RESEARCH ARTICLE

Antipathogenic Activity of Acroporid Bacterial Symbionts Against Brown Band Disease-Associated Bacteria

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Abstract

The coral reefs' condition in most regions in Indonesia has been declining due to coral diseases, such as Brown Band Disease (BrBD). A treatment for BrBD involves the use of biological control agents that have antagonistic properties against disease-causing agents. This study aimed to isolate bacteria from healthy hard coral, those associated with BrBD, and those that had bioactivities against BrBD. Sampling and identification of corals and BrBD were carried out in March 2015 at the Marine National Park of Karimunjawa. Bacteria from healthy and infected corals were isolated and purified. The isolates were subjected to antipathogenic assay using overlay and agar diffusion methods. Finally, molecular identification of active bacteria was carried out using the 16S rRNA gene amplification. As many as 57 bacterial isolates were obtained from healthy coral, as well as four bacterial isolates from coral with BrBD symptoms. A total of 15 bacterial isolates (26%) showed antipathogenic activity against BrBD-associated bacteria. Three isolates with the strongest antipathogenic activities, i.e., GAMSH 3, KASH 6, and TAPSH 1 were identified by 16S rRNA gene sequences. The results showed that they were aligned to *Virgibacillus marismortui* (97%), *Oceanobacillus iheyensis* (97%), and *Bacillus cereus* (96%), respectively.

Keywords: antibacterial, coral disease, holobiont, pathogen, molecular identification

Introduction

A disease occurred by the interaction among a host, pathogen (disease), and the environment. Infectious biotic diseases are caused by a microbial agent (bacterium, fungus, virus, or protist) that can be spread between host organisms. Consequently, it has a negative impact on the host's health (Raymundo et al., 2008). Coral is a holobiont composed of coral microbiome animals, including zooxanthellae, bacteria, fungi and archaea (Bang et al., 2018; Bosch & McFall-Ngai, 2011). Environmental changes, global warming, and water quality greatly affect the composition of the microbiome in the corals (Maynard et al., 2015; Ziegler, Seneca, Yum, Palumbi, & Voolstra, 2017). This condition triggers pathogens to multiply and cause coral disease. Research from Sweet et al. (2019) reported that disease triggers chaotic dynamics in microbial communities and increases biodiversity. There was a significantly higher community (compositional homogeneity) in the pathobiome of diseased corals compared to that of the microbiome associated with healthy tissue. This composition illustrates a strong competition between the pathogenic community and those associated with the 'healthy' coral holobiont. Some bacteria such as, such as *Aurantimonas coralicida, Vibrio carchariae, Serratia marcescens, Aspergillus sydowii* as well as *V. corallilyticus,* and *V. shiloi* act as coral pathogenic agents. These bacteria cause coral diseases, i.e., White Plague II; White Band II; White pox; Aspergilosis and bacterial bleaching, respectively (Raymundo et al., 2008).

The coral disease significantly affects the changes in reproduction rates, growth rates, community structure, species diversity, and abundance of reef-associated organisms (Hobbs, Frisch, Newman, & Wakefield, 2015; Walton, Hayes, & Gilliam, 2018).

Infectious coral diseases had increased in frequency and distribution since the 1970's when a white band disease outbreak massively damaged Caribbean acroporids (Dustan, 1977). Since that time, there has been exponential increased number of reported disease, host species and locations with disease observations. This rate of change is abnormal and has resulted in a significant loss of coral cover. Various studies have reported coral disease outbreaks, such as in the Great Barrier Reef in 2003 (Willis, Page, & Dinsdale, 2004) and Florida in 2014-2015 (Aeby et al., 2019). Coral diseases also occur in Indonesia such as in Karimunjawa (Wijayanti et al., 2020), Wakatobi National Park (Haapkylä, Seymour, Trebilco, & Smith, 2007), the Spermonde and Wakatobi Islands of Sulawesi (Muller et al., 2012). Moreover, they are also found in Seribu Islands (Johan, Zamany, Smith, & Sweet, 2016), Bangka Island of Sulawesi (Ponti et al., 2016), Panjang Island of Central Java (Sabdono, Sawonua, Kartika, Amelia, & Radjasa, 2015), and Raja Ampat of Papua (Subhan et al., 2020). There are several coral diseases known to date, i.e., pigmented band diseases, black band disease, skeletal eroding band, and brown band disease (BrBD).

BrBD is characterized by the varied size of a brown band between healthy tissue and exposed white skeleton on branching Acropora (Raymundo et al., 2008; Sweet & Bythell, 2012). BrBD was first discovered in 2003 in the Great Barrier Reef (Willis et al., 2004). Loss of coral tissue caused by BrBD in Mansuar Island, Indonesia, has been found in Porites, Fungia, Pachyseris, etc. (Subhan et al., 2020). BrBD also infected the branching of *Acropora* sp. in Spermonde and Wakatobi, Sulawesi (Muller et al., 2012) and Karimunjawa National Park Island (Sabdono, Wijayanti, & Sarjito, 2017). BrBD is caused by a bacterial pathogen that attacks the necrotic tissue and allows for secondary infection by ciliates (Boyett, 2006; Sweet & Bythell, 2012). *Acinetobacter* sp. RA3849 ORF, *Streptococcus* sp. YM395, *Enterobacter faecalis* C56, and *V. alginolyticus* H2X5 have been reported as the causal agents of BrBD infection (Rahmi, Jompa, & Tahir, 2019). At Davies Reef, the level of tissue loss due to BrBD ranges from 0.3 to 9 cm/day with rapid transmission (Nash, 2003; Willis et al., 2004).

There have been no satisfactory results on the prevention and treatments of coral diseases (Efrony, Loya, Bacharach, & Rosenberg, 2007; Pollock, Morris, Willis, & Bourne, 2011). Aeby et al. (2019) reported the use of antibiotics to treat different types of tissue loss lesions in *Montastraea cavernosa*. The use of antibiotics to cure coral disease may cause pathobiome resistance in the future. Also, they potentially increase the antimicrobial resistance (AMR) in the aquatic environment. Therefore, studies are needed regarding alternative treatments of coral disease using biological control agents. Biological control agents are disease control management strategies against the causative disease agent using micro or macro-organisms with antagonistic properties. Their working system against coral disease employs the production of antimicrobial compounds to inhibit the growth or kill the pathogens (Sabdono, Trianto, Radjasa, & Wijayanti, 2019; Teplitski & Ritchie, 2009; Wijayanti, Sabdono, Widyananto, Dirgantara, & Hidaka, 2018). This study aimed to explore alternative treatments of BrBD-associated bacteria using bacteria from healthy coral.

Material and Methods

Study Area and Specimen Collection

The study was conducted at the Marine National Park of Karimunjawa, Jepara, Central Java, Indonesia in March 2015. The specimens collection was conducted after receiving legal permission from the conservation authority. The three hard coral specimens were collected near Kemujan Island, Tengah Island, and Sambangan Island, as shown in Figure 1. We took samples of healthy and BrBD-symptomatic hard coral specimens from the *Acroporidae* family. All specimens were collected from 1-5 m depth by skin diving or scuba diving. Some *Acropora* specimens were separated from the colony with a hammer. The samples were then placed in plastic zip-lock bags with seawater from the sampling sites. The plastic bags were kept in a cool box during transportation to the laboratory of Marine Biotechnology (TMB) at Diponegoro University, Semarang.

Coral Sampling

Hard coral specimens were documented down to the genus level, according to Veron (2000). To identify hard coral with BrBD infections, we used the Handbook of Underwater Cards for Assessing Coral Health on Indo-Pacific Reefs (Beeden, Willis, Raymundo, Page, & Weil, 2008). *Acropora* sp. with a BrBD infection is characterized by a brown circular band on their branches. This band separates the dead tissue from the healthy one (Figure 2).

Isolation of Bacteria from Coral Samples and Antipathogenic Assay

Healthy (Figure 3) and BrBD-infected coral samples (Figure 2) were rinsed with sterilized seawater to clean their surfaces from dirt. Each tissue sample was then scraped off with a sterile knife and crushed with a sterile mortar and pestle. Approximately 1 g of each tissue paste was prepared in sterile seawater with a

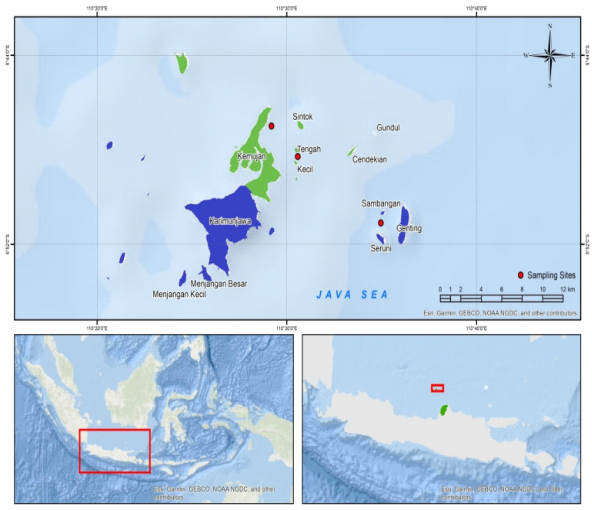

Figure 1. The sampling sites of healthy and BrBD-symptomatic Acroporidae hard coral. Site 1 (05p 48' 654'' S, 110p 30' 483'' E), site 2 (05p 50' 643'' S, 110p 35' 815'' E), and site 3 (05p 48' 779'' S, 110p 29' 955'' E), at the Marine National Park of Karimunjawa, Java Sea, Central Java, Indonesia

Figure 2. *Acropora* sp. infected by BrBD (see black circle) in Karimunjawa Island, Java Sea, Central Java, Indonesia as the source of pathogenic bacteria

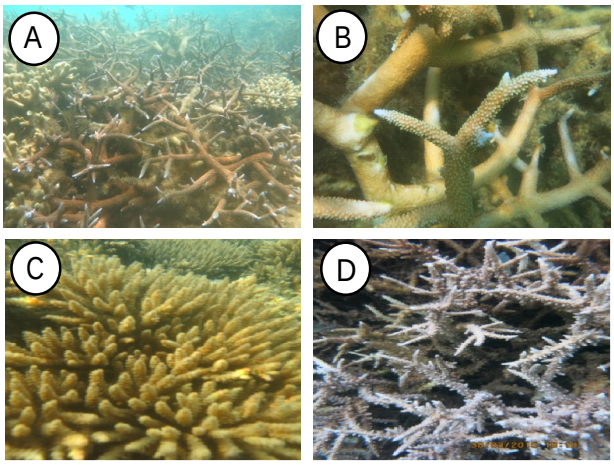

Figure 3. Healthy *Acropora* spp. corals (A-D) collected from Karimunjawa Island, Java Sea, Central Java, Indonesia as the source of antipathogenic bacteria

serial dilution. Each solution was then spread and cultivated on a ZoBell 2216E agar medium.

The antipathogenic assay of bacterial isolates from healthy hard coral against BrBD-associated bacteria was performed using an overlay method. The overlay method of healthy coral bacterial symbionts being grown in the ZoBell 2216E agar medium and incubated at 37 °C for 48 h as dotting plates. The isolated BrBD-infected hard corals bacteria were cultured for 48 h on the ZoBell 2216E and incubated in an orbital shaker at 120 rpm. About 1% (v/v) of the liquid culture was subsequently suspended in the ZoBell 2216E soft-medium agar (1.5% agar/1L) and poured onto the dotting plates. The plate was then re-incubated for 48 h at 37 °C Antagonistic interaction between the healthy and infected bacteria was indicated by the formation of a clear zone around the dotting. The clear zone that appeared was measured with a veneer caliper and recorded in millimeters in triplicates.

The dotting bacterial isolates with antipathogenic activity were confirmed with an agar disk diffusion method modified from Sabdono, Sawonua, Kartika, Amelia, and Radjasa (2015). With this method, the BrBD-associated bacterial isolates were grown and shook as with the previously described overlay method. Next, 30 µl of *Acroporid* bacterial symbionts that showed antipathogenic activity was placed onto sterile paper disks (d: 8 mm, from Advantec Toyo Roshi, Ltd, Japan). The disks were then placed into the plates containing BrBD-associated pathogenic bacteria and incubated at 37 °C for 48 h. The diameter of the clear zone (inhibition zone) surrounding the paper disk was measured in millimeters. The inhibition zone indicated the antagonistic interaction between the healthy and BrBD-associated pathogenic bacteria.

Biochemical Characterizations of Antipathogenic Bacterial

Bacterial isolates from the second antipathogenic assay that retained activity against BrBD-associated bacteria were identified according to their biochemical characteristics. Bacterial colony characteristics were then compared to those described in the references of the identification of bacteria (Breed, Murray, & Smith, 1957; Cowan & Steel's, 1993). Gram staining was conducted to observe the gram-positive and gram-negative of group bacteria. Biochemical characterizations were identified with tests on oxidation, catalase, motility, indole, methyl red, VP assay, and more. To support the identification of bacteria up to the species level, the molecular method was employed.

Bacterial Identification by 16S rRNA Gene Amplification and Phylogenetic Analysis

DNA Extraction

The genomic DNA of active bacterial isolates was extracted using a Chelex DNA Extraction method 100 (Lamballerie, Zandotti, Vignoli, Bollet, & Micco, 1992; Walsh et al., 2013). The obtained DNA samples were then measured and qualified with a NanoDrop 2000 spectrophotometer (Thermo Scientific). Ratios of 260/280 nm and 260/230 nm were used to observe DNA purity and concentration between ranges 1.8 and 2.0 (Susilowati, Sabdono, & Widowati, 2015).

PCR Amplification and Sequencing of 16S rRNA Gene Fragments

The DNA was amplified for the 16S rRNA gene by means of polymerase chain reaction (PCR), with the following universal primer: 27F (5'AGAGTTT GAT CMTGGCTCAG-3') and the following specific eubacteria primer: 1492 R (5'TACGGT TAACC TTG TTACGACTT-3') (Long & Azam, 2001; Rohwer, Seguritan, Azam, & Knowlton, 2002). The 50 µL PCR reaction consisted of 2.0 µL of each primer, 18.5 µL of ddH_2O, 2.5 µL of DNA template, and 25 µL of KAPATaq Extra. The PCR amplification process on 16S rRNA genes was carried out with an MJ Mini *Personal Thermal Cycler* (BIO-RAD). The PCR conditions were as follow: initial temperature at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 1 min, then annealing at 55 °C for 1 min, followed by extension at 72 °C for 1 min, and final extension at 72 °C for 7 min after which it was stored at 4 °C . The PCR products were visualized with 1% agarose gel electrophoresis, and the result was observed with a UVIDoc HD5 (UVITEC Cambridge). PCR products with an estimated ~1,500 bp were sequenced at 1st Base Malaysia. These sequencing results were then compared to other sequences from the GenBank database at the National Center for Biotechnology Information (NCBI), National Institute for Health (NIH), USA (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997). BLAST was used to identify similarity levels with other bacteria (Sabdono et al., 2015).

Phylogenetic Analysis

Phylogenetic analysis of active bacterial isolates was constructed by MEGA X software. The Clustal W and a neighbor-joining tree with a bootstrap method based on 1,000 replications were performed (Kumar, Nei, Dudley, & Tamura, 2018). The genetic codes of the 16S rRNA gene sequencing have been submitted to GenBank DDBJ (DNA Data Bank of Japan). The accession numbers are as follow: LC099529 (GAMSH 3), LC099530 (KASH 6), and LC099531 (TAPSH 1) (Table 3).

Results and Discussion

Hard Coral Identification

The coral sampling at the three sites in Karimunjawa Island provided several healthy and BrBD-infected coral samples identified as *Acropora* sp. (Figure 3). The *Acroporidae* family has four genera, namely *Acropora, Montipora, Anacropora,* and *Astreopora*. The first three genera have similar small corallite characteristics, such as an absent columella, simple septa, an aspecific structure, and extra-tentacular formed corallites. Conversely, the corallites of the genus *Astreopora* are more extensive, and the septa are well developed with a simple column (Suharsono, 2008; Johnson et al., 2011).

Isolation and Antipathogenic Activity of Bacterial Against BrBD Coral Bacteria

A total of 57 bacterial isolates were successfully isolated from healthy *Acroporid* corals and four others from *Acropora* spp. with BrBD infection. These four bacterial isolates were further named BrBD-associated bacteria (BrBD 1, BrBD 2, BrBD 3 and BrBD 4). Antipathogenic activity against four BrBD-associated bacteria was observed in 15 (26%) of the 57 isolates (Figure 4). After further confirmation test, the number of active isolates was decreased. Presumably, during the agar disk diffusion test, healthy coral-associated bacteria first diffused with the paper disk, resulting in decreased BrBD-associated bacteria inhibition. Only bacteria with high antipathogenic activity were consistent in inhibiting the growth of BrBD-associated bacteria. Previous results have also reported the decrease in antipathogenic activity from the overlay test (23 isolates) to agar diffusion (10 isolates) (Sabdono et al., 2015). Moreover, Ayuningrum et al. (2019) also reported half of the tunicate-associated bacteria had an antibacterial activity that can decrease harmful pathogens from screening. The three isolates with the highest antipathogenic activity were GAMSH 3, KASH 6, and TAPSH 1 (see Table 1 and Figure 5).

The agar disk diffusion activities from bacterial isolates GAMSH3, KASH 6 and TAPSH 1 against BrBD-associated bacteria are shown in Figure 5.

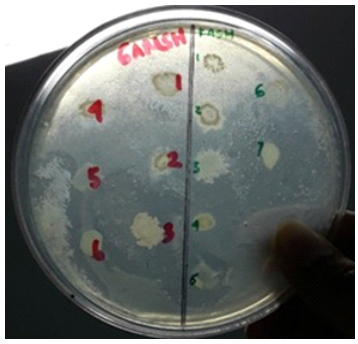

Figure 4. The screening result of healthy bacterial isolates (GAMSH 1-6 and KASH 1-7) against pathogenic bacteria using the overlay method showed that isolates GAMSH 6 and KASH 6 had the strongest antipathogenic activity.

It is suspected that not all bacteria associated with healthy hard coral produce secondary metabolites antagonistic properties against BrBD-associated bacteria. Results from Kvennerfors et al. (2012) show that about 25% to 70% of bacterial associations cultured from coral mucus have antibacterial activity. The ability of antipathogenic bacteria to inhibit BrBD-associated bacteria might be influenced by the spectrum of antibacterial compounds present. According to Volk and Wheeler (1993), antibacterial compounds can be classified into two classes based on their ability to kill bacteria. They are known as "broad-spectrum antibacterial" (kill Gram-positive or Gram-negative bacteria) and "narrow-spectrum antibacterial" (kill Gram-positive bacteria or Gram-negative bacteria only). The inhibition zone sizes, as shown in Table 1, were also influenced by the concentration of the compound produced by active bacteria. The inhibition amount is linked to the concentration of antimicrobial compounds, number and types of microbes, temperature, time, pH, and organic substances (Lorian, 2005).

The inhibitory activity of bacterial isolates against BrBD-associated bacteria is characterized by the formation of a clear zone around the paper disk. This clear zone has resulted from the antagonistic activity between the healthy hard coral symbiont bacteria and the BrBD-associated bacteria. During this antagonistic activity, secondary metabolite compounds derived from coral symbionts are produced as a result of the competition of space and nutrition. Secondary metabolites are produced by organisms in response to their environment. They play an important role in the process of attachment and colonization of targets (Long & Azam, 2001; Romanengko et al., 2008). Some hard coral symbiont bacteria showed antagonistic ability against Black Band Disease (Sabdono & Radjasa, 2006; Sabdono et al., 2017; Wijayanti et al., 2018) and White Pox Disease (Ritchie, 2006). Other hard coral bacteria also inhibit coral diseases, such as White Plague, Yellow Band, Pink Line Syndrome, and Yellow Blotch (Sabdono et al., 2015). Our recent study reported that secondary metabolites from bacteria-associated with healthy hard coral can also be used against the human pathogen (Ayuningrum, Muchlissin, Sabdono, Trianto, & Radjasa, 2020).

This study showed that bacterial isolates GAMSH 3, KASH 6, and TAPSH 1 were bacterial isolates in symbiosis with *Acropora* sp. Another research also reported that Acroporid bacterial isolates have antipathogenic activity against the coral bacterial pathogen (Willis et al., 2004). Furthermore, Acroporid corals from Karimunjawa, Indonesia was the source of antipathogenic bacteria against Black Band Disease (Wijayanti et al., 2018) and White Patch Disease (Wijayanti et al., 2020).

Table 1. Selected isolates of antipathogenic assay from the overlay and agar disk diffusion method

No	Isolate code	Overlay result	Agar disk diffusion (mm)			
			BrBD 1	BrBD 2	BrBD 3	BrBD 4
1	GAMSH 3	++++	4.12±0.46	4.38±0.15	5.58±0.53	8.12±0.31
2	KASH 6	++++	4.60±0.42	3.85±0.19	6.90±0.40	5.38±0.46
3	TAPSH 1	+++	4.35±0.59	4.05±0.13	-	7.35±0.45

Note: - : no activity on BrBD isolate; +++ : active on three BrBD isolates; ++++ : active on four BrBD isolates.

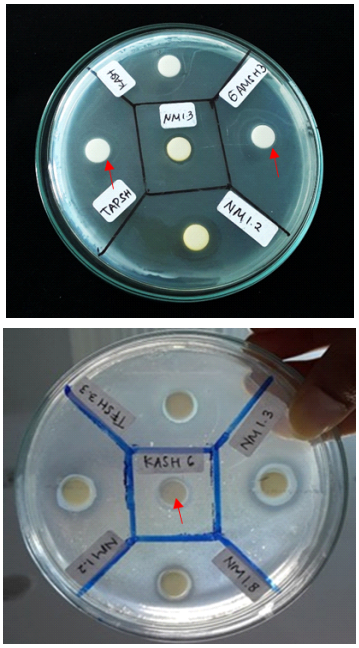

Figure 5. The agar disk diffusion result of bacterial isolates GAMSH 3, KASH 6, and TAPSH 1 against BrBD-associated bacteria. The clear zone was indicated by the red arrow.

Table 2. Biochemical characterization of antipathogenic BrBD coral bacteria

Characteristics	Antipathogenic of BrBD coral bacteria		
	GAMSH 3	KASH 6	TAPSH 1
Gram	+	+	+
Motility	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
Glucose	+	+	+
TSIA	+	+	+
Ornithine	+	+	+
Indole	-	-	-
Urease	-	-	-
Simmon's Citrate	-	-	-
MR Test	-	-	-
VR Reaction	-	-	-
Gelatine	+	+	+
Arabinose	-	-	-
Lactose	-	-	-
Mannitol	-	-	-
Maltose	-	-	-
Sorbitol	-	-	-
Mannose	-	-	-
Xylose	-	-	-
Sucrose	-	-	-

Note: + : positive reaction; - : negative reaction

Biochemical Characteristics of Antipathogenic of BrBD Coral Bacteria

Gram staining results showed that isolates GAMSH 3, KASH 6, and TAPSH 1 with the highest antipathogenic activities were Gram-positive. Based on biochemical characterization, all bacterial isolates were motile and reacted positively to catalase and oxidase. They were able to produce ornithine enzymes but were unable to produce citrate and urease enzymes. All isolates reacted negatively in the sugars test but produced fermented glucose. The biochemical characterization results can be seen in Table 2.

Molecular Identification of Antipathogenic Activity of Bacterial Against BrBD Coral Bacteria

Isolates GAMSH 3, KASH 6, and TAPSH 1 were identified using a molecular method. The molecular identification was conducted by comparing the genetic features of the isolates to those of similar bacteria. As shown in Figure 6, the three isolates have PCR products with approximately 1,500 bp in length. Further sequencing analysis indicated that GAMSH 3, KASH 6, and TAPSH 1 have 1,471 bp, 1,461 bp, and 1,455 bp in length, respectively (Table 3).

The result of BLAST homology was different from the biochemical test done previously. The biochemical identification showed that all three bacterial isolates belonged to the same genus. It is indicated that another biochemical identification specific to species level was needed to assign the isolates. Further 16S rRNA sequence analysis showed that the three bacteria were from different genera: *Virgibacillus*, *Oceanobacillus* and *Bacillus*. These genera are in the same family level Bacillaceae. Croci et al. (2007) and Moraes, Perin, Silva Júnior, and Nero (2013) stated that the molecular method is more accurate and reliable than biochemical identification. Comparison of the 16S rRNA sequence and the phylogenetic tree (Figure 5) with those in the GenBank resulting close similarity to three genera. The result showed that GAMSH 3 was closely related to *Virgibacillus marismortui* with a homology of 97%. KASH 6 was similar to *Oceanobacillus iheyensis* from deep-sea sediments (Lu, Nogi, & Takami, 2001; Takami, Takaki, & Uchiyama), whereas TAPSH 1 was closely related to *Bacillus cereus* with 96% homology.

Previous studies have reported the bioactivities of *V. marismortui, O. iheyensis* and *B. cereus.* Sulistiyani, Nugraheni, Khoeri, Sabdono, and Radjasa (2010) showed that *V. marismortui* bacteria associated with soft coral *Sinularia* sp. were active against MDR bacteria *Staphylococcus aureus*. *V. marismortui* isolated from salt pans in Cavellosim, India, by Kamat, Kiran, and Kerkar (2011) also inhibited *S. aureus*. Additionally, *V. marismortui* was found to have antipathogenic potency against Black Band Disease in Karimunjawa (Wijayanti et al., 2018). *Oceanobacillus iheyensis* is a gram-positive, aerobic, rod-shaped, motile bacterium with peritrichous flagella that can form spores (Lu et al.,2001; Takami et al., 2002). *Oceanobacillus iheyensis* has been reported to inhibit the growth of *E. coli, K. pneumoniae, S. typhi, S. aureus, P. aeruginosa, P.*

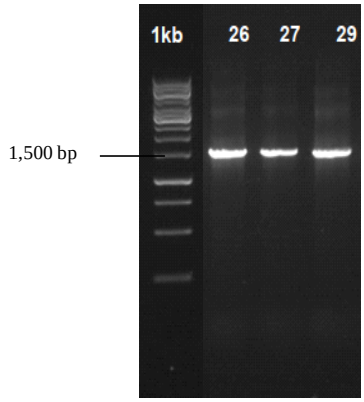

Figure 6. Evaluation of 16S rRNA gene amplification using electrophoresis gel of antipathogenic BrBD coral isolates 26 (GAMSH 3), isolates 27 (KASH 6), isolates 29 (TAPSH 1); 1 kb: DNA markers.

Table 3. BLAST homology analysis of antipathogenic bacterial isolates against BrBD-associated bacteria

Isolate code	Length of nucleotide (bp)	Accession number	Closest relatives	Homology	BLAST
GAMSH 3	1,471	LC099529	*Virgibacillus marismortui*	97%	NR_028873.1
KASH 6	1,461	LC099530	*Oceanobacillus iheyensis*	97%	NR_075027.1
TAPSH 1	1,455	LC099531	*Bacillus cereus*	96%	NR_115714.1

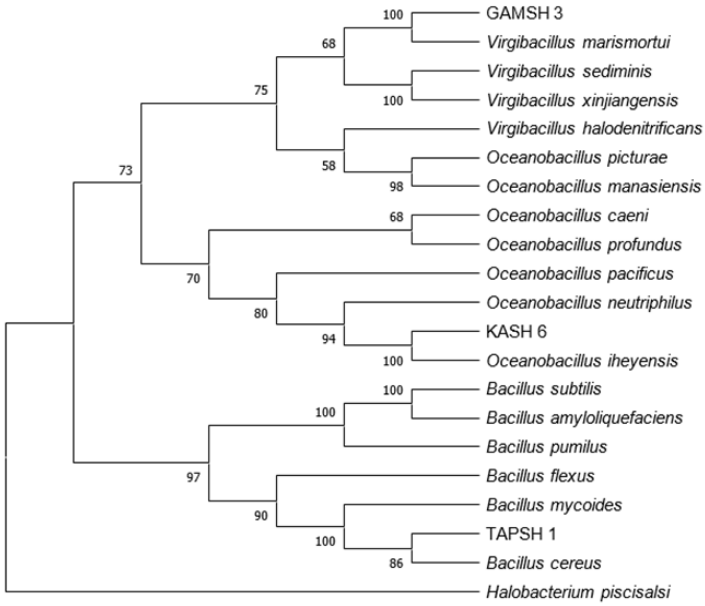

Figure 7. Phylogenetic tree based on 16S rRNA gene sequence analysis of antipathogenic bacterial isolates from BrBD coral (GAMSH 3, KASH 6, and TAPSH 1), using the Neighbour Joining Approach. *Halobacterium piscisalsi* was used as an outgroup.

vulgaris and *E. aerogenes* (Tambekar & Dhundale, 2013). According to Anand et al. (2006), *Bacillus* sp. is a gram-positive, rod-shaped, sporulating, and motile bacteria. A similar study showed that *Bacillus* sp. associated with corals produced secondary metabolites that can inhibit BrBD (Sabdono & Radjasa, 2006). *Bacillus* from sponges also inhibited the growth of *S. aureus, V. harveyii, E. coli, P. aeruginosa*, EPEC K-11, *Candida albicans,* and *C. tropicalis* (Abubakar, Wahyudi, & Yuhana, 2011).

Conclusion

A total of 57 bacterial isolates were successfully isolated from healthy Acroporid corals and four others from *Acropora* spp. with BrBD infection. Bacterial isolates with the highest antipathogenic activity against bacteria isolated from the BrBD-infected hard corals were GAMSH 3, KASH 6, and TAPSH 1. Further analysis indicated that these bacteria belonged to the Gram-positive bacteria. According to the 16S rRNA gene BLAST, these bacteria are closely related to *V. marismortui, O. iheyensis,* and *B. cereus,* respectively. Further research is needed to test the capability of these potential strains to combat the BrBD-causing agent on a field scale.

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