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Pathogenic *Vibrio* spp. identified for white syndrome coral disease in Tioman Island Marine Park, Malaysia

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ABSTRACT

Aims: Coral diseases have emerged over the last several decades, causing a loss of live coral cover in the Caribbean and Indo-Pacific reefs. Hence, microbiological and disease cultural techniques are commonly used to investigate their causative microbial agents. This is the first study to identify the potential of pathogenic *Vibrio* spp. isolated from apparently white syndrome (WS) coral disease in Tioman Island Marine Park using biochemical and molecular techniques.
Methodology and results: The *Vibrio* colonies were isolated from 108 samples of WS infected corals (*Acropora cytherea* and *Montipora aequituberculata*) including seawater, sediment and algae found adjacent to infected coral colonies. A total of one hundred representative *Vibrio* isolates were characterized and most of them (n=50) were identified as *V. vulnificus*, *V. alginolyticus* and *Photobacterium damselae* following biochemical analysis. The molecular analysis revealed six *Vibrio* spp. (*V. coralliilyticus*, *V. hepatarius*, *V. brasiliensis*, *V. tubiashi*, *V. campbellii*, *V. ishigakensis*) and one *Photobacterium rosenbergii*. *V. coralliilyticus* isolated from all infected coral samples may be highly responsible for the sign of WS disease.
Conclusion, significance and impact of study: The findings of this study provide baseline data and information on potential coral pathogens identified in the coastal waters of Tioman Island. Etiological disease study is suggested to validate their severity and virulence factors in the future.

Keywords: Coral disease, white syndrome, *Vibrio* bacteria, marine park, Peninsular Malaysia

INTRODUCTION

White syndrome (WS) has been characterized as any distinctive patches, bands or plagues of the exposed white coral skeleton (Work and Aeby, 2011). The epizootic is notably known for the mass mortality of scleractinian hard corals in the Caribbean (Gil-Agudelo *et al*., 2006) and Indo-Pacific (Willis *et al*., 2004; Bourne *et al*., 2015). From 1970 to 1990, the Caribbean WS outbreak has debilitated by approximately 95% of the common acroporid corals from species *Acropora palmata* and *A. cervicornis* (Aronson and Precht, 2001; Pandolfi and Jackson, 2006). Over the following years from 1999 until 2003, WS has been observed to rapidly spread in the Great Barrier Reef with more than 50 reported cases per reef site (Willis *et al*., 2004). It has been affected multiple coral species (Dalton *et al*., 2010) with *A. hyacinthus* and *A. cytherea* being the most susceptible (Willis *et al*., 2004; Aeby *et al*., 2011). Despite having widespread distribution and causing regional reef decline, the causative agents for WS are yet to be comprehensively determined.

To date, several microbial pathogens have been identified as causative agents for WS in the Caribbean and Indo-Pacific. These include Gram-negative bacterium *Serratia marcescens* (Patterson *et al*., 2002), *Aurantimonas coralicida* (Denner *et al*., 2003) and *Thalassomonas loyana* (Thompson *et al*., 2006). Other Gram-negative *Vibrio* bacterial species such as *V. coralliilyticus* (Sussman *et al*., 2008; Ushijima *et al*., 2014), *V. owensii* (Ushijima *et al*., 2012), *V. harveyi* (Luna *et al*., 2010) and *V. alginolyticus* (Zhenyu *et al*., 2013) have been characterized as the causative agents of Indo-Pacific WS coral disease. In addition to causative agents, environmental stressors such as elevated seawater temperatures, nutrient pollution and sedimentation have also been linked with the outbreak of WS in the

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Caribbean and Indo-Pacific coral communities (Ruiz-Moreno *et al*., 2012; Couch *et al*., 2014, Pollock *et al*., 2014).

In Malaysia, coral reefs are continuously threatened due to multiple environmental stressors such as sedimentation, high coastal development and widespread of tourism activities (Toda *et al*., 2007; Praveena *et al*., 2012; Shahbudin *et al*., 2017; Hanapiah *et al*., 2019; Akmal *et al*., 2019). These are further compounded by the bleaching events recorded in 1998 and 2010, causing from mild to severe bleaching impacts of hard coral colonies (Tan and Heron, 2011). Such natural and human disturbances are predicted to increase microbial communities, leading to the occurrence of coral disease around Malaysian reefs. However, there has been a scarcity of coral disease research in this peninsular region. Preliminary coral surveys at several reef sites around Tioman Island indicated that WS was commonly found afflicted *Acropora* and *Montipora* corals (Akmal and Shahbudin, 2019). Therefore, this study was primarily aimed to identify the potential of pathogenic *Vibrio* spp. isolated from WS coral disease using biochemical and molecular techniques. The outcomes of this study contribute to the baseline information on microbial pathogens that facilitate the development of WS coral disease in marine protected area of Tioman Island.

MATERIALS AND METHODS

Sample collection

Four times samplings were conducted in July 2018, October 2018, March 2019 and June 2019. Samples were collected at three selected reef sites, namely Sanggit Bay (02° 45' 08.6" N, 104° 13' 13.8" E), Salang Bay (02° 53' 34.4" N, 104° 09' 24.0" E) and Bakau Bay (02° 54' 61.4" N, 104° 06' 74.6" E) in Tioman Island Marine Park (Figure 1). A total of six coral fragments (1–2 cm in diameter), displaying the WS sign were collected from depths between 3 and 15 m at each reef site. The targeted corals for WS were tabular species of *Acropora cytherea* and foliose species of *Montipora aequituberculata* (Figure 2). Other samples including seawater, sediment and algae found in adjacent diseased coral colonies were also collected in the study area. In total, 27 samples were collected (n = 9 per site) for each month of sampling. Throughout the sampling period, a total of 108 samples were collected from 72 diseased corals, 12 water, 12 sediment and 12 algae. All samples were collected under permit from the section of Marine Park Malaysia (permit number: JTLM 630 - 7 Jld. 8 - 49).

Bacterial isolation and biochemical analysis

Bacteria were isolated from all samples (coral mucus, seawater, sediment and algae) using sterile cotton swabs and spreading onto universal bacterial medium tryptic soy agar (TSA). The obtained bacterial colonies were then sub-cultured onto selective *Vibrio* growth medium thiosulfate citrate bile salt-sucrose (TCBS) using the

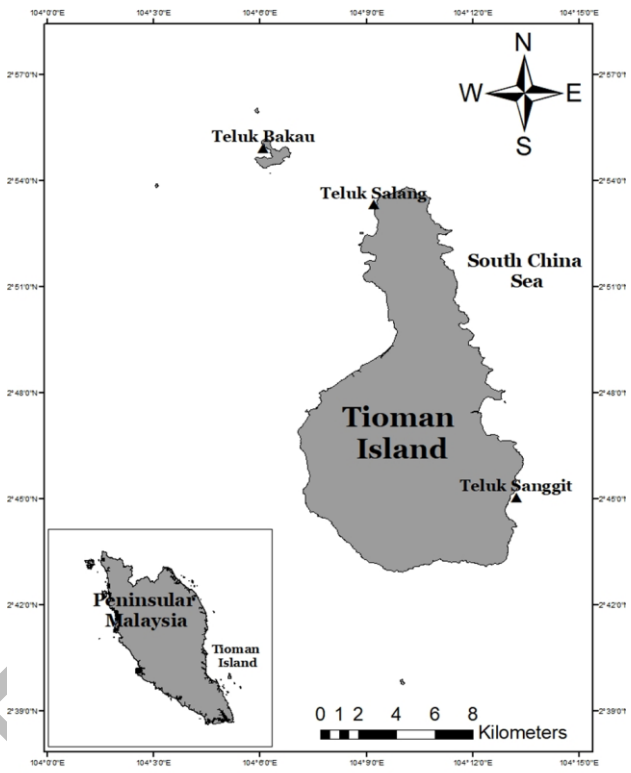

Figure 1: Three sampling locations in this study which located in Tioman Island Marine Park.

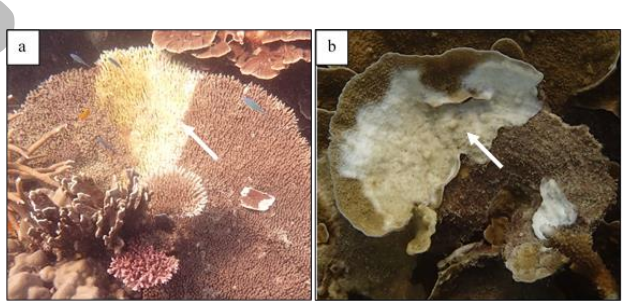

Figure 2: a) *Acropora cytherea* and b) *Montipora aequituberculata* show a diffuse pattern of tissue loss (as indicated by white arrow), leaving white colour on the active lesion fronts and yellow to brown colours on the exposed skeleton.

streaking technique. Presumably, 2–4 mm in diameter of uniform yellow and green colonies were selected as *Vibrio* isolates. Parafilm tapes were used to seal agar plates and placed upside down in a warm and dark place during the sampling period. In the laboratory, all *Vibrio* isolates were sub-cultured onto TSA agar supplemented with 10% of sodium chloride (NaCl) and incubated at 30 °C for about 18 h. Pure bacterial colonies were then morphologically observed through Gram staining, oxidase and catalase tests before further subjected to the API

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Table 1: Sample ID, location, isolation source and accession number of sample sequences.

No.	Sample ID	Location	Isolation source	Accession No.
1	TSGP1	Sanggit Bay	WS *A. cytherea*	MN339960
2	TSGP2	Sanggit Bay	WS *A. cytherea*	MN339962
3	TSGP3	Sanggit Bay	WS *M. aequituberculata*	MN339964
4	TSGP4	Sanggit Bay	Seawater	MN339954
5	TSGP5	Sanggit Bay	Sediment	MN339953
6	TSGP6	Sanggit Bay	Algae	MN339957
7	TBKP7	Bakau Bay	Seawater	MN339958
8	TBKP8	Bakau Bay	Sediment	MN339950
9	TBKP9	Bakau Bay	Algae	MN339951
10	TBKP10	Bakau Bay	WS *M. aequituberculata*	MN339956
11	TBKP11	Bakau Bay	WS *A. cytherea*	MN339961
12	TBKP12	Bakau Bay	WS *A. cytherea*	MN339965
13	TBKP13	Bakau Bay	WS *M. aequituberculata*	MN339955
14	TSKP14	Salang Bay	Seawater	MN339959
15	TSLP15	Salang Bay	Sediment	MN339949
16	TSLP16	Salang Bay	Algae	MN339952
17	TSLP17	Salang Bay	Sediment	MN339948
18	TSLP18	Salang Bay	WS *M. aequituberculata*	MN339967
19	TSLP19	Salang Bay	WS *M. aequituberculata*	MN339963
20	TSLP20	Salang Bay	WS *A. cytherea*	MN339966

20NE kit (standardized identification system for non-enteric Gram-negative rods; BioMérieux, France) for species identification.

Molecular analysis

Genomic DNA was extracted from pure cultured of *Vibrio* isolates using the Wizard Genomic DNA kit (Promega, USA), following the manufacturer's instructions. The extracted DNA template was quantified using the Nanodrop quantification method (Desjardins and Conklin, 2011) and stored at –20 °C for longer storage. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was performed using universal primers 8F and 1492R; 5'- AGAGTTTGATCCTGGCTCAG - 3' and 5'- GGTTACCTTGTTACGACTT - 3' (Lane, 1991). PCR reactions (50 µL) were performed on the Eppendorf Mastercycler with a reaction mixture consisting of 1 µL of DNA template, 25 µL of MyTaq™ (Bioline, USA), 1 µL of 20 µM of forward and reverse primers and 23 µL of sterile distilled water. Cycling conditions comprised of 3 min for initial denaturation at 94 °C followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and concluded by 10 min extension step at 72 °C. PCR products were visualized using a 1% agarose gel under the UV Transilluminator (Alphaimager™ 2200, Germany). All successful single band PCR products were sent to the First Base, Malaysia for DNA sequencing process.

Phylogenetic analysis

All sample sequences were checked for low quality bases using Sequence Scanner ver. 1.0 (Gene Codes Corporation). Multiple sequence alignment between forward and reverse sequences was then performed using ClustalX ver. 2.1 (Larkin *et al*., 2007). Next, sequence data was aligned with the most similar sequences retrieved from the Genbank database using the NCBI BLAST program (Altschul *et al*., 1997) and further analyzed using the EzTaxon-e database (Kim *et al*., 2012). A total of 20 sample sequences from seven bacterial strains were identified and further submitted to Genbank (www.ncbi.nih.nlm.gov/Genbank/). Details on sample ID, isolation source and accession number of samples sequences were listed in Table 1. In addition, 12 reference sequences of 16S rRNA gene retrieved from the Genbank database were also used to construct the phylogenetic tree as follows: NR117887 (*Vibrio brasiliensis*), NR025491 (*V. hepatarius*), NR118093 (*V. tubiashii*), NR113784 (*V. harveyi*), NR121709 (*V. alginolyticus*), NR114632 (*V. parahaemolyticus*), NR113782 (*V. campbelli*), NR036888 (*V. vulnificus*), NR025476 (*V. neptunius*), NR117892 (*V. coralliilyticus*), NR156028 (*V. ishigakensis*) and NR042343 (*Photobacterium rosenbergii*). *Agrobacterium tumefaciens* (NR041396) was used as an outgroup sequence. In total, 33 nucleotide sequences (samples, reference and outgroup sequences) were aligned using Clustal W, which

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was incorporated in the Molecular Evolutionary Genetics Analysis (MEGA) software ver. 6 using default settings (Tamura *et al*., 2013). MEGA ver. 6 software was also used to construct the phylogenetic tree based on neighbor joining (NJ) and maximum likelihood (ML) evolutionary distance analyses. NJ was computed using the Tamura-3 parameter (Tamura, 1992). Meanwhile, ML was computed using General Time Reversible + Gamma + Proportion Invariant (GTR + G + I) (Nei and Kumar, 2000) based on the best-fit model estimated using MrModeltest ver. 2.3 program (Nylander, 2004). Both evolutionary distance analyses were performed with 1000 bootstrap (Tamura, 1992).

RESULTS

Bacterial isolation

Four times samplings (July 2018, October 2018, March 2019 and June 2019) were collected with a total of 108 samples from diseased corals including seawater, sediment and algae found adjacent to WS infected corals. In total, 159 pure colonies were isolated from all samples, consisting of 100 Gram-negative *Vibrio* isolates and 59 Gram-positive isolates. Phenotypically, *Vibrio* isolates appeared as large yellow and green colonies, indicating the absence of sucrose fermentation. Additionally, they grew on a selective TCBS medium and were able to grow on a TSA medium supplemented with 10% NaCl (Figure 3). Gram staining result also revealed the form of Gram-negative bacilli with curve and rod shapes for *Vibrio* isolates (Figure 4).

Bacterial identification based on API20 NE kit

In total, 50 identical pure colonies were selected from 100 identified Gram-negative *Vibrio* isolates. All pure colonies were determined to be oxidase-positive (indicated by purple colour formation in oxidase strip) and catalase-negative (indicated by gas production in 3% H_2O_2). Based on the API 20NE identification system, two *Vibrio* spp. (*V. vulnificus* and *V. alginolyticus*) and one *Photobacterium damselae* were identified following their phenotypic characteristics (Table 2). The results of biochemical reactions showed that *Vibrio* spp. were reduced nitrate to nitrite and produced indole. They were also showed to be the glucose fermentation-positive and arginine dihydrolase-negative. However, *V. vulnificus* (GEL +/ PNPG + /GLU –) and *V. alginolyticus* (GEL -/ PNPG - /GLU +) showing the different for gelatin hydrolysis (GEL), β-galactosidase activity (PNPG) and assimilation of glucose reaction (GLU). As similar to *Vibrio* spp, *P. damselae* was also showed a positive reaction for nitrate reduction and glucose fermentation. However, it was differed from the *Vibrio* spp. by having indole production-negative and arginine dihydrolase-positive.

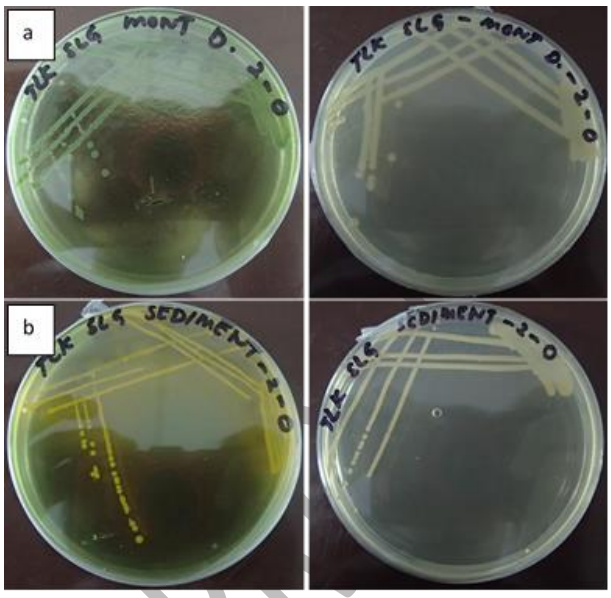

Figure 3: Green (a) and yellow (b) colonies of *Vibrio* isolates on TCBS (left) and TSA (right) media.

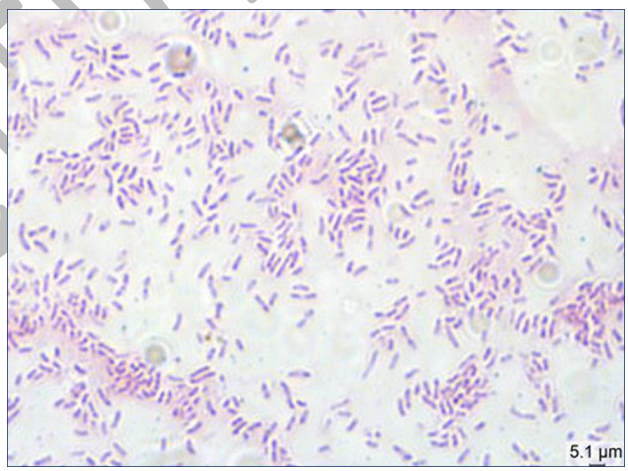

Figure 4: Morphology of Gram-negative bacilli with curve and rod shapes for *Vibrio* isolates after Gram staining under light microscope with 40× objective.

Bacterial identification based on 16S rRNA gene sequencing

A total of six *Vibrio* spp. (*V. coralliilyticus*, *V. ishigakensis*, *V. brasiliensis*, *V. campbellii*, *V. hepatarius* and *V. tubiashii*) and one *Photobacterium rosenbergii* were retrieved from the Genbank with percentage of similarity ranged from 99.3% until 100% (Table 3). All bacterial species were similarly identified by EzTaxon server with the percentage of identification ranged from 99.4% until 100%. Sample sequences from WS infected corals (*A. cytherea* and *M. aequituberculata*) were mostly identified

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Table 2: Phenotypic characterization of bacterial isolates using the API 20NE kit.

Phenotypic characteristics	*Vibrio* Isolates		
	Vibrio vulnificus	*Vibrio alginolyticus*	*Photobacterium damselae*
Samples (n = 50)	n = 15	n = 15	n = 20
Gram's stain	-	-	-
Cell morphology	cocci	cocci	cocci
Oxidase	+	+	+
Catalase	-	-	-
Nitrate reduction (NO_3)	+	+	+
Indole production (TRP)	+	+	-
Glucose fermentation (GLF)	+	+	+
Arginine dihydrolase (ADH)	-	-	+
Hydrolysis of:			
Aesculin (ESC)	+	+	-
Gelatin (GEL)	+	-	-
Enzyme activity of β-galactosidase (PNPG)	+	-	+
Assimilation of:			
Glucose (GLU)	-	+	+
Mannose (MNE)	-	-	-
Malate (MLT)	+	+	+

+: indicates a positive result, −: indicates a negative result.

Table 3: Bacterial species identified by the GenBank and Eztaxon databases.

No.	Sample ID	Strains	Genbank	BLAST similarity (%)	EzTaxon	Similarity (%)
1	TSGP1	ATCC BAA-450	*V. coralliilyticus*	100%	*V. coralliilyticus*	99.4%
2	TSGP2	ATCC BAA-450	*V. coralliilyticus*	100%	*V. coralliilyticus*	99.4%
3	TSGP3	JCM 19231	*V. ishigakensis*	99.3%	*V. ishigakensis*	99.4%
4	TSGP4	LMG 20546	*V. brasiliensis*	99.9%	*V. brasiliensis*	99.7%
5	TSGP5	LMG 20546	*V. brasiliensis*	99.9%	*V. brasiliensis*	99.7%
6	TSGP6	CAIM 519	*V. campbellii*	99.7%	*V. campbellii*	100%
7	TBKP7	CAIM 519	*V. campbellii*	99.7%	*V. campbellii*	100%
8	TBKP8	LMG 22223	*P. rosenbergii*	99.6%	*P. rosenbergii*	100%
9	TBKP9	LMG 20546	*V. brasiliensis*	99.9%	*V. brasiliensis*	99.7%
10	TBKP10	LMG 20546	*V. brasiliensis*	99.9%	*V. brasiliensis*	99.7%
11	TBKP11	ATCC BAA-450	*V. coralliilyticus*	100%	*V. coralliilyticus*	99.4%
12	TBKP12	JCM 19231	*V. ishigakensis*	99.3%	*V. ishigakensis*	99.4%
13	TBKP13	LMG 20546	*V. brasiliensis*	99.3%	*V. brasiliensis*	99.7%
14	TSKP14	CAIM 519	*V. campbellii*	99.7%	*V. campbellii*	100%
15	TSLP15	LMG 22223	*P. rosenbergii*	99.6%	*P. rosenbergii*	100%
16	TSLP16	LMG 20546	*V. brasiliensis*	99.9%	*V. brasiliensis*	99.7%
17	TSLP17	LMG 22223	*P. rosenbergii*	99.5%	*P. rosenbergii*	99.9%
18	TSLP18	LMG 20362	*V. hepatarius*	99.8%	*V. hepatarius*	100%
19	TSLP19	ATCC BAA-450	*V. coralliilyticus*	100%	*V. coralliilyticus*	99.4%
20	TSLP20	ATCC 19109	*V. tubiashii*	99.7%	*V. tubiashii*	99.7%

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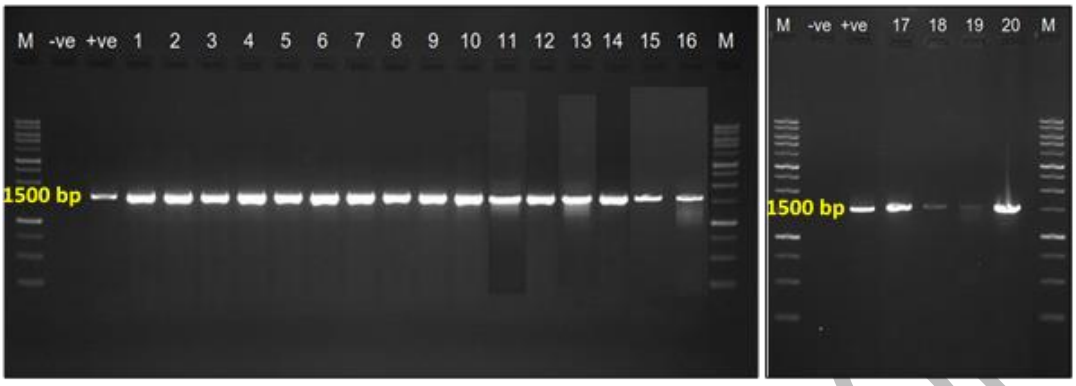

Figure 5: Amplification of PCR products using universal primer: 8F and 1492R on 1% agarose gel electrophoresis. Lane M: 1 kb ladder; lane –ve: PCR no template control; lane +ve: positive control, lane 1 - 20: positive samples indicate the targeted size of 1500 bp for 16S rRNA gene.

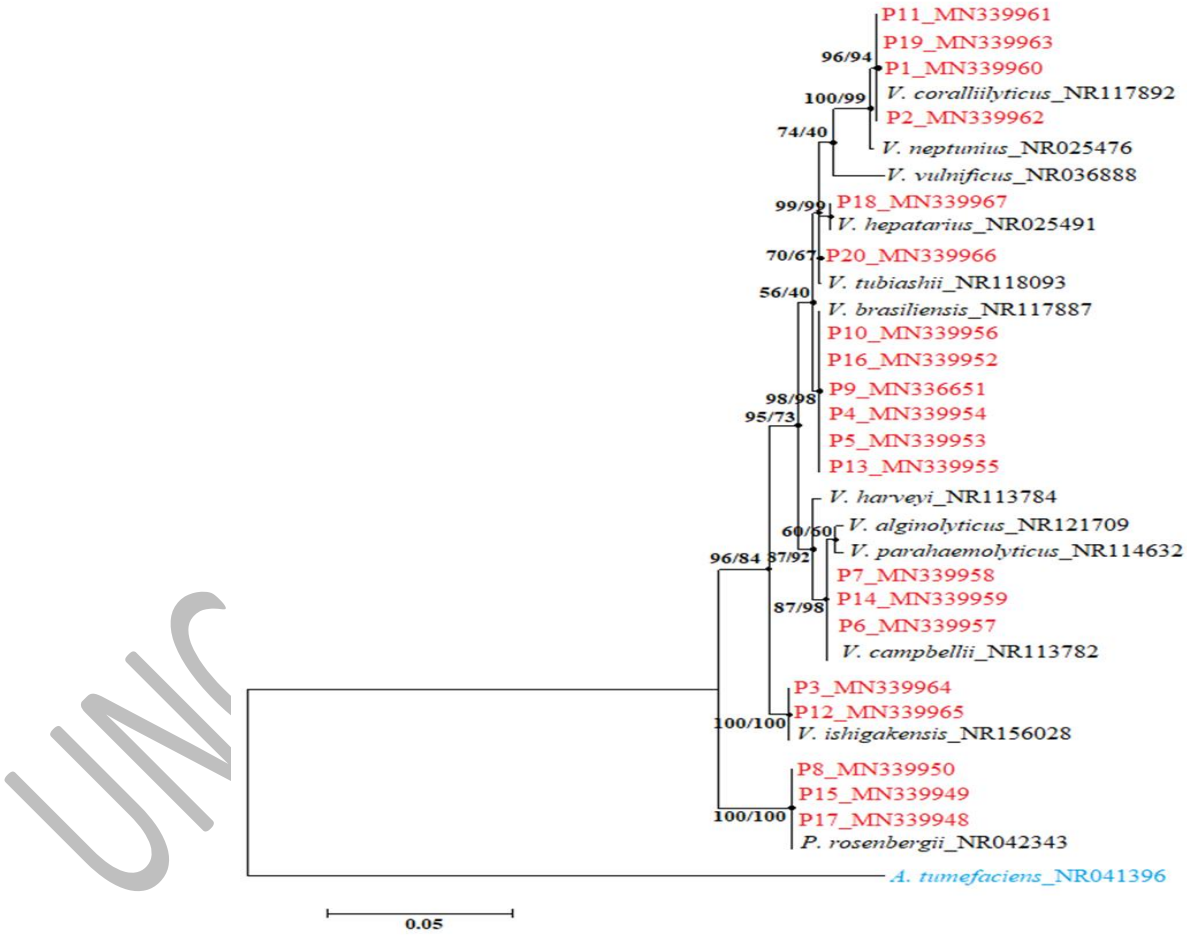

Figure 6: Evolutionary distance ML phylogenetic tree based on 16S rRNA gene sequences of isolates obtained in this study. Coral pathogens are marked in red and labeled from P1 until P20. Reference strains are marked in black and outgroup marked in blue. Nodes represent bootstrap values ≥ 50% based on 1000 replicates generated from ML and NJ distance analyses. Scale bar corresponds to 5% estimated sequence divergence.

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as a species of *V. coralliilyticus* with 100% similarity. 16S rRNA gene amplicons bands with 1500 base pairs (bp) were visualized in 1% of agarose gel electrophoresis (Figure 5).

Molecular phylogenetic tree of *Vibrio* species

The molecular phylogenetic tree consists of 33 nucleotide sequences which include sample, reference and outgroup sequences (Figure 6). Sequence from species *A. tumefaciens* was used as an out-group for the tree topology. All samples sequences were clustered with seven strains of coral pathogens namely *V. brasiliensis* (NR117887), *V. hepatarius* (NR025491), *V. tubiashii* (NR025491), *V. campbellii* (NR113782), *V. coralliilyticus* (NR117892), *V. ishigakensis* (NR156028), and *P. rosenbergii* (NR042343), sharing high sequence identities ranged from 98 until 100% similar. Out of 20 samples sequences, six samples were shared sequence identities in 99% similar with *V. brasiliensis* that has been characterized from marine sponge *Scleritoderma cyanea* (Hoffman *et al*., 2012). Meanwhile, four samples sequences from WS diseased corals were clustered in 99% similar with a previously characterized coral pathogen *V. corallilyticus*, causing bleaching to *Pocillopora damicornis* (Ben-Haim *et al*., 2003) and WS disease to *Acropora*, *Montipora* and *Pachyseris* corals (Sussman *et al.*, 2008). Additionally, three sediment samples were clustered in 100% similar with *P. rosenbergii* associated with the bleaching corals (Thompson *et al*., 2005). Other *Vibrio* spp. (*V. hepatarius*, *V. tubiashi*, *V. campbellii* and *V. ishigakensis*) identified in this study were also involved in disease causation to aquatic animals such as corals, fish and bivalves (Thompson et al., 2003a; 2003b).

DISCUSSION

Bacterial isolation and biochemical identification of *Vibrio* spp.

This study successfully cultured a hundred representative *Vibrio* isolates, belonging to the Vibrionaceae family from WS diseased corals including seawater, sediment and algae obtained from adjacent diseased coral colonies. The morphological characteristics were confirmed by the presence of Gram-negative bacilli with curve and rod shapes of *Vibrio* isolates, along with the capacity to form green or yellow colonies on TCBS medium (Mustapha *et al*., 2013). The ability to grow on TSA medium supplemented with 10% NaCl also showed a common halophilic characteristic of *Vibrio* spp. to adapt in a high salinity water condition (Jayasinghe *et al*., 2010). This high-salt adaptation can improve the survival of pathogenic *Vibrio* spp. under adverse environmental conditions (Gomathi *et al*., 2013; Kalburge *et al*., 2014).

Based on the biochemical reactions, two *Vibrio* spp. (*V. vulnificus* and *V. alginolyticus*) and one *Photobacterium damselae* (formerly known as *V. damsela*) were identified with a high percentage of 99.9% similarity. These bacterial species are generally populated around coastal areas (Thompson *et al*., 2004; Pruzzo *et al*., 2005). Over 80 *Vibrio* spp. identified worldwide, 12 species in particular *V. cholera*, *V. vulnificus*, *V. alginolyticus* and *V. parahaemolyticus* are clinically implicated in human diseases, including severe diarrheal disease (known as vibriosis) linked with contaminated water and raw seafood consumption (Vezzulli *et al*., 2013). Moreover, the pathogenesis of *V. alginolyticus* and *P. damselae* may result in the mass mortality of cultivated fish, oysters and other bivalves in the aquaculture industry (Beleneva *et al*., 2007; Chatterjee and Haldar, 2012; Najwa *et al*., 2015). Since a large number of *Vibrio* spp. are causing severe mortality of aquatic species, their occurrence might also be facilitated the emergence of WS coral disease in Tioman Island.

Nonetheless, species identified using the biochemical test did not substantially support the sequence findings from the samples of the molecular 16S rRNA gene. The API 20NE kit is usually used for rapid identification of clinical and environmental bacterial species (O'Hara *et al*., 2003; Israil *et al*., 2003). However, the use of this miniaturized test is ineffective in the detection of certain bacteria belonging to the Vibrionaceae family (Colodner *et al*., 2004; Martinez-Urtaza *et al*., 2006), suggesting an optimization of temperature, incubation time and media composition for correct identification (Ottaviani *et al*., 2003). Besides, there are limited taxa with only five *Vibrio* spp. (*V. vulnificus*, *V. alginolyticus*, *V. cholera*, *V. metschnikovii* and *V. parahaemolyticus*) and a single species of *P. damselae* in the APIWEB™ system database. This may result in a misidentification of bacterial species detected based on a biochemical test. Hence, the molecular method utilizing the 16S rRNA primer was used to further identify *Vibrio* spp. in the study area. It has been documented that the 16S rRNA gene provides the most accurate phylogenetic classification of microorganisms (Kang *et al*., 2003; Kim and Bang, 2008) and is commonly used to detect novel pathogens (Clarridge, 2004)

Molecular identification of *Vibrio* bacterial pathogens

Molecular findings based on nearly complete 16S rRNA gene sequences (1500 bp) revealed six *Vibrio* spp. (*V. coralliilyticus*, *V. hepatarius*, *V. brasiliensis*, *V. tubiashi, V. campbellii* and *V. ishigakensis*) and one *Photobacterium rosenbergii*. These bacterial species were closely related, ranging from 99.3 until 100% (following the Genbank and Eztaxon databases) to bacterial pathogens associated with severe diseases in coral species and other aquatic animals. A taxonomic cluster showed that all sequences retrieved from WS diseased corals had a phylogenetic affiliation with *V. coralliilyticus* at 96% bootstrap value. This *Vibrio* sp. is known as a common coral pathogen in the Indo-Pacific reefs.

V. coralliilyticus has previously been described as a novel temperature-dependent pathogen due to rapid degradation of coral tissue within 2 weeks when the water temperatures rise over 25 °C (Ben-Haim and Rosenberg,

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2002). It has also been recognized as an etiological agent for the bleaching of *Pocillopora* coral in the Indian Ocean and Red Sea regions (Ben-Haim *et al*., 2003). In other localities at the Republic of the Marshall Islands, Palau and the GBR, *V. coralliilyticus* has been reported as a potential microbial agent, causing WS sign for *Acropora*, *Pachyseris* and *Montipora* corals (Sussman *et al*., 2008). Besides, the high prevalence of *V. coralliilyticus* was attributed to gorgonian mortality in the Mediterranean Sea (Bally and Garrabou, 2007) as well as to diseased oyster larvae (*Crassostrea gigas*) in UK and scallop (*Nodipecten nodosus*) in Brazil (Ben-Haim *et al*., 2003).

The molecular phylogenetic tree also revealed that some sequences of WS diseased corals were grouped with *V. hepatarius* (99% bootstrap value), *V. brasiliensis* (98%), *V. campbellii* (87%) and *V. ishigakensis* (100%). *V. hepatarius* and *V. brasiliensis* have previously been described as the causative microbial agents for diseased aquatic organisms, including bivalves, fish, rotifers and shrimps (Thompson *et al*., 2003a; 2003b), while *V. campbellii* and *V. ishigakensis* have been found to be dominated within sediments and seawater in coral reef areas (Thompson *et al*., 2004; Gao *et al*., 2016). Besides that, *V. campbellii* has also been identified as one of the opportunistic pathogens for corals, shrimp and oysters (Lin *et al*., 2010). In comparison, the molecular phylogenetic tree derived from the 16S rRNA gene was clustered all sediment sequences with a previously identified coral pathogen *P. rosenbergii* (Thompson *et al*., 2005) in 100% bootstrap support. This *Photobacterium* sp. has been isolated from diseased and healthy corals around Magnetic Island, Australia (Thompson *et al*., 2005). Therefore, concerning the current findings and supported by previous literature, it can be ascertained that these identified bacterial species, particularly *V. coralliilyticus* could become the primary pathogens causing WS coral disease in the marine protected area of Tioman Island.

CONCLUSION

In conclusion, this study provides baseline data on potential coral pathogens identified in Tioman Island Marine Park. The biochemical and molecular findings of this study revealed the dominant of *Vibrio* spp. as the causative microbial pathogens for white syndrome coral disease. Throughout the culture period, six *Vibrio* spp. (*V. coralliilyticus*, *V. hepatarius*, *V. brasiliensis*, *V. tubiashi, V. campbellii* and *V. ishigakensis*) and one *Photobacterium rosenbergii* were identified with *V. coralliilyticus* predominantly found in infected coral colonies. This identified *Vibrio coralliilyticus* was closely related to the coral pathogen identified in the Indo-Pacific reefs. Accordingly, other bacterial species found within Tioman reefs were also closely related to bacterial pathogens, causing mortality in aquatic animals at different reef localities. Current data and information could be valuable for a future etiological study of coral disease in the coastal waters of Tioman Island.

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