ISOLATION AND IDENTIFICATION OF BACTERIAL COMMUNITIES FROM CORAL TISSUE AFFECTED BY BLACK BAND DISEASE AT PULAU REDANG, TERENGGANU, MALAYSIA

JAAFAR, S. N. T.[1,2,3*] – SUHAIMI, M. N. A.[1] – MAZNAN, N. A.[1] – SAFUAN, C. D. M.[2] – TAN, C. H.[1,2,3] – YUCHAROEN, M.[4] – SAIDIN, J.[5] – BACHOK, Z.[2]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[2]*Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[3]*Research and Education on Environment for Future Sustainability (REEFS) Research Interest Group (RIG), Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[4]*Marine and Coastal Resources Institute, Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Thailand*

[5]*Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[*]*Corresponding author*
e-mail: tahirah@umt.edu.my

(Received 11th Dec 2022; accepted 10th Mar 2023)

Abstract. Coral reefs in Southeast Asia are becoming progressively degraded due to the natural and anthropogenic disturbances which lead to poor water quality threatening the reefs' health. Coral disease is one of the threats to the coral reefs worldwide but research on coral disease in Southeast Asia is relatively scarce especially on bacterial community associated with the disease. The destructive coral black band disease (BBD) can be found widely in the southern region of the South China Sea, Malaysia. This study aims to compare the microbial communities of healthy and BBD-infected coral tissue from two different coral species *Montipora turtlensis* and *Acropora spicifera* collected at the Shark Point of Pulau Redang, Terengganu. The bacterial isolated from corals nubbins were examined by combining culture-dependent method and bacterial sequencing of 16S rDNA. A total of 18 bacteria isolates were identified from both species of coral tissue samples prior to sub-culturing colonies samples. The sequences detected were derived from a wide taxonomic range, including representatives of Actinobacteria, Furmicutes and Proteobacteria phyla. Bacteria belonging to the phyla Proteobacteria were further divided into alpha and gammaproteobacteria. Most of the bacteria identified can be found on healthy and BBD-affected coral tissue samples; however, there are few bacteria which can be only found on BBD-affected tissue of respective coral species such as *Lelliottia* sp., *Salnicola salarius* and *Microbacterium petrolearium* for *M. turtlensis* while *Pseudomonas stutzeri* and *Erythrobacter* sp. for *A. spicifera.*
Keywords: *coral disease, pathogenic bacteria, Montipora turtlensis, Acropora spicifera, coral reefs, Southeast Asia*

Introduction

Over the last thirty years, scale deterioration of environmental situations has become progressively worse on both local and a global scale, affecting the status of coral health and hence compromising the ecosystem of coral reef entirely (Lesser et al., 2009). The

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

springing up of coral diseases issue has become one of the most significant causes of the dramatic loss of reef's global decline. Over 20 recorded diseases were known affecting the coral and three of them such as white band disease (WBD), white plague disease (WPD) and Black band disease (BBD) was reported more than two thirds of occurrence at the Caribbean (Weil et al., 2006). Bacteria communites that affecting the coral reefs are quite comman. For instance, some of bacteria were isolated from putative WBD have been identified as Vibrio and Rickettsia (Gignoux-Wolfsohn and Vollmer, 2015). The other bacteria identifiend from WPD such as from the families of Alteromonadaceae, Rhodobacteraceae, Vibrionaceae are known as coral pathogens (Roder et al., 2014).

According to some early studies regarding the disease on corals, BBD was the earliest been reported to affect corals as reported by Antonius (1985) on the reef of Florida Keys as well as in Belize. The occurance of the disease was futher reported by other researchers such as Garret and Ducklow (1975), Rutzler et al. (1983) and Edmund (1991). Since then, until recently, many researchers taking part in conducting study on BBD regionally including in Sabah Marine Park (Miller et al., 2015), and Tioman Island Marine Park (Akmal and Shahbudin, 2020) as well as in global scale such as in Hawaiian Archipelago, Red Sea, Maldivian Archipelago and Seribu Archipelago Indonesia (Montano et al., 2013; Aeby et al., 2015; Johan et al., 2015; Hadaidi and Voolstra, 2020).

Other than symbiotic algae zooxanthellae, there are abundant and diverse bacterial communities which incredibly well associate on corals. These corals rely on bacteria as particularly in nitrogen and carbon cycle, dissolved organic matter and immunity (Borneman, 1998). A bacterium plays a vital role against competitors or predators. Coral tissue was thought to serve as first-line defense in counter with any foreign organisms from trying to invade into it. It will be difficult task to puzzle out the nature of these associations; therefore, numerous previous studies on bacterial community associated with BBD are documented. For example, presence of boring cyanobacteria and novel bacteria on infected coral in Florida, USA (Miller et al., 2011), changes in bacterial community associated with BBD in coral *Favia* sp. and microbial community characterization of BBD microbial mat in nine coral genera in Red Sea (Arotsker et al., 2015; Hadaidi et al., 2018), classification of BBD cyanobacteria associated with Okinawan BBD, Japan (Hutabarat et al., 2018) and identification of main pathogenic bacteria stimulating BBD in hard coral *Pachyseris speciosa* from island around south Sulawesi, Indonesia (Jompa et al., 2020). However, study conducted on the bacterial communities of diseased and healthy corals are very few especially in Malaysia (Kalimutho et al., 2007).

To date, assessment on coral disease have gained interest in Malaysia but the studies (e.g., Miller et al., 2015; Browne et al., 2019; Akmal and Shahbudin, 2020) only limited to the quantitative distribution of hard coral affected by the disease. To further understand the bacteria communities that associated with the coral disease, this study aims to isolate and identify bacterial communities for healthy and BBD-infected coral tissue. Isolation and identification of microbial communities allied with BBD is essential to determine as it will give access to future researchers on the potential bacteria associated with the coral disease. Additionally, we also compared the bacteria species presence on healthy and BBD-infected tissue from coral samples.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

Materials and methods

Coral sampling collection

The coral specimens (*Montipora turletensis* and *Acropora spicifera*) used for this study were collected from Pulau Redang, Terengganu (*Fig. 1*) which are located in the east coast of Peninsular Malaysia. Shark Point (05˚46'43" N, 103˚0" E) has been selected as study area and monitoring have been conducted for four consecutive months started in July until October 2019, in order to observe the disease progression on infected coral colonies.

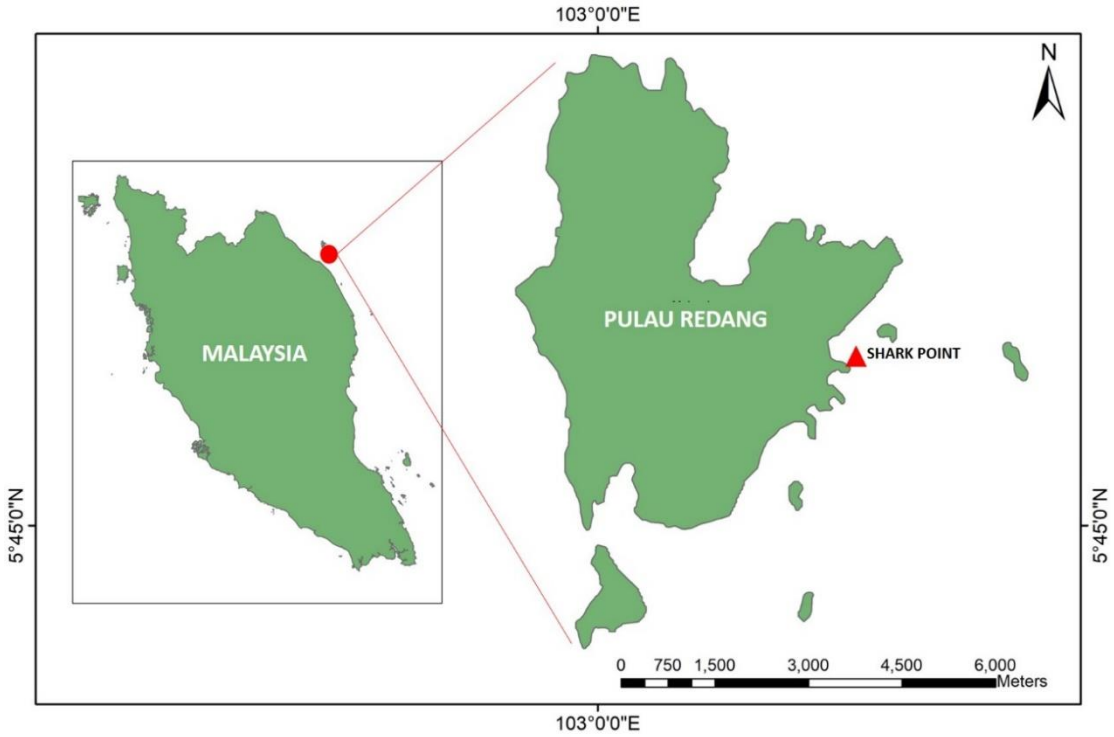

Figure 1. *The map of Pulau Redang, Terengganu, Malaysia. Red triangle indicate Shark Point where the study of corals infected with black band disease took place*

Coral specimens were collected in monthly between 1 m to 3 m depth at Shark Point, Pulau Redang from July to October 2019. The coral species *Montipora turtlensis* and *Acropora spicifera* were identified according to their corallite's characteristics (Veron, 2000). Six coral colonies; three each from healthy and BBD infected of the two identified coral species (*Fig. 2*) were selected and tagged with plastic tag for the repetitive sampling. During each sampling, one coral fragment with a size approximately of 1.5 cm x 1.5 cm was collected using a chisel and hammer from each tagged coral colony. The corals tissue layers have then been scraped from the healthy and diseased area using sterile dissecting knife and the tissues were kept separately in 1 ml labelled Eppendorf tubes. The samples then stored in ice chest and transporting back to the laboratory immediately after sampling.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

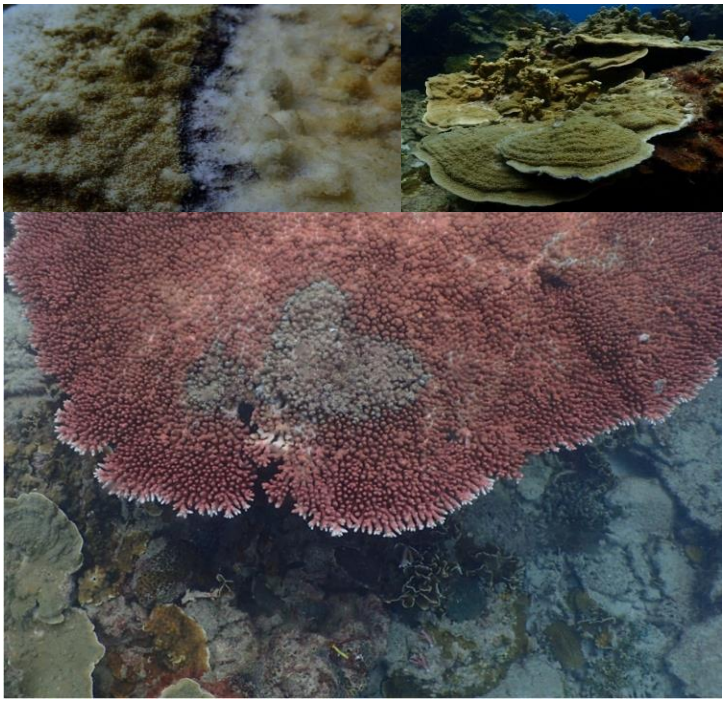

Figure 2. *Photos of the targeted coral colonies. Photo of BBD distinguished between healthy, affected and death area on Montipora turtlensis (top left). A healthy colony of M. turtlensis without infected by BBD (top right). Lesion progression; a black band on an Acropora specifiera spreading out from the centre of the colony (below)*

Bacteria isolation and purification

Ten-fold serial dilution was performed on each sample from three different coral colonies before being poured onto marine agar. All plates then were placed in the incubator at 28°C for seven days with daily observation on colonies presence. Each bacterial colony was morphologically characterized by shape, margin, elevation, size, texture, appearance, pigmentation and optical property. After seven days incubation period, colonies' displaying different morphologies was systematically purified and preserved in 40% glycerol at -80°C (Cárdenas et al., 2012).

Extraction of DNA and 16s rRNA gene amplification

Colonies possess different characteristics morphologically, varies between the abundance of affected or healthy coral tissue, absent in healthy condition but present in the infected tissue or the other way round, were selected for further analysis to identify the bacterial species by sequencing full length 16S rRNA gene. The extractions of DNA on isolated bacteria took place by boiling the colony in 100 µl of sterile water for 10 minutes at 94°C then immediately cool the sample in ice for 5 minutes (Lane, 1991). The supernatant then served as a template to amplify the 16S rDNA using universal primers 27F(5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R(5′-GGTTACCTTGTTAC-GACTT-3′) in a final volume of 25 µl containing 5 m application buffer, 0.1 U of Platinum Pfx Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.4 µ of each primer and 0.6 m $MgSO_4$, 0.4 m deoxyribonucleotide triphosphates. Amplification process was carried out at an initial cycle at 94°C for 5 minutes, after that 1 minute of 30

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

cycles at 94°C, 1 minute at 52°C and 72°C, with a last extension step at 72°C for 7 minutes. The PCR product then outsourced to 1st Base DNA Sequencing Division, Selangor, Malaysia,company and MEGA-X software was used for editing the chromatogram. Lastly, sequence which match the values of 97.5-100% in EZ Bio Cloud 16S Database were used to assign species (Stackebrandt and Goebel, 1994) and establishing Venn diagram for bacteria species distribution present on healthy, affected or both on coral tissue.

Results

Disease progression on affected coral colonies

Observation on coral colonies infected by BBD shows dark, distinctive band which move across the surface of the coral, separating live tissue and exposed skeleton and spread out over short time leaving behind coral skeleton covered with algal. *Fig. 3* shows BBD progression on affected coral colonies were captured during the sampling time. During the active phase of lesion progression, the black band manifest on coral showing the microbial assemblage adjacent to healthy tissue, penetrating and covering both intact and death coral tissues in the infected area thus showed BBD lesion external macroscopic sign.

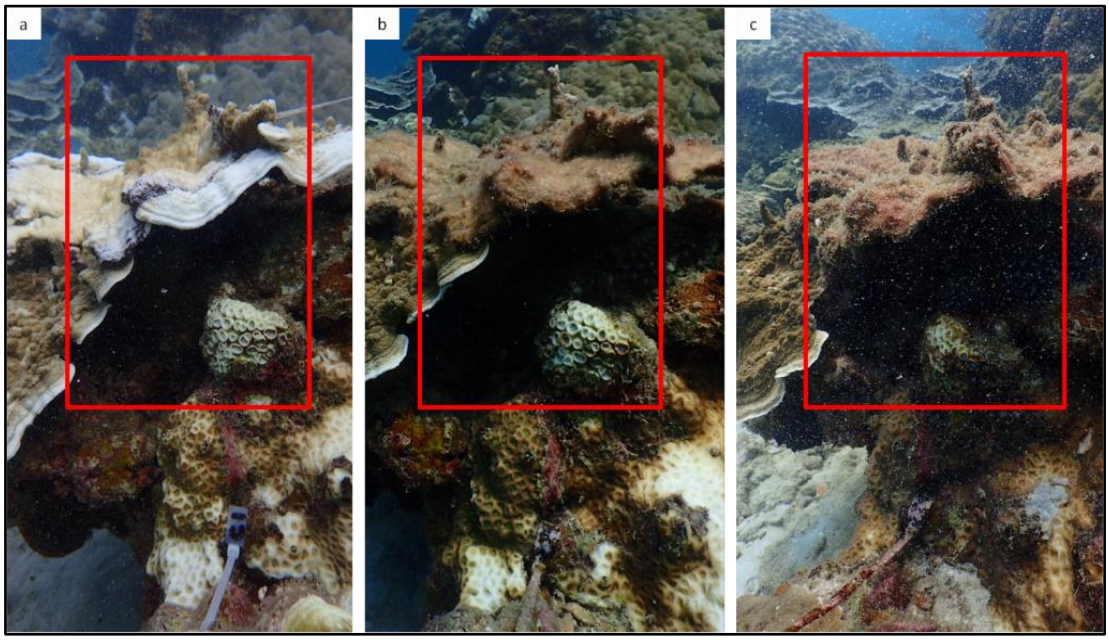

Figure 3. *Colony 1 of Montipora turtlensis. (a) July, 2019 (b) August, 2019 (c) September, 2019*

Bacteria identification based on 16s rRNA sequence

A total of 18 bacteria isolates were selected based on their morphological characteristics (*Fig. 4*). In all, 50% of the colonies were cream-colored, circular and with creamy texture (*Fig. 4a,b,c etc.*). The colonies abundance between healthy and affected coral tissues were differentiate based on its morphological characteristics and identified taxonomically by 16S rDNA sequencing.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

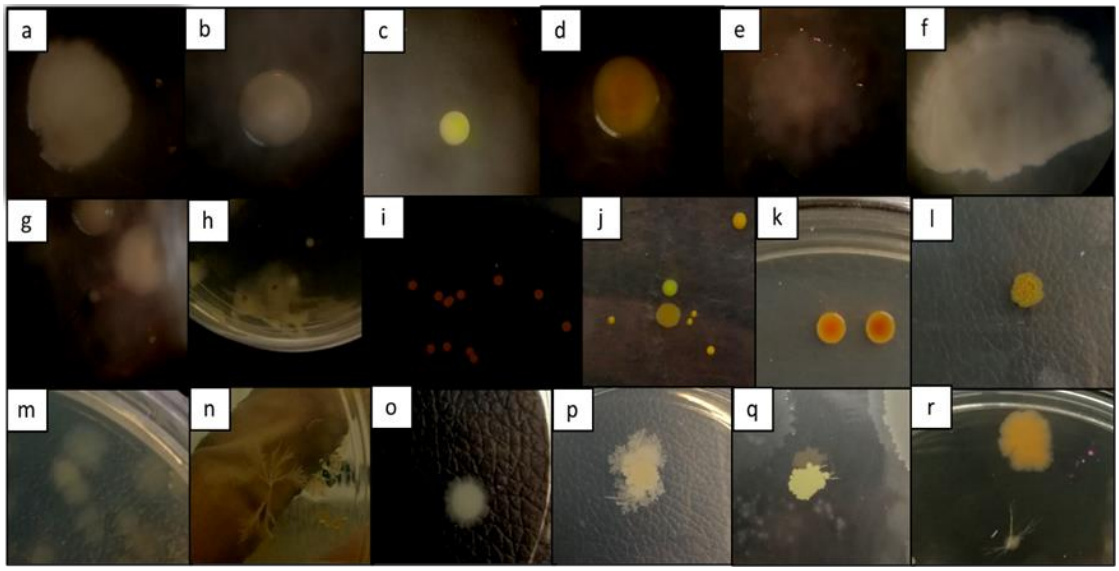

Figure 4. *Morphology and colonial character of different bacterial isolates from healthy and BBD coral tissue. The morphological characteristics are irregular, undulate, flat, large, smooth, dull, cream, opaque (a)* ***GM****; circular, entire, flat moderate, smooth, dull, cream, opaque (b)* ***RM****; circular, entire, flat, punctiform, smooth, dull, yellow, opaque (c)****YM****; circular, entire, flat, moderate, smooth, dull, orange, opaque (d)****OM****; irregular, undulate, flat, moderate, smooth, dull, white, translucent (e)* ***BLM****; irregular, undulate, flat, large, rough, dull, cream, opaque (f)****LGM****; circular, entire, flat, punctiform, smooth, dull, cream, opaque (g)****BM****; irregular, undulate, flat, swarm, smooth, dull, white, translucent(h)****BRM****; circular, entire, flat, punctiform, smooth, dull, red, opaque (i)****RDM****; circular, entire, flat, small, smooth, shiny, yellow, translucent (j)****RRCA****; circular, entire, flat, small, smooth, shiny, orange, translucent (k)****OGM****; irregular, undulate, flat, small, rough, dull, yellow, opaque (l)****YRA****; irregular, undulate, flat, moderate, smooth, dull, white, transparent (m)****RSA****; irregular, undulate, flat, swarm, smooth, dull, white, translucent (n)****STA****; circular, undulate, flat, moderate, smooth, dull, white, opaque (o)****ROSA****; irregular, undulate, flat, moderate, smooth, dull, cream, opaque (p)****OCA****; irregular, undulate, flat, small, smooth, dull, light yellow, opaque (q)****YIA****; and irregular, undulate, flat, moderate, smooth, dull, orange, opaque (r)****OIA****; colony. Refer Table 1 for the full names of the bacteria species*

Bacteria community isolates from healthy and BBD corals tissue

These samples were sequenced at 1st BASE DNA Sequencing Division and analysed by Ez Bio Cloud (*Table 1*). It should be noted that bacteria belong to the phyla Proteobacteria were further divided into alpha and gammaproteobacteria. *Table 1* represent the detected sequences derived from a wide taxonomic range as showed that the isolates included representatives of Actinobacteria, Firmicutes and Proteobacteria phyla (alpha and gamma) whereas *Fig. 5* represent the percentage of bacterial abundance of coral tissue *Montipora turtlensis* and *Acropora spicifera* derived from the sequencing data.

The distributions of the cultured bacteria were spread between healthy and BBD-infected tissue from two coral species (*Fig. 6*). There were no bacteria common to both healthy coral species only; however, *Microbacterium aquimaris* strain YM was a single bacterium that present to both BBD-affected tissues for both coral species. Nevertheless, this study is considered impetuous to derive any conclusion about the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

character of this bacterium in the pathogenesis of the disease. A formal confirmation test of such causal association by applying *in vitro* infection assays would be suggested. For *Montipora turtlensis*, there are only 3 bacteria that are present in BBD-affected tissue sample; *Lelliottia* sp. strain OCA, *Salinicola salarius* strain YRA and *Microbacterium petrolearium* strain ROSA and BM, while *Pseudomonas stutzeri* strain RSA and *Erythrobacter* sp. strain STA were found only in BBD-affected tissue sample of *Acropora* sp. In addition, *Citrobacter koseri* strain YIA and *Paracoccus* sp. strain OGM were isolated from both healthy and affected sample of *A.spicifera*. Further, there are total of seven bacteria which can be found in both condition healthy and affected tissue from both *Montipora turtlensis* and *A.spicifera*; *Gordinia didemni* strain RRCA, *Barrientosiimonas humi* strain RM, *Virgibacillus pantothenticus* strain GM, *Staphylococcus warneri* strain OM, *Virgibacillus* sp. strain LGM, *Pseudomonas* sp. strain OIA, and *Bacillus* sp. strain BLM. Unfortunately, bacterial strain labelled BRM could not be identified because of the bacterial base pair sequence was too short.

Table 1. *16S rDNA sequencing results for healthy and BBD affected coral of Montipora turtlensis and Acropora spicifera colony from Shark Point in Pulau Redang, Terengganu*

Label	ID%	Accession no.	Nearest phylogenetic relative
GM	98.9	OM243119	*Virgibacillus pantothenticus*
RM	98.6	OM243115	*Barrientosiimonas humi*
YM	98.3	OM243123	*Microbacterium aquimaris*
OM	99.5	OM243117	*Staphylococcus warneri*
BLM	82.7	AE016877	*Bacillus* sp.
LGM	98.8	OM243124	*Virgibacillus* sp.
RRCA	97.7	OM243111	*Gordinia didemni*
OGM	91.4	OM243122	*Paracoccus* sp.
YRA	99.4	OM243116	*Salinicola salarius*
RSA	98.7	CP002881	*Pseudomonas stutzeri*
STA	97.2	jgi.1058038	*Erythrobacter* sp.
ROSA	99.1	OM243118	*Microbacterium petrolearium*
OIA	89.1	OM243112	*Pseudomonas* sp.
BRM	-	-	Undefine
OCA	89.6	KX709881	*Lelliottia* sp.
YIA	98.8	OM243113	*Citrobacter koseri*
BM	98.9	KF697702	*Microbacterium petrolearium*
RDM	87.9	DQ119293	*Microbacterium* sp.

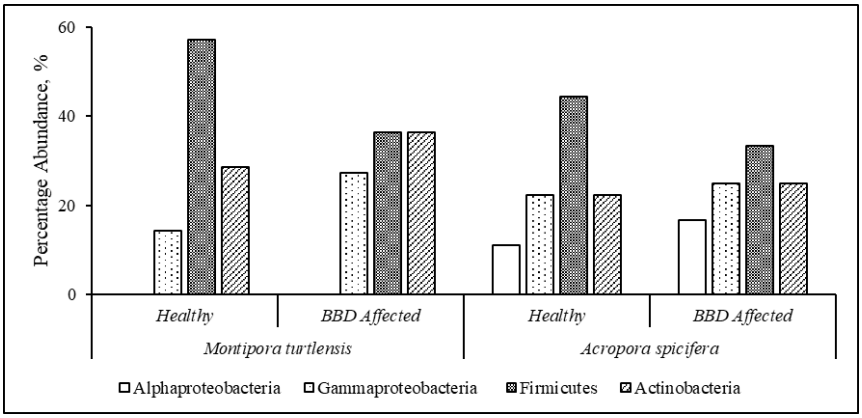

Figure 5. *Percentage of bacterial abundance of coral tissue Montipora turtlensis and Acropora spicifera from Pulau Redang*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

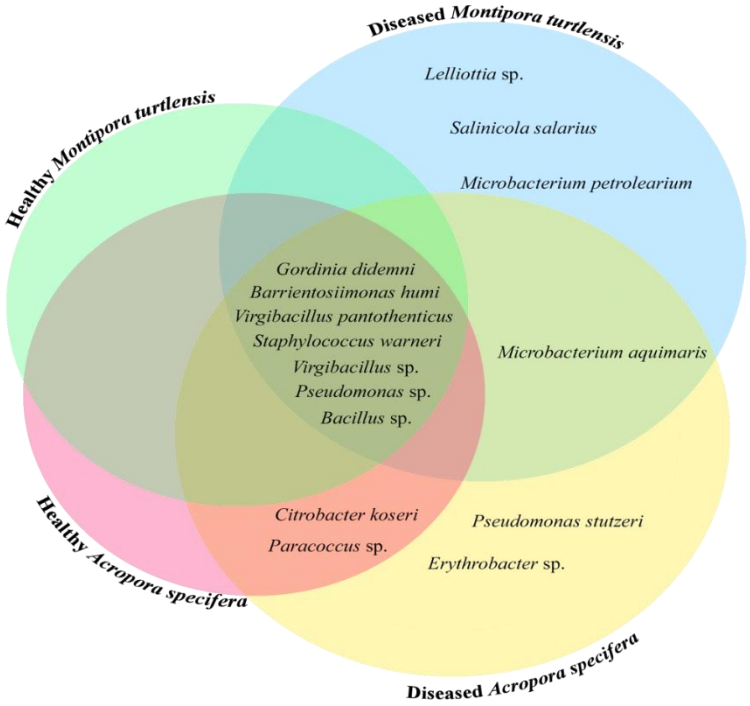

Figure 6. *Venn diagram of cultured bacterium species showing their distribution in healthy, and BBD affected coral tissue samples*

Discussion

The relationship between the host immunity, the microorganisms and the environmental factors creates synergy to balance the coral homeostasis, and any of three factors affected could resulting the development and progression of diseases. Further, the disease will give rise to secondary change in the composition of microbial communities that associated with coral host together with the various immune parameters. The present work studied the comparison of microbial communities from two coral species *Montipora turtlensis* and Acropora specifera infected with black band disease (BBD) collected from Shark Point, Pulau Redang, Terengganu. For both coral species, there was no difference in bacterial variations for the healthy coral tissue. On the other hand, there are slightly differences in bacteria identified on BBD infected tissue. This indicates that the diversity of bacteria is rely upon the basal community composition specific for each coral species and are not represent the disease condition (Cárdenas et al., 2012).

The polymicrobial mat of BBD includes filamentous cyanobacteria (no result), together with various heterotrophic bacteria which lace up with disrupted and necrotic coral tissues fragments. To date, there are yet comprehensive study conducted particularly on microbial communities of BBD on coral tissue in Pulau Redang, Malaysia to compare the findings of molecular and identification of the bacteria species on the area. Dunphy et al. (2019) suggested that local host are responsible in structuring the microbial community, this is because the dissimilarity increased with geographical distance which there are significant differences could be observed in microbial community composition across space and time.

In total, proteobacteria was the predominant bacteria phylum with percentage of 41% bacterial strain identified in both healthy and BBD infected coral tissue, this finding

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

consistent with previous survey in other reef-building corals based on culture-independent methods (Santiago-Vazquez et al., 2007; Kvennefors et al., 2010), followed by Actinobacteria and Firmicutes with percentage of 35% and 24% respectively. Although strain of cyanobacteria (*Phormidium corallyticum*) are commonly reported in previous studies to be the dominant causative agent of BBD towards the corals (Rutzler et al., 1983; Carlton and Richardson, 1995; Richardson and Kuta, 2003; Klaus et al., 2011), the finding from this study could be beneficial to determine other microbial consortium comprise of various strain of bacteria which possibly pathogenic and associated with the disease since the methods used were not adequate to isolate and extract of cyanobacteria.

According to Stackebrandt and Goebel (1994), 16S rDNA sequences identified that have similarities <97.5% are unlikely to be related at the species level. Further, nine from 18 bacterial strain with similarity >97.5% were identified including *Virgibacillus pantothenticus*, *Barrientosiimonas humi*, *Microbacterium aquimaris*, *Staphylococcus warneri*, *Godinia didemni*, *Salinicola salarius*, *Pseudomonas stutzeri*, *Microbacterium petrolearium*, and *Citrobacter koseri*. The problem encountered with the remaining of the bacterial strain which below 97.5% are mainly because of low percentage of completeness due to the short length of base pair sequences. To reconfirm the bacterial strain below accepted percentage of similarity, the bacterial isolates should be culture again and undergoing DNA extraction step until gel electrophoresis before outsourced for identification. The other possibility was that the isolates might be not yet been describe species elsewhere. This statement needs further identification studies such as DNA base composition (mol% G+C content) and whole genome DNA-DNA hybridization (DDH) (Wayne et al., 1987).

Results shown that all bacteria (i.e., *Lelliottia* sp., *Salinicola salarius*, *Microbacterium petrolearium*, *Microbacterium aquimaris*, *Pseudomonas stutzeri* and *Erythrobacter* sp.) identified from the BBD infected coral tissue could be found in seawater, but there is no study been performed previously to confirm the pathogenicity of those bacteria in causing the BBD. *Lelliottia* sp. is one of the genus in the *Enterobacteriaceae* family which has been known associated with coral disease. For example, *Enterobacteriaceae* found to be more abundant in coral samples with White Plague Disease (WPD) in *Montastraea faveolata* (Sunagawa et al., 2009) and building coral *Orbicella faveolata* (Daniels et al., 2015). The specific role of *Lelliottia* sp. in coral disease is not yet fully understood, however, report of *Lelliottia* in insect guts have been found, where the authors suggest a protective antimicrobial role of this bacterium (Fang et al., 2020) suggesting a possible role of this bacteria in managing coral disease. Meanwhile, a study suggested that *Salinicola* sp. may play a beneficial role by solubilizing phosphate (Zhang et al., 2021) suggesting the presence of *Salinicola salarius* could be due to the stress condition such as low levels of phosphorus that resulted to the disease.

Next, *Microbacterium* sp. phylum of *Actinobacteria* is the only bacteria identified present to both BBD-infected tissues for both coral species sample. This bacterium is widely distributed in the marine ecosystem and known to associate with one soft coral *Alcyonium gracllimum* and one stony coral *Tubastrea coccinea* (Yang et al., 2013). *Microbacterium* sp. was proven to frgshave the ability in producing the porphyrin that causing lesion to human in cutaneous tissue called erythrasma (Yasuma et al., 2011), thus may presumed the potential of this bacteria give rise to substantial tissue loss which eventually led to necrotic coral tissue.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

Genus *Erythrobacter* is known to be one of the marine aerobic anoxygenic phototrophs, although this bacterium was not previously found in other studies related to black band disease to confirm the pathogenicity, it was shown that *Erythrobacter* sp. was present in the diseased tissue of *Montastrea annularis* in conjunction of the study conducted in Caribbean Sea on White Plague (WP)-like disease (Pantos et al., 2003). Despite the presence in *Acropora spicifera* BBD affected coral tissue sample, *Pseudomonas stutzeri* are documented in other study to have the ability to inhibit the growth of diseased isolates by exhibiting their anti-pathogenic property (Sabdono et al., 2015). In addition, *Pseudomonas* sp. which categorised as heterogeneous and ubiquitous group is commonly can be found in many habitats including water and soil, study conducted by Cervino et al. (2006) found that several *Pseudomonas* sp. are associated with a disease affecting marine sponge *Ianthella basta*.

As for the bacteria of phylum Firmicutes identified present on both healthy and affected tissue sample which is *Virgibacillus* sp. and *Bacillus* sp. have formerly been documented in coral BBD consortium members (Cooney et al., 2002; Barneah et al., 2007; Klaus et al., 2011). In addition, the Firmicute associated with the BBD in the data as well known to be associated with other coral disease for instance in WP disease (Sunagawa et al., 2009; Roder et al., 2014) and *Porites* white patch syndrome (Séré et al., 2013). Unfortunately, out of the 18 bacterial isolates identified, there was one strain of BRM unsuccessfully to be identified in EZ Bio Cloud due the shortness of the bacteria strain. The observation performed during the incubation period of the bacteria culture shown that the BRM strain was presently available in both healthy and BBD affected corals tissue samples.

Conclusions

As the first study conducted to isolate and identify the bacterial communities from coral tissues infected by black band disease on coral reef of Pulau Redang, Terengganu, this finding requires further experimental investigation by introducing those successfully bacterial isolates on another healthy coral tissue to validate that it relates to specific pathogenic feature. Despite of no causality explanations can be acquired from this observation, finding from this study provides the first estimate of the bacterial diversity associated with healthy and BBD affected coral tissue and may serve as a baseline data for future reference particularly in Pulau Redang, Malaysia. Better comprehensive experimental approaches should take into consideration in determining bacterial communities from healthy and diseased tissues such as metagenomics methods, evaluation of antibiotic production and evaluation of antagonistic behaviours.

Acknowledgements. Authors acknowledge the Ministry of Higher Education (MOHE) for funding under the Fundamental Research Grant Scheme (FRGS) (FRGS/1/2018/WAB09/UMT/03/3) and also PSU-TUYF Charitable Trust Fund under a project “Coral reef biodiversity conservation and connectivity in southern Gulf of Thailand to support reef resilience and sustainable use’. The Authors also would like to express their gratitude to all the researchers and staff for their invaluable assistance during the research period.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

REFERENCES

[1] Aeby, G. S., Work, T. M., Runyon, C. M., Shore-Maggio, A., Ushijima, B., Videau, P., Beurmann, S., Callahan, S. M. (2015): First record of black band disease in the Hawaiian archipelago: response, outbreak status, virulence, and a method of treatment. – PLoS ONE 10(3): e0120853.

[2] Akmal, K. F., Shahbudin, S. (2020): Baseline assessment of coral health and disease in Tioman Island Marine Park, Malaysia. – Community Ecology 21(3): 285-301.

[3] Antonius, A. (1985): Coral diseases in the Indo-Pacific: A first record. – Marine Ecology 6(3): 197-218.

[4] Arotsker, L., Kramarsky-Winter, E., Ben-Dov, E., Siboni, N., Kushmaro, A. (2015): Changes in the bacterial community associated with black band disease in a Red Sea coral, Favia sp., in relation to disease phases. – Diseases of aquatic organisms 116(1): 47-58.

[5] Barneah, O., Ben-Dov, E., Kramarsky-Winter, E., Kushmaro, A. (2007): Characterization of black band disease in Red Sea stony corals. – Environmental Microbiology 9(8): 1995-2006.

[6] Borneman, E. (1998): Bacteria and Coral: Good or Bad? – online available at: http://www.reefs.org/library/talklog/e_borneman_051098.html (Accessed 5th October 2019).

[7] Browne, N., Braoun, C., McIlwain, J., Nagarajan, R., Zinke, J. (2019): Borneo coral reefs subject to high sediment loads show evidence of resilience to various environmental stressors. – PeerJ 7: e7382.

[8] Cárdenas, A., Rodriguez, L. M., Pizarro, V., Cadavid, L. F., Arévalo-Ferro, C. (2012): Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. – The ISME Journal 6(3): 502.

[9] Carlton, R. G., Richardson, L. L. (1995): Oxygen and sulfide dynamics in a horizontally migrating cyanobacterial mat: black band disease of corals. – FEMS Microbiology Ecology 18(2): 155-162.

[10] Cervino, J. M., Winiarski-Cervino, K., Polson, S. W., Goreau, T., Smith, G. W. (2006): Identification of bacteria associated with a disease affecting the marine sponge Ianthella basta in New Britain, Papua New Guinea. – Marine Ecology Progress Series 324: 139-150.

[11] Cooney, R. P., Pantos, O., Le Tissier, M. D. A., Barer, M. R., O'Donnell, A. G., Bythell, J. C. (2002): Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. – Environmental Microbiology 4(7): 401-413.

[12] Daniels, C. A., Baumgarten, S., Yum, L. K., Michell, C. T., Bayer, T., Arif, C., Roder, C., Weil, E., Voolstra, C. R. (2015): Metatranscriptome analysis of the reef-building coral Orbicella faveolata indicates holobiont response to coral disease. – Frontiers in Marine Science 2: 62.

[13] Dunphy, C. M., Gouhier, T. C., Chu, N. D., Vollmer, S. V. (2019): Structure and stability of the coral microbiome in space and time. – Scientific reports 9(1): 1-13.

[14] Edmunds, P. J. (1991): Extent and effect of black band disease on a Caribbean reef. – Coral Reefs 10(3): 161-165.

[15] Fang, J. X., Zhang, S. F., Liu, F., Zhang, X., Zhang, F. B., Guo, X. B., Zhang, Z., Zhang, Q. H., Kong, X. B. (2020): Differences in Gut Bacterial Communities of Ips typographus (Coleoptera: Curculionidae) Induced by Enantiomer-Specific α-Pinene. – Environmental Entomology 49(5): 1198-1205.

[16] Fidalgo, C., Proença, D. N., Morais, P. V., Henriques, I., Alves, A. (2019): The endosphere of the salt marsh plant Halimione portulacoides is a diversity hotspot for the genus Salinicola: description of five novel species (Salinicola halimionae sp. nov., Salinicola aestuarinus sp. nov., Salinicola endophyticus sp. nov., Salinicola halophyticus sp. nov. and Salinicola lusitanus sp. nov). – International journal of systematic and evolutionary microbiology 69(1): 46-62.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

[17] Garrett, P., Ducklow, H. (1975): Coral diseases in Bermuda. – Nature 253(5490): 349.
[18] Gignoux-Wolfsohn, S. A., Vollmer, S. V. (2015): Identification of Candidate Coral Pathogens on White Band Disease-Infected Staghorn Coral. – PLoS One 10(8): e0134416. doi: 10.1371/journal.pone.0134416.
[19] Hadaidi, G., Ziegler, M., Shore-Maggio, A., Jensen, T., Aeby, G., Voolstra, C. R. (2018): Ecological and molecular characterization of a coral black band disease outbreak in the Red Sea during a bleaching event. – PeerJ 6: e5169.
[20] Hadaidi, G. A., Voolstra, C. R. (2020): Corals Are Sick: Black Band Disease Is Attacking. – Frontiers for Young Minds 8: 6.
[21] Hutabarat, P. U. B., Nguyen, X. H., Suda, S. (2018): Black Band disease-related (BBD) cyanobacterium from Okinawan corals. – Journal of Applied Phycology 30(6): 3197-3203.
[22] Johan, O., Bengen, D. G., Zamani, N. P., Sweet, M. J. (2015): The distribution and abundance of black band disease and white syndrome in Kepulauan Seribu, Indonesia. – HAYATI Journal of Biosciences 22(3): 105-112.
[23] Jompa, J., Tahir, A., Malina, A. C., Rantetondok, A. (2020): In vitro analysis of pathogenic bacteria causing black band disease on Pachyseris speciosa (Dana, 1846). – Aquaculture, Aquarium, Conservation & Legislation 13(4): 1865-1876.
[24] Kalimutho, M., Ahmad, A., Kassim, Z. (2007): Isolation, characterization and identification of Bacteria associated with mucus of Acropora cervicornis Coral from Bidong Island, Terengganu, Malaysia. – Malaysian Journal of Science 26(2): 27-39.
[25] Klaus, J. S., Janse, I., Fouke, B. W. (2011): Coral black band disease microbial communities and genotypic variability of the dominant cyanobacteria (CD1C11). – Bulletin of Marine Science 87(4): 795.
[26] Kvennefors, E. C. E., Sampayo, E., Ridgway, T., Barnes, A. C., Hoegh-Guldberg, O. (2010): Bacterial communities of two ubiquitous Great Barrier Reef corals reveals both site-and species-specificity of common bacterial associates. – PLoS ONE 5(4): e10401.
[27] Lane, D. J. (1991): 16S/23S rRNA Sequencing. – In: Stackebrandt, E., Goodfellow, M. (eds.) Nucleic acid techniques in bacterial systematics John Wiley & Sons: New York; pp. 115-175.
[28] Lesser, M. P., Slattery, M., Leichter, J. J. (2009): Ecology of mesophotic coral reefs. – Journal of Experimental Marine Biology and Ecology 375(1-2): 1-8.
[29] Miller, A. W., Blackwelder, P., Al-Sayegh, H., Richardson, L. L. (2011): Fine-structural analysis of black band disease-infected coral reveals boring cyanobacteria and novel bacteria. – Diseases of aquatic organisms 93(3): 179-190.
[30] Miller, J., Sweet, M. J., Wood, E., Bythell, J. (2015): Baseline coral disease surveys within three marine parks in Sabah, Borneo. – PeerJ 3: e1391.
[31] Montano, S., Strona, G., Seveso, D., Galli, P. (2013): Prevalence, host range, and spatial distribution of black band disease in the Maldivian Archipelago. – Diseases of Aquatic Organisms 105(1): 65-74.
[32] Pantos, O., Cooney, R. P., Le Tissier, M. D., Barer, M. R., O'Donnell, A. G., Bythell, J. C. (2003): The bacterial ecology of a plague-like disease affecting the Caribbean coral Montastrea annularis. – Environmental Microbiology 5(5): 370-382.
[33] Richardson, L. L., Kuta, K. G. (2003): Ecological physiology of the black band disease cyanobacterium Phormidium corallyticum. – FEMS Microbiology Ecology 43(3): 287-298.
[34] Roder, C., Arif, C., Bayer, T., Aranda, M., Daniels, C., Shibl, A., Voolstra, C. R. (2014): Bacterial profiling of White Plague Disease in a comparative coral species framework. – The ISME Journal 8(1): 31-39.
[35] Rutzler, K., Santavy, D. L., Antonius, A. (1983): The black band diseases of Atlantic reef corals. III. Distribution, ecology, and development. – PSZNI Marine Ecology 4: 329-358.
[36] Sabdono, A., Sawonua, P. H., Kartika, A. G. D., Amelia, J. M., Radjasa, O. K. (2015): Coral diseases in Panjang Island, Java Sea: diversity of anti–pathogenic bacteria coral symbionts. – Procedia Chemistry 14: 15-21.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835

[37] Santiago-Vázquez, L. Z., Brück, T. B., Brück, W. M., Duque-Alarcon, A. P., McCarthy, P. J., Kerr, R. G. (2007): The diversity of the bacterial communities associated with the azooxanthellate hexacoral Cirrhipathes lutkeni. – The ISME journal 1(7): 654-659.
[38] Séré, M. G., Tortosa, P., Chabanet, P., Turquet, J., Quod, J. P., Schleyer, M. H. (2013): Bacterial communities associated with Porites white patch syndrome (PWPS) on three Western Indian Ocean (WIO) coral reefs. – PLoS ONE 8(12): e83746.
[39] Stackebrandt, E., Goebel, B. M. (1994): Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. – International journal of systematic and evolutionary microbiology 44(4): 846-849.
[40] Sunagawa, S., DeSantis, T. Z., Piceno, Y. M., Brodie, E. L., DeSalvo, M. K., Voolstra, C. R., Weil, E., Andersen, G. L., Medina, M. (2009): Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral Montastraea faveolata. – The ISME journal 3(5): 512-521.
[41] Veron, J. E. N. (2000): Corals of the World. – Australian Institute of Marine Science, Townsville, Australia, 489p.
[42] Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P. (1987): Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. – International Journal of Systematic and Evolutionary Microbiology 37(4): 463-464.
[43] Weil, E., Smith, G., Gil-Agudelo, D. L. (2006): Status and progress in coral reef disease research. – Diseases of Aquatic Organisms 69(1): 1-7.
[44] Yang, S., Sun, W., Tang, C., Jin, L., Zhang, F., Li, Z. (2013): Phylogenetic diversity of actinobacteria associated with soft coral Alcyonium gracllimum and stony coral Tubastraea coccinea in the East China Sea. – Microbial Ecology 66(1): 189-199.
[45] Yasuma, A., Ochiai, T., Azuma, M., Nishiyama, H., Kikuchi, K., Kondo, M., Handa, H. (2011): Exogenous coproporphyrin III production by Corynebacterium aurimucosum and Microbacterium oxydans in erythrasma lesions. – Journal of medical microbiology 60(7): 1038-1042.
[46] Zhang, Y., Yang, Q., Ling, J., Long, L., Huang, H., Yin, J., Wu, M., Tang, X., Lin, X., Zhang, Y., Dong, J. (2021): Shifting the microbiome of a coral holobiont and improving host physiology by inoculation with a potentially beneficial bacterial consortium. – BMC Microbiology 21(1): 1-14.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_18231835