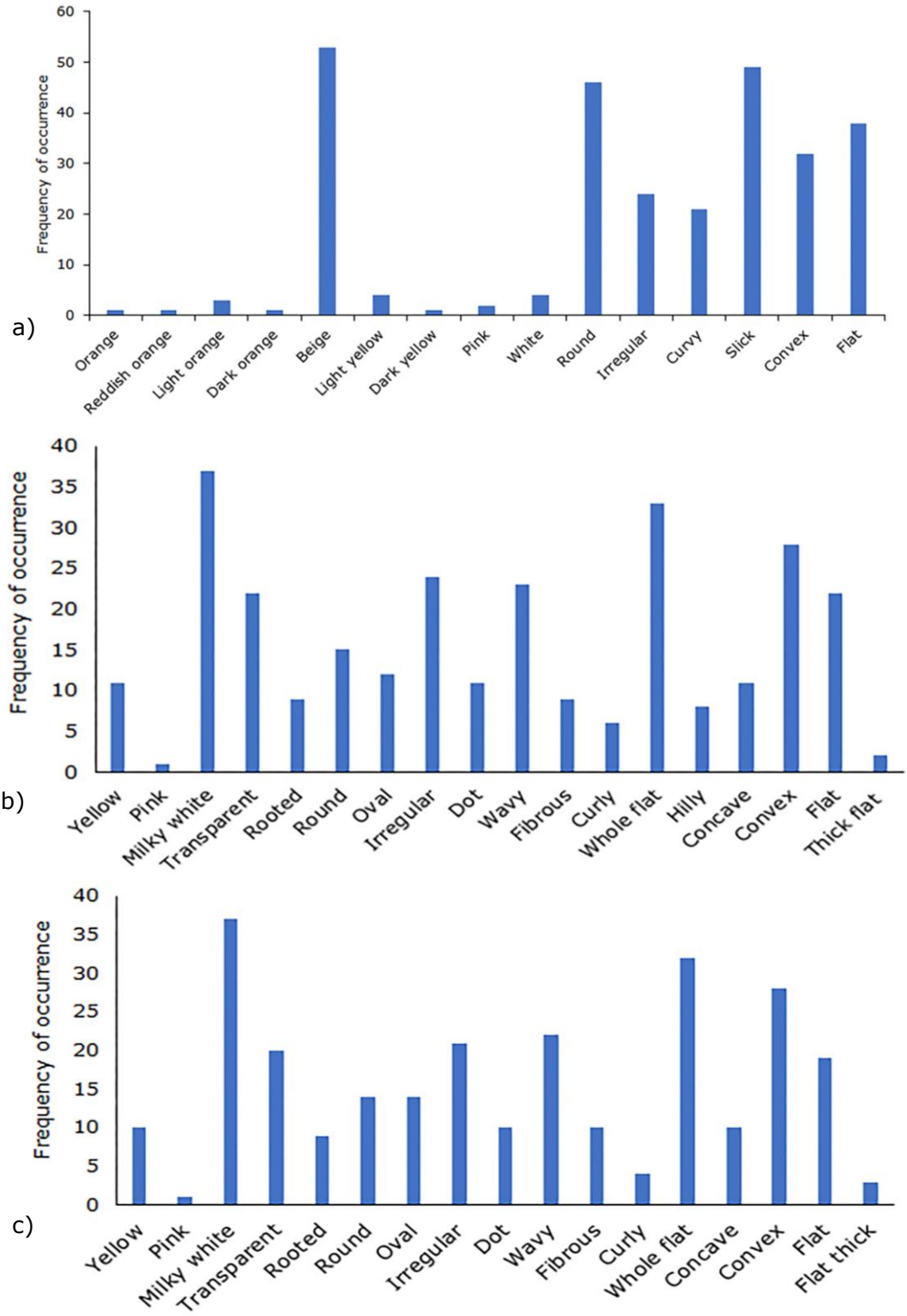

Figure 2. Frequency of occurrence of the morphology of sponge bacteria colonies in coral ecosystems (a), seagrass (b) and mangrove (c).

Based on the results of the study, it was shown that the sponge association bacteria had the activity of hydrolyzing organic matter of fat, protein, ammonia and cellulose (Figure 3).

Sponge symbiont bacteria have antibacterial activity and extracellular enzymatic activity (Table 2).

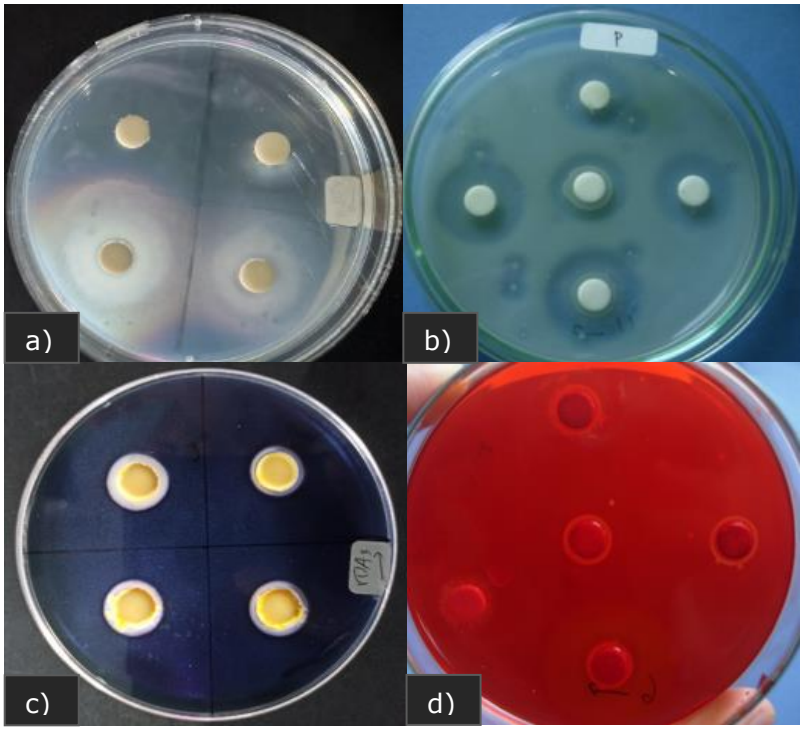

Figure 3. Test of extracellular enzymatic activity of sponge-associated bacteria (a: Lipolitic, b: Proteolytic, c: Amylolytic, d: Cellulolytic).

Table 2

Diameter of antibacterial activity zones and enzymatic hydrolysis of sponge bacteria

Isolates	*Vibriosis (cm)*				*MDR (cm)*				*Enzymatic (cm)*		
	Vh	*Vp*	*Va*	*Vv*	*Sa*	*Se*	*Ec*	*Pa*	*L*	*A*	*S*
K.S1.B2	0.47	-	1.27	-	-	-	1.70	-	-	-	-
K.S1.B8	-	-	-	1.76	-	-	-	-	1.16	-	1.76
K.S2.B11	-	-	-	-	1.13	-	-	1.60	-	-	-
K.S3.B24	-	1.56	-	2.04	1.54	-	-	-	-	-	1.83
K.S4.B29	0.12	-	1.10	1.28	-	1.14	-	1.75	-	-	-
K.S7.B57	-	-	-	-	1.36	-	-	1.49	-	2.10	-
K.S7.B58	-	-	-	-	-	1.21	-	-	-	1.84	-
K.S7.B61	0.17	2.16	-	1.25	-	-	-	-	-	1.67	-
L.S1.B6	0.14	1.89	-	-	1.89	-	1.60	1.48	-	1.57	-
L.S3.B18	-	-	2.55	1.06	-	1.22	-	-	-	-	-
L.S4.B30	0.83	-	1.54	1.69	-	-	-	-	-	-	1.94
L.S6.B51	-	2.15	-	-	-	-	-	-	2.72	2.32	-
L.S9.B63						1.65			1.87	-	1.78
M.S1.B5	0.93	-	2.45	-	-	-	-	1.50	-	-	1.76
M.S2.B12	-	-	2.21	-	1.48	1.81	-	-	2.60	-	-
M.S5.B25	-	-	-	-	1.21	-	-	-	1.71	-	1.60
M.S7.B39	-	-	-	-	-	1.42	1.14	-	-	-	-
M.S7.B46	-	1.40	-	-	-	-	1.64	-	-	2.35	1.97
M.S7.B51	-	-	1.99	-	-	-	-	1.49	3.20	-	1.49
M.S9.B67	0.72	-	1.85	-	-	1.71	-	-	2.07	-	-

Vh-*Vibrio harveyi*; Vp-*V. parahaemolyticus*; Va-*V. alginolyticus*; Vv-*V. vulnificus*; Sa-*Staphylococcus aureus*; S-*S. epidermidis*; Ec-*Escherichia coli*; Pa-*Pseudomonas aeruginosa*; Pa-Proteolytic; L-Lipolytic; A-Amilolytic; S-Cellulolytic.

The antagonist activity test against the pathogenic bacterium *Vibrio harveyi* contained 7 isolates. The isolate M.S1.B5 had the highest zone diameter (0.93 cm) against the

bacterial pathogen *V. harveyi*. Antagonistic activity test against pathogenic bacteria *V. parahaemolyticus* showed that 5 isolates were antagonistic. Isolate K.S7.B61 had the highest antagonistic zone diameter (2.16 cm) against *V. parahaemolyticus*.

Antagonistic activity test against pathogenic bacteria *V. alginolyticus* showed that 8 isolates were antagonistic, which included 2 isolates of coral ecosystem sponges, 2 isolates of seagrass and 4 isolates of mangroves. Seagrass isolate L.S3.B18 had the highest antagonist zone diameter (2.55 cm) against *V. alginolyticus*. Antagonistic activity test against pathogenic bacteria *V. vulnificus* showed that 6 isolates were antagonistic: 4 isolates of coral ecosystem sponges and 2 isolates of mangroves. K.S3.B24 isolate had the highest antagonistic zone diameter (2.04 cm) against *V. vulnificus*.

Furthermore, the antagonistic activity test against the pathogenic bacteria *S. aureus* showed that 6 isolates were antagonistic: 3 isolates of coral ecosystem sponges, 1 isolate of seagrass 2 isolates of mangrove. L.S1.B6 isolate had the highest antagonistic zone diameter (1.89 cm) against *S. aureus*. Antagonistic activity test against pathogenic bacteria *S. epidermidis* showed that 7 isolates were antagonistic: 2 isolates of coral ecosystem sponges, 3 isolates of seagrass 2 isolates of mangroves. M.S2.B12 isolate had the highest antagonist zone diameter (1.81 cm) against *S. epidermidis*.

Antagonistic activity test against pathogenic bacteria *E. coli* showed that 4 isolates were antagonistic: 1 isolate of coral ecosystem sponge, 1 isolate of seagrass and 2 isolates of mangrove. K.S1.B2 isolate had the highest antagonist zone diameter (1.70 cm) against *E. coli*. Antagonistic activity test against pathogenic bacteria *Pseudomonas aeruginosa* showed that 6 isolates were antagonistic: 3 isolates of coral ecosystem sponges, 3 isolates of seagrass and 2 isolates of mangroves. K.S4.B29 isolate had the highest antagonist zone diameter (1.75 cm) against *P. aeruginosa*.

The proteolytic extracellular enzymatic activity test showed that there were 9 active proteolytic isolates: 4 isolates of coral ecosystem sponges, 2 isolates of seagrass and 3 isolates of mangroves. M.S1.B5 isolate had the highest proteolytic hydrolysis zone diameter (2.49 cm). The lipolytic extracellular enzymatic activity test showed that there were 7 lipolytic active isolates: 1 isolate of coral ecosystem sponge, 2 isolates of seagrass and 4 isolates of mangrove. The isolate M.S7.B51 had the highest diameter of the lipolytic hydrolysis zone (3.20 cm).

Amylolytic extracellular enzymatic activity test showed that there were 7 active amylolytic isolates: 3 of coral ecosystem sponges, 2 of seagrass 2 of mangrove isolates. M.S7.B46 isolate had the highest amylolytic hydrolysis zone diameter. Cellulolytic extracellular enzymatic activity test showed that there were 8 active cellulolytic isolates: 2 isolates from sponge coral, 2 isolates of seagrass 4 isolates of mangrove ecosystems. M.S7.B46 isolate had the highest cellulolytic hydrolysis zone diameter (1.97 cm).

The Rep-PCR test results of 20 bacterial isolates that had extracellular antibacterial and enzymatic bioactivity showed that the gel doc was amplified and well visualized (Figure 4). The rep PCR results showed that bands were formed continuously. The band pattern formed from each isolate looked different. Tests of the gel doc rep PCR visualization were then carried out for a binomial analysis, then for a cluster analysis, using the SPSS program, as presented in Figure 5.

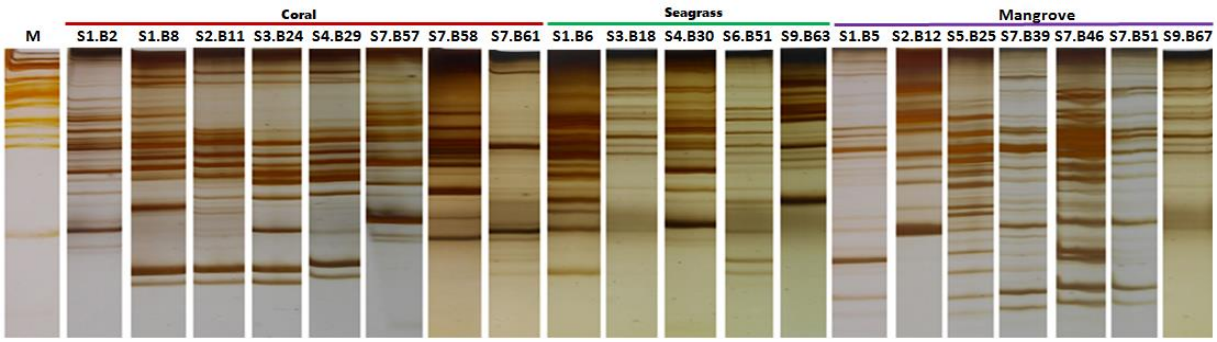

Figure 4. Gel doc results of electrophoresis rep-PCR analysis of sponge association bacteria.

Based on the results of the cluster analysis and bioactivity tests, 10 isolates from 20 isolates were then selected and 16 S-rDNA were identified. The isolates were K.S1.B2; K.S3.B24; K.S4.B29; K.S7.B61; L.S1.B6; L.S3.B18; M.S1.B5; M.S2.B12; M.S7.B46 and M.S7.B51.

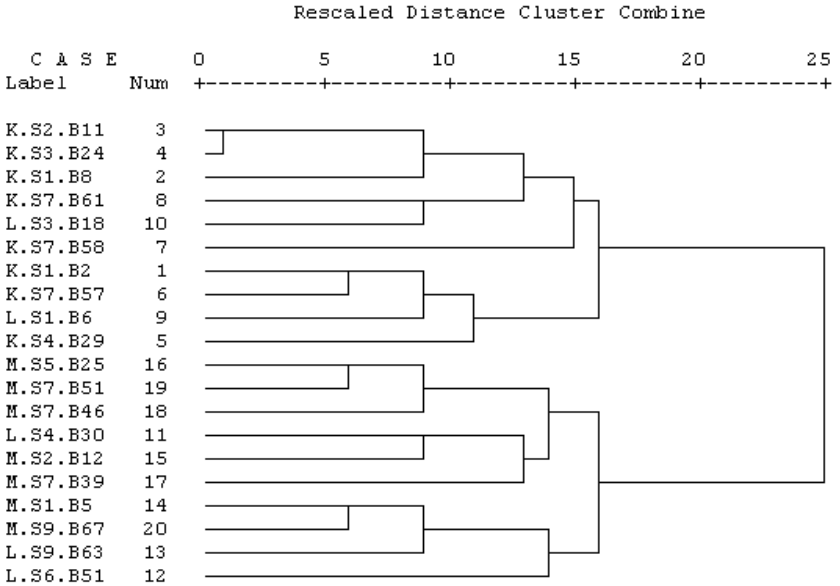

Figure 5. Cluster analysis of binary data from rep-PCR results for the sponge-associated bacteria.

The results of the cluster analysis showed similarities between different isolates (Figure 5): bacteria were grouped in 10 clusters, based on binary rep-PCR data. Cluster 1 consisted of 3 isolates, namely K.S2.B11, K.S3.B24 and K.S1.B8. Cluster 2 consisted of 2 isolates, namely K.S7.B61 and L.S3.B18. Cluster 3 consisted of 1 isolate, namely K.S7.B58. Cluster 4 consisted of 3 isolates, namely K.S1.B2 K.S7.B57 and L.S1.B6 isolates. Cluster 5 consisted of 1 isolate, namely K.S4.B29. Cluster 6 consisted of 3 isolates, namely M.S5.B25, M.S7.B51 and M.S7.B46. Cluster 7 consisted of 2 isolates, namely L.S4.B30 and M.S2.B12. Cluster 8 consisted of 1 isolate, namely M.S7.B39. Cluster 9 consisted of 3 isolates, namely M.S1.B5, M.S9.B67 and L.S9.B63. Cluster 10 consisted of 1 isolate, namely L.S6.B51.

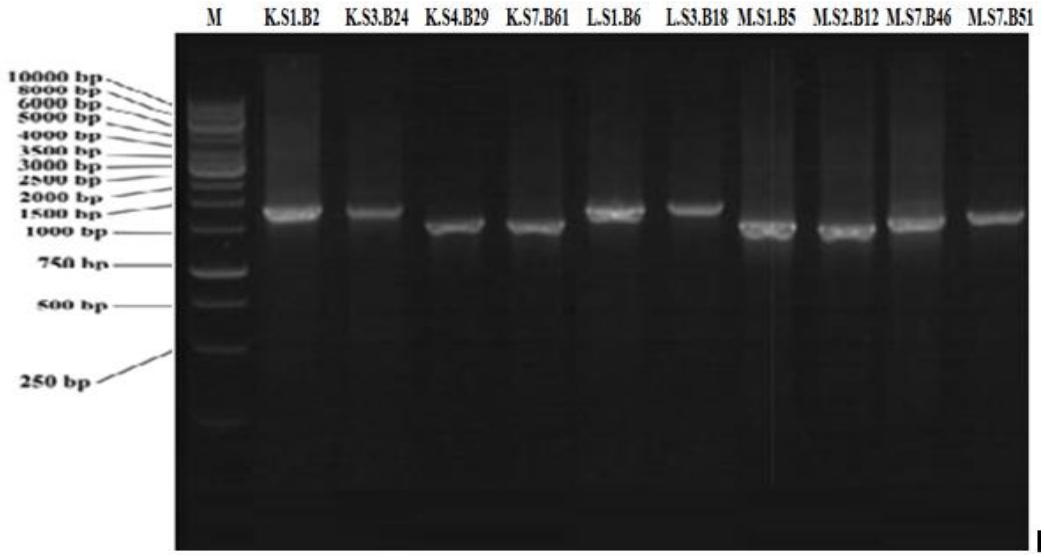

Figure 6. Gel doc result of the electrophoresis PCR 16 S rDNA of the sponge association bacteria.

The PCR analysis was successfully carried out. Results of the electrophoresis showed that the 10 samples revealed band formation in the range of 1,000-1,500 bp. In addition, the PCR results show a single and uncontaminated band (Figure 6). Furthermore, the results of the sequencing analysis were successful for homology BLAST, as listed in Table 3.

Table 3

Results of the blast homology for the identification of sponge-associated bacteria

Isolates	*Sequences (bp)*	*Bacteria species name*	*Homology*	*Access no.*
K.S1.B2	1,456	*Bacillus firmus* strain Xmb067	100	KT986192.1
K.S3.B24	1,458	*Bacillus oceanisediminis* strain Xmb065	100	KT986190.1
K.S4.B29	1,417	*Bacillus flexus* strain RP-UL	97	GU592213.1
K.S7.B61	1,404	*Halomonas venusta* partial	100	LN794812.1
L.S1.B6	1,436	*Halomonas* sp. MD5	99	KM362826.1
L.S3.B18	1,368	*Brevundimonas* sp. N5	98	JN003651.1
M.S1.B5	1,490	*Salibacillus* sp. NT N53	99	AB166991.1
M.S2.B12	1,456	*Bacillus firmus* strain Xmb067	98	KT986192.1
M.S7.B46	1,438	*Bacillus* sp. M-237-24	97	KF746906.1
M.S7.B51	1,451	*Bacillus pumilus* strain Lmb061	100	KT986126.1

There were 10 isolates that have a high homology percentage, between 97-100%. The isolates with a homology value of 97% were the K.S4.B29 and M.S7.B46, both with sequence values of 1,417 and 1,438, which were identified as *Bacillus flexus* strains RP-UL and *Bacillus* sp. M-237-24, with the access numbers GU592213.1 and KF746906.1, respectively. The isolates with a homology value of 98% were the L.S3.B18 and M.S2.B12, both with sequence values of 1,368 and 1,456, which were identified as *Brevundimonas* sp. N5 and *Bacillus firmus* strain Xmb067, with the access numbers JN003651.1 and KT986192.1, respectively.

The isolates with homology value of 99% were the L.S1.B6 and M.S1.B5, both with sequence values of 1,436 and 1,490, which were identified as *Halomonas* sp. MD5 and *Salibacillus* sp. NT N53, with the access numbers KM362826.1 and AB166991.1, respectively. The isolates with 100% homology value were K.S1.B2, K.S3.B24, K.S7.B61 and M.S7.B51, with sequence values of 1456, 1458, 1404 and 1451, which were identified as *Bacillus firmus* strain Xmb067, *Bacillus oceanisediminis* strain Xmb065, *Halomonas venusta* partial and *Bacillus pumilus* strain Lmb061, with the access numbers KT986192.1, KT986190.1, LN794812.1 and KT986126.1.

Discussion. The rep-PCR results in Figure 4 show that the band was formed continuously. The band pattern formed from each isolate looked different, so it can be assumed that the difference in the band pattern came from the different types of bacteria. Based on the cluster analysis and bioactivity test results, 10 isolates were selected as potentially antibacterial and have the activity to hydrolyze organic matter of fat, protein, ammonia and cellulose. The identification results of the 10 selected isolates showed that 6 isolates were identified as bacteria of the genus Bacillus, namely *Bacillus firmus* of the strain Xmb067, *Bacillus oceanisediminis* of the strain Xmb065, *Bacillus flexus* of the strain RP-UL, *Salibacillus* sp. of the strain NT N53, *Bacillus* sp. of the strain M-237-24 and *Bacillus pumilus* of the strain strain Lmb061. Two bioactive bacteria were identified in the genus Halomonas, namely *Halomonas venusta* with partial sequence and *Halomonas* sp. MD5, and *Brevundimonas* sp. N5. The results show that there were 2 different isolates: from coral ecosystems (K.S1.B2) and from mangrove ecosystems (M.S2.B12), which were identified in the same bacterial name, *Bacillus firmus* strain of the strain Xmb067. Examples of bacteria types which can be found in different hosts (Pringgenies et al 2021) and in mangrove sediments (Setyati et al 2019) are: *Bacillus oceanisediminis* isolated from the sea cucumber *Holothuria atra* (Jaeger, 1833) (Santosa et al 2020), *Bacillus aquimaris* and *Virgibacillus chiguensis* on *Holothuria atra* and *Holothuria leucospilota*. This condition occurred because the bacterium *Bacillus*

oceanisediminis is a bacterium commonly found in sediments (Lee et al 2012). The type of bacteria *B. oceanisediminis* was also found in isolates from coral ecosystems, namely the isolate K.S3.B24. *B. oceanisediminis* bacteria are known to have an antibacterial activity against the vibriosis bacteria and the *Staphylococcus aureus* MDR bacteria, and have proteolytic and enzyme activities. As found in symbiotic bacteria, *B. oceanisediminis* bacteria have antagonistic zone diameter or anti-bacterial potential against MDR *S. aureus* and *E. coli*. The results showed that the bacteria were gram-positive, rod-shaped and spore-forming. *Staphycococcus aureus* was found in sediment mangrove (Ariyanto 2019). GC-MS analysis of *B. oceanisediminis* bacteria found methyl hexadecanoate/methyl palmitate, bis(2-ethylhexyl)-1,2-benzene dicarboxylate, 9-octadesenal, glycerol-1,3-dihexadecanoate and diisooctyl-1,2-benzene dicarboxylate in bacteria from the stomach contents of sea cucumbers, all having an antibacterial potential (Santosa et al 2020).

Results of the study found that most of the symbiont sponge bacteria were identified from the genus Bacillus bacteria, which have potential as antibacterial, and are active in the production of extracellular enzymes. Results of this study were similar to (Raveendran et al 2018), who said that Bacillus bacteria is naturally found in various environments, including those that live freely or are pathogenic. Some Bacillus species produce extracellular enzymes such as protease, lipase, amylase and cellulase that play an important role in the digestive system of animals. Thus, the results of this study can be applied in the field of bioindustry. For example, *Bacillus firmus* is known as a probiotic useful for controlling *Aeromonas hydrophila* disease in African catfish, *Clarias gariepinus* (Lusiastuti et al 2014). Research studying the antibacterial potential and hydrolytic activities of Bacillus bacteria from the Dead Sea black mud found that there were 8 types of Bacillus which are halotolerant bacteria, based on their morphological, physiological and biochemical properties. These bacteria were *B. oceanisediminis, B. subtilis, B. firmus, B. paralicheniformis*, *B. methylotrophicus*, *B. amyloliquefaciens*, *B. sonorensis* and *B. malikii* (Maher 2017). Furthermore, Roy et al (2020) stated that *Bacillus flexus* bacteria utilize fructose, xylose, lactose, cellobiose, dextrose and mannose as carbon sources. Its starch hydrolysis properties can be explored in agricultural land: it has the potential to improve soil and plant health. *B. flexus* can hydrolyze lipids, gelatin and starch, which is very promising for applications in the bioindustry field.

Bacillus pumilus bacteria are also useful in the field of bioindustry: it can secrete cellulase enzymes which degrade cellulose into glucose or oligosaccharides (Ariffin et al 2006). Cow dung fermentation using *B. pumilus* increases the culture media nutrients. *B. pumilus* isolates were biochemically identical, exhibited protease and lipase activity and were uniformly sensitive to the antibiotics tested in the study (Parvathi et al 2009). The bacterium *B. pumilus* HR10 not only provides a reference for further research on the related action between biofilm formation and multiple biocontrol mechanisms of *B. pumilus* HR10, but also improves understanding of the regulatory pathways for biofilm formation by *B. pumilus* HR10. These observations not only provide a reference for further research about the coordinated action between biofilm formation and the multiple biocontrol mechanisms of *B. pumilus* HR10 but also improve the understanding of the regulatory pathway of biofilm formation by *B. pumilus* HR10 (Zhu et al 2020).

Brevundimonas vesicularis is a genus of non-fermenting Gram-negative bacteria. *Brevundimonas* sp. N5 was found to have an antimicrobial effect against Vibrio species and against the bacteria with MDR, as shown in the Table 2. Meanwhile, in the case of the marine bacterium *Halomonas venusta*, studies have demonstrated amazing potential for bioremediation, for biosynthesis of Se nanoparticles (SeNPs), as free radical scavenger, as an anti-biofilm, chemotherapeutic and larvicidal agent (Vaigankar et al 2020). Lee et al (2015) stated that *Halomonas* bacteria were Gram-negative, motile (through their lophotrichous flagella) and highly aerobic. Their colonies were cream, round and convex with all margins. *Halomonas* bacteria were susceptible to amikacin, lincomycin, nalidixic acid, kanamycin, gentamicin, neomycin, rifampin, polymyxin B, erythromycin, tetracycline, apramycin, sisomycin and chloramphenicol.

Conclusions. A number of 209 symbiotic sponge bacteria were isolated from coral, seagrass and mangrove ecosystems. Among the isolates, 9 bacteria had potential as anti-vibriosis and antibacterial against the bacteria with MDR, also having lipolytic, proteolytic, amylolytic and cellulolytic activities. Six types of bacteria were identified from the genus Bacillus, namely *Bacillus firmus* strain Xmb067, *Bacillus oceanisediminis* strain Xmb065, *Bacillus flexus* strain RP-UL, *Salibacillus* sp. NT N53, *Bacillus* sp. M-237-24 and *Bacillus pumilus* strain Lmb061. Two bioactive bacteria were identified in the genus Halomonas, namely *Halomonas venusta* partial and *Halomonas* sp. MD5 and one in the genus Brevundimonas, namely the *Brevundimonas* sp. N5. The types of bacteria found are promising for applications in the bioindustry field.

Conflict of interest. The authors declare that have no conflict of interest.

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Received: 30 June 2021. Accepted: 12 August 2021. Published online: 26 August 2021
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How to cite this article:
Zainuddin M., Pringgenies D., Radjasa O. K., 2021 Bioactive antibacterial compounds against bacterial pathogens and potential production of extra cellular enzymes from sponge associations in Karimunjawa Island, Indonesia. AACL Bioflux 14(4):2304-2316.