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# Bacterial community associated with *Pyrodinium bahamense* var. compressum during late exponential growth phase based on 16S rRNA gene sequence analysis

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## ABSTRACT

**Aims:** *Pyrodinium bahamense* var. compressum is one of the principal causal agents of harmful algal blooms (HABs) in the coastal waters of Sabah, Malaysia. Seafood and aquaculture products tainted with lethal concentrations of the principal neurotoxin, saxitoxin, have been implicated in mortality and morbidity. The bacteria-algae association may play a key role in paralytic shellfish toxin (PST) production during a toxic bloom event. The production of PST during a harmful bloom is unclear and research on the bacterial diversity associated with Sabah *P. bahamense* is scarce. The present study examined the cultivable bacteria diversity associated with *P. bahamense* through 16S ribosomal RNA (rRNA) gene sequence analysis.
**Methodology and results:** The V3-V4 region of the 16S rRNA gene sequence was amplified and used to identify bacterial populations associated with *P. bahamense* var. compressum. A total of 62 isolates were successfully isolated, belonging to three different phyla, which were Proteobacteria; 55 (89%), Bacteroidetes; 6 (10%) and Actinobacteria; 1 (1%). Out of 55 Proteobacteria, 27 isolates were gamma-Proteobacteria (*Marinobacter salsuginis*) and 28 of the isolates were alpha-Proteobacteria; *Mameliella atlantica* (13), *Roseibium denhamense* (10) and *Roseibium hamelinense* (5). The remaining bacteria isolates from the phyla Bacteroidetes and Actinobacteria were identified as *Muricauda lutimaris* (6) and *Micrococcus luteus* (1), respectively.
**Conclusion, significance and impact of study:** The analysis of the bacterial 16S rRNA gene revealed multiple bacterial taxa associated with the toxic *P. bahamense* var. compressum bloom. The findings of the present work will pave the way for further studies aimed at isolating and characterizing genes involved in the saxitoxin biosynthesis in the associated bacteria.

*Keywords:* 16S rRNA gene, bacteria, harmful algal bloom, paralytic shellfish poisoning, *Pyrodinium bahamense* var. compressum

## INTRODUCTION

Harmful algal blooms (HABs) have become a routine occurrence in the coastal waters around the world, posing a threat to human health, fisheries and the aquatic ecosystem (Trottet *et al.*, 2021). Other ecological effects include changes to marine trophic formations, the mortality of marine mammals, fish and seabirds, and the demise of livestock in freshwaters (Ben-Gigirey *et al.*, 2021). Blooms occur when an alga population rapidly increases to the degree where it dominates the local benthic or local water bodies (Vadeboncoeur *et al.*, 2021). These blooms become harmful because some of the causative organisms produce toxins that are lethal to humans and marine life, as well as because of dense overgrowth that can occur in localized areas, depleting oxygen for marine life beneath (He, 2015).

In Sabah, Malaysia, HAB has become an annual event along the coastal parts of West Sabah with one of the contributing organisms being the dinoflagellate, *Pyrodinium bahamense* var. compressum (Law *et al.*, 2020). The first occurrence was reported in January 1976, when two people died and another 17 others were hospitalized due to paralytic shellfish poisoning (PSP) after eating contaminated shellfish (Jipanin *et al.*, 2019). *Pyrodinium bahamense* blooms are prevalent in the Southeast Asian region, with reports from Malaysia (Law *et al.*, 2020), Brunei (Ranjith and De Silva, 1998), Indonesia (Likumahua *et al.*, 2021) and the Philippines (Yñiguez *et al.*, 2021) as well as the Pacific coast of Central America (Müller *et al.*, 2020).

Saxitoxin (STX) is a neurotoxin produced by *P. bahamense* that causes PSP, a potentially fatal neurological disorder in humans. Paralytic shellfish toxins

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(PST) reversibly bind to the voltage-dependent sodium channel and block its pores, preventing nerve impulse transmission and resulting in neuromuscular paralysis (Bricelj *et al.*, 2005). Globally, the accumulation of STX in shellfish has caused a 15% yearly mortality rate with the assessment of 2,000 reports on human PSP for nearly over the past four decades; however, since the surveillance is limited, this may be an underestimate (Havelaar *et al.*, 2015; Edwards *et al.*, 2018).

There is considerable debate surrounding the origin of the PST produced by *Pyrodinium bahamense* var. compressum, *Gymnodium catenatum* and *Alexandrium* spp., especially the role of the associated bacteria may play in bloom toxicity. Previous studies by Azanza *et al.* (2006) and Espino *et al.* (2007) showed promising results on the tropical *P. bahamense* endosymbionts autonomous production of PSTs. The endosymbionts associated with the Philippines *P. bahamense* strain isolated from Masinloc Bay, Zambales which included *Moraxella* spp., *Erythrobacter* spp. and *Bacillus* spp., were shown to contain either saxitoxin or neo-saxitoxin or both at levels ≤73 ng/$10^7$ bacterial cells based on high performance liquid chromatography (HPLC) analysis (Azanza *et al.*, 2006). Meanwhile, the HPLC-Fluorometry analysis of the purified toxin extracted from a bacterium, *Micrococcus luteus* associated with a different *P. bahamense* strain collected from another harmful algal bloom in Masinloc, Zambales indicated the presence of gonyautoxins 1 and 3. The presence of toxin in three *Micrococcus* isolates suggested that the toxin was produced by the associated bacteria rather than the dinoflagellate itself (Espino *et al.*, 2007). These previous studies suggested that the microbial community associated with a toxic harmful algal bloom event may play a role in the toxin biosynthesis pathway.

In the previous study by Law *et al.* (2020), the bacteria community associated with the same *P. bahamense* var. compressum culture was assessed by using the high-throughput sequencing of the 16S metagenomes. The study discovered various interesting bacterial taxa associated with *P. bahamense*, including some that are potentially putatively toxic or are involved in toxin biosynthesis. Although more advanced techniques has been applied to characterize the bacterial taxa associated with *P. bahamense*, this study aimed to comprehensively document the cultivable bacterial flora associated with the tropical PST-producing dinoflagellate *P. bahamense* as a first step to understand the role of bacteria in toxin biosynthesis during the late exponential growth phase where the toxin is the highest, so that future research investigating the role of the bacteria in toxin production can be carried out on the cultivable organisms. This study reports the use of the V3 and V4 variable regions of the 16S rRNA gene to identify the marine bacteria associated with *P. bahamense*.

## MATERIALS AND METHODS

### *Pyrodinium bahamense* culture and cell maintenance

The microalgal sample was taken from the Unit for Harmful Algal Bloom Studies, Borneo Marine Research Institute, Universiti Malaysia Sabah. The *P. bahamense* strain labeled as CC-UHABS-040(M) was isolated from Sepanggar Bay in December 2012 at the highest PST concentration in the coastal waters of Sabah (The Star, 2013). Monoalgal culture of *P. bahamense* was established from a single dinoflagellate cell and maintained through continuous laboratory culture in sterile seawater-based enriched f/2 medium. The culture medium (f/2 medium) was prepared according to Guillard and Ryther (1962). The *P. bahamense* culture was maintained in an environmental chamber (Sanyo) at 25 °C in a light/dark cycle of 12:12 h and light intensity of 200 µE $m^{-2}$ $s^{-1}$.

### Estimation of growth characteristics of *P. bahamense*

The growth estimation experiment was conducted in triplicate. Approximately 150 mL of f/2 media were prepared in 250 mL conical flasks. The media was inoculated with 30 mL of a two-week-old *P. bahamense* culture and incubated at 25 °C in a light/dark cycle of 12:12 h with a light intensity of 200 µE $m^{-2}$ $s^{-1}$. Enumeration of the cell concentrations in the starting inoculum was carried out by using a Sedgewick-Rafter Counting Chamber. Development of the culture was monitored every two days (48 h) for 30 days as recommended by Hold *et al.* (2001). For each replicate, cells in a 1 mL aliquot of *P. bahamense* culture were fixed using Lugol's solution in the counting chamber and directly counted under a CX31 upright routine light microscope (Olympus, Japan) at 100× magnification. Estimates of cell counts were calculated based on the formula by Wetzel and Likens (1991), as shown in Equation (1). After 30 days of incubation and monitoring, cell counts of the triplicate cultures were averaged, and a graph of the growth estimation (cell counts per two-day interval) was plotted.

No. of cells per mL = $[(C)(1{,}000\ mm^3)]/[(L)(D)(W)(S)]$....(1)

Where, C was the number of cells counted, L was the length of Sedgewick Rafter chamber in mm, D was the depth of Sedgewick-Rafter chamber in mm, W was the width of Sedgewick-Rafter chamber in mm and S was the number of strips used to count the cells.

### Bacterial isolation from *P. bahamense*

Isolation of the bacteria associated with *P. bahamense* was carried out according to Hold *et al.* (2001). A total of 1 mL of *P. bahamense* culture, from the late exponential (16th day) was transferred into separate 15 mL sterile centrifuge tubes. The tubes were then sonicated using Ultrasonic Homogenizer (Qsonica Sonicators Q125) to


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break the dinoflagellate cells. Each sample tube was sonicated for 3 min (pulse: 20 sec on 30 sec off) at 40% amplitude. The tubes were put on ice during the sonication process to prevent sample from overheating. The experiment was conducted in triplicates and diluted serially in ten-fold dilution in sterile seawater. A 100 µL of each serially diluted inoculum were plated in triplicates onto sterile marine agar (Difco, USA) and incubated for at least 72 h at 37 °C. The plates containing 50 to 100 colonies were chosen and all single colonies were sub-cultured individually onto a fresh sterile marine agar. The plates were then incubated at 37 °C overnight to obtain pure cultures. Each pure culture was stored in marine broth containing 50% (v/v) glycerol at –80 °C until further use.

**DNA extraction of bacterial isolates**

Each isolate was cultured overnight at 37 °C in 10 mL marine broth (Difco, USA) in a shaking incubator. A total of 1.5 mL of the bacterial culture were used to perform the DNA extraction using the DNeasy® Blood & Tissue DNA Isolation Kit (Qiagen Biotechnology, Germany), following the manufacturer's instructions. Total DNA concentration was measured using a Nanodrop 1000 UV-Vis spectrophotometer and the samples were stored at –20 °C prior to PCR amplification.

**Polymerase chain reaction (PCR) amplification of the 16S ribosomal DNA (16S rDNA)**

The amplification of the 16S rRNA gene was carried out using the Phusion Flash PCR Master Mix (Thermo Fisher Scientific, USA). For each reaction, 1 µL of DNA template was added into 19 µL of PCR reaction mixture consisting of 1× Phusion Flash PCR Master Mix and 0.5 µM of each of the forward and reverse primers. The 16S primers used for the amplification of the V3-V4 region were S-D-Bact-0341-b-S-17, 5′-CCTACGGGNGGCWGCAG-3′ and S-D-Bact-0785-a-A-21, 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth *et al.*, 2013). The amplification condition was as follows: 10 sec of initial denaturation step at 98 °C, followed by 30 cycles of denaturation at 98 °C for 10 sec, annealing at 55 °C for 5 sec and extension at 72 °C for 8 sec, and a final extension at 72 °C for 1 min. The PCR was carried out on a PTC-200 Thermal Cycler (MJ Research, USA). Bands corresponding to the partial 16S rDNA amplicons were excised from the agarose gel and purified using QIAquick Gel Extraction Kit (Qiagen Biotechnology, Germany). The purified PCR products were sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems) using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems).

**16S rDNA sequence determination of bacteria associated with *P. bahamense***

The raw sequences of the isolates of bacteria were pre-analyzed using the GeneStudio™ Professional (http://genestudio.com). The sequences were viewed and analyzed with the chromatogram file as references to trim and edit the sequences by removing high noise at the 5' or 3' end, then compiled into FASTA formatted sequences. The identity of bacterial isolates was determined by aligning the sequences against the 16S Prokaryotic Ribosomal RNA Database of the National Centre for Bioinformatics Information (NCBI) GenBank. All the 16S rRNA gene sequences of the isolates used in this study were deposited in the NCBI GenBank nucleotide sequences database (https://www.ncbi.nlm.nih.gov/nucleotide) with the accession numbers MH507080-MH507141 (Table S1).

**Morphology characterization of the bacterial isolates**

One bacterial isolate representing each identified species was subjected to morphology characterization. Morphology characterization using Gram-staining was carried out following the method described by Beveridge and Davies (1983). The stained bacterial smear was observed using the 20× objective lens under a standard light microscope.

**RESULTS**

**Growth characteristic of *P. bahamense***

An experiment to determine the growth characteristic of *P. bahamense* culture was carried out to ascertain the development time of the cultured *P. bahamense* so that the timing for harvesting the cells is optimal. Based on the growth estimation graph (Figure 1), the growth of Sabah *P. bahamense* isolate in f/2 medium was observed to increase exponentially within a sixteen-day interval starting from day 0. The early exponential stage of growth was achieved after the sixth day. The late exponential phase was between day 12 to 16. After day 16, the cultured cells entered the stationary phase for about five days before entering the death phase on day 21 onwards.

**Molecular and morphology characterization of the bacterial isolates**

A total of 68 single colonies (labelled as SY1 to SY68) isolated from the late exponential phase (16th day of culture) of the *P. bahamense* culture were obtained, accounting for 90% of the plated colonies that could thrive in the marine agar. However, only 62 associated bacteria were successfully identified from the 16S rRNA gene analysis, with approximately 97 to 99% similarity with sequences from the NCBI GenBank nucleotide sequence database. The remaining six isolates were excluded since the sequence similarity search revealed no similarity, which could be new species obtained from this study. The six unidentified bacterial isolates were stored in glycerol stock for further investigation in a follow-up study. The sequence similarity search results for the 16S rRNA sequences obtained in this study are illustrated in Figure 2a (phylum-level distribution) and Figure 2b (species-level distribution). Details on the sequence similarity results of

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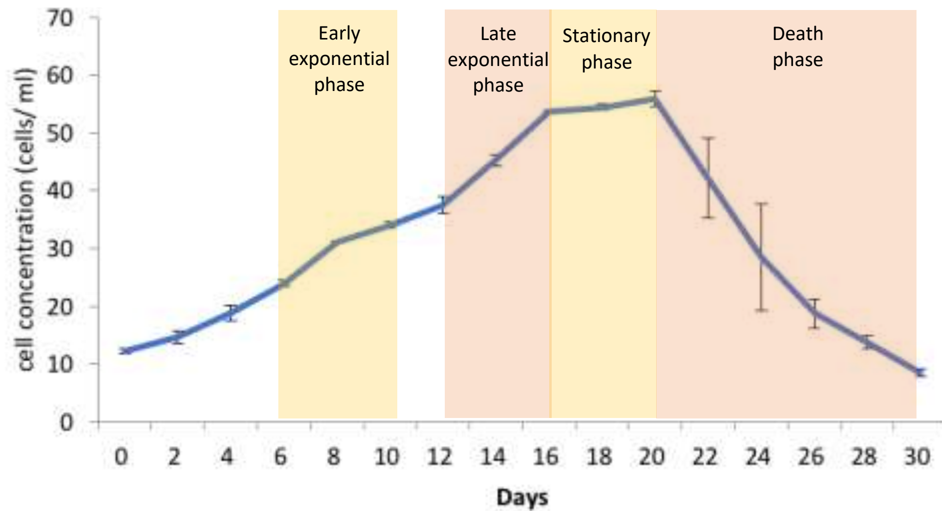


**Figure 1:** Growth characteristic of *P. bahamense* cultured in f/2 medium for 30 days in which the early exponential phase was from day 6 to 10, late exponential from day 12 to 16, stationary phase from day 17 to 20 and death phase occurring between day 21 to 30.

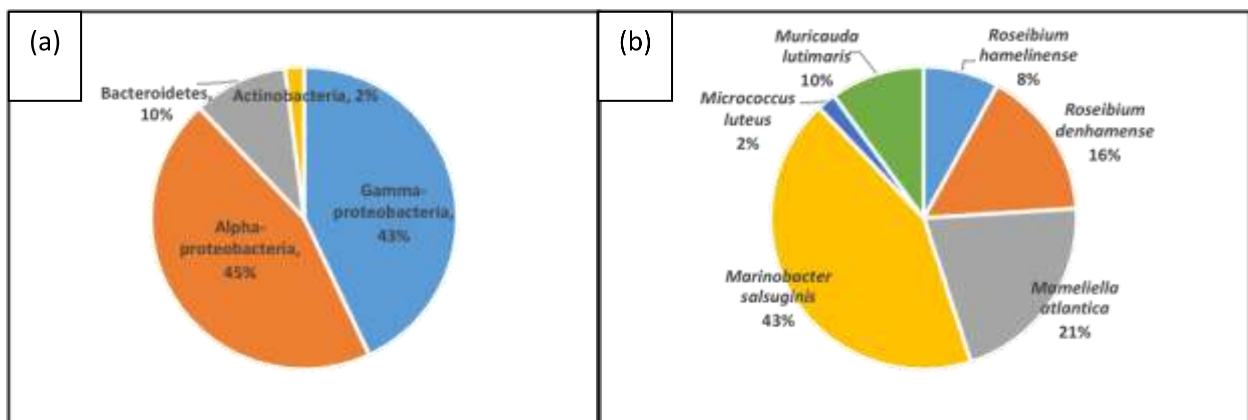


**Figure 2:** Distribution of the cultivable bacterial isolates associated with *P. bahamense* based on its 16S rRNA gene sequences. (a) Phylum level, (b) Species level.

the 16S rRNA gene of the 62 bacterial isolates can be accessed in Table S1.

Figure 2a shows the bacteria community associated with *P. bahamense* is made up of three different phyla: Proteobacteria; 55 (88%), Bacteroidetes; 6 (10%) and Actinobacteria; 1 (2%). Figure 2b shows that 27 of the 55 Proteobacteria were gamma-Proteobacteria (*Marinobacter salsuginis*) and 28 were alpha-Proteobacteria [*Mameliella atlantica* (13), *Roseibium denhamense* (10) and *Roseibium hamelinense* (5)]. *Muricauda lutimaris* (6) and *Micrococcus luteus* (1) were the remaining bacterial isolates identified under the phyla Bacteroidetes and Actinobacteria, respectively (Figure 2b).

In addition, the morphology of the identified bacteria was determined. The results of Gram-staining of the associated bacteria are shown in Figure 3. Most of the isolates were found to be rod-like shapes of varying lengths (Figure 3a-e), except for the bacterium *M. luteus*, which was spherical or coccus-shaped (Figure 3f). Based on the Gram-staining results, the majority of the isolated bacteria, *M. salsuginis*, *R. denhamense*, *R. hamelinense*, *M. lutimaris* and *M. atlantica* were Gram-negative (Figure 3a-e). Only one bacterium, *M luteus*, was Gram-positive, staining the bacteria cells purple (Figure 3f). Besides that, based on the observation of the colony morphology, all the colonies were smooth, slightly convex and round. Yellow-pigmented colonies were observed for bacteria *M. lutimaris* and *M. luteus*, whereas the colony colour of the remaining four bacteria was cream.

## DISCUSSION

The 62 associated bacteria successfully identified were from the late exponential growth phase (16th day) of the *P. bahamense* culture (Figure 1). During the late


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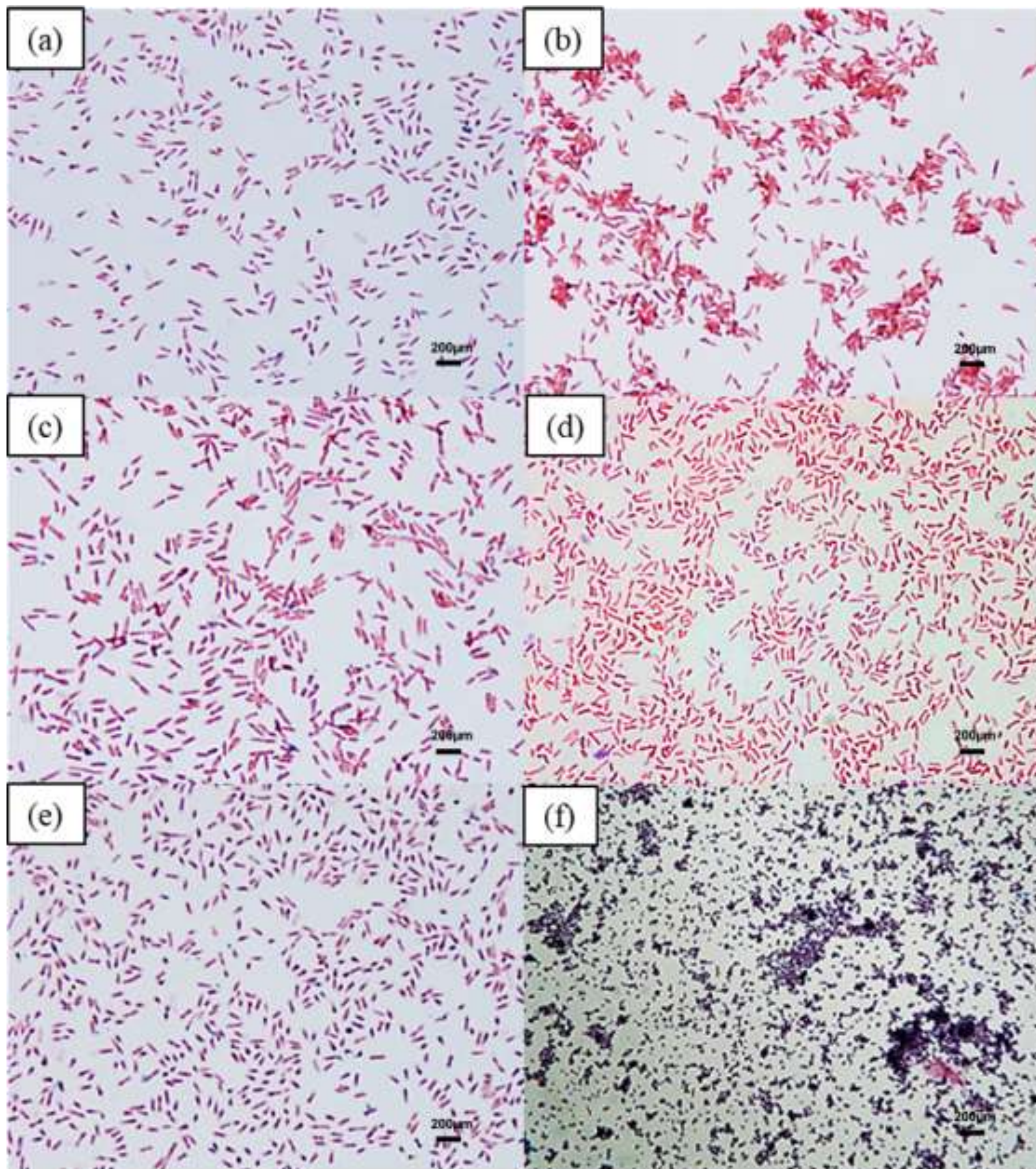


**Figure 3:** Gram-staining observation of the bacterial isolates under 20× magnification (Scale bar: 200 µm). (a) *Marinobacter salsuginis*, (b) *Roseibium denhamense*, (c) *Roseibium hamelinense*, (d) *Muricauda lutimaris*, (e) *Mameliella atlantica* and (f) *Micrococcus luteus*.

exponential phase, as the multiplication of the microalgae starts slowing down, the toxin is produced mostly maybe due to the stress from limited resources (Gedaria *et al*., 2007; Mujtaba *et al*., 2012; Yen *et al*., 2013). In a study that used the similar conventional 16S rDNA identification method (Azanza *et al*., 2006), the culture phase during which the bacteria were harvested appeared to affect the types of isolated bacteria. The overall phylum of the bacterial community in this study is similar to that of Azanza *et al.* (2006), which could be attributed to the similar harvesting period (late exponential to mid-stationary phase). The bacterial community of *P. bahamense* in Chin *et al.* (2013) and Law (2017) studies was dominated mainly by Proteobacteria as it was harvested during the culture's exponential phase when the *P. bahamense* cells were still in an active state of growth.

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Figure 2b depicts the cultivable bacteria isolated from *P. bahamense* cultures to be dominated by bacteria from the genus *Marinobacter* (43%), where all of the sequences showed 99% similarity to *Marinobacter salsuginis* (Accession no.: NR_044044). Its high abundance in the *P. bahammense* culture could be related to the richness of fatty acids, sterols, lipids and oils of the dinoflagellate (Buchan *et al*., 2014). *Marinobacter* strains are renowned for their metabolic ability to utilize various hydrocarbons as alternate nitrogen, phosphorus and carbon sources. Hence, they could be responsible for the growth enhancement in the dinoflagellate, which is actively happening during the exponential growth phase of the *P. bahamense* culture. This genus is often found in tight association with other dinoflagellate species such as *Alexandrium tamarense*, *Gymnodinium catenatum*, *Scrippsiella trochoidea*, *Noctiluca scintillans*, *Scrippsiella* sp. and *Pfiesteria* sp. (Jasti *et al*., 2005), including *P. bahamense* (Law, 2017). In a recent study by Albinsson *et al.* (2014), this species was reported to physiologically affect the intracellular saxitoxin production in its dinoflagellate host in *G. catenatum*. Furthermore, this genus has been reported to promote dinoflagellate growth by supplying bioavailable iron via the siderosphore vibrioferrin (Yarimizu *et al*., 2018) and is capable of supporting the survival of the dinoflagellate *G. catenatum* (Albinsson *et al*., 2014).

The second primary isolate (21%) has a 97 to 99% similarity to *Mameliella atlantica* (Accession no.: NR_136489), a Gram-negative short rod-shaped bacterial (Figure 3e). This species belongs to the Rhodobacteraceae family, a marine bacterium of the *Roseobacter* clade (Xu *et al*., 2015). This specific clade is considered to be responsible for the degradation of organic sulfur-containing molecules such as dimethyl-sulfoniopropionate (DMSP), which is produced by dinoflagellates and then processed by *Roseobacter* bacteria to create dimethylsulfide (DMS) (Osorio-Rodriguez *et al*., 2021). Dungca-Santos *et al.* (2019) reported that the same bacterium *M. atlantica* showed algicidal activity (approximately 82%) towards the Philippines *P. bahamense*. The presence of *M. atlantica* in this study maybe because the *P. bahamense* culture was harvested at the end of the late exponential phase (day 16), approaching the stationary and death phase of the culture. In the stationary phase, the rate of the microalgal cell growth is equal to the rate of microalgal cell death. Therefore, the size of the microalgal population remains constant. Even though some microalgal cells continue to divide, others begin to die due to the presence of algicidal bacteria or other factors.

The third (16%) and fifth (8%) most isolated bacteria are both from the same *Roseibium* genus (Figure 2b), which were Gram-negative rod-shaped bacteria (Figure 3b and 3c). The third and fifth most abundant bacteria successfully isolated from *P. bahamense* culture showed 99% similarity to *Roseibium denhamense* (Accession no.: NR_025885) and *Roseibium hamelinense* (Accession no.: NR_043436), respectively. Both of these species were bacteriochlorophyll-containing bacteria, previously isolated from the east and west coasts of Australia (Suzuki *et al*., 2000) and belonged to the alpha-2 subclass of the Proteobacteria. One characteristic that distinguishes both of these strains is that *R. denhamense* has the ability to utilize acetate, meanwhile, *R. hamelinense* cannot. Based on bioinformatics predicted saxitoxin biosynthesis pathway from several studies, the first reaction suggested to occur is Claisen condensations where arginine, acetate and methionine methyl act as the precursors (Mihali *et al*., 2011). The strain *R. denhamense* may aid in utilizing acetate as the initiation step in toxin production in this particular dinoflagellate.

The third least isolated bacteria comprised 10% of the bacterial community associated with *P. bahamense* (Figure 2b). The bacterium is also a Gram-negative rod-shaped bacterium (Figure 3d) and produced yellow-pigmented colonies. The bacteria showed 97 to 99% similarity to *Muricauda lutimaris* (Accession no.: NR_044423), which was previously isolated from the tidal flat of the Yellow Sea (Yoon *et al*., 2008). *Muricauda* sp. possesses algicidal activities towards non-toxic HAB dinoflagellate; *Skeletonema costatum* (Shi *et al*., 2013) by attaching to them and decomposing them directly. Similar to *M. atlantica*, the bacterium *M. lutimaris* was probably present because the *P. bahamense* culture was approaching the stationary and death growth phase, where cell death has started commencing.

Lastly, only one isolate exhibited 99% sequence homology with *Micrococcus luteus* (Accession no.: NR_075062) of the Actinobacteria phylum. This isolate was the only isolate that was Gram-positive and coccus in shape (Figure 3f). The bacteria also produced a brighter yellow colony than the colony of *Muricauda* isolate. This particular strain was often associated with tropical *P. bahamense* (Azanza *et al*., 2006; Chin *et al*., 2008; this study) but in low quantity and only at the late exponential to stationary phase. Although *M. luteus* were reported to produce secondary metabolites (not identified) with strong algicidal activity against HAB dinoflagellates such as *Alexandrium catenella*, *Prorocentrum micans* and *Heterosigma akashiwo* (Seong and Jeong, 2013), the *M. luteus* isolated from several cultures of tropical *P. bahamense* strains was reported can autonomously produce PST derivatives; gonyautoxin 1 and gonyautoxin 3 in the purified toxin extracts (Espino *et al*., 2007). This particular strain could be the best candidate for a follow-up study to determine the relationship between the associated bacterium and *P. bahamense* in toxin production, as this species is constantly linked with the *P. bahamense* toxin production.

A metagenomic study of bacterial communities associated with the similar *P. bahamense* strain by Law *et al.* (2020) was able to classify 10 phyla with 105 different genera of bacteria. Within the broad range of genera, our traditional PCR-based 16S rRNA identification methodology was able to isolate two species reported in their metagenome data, which are *M. salsuginis* and *R. hamelinense*. In comparison between identification through culture-independent method (Law *et al*., 2020) and culture-dependent method (this study; Chin *et al*.,

2013; Law, 2017) of associated bacteria using the same *P. bahamense* culture, only approximately two percent of the isolates were found using PCR-based method. It is most likely related to the fact that some bacteria, dubbed as "uncultivable", cannot be grown under standard culture protocols (Stewart, 2012). Even though the metagenomes were not able to characterize up to the species level, metagenomics data from Law *et al.* (2020) acquired a wider taxonomic distribution than this study, which only had five different species from four different genera and phylum.

In terms of isolation technique on the same *P. bahamense* dinoflagellate, Azanza *et al.* (2006) developed a method that effectively obtained intracellular bacteria (axenic culture sonication), meanwhile Law (2017) obtained extracellular bacteria (sonication using Ultrasonic Cleaner to disrupt bacterial-dinoflagellate attachment). The *P. bahamense* cells in this study were broken using a homogenizer, which breaks the cells and releases the contents within the cells. Since the *P. bahamense* culture was not treated with any antibiotics, the free-living and associated extracellular bacteria were included in the isolation process along with the intra-cellular bacteria. Different isolation techniques could result in isolating different type of bacteria species.

The identification of the bacteria associated with *P. bahamense* in this study has helped examine potential bacteria associated with the HAB process, as well as putatively toxin-producing bacteria associated with toxic dinoflagellate. Despite the hypothesis of the association between marine bacteria and toxic harmful algal bloom, the role of the marine bacteria during a toxic bloom event is still uncertain. As a result, further study can be conducted on the isolated associated bacteria in this study to provide more insights into the roles of bacteria in HAB and toxin production during a bloom.

## CONCLUSION

This study revealed the microbial diversity associated with an important paralytic shellfish toxin-producing species in Malaysia, *Pyrodinium bahamense* var. compressum, which was from three phyla: Proteobacteria, Bacteroidetes and Actinobacteria. The major bacterial species identified included *Marinobacter salsuginis*, *Mameliella atlantica* and *Roseibium denhamense*, whereas *Muricauda lutimaris*, *Roseibium hamelinense* and *Micrococcus luteus* were identified as minor isolates. Based on previous studies, the bacterium from the present study, *M. luteus* was revealed to be a potential bacterium linked with paralytic shellfish toxin production. As a result of the findings provided here, we now have a better grasp of the diversity of bacteria associated with toxic *P. bahamense*, which may help us understand their role in toxin production during a bloom event. As a follow-up to this study, additional genomic analysis of the probable paralytic shellfish toxin-producing bacteria, *M. luteus* will be carried out.

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**SUPPLEMENTARY INFORMATION**

**Table S1:** Sequence similarity results of the 16S rRNA gene of the 62 bacteria isolated from PBVC culture against the NCBI database, showing the closest relative, the accession number of the reference in the NCBI Genbank and the percentage similarity.

| Strain ID | Accession no. | Isolates length | Closest match in GenBank (Accession No.) | ID (%) |
|---|---|---|---|---|
| SY2 | MH507081 | 435 | Gamma-Proteobacteria | 99 |
| SY3 | MH507082 | 433 | *Marinobacter salsuginis* | |
| SY4 | MH507083 | 431 | (NR_044044) | |
| SY5 | MH507084 | 432 | | |
| SY6 | MH507085 | 432 | | |
| SY12 | MH507090 | 422 | | |
| SY13 | MH507091 | 425 | | |
| SY14 | MH507092 | 427 | | |
| SY20 | MH507096 | 428 | | |
| SY27 | MH507102 | 429 | | |
| SY28 | MH507103 | 433 | | |
| SY29 | MH507104 | 424 | | |
| SY30 | MH507105 | 453 | | |
| SY31 | MH507106 | 434 | | |
| SY32 | MH507107 | 450 | | |
| SY33 | MH507108 | 425 | | |
| SY34 | MH507109 | 418 | | |
| SY35 | MH507110 | 419 | | |
| SY39 | MH507112 | 434 | | |
| SY42 | MH507115 | 435 | | |
| SY43 | MH507116 | 430 | | |
| SY44 | MH507117 | 430 | | |
| SY45 | MH507118 | 428 | | |
| SY46 | MH507119 | 425 | | |
| SY47 | MH507120 | 425 | | |
| SY48 | MH507121 | 432 | | |
| SY50 | MH507123 | 431 | | |
| SY1 | MH507080 | 410 | Alpha-Proteobacteria | 97–99 |
| SY9 | MH507087 | 404 | *Mameliella atlantica* | |
| SY11 | MH507089 | 409 | (NR_136489) | |
| SY18 | MH507095 | 395 | | |
| SY41 | MH507114 | 428 | | |
| SY49 | MH507122 | 405 | | |

(Continued)

| Strain ID | Accession no. | Isolates length | Closest match in GenBank (Accession No.) | ID (%) |
|---|---|---|---|---|
| SY53 | MH507126 | 411 | | |
| SY56 | MH507129 | 402 | | |
| SY60 | MH507133 | 399 | | |
| SY61 | MH507134 | 408 | | |
| SY62 | MH507135 | 399 | | |
| SY63 | MH507136 | 400 | | |
| SY68 | MH507141 | 399 | | |
| SY16 | MH507093 | 403 | Alpha-Proteobacteria | 99 |
| SY17 | MH507094 | 411 | *Roseibium denhamense* | |
| SY51 | MH507124 | 409 | (NR_025885) | |
| SY52 | MH507125 | 408 | | |
| SY54 | MH507127 | 413 | | |
| SY55 | MH507128 | 406 | | |
| SY57 | MH507130 | 409 | | |
| SY64 | MH507137 | 410 | | |
| SY65 | MH507138 | 408 | | |
| SY66 | MH507139 | 411 | | |
| SY10 | MH507088 | 400 | Alpha-Proteobacteria | |
| SY36 | MH507111 | 402 | *Roseibium hamelinense* | |
| SY40 | MH507113 | 403 | (NR_043436) | |
| SY58 | MH507131 | 400 | | |
| SY59 | MH507132 | 415 | | |
| SY8 | MH507086 | 433 | Bacteroidetes | 97–99 |
| SY21 | MH507097 | 416 | *Muricauda lutimaris* | |
| SY23 | MH507098 | 415 | (NR_044423) | |
| SY24 | MH507099 | 419 | | |
| SY25 | MH507100 | 430 | | |
| SY26 | MH507101 | 428 | | |
| SY67 | MH507140 | 412 | Actinobacteria<br>*Micrococcus luteus*<br>(NR_075062) | 99 |

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