DETECTION OF ANATOXIN-A ENCODING GENE IN ISOLATED CYANOBACTERIAL STRAINS FROM MALAYSIA

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ABSTRACT

Cyanobacterial blooms have been occasionally observed in most of water bodies. These blooms represent a major ecological and human health problems around the world due to the ability of some cyanobacteria can produce toxins. The production of the cyanobacterial toxins are gene-specific, not species-specific. Thus morphological identification is not reliable to determine the toxicity of the cyanobacteria. As part to determine the status of cyanobacterial toxins in Malaysia, cyanobacterial strains were isolated from different environments in Malaysia and identified using cyanobacterial 16S rRNA gene sequence. PCR assay was carried out to detect the presence of cyanobacterial toxin-encoding genes in these isolated strains by amplifying genes encoded for microcystin, anatoxin-a, cylindrospermopsin and saxitoxin. Using molecular identification of 16S rRNA gene sequences, a total of forty-two cyanobacterial strains were identified, which belongs to eighteen different genera of *Synechococcus*, *Cyanobium*, *Synechocystis*, *Chroococcidiopsis*, *Leptolyngbya*, *Nodosilinea*, *Limnothrix*, *Pseudanabaena*, *Cephalothrix*, *Aerosakkonema*, *Oscillatoria*, *Alkalinema*, *Pantanalinema*, *Planktolyngbya*, *Scytonema*, *Nostoc*, *Hapalosiphon* and *Symphyonemopsis*. The toxicity of these strains were tested using PCR amplification of toxin-encoding genes using specific primers. Anatoxin-a (ATX) gene, which involved in the biosynthesis of anatoxin-a was detected in two isolated strains namely *Limnothrix* sp. B15 and *Leptolyngbya* sp. D1C10. Thus, toxin-encoding gene was successfully detected in the isolated cyanobacterial using PCR amplification of specific sequences. Cyanobacteria producing toxin and non-toxin producing in these isolated strains can also be distinguished using this technique. Through this study, the presence of cyanobacterial toxin in Malaysia can now be determined as potential threat because anatoxin-a-encoding gene was detected in this study and the status of cyanobacterial toxins in Malaysia can now be clarified.

Keywords: Anatoxin-a, cyanobacterial 16S rRNA, cyanobacterial toxins, PCR amplification, toxin-encoding gene.

INTRODUCTION

Cyanobacteria also known as blue-green algae is a photosynthetic bacteria and can be found in the most of water column. They can multiply rapidly in water surface and form blooms when in large population. Cyanobacterial blooms can be found in eutrophic water bodies of freshwater, estuarine as well as marine ecosystem. As an examples, cyanobacterial blooms in freshwater ecosystem mainly consisted of *Microcystis*, *Anabaena* or *Cylindrospermopsis,* while estuarine ecosystems mainly consisted of *Nodualria* and *Aphanizomenon.* Cyanobacterial blooms in marine ecosystem consisted of *Lyngbya*, *Synechococcus* and *Trichodesmium* [9].

The blooms can affect water quality to be degraded as their presence will cause the foul odors and tastes, deoxygenation of bottom waters, toxicity, aquatics kills and disruption of food web [16]. They also can produce toxins also known as cyanotoxins that severely affect animals as well as human health [10]. A wide range of toxins produced by cyanobacteria can be classified into few distinct categories in terms of their structure and mode of action. The toxins can be divided into hepatotoxins; microcystins and nodularins, cytotoxins; cylindrospermopsins, neurotoxins; anatoxins and saxitoxin, and dermatotoxins; lyngbyatoxins [4]. The problems with the cyanobacterial toxins is that the waters is unsuitable for multi-purposes even for irrigation because the toxins is persists in waters even after the disappearance or death of the algal blooms [13].

Identification of cyanobacterial strains based on morphological characteristics and 16S rRNA gene sequences can be used to detect the presence of cyanobacteria in the samples as well as to identify unknown cyanobacterial strains, but does not recognized whether the strains are toxin-producing or non-toxin-producing cyanobacteria because production of cyanotoxin is not specific to species, but gene-specific. Thus, to identify the potential toxin producers in cyanobacteria, detection of toxin-encoding gene using PCR amplification of specific sequences is likely the best options.

METHODOLOGY

Sampling locations

The sampling locations in this study involved three states in Malaysia which were Penang, Perak, and Sarawak due to the easy accessibility to the sampling locations. Sampling points were selected based on the place that potentially exposed to human activity either for recreational or drinking water purposes. The Air Itam Dam, Teluk Bahang Dam, Waterfall Reservoir, Mengkuang Dam, Banding Lake, Teluk Intan, Kuala Gula Sanctuary, Bukit Merah Lake and Miri, Sarawak.

Isolation and culturing of cyanobacterial strains

Sample was collected from selected sampling sites. Cyanobacteria were isolated by numerous streaking on BG 11 agar plates until single species was obtained. Isolates were maintained in sterile liquid or slanted agar BG 11 media at ambient temperature.

Extraction and purification of genomic DNA

The methods used to extract genomic DNA from cyanobacterial was identical to other eubacteria. To extract DNA from the sample, a commercial kit; Bacterial DNA Extraction Kits (Vivantis Technologies, Malaysia) was used. The

procedures were carried out according to handbook provided by the manufacturer. Extracted DNA was stored at -20^{o}C until required to prevent DNA degradation in the absence of buffering agent.

Molecular identification using cyanobacterial 16S rRNA gene

Isolated strains was identified using 16S rRNA gene sequence analysis using primer pairs CYA106F, CYA781R(a) and CYA781R(b). PCR protocols to amplify cyanobacterial 16S rRNA involved an initial denaturation for 2 min at 95°C; followed by 30 cycles, each consisting of 60 s at 94°C, 60 s at 60°C, and 60 s at 72°C; and a final extension of 7 min at 72°C [8].

Molecular detection of toxin-encoding genes

The samples were tested for the presence of generic microcystin (*mcyE*) gene, polyketide synthase (PKS) and peptide synthetase (PS) genes, the genes encoding anatoxin-a, cylindrospermopsin and saxitoxin. Generic microcystin (*mcyE*) gene amplified using forward primer; mcyE-F2 combined with reverse primer; mcyE-R4 [11, 12]. PCR protocols was performed as followed: The first step was an initial denaturation step of 2 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C and a final extension of 10 min at 72°C [12]. Polyketide synthase regions (PKS) gene was amplified using degenerate oligonucleotide forward primer; DKF and reverse primer; DKR [6]. PCR protocols to amplify PKS gene involved an initial denaturation for 2 min at 95°C; followed by 30 cycles, each consisting of 10 s at 94°C, 20 s at 50°C, and 60 s at 72°C; and a final extension of 7 min at 72°C (Schembri et al., 2001). For samples positive general PKS gene, PKS specific primer pairs were used to detect genes encodes for anatoxin-a and cylindrospermopsin. The PKS specific primer pair of atxoaf and atxar was used to amplify the gene encodes for the PKS fragment of the putative anatoxin-a (ATX) biosynthesis gene cluster. PCR protocols was performed as followed: The first step was an initial denaturation step of 2 min at 95°C followed by 30 cycles of 10 s at 94°C, 20 s at 55°C and 60 s at 72°C and a final extension of 10 min at 72°C [1]. Another PKS specific-cylindrospermopsin primers, M4 and M5 were used to detect the presence of cylindrospermopsin gene. PCR protocols was performed as followed: The first step was an initial denaturation step of 2 min at 95°C followed by 30 cycles of 10 s at 94°C, 20 s at 55°C and 60 s at 72 °C and a final extension of 7 min at 72°C [14]. Peptide synthetase (PS) degenerate primers; MTF2 and MTR were used to amplify the general PS regions. PCR protocols was performed as followed: The first step was an initial denaturation step of 4 minutes at 94°C followed by 30 cycles of 10 s at 94°C, 20 s at 50°C and 60 s at 72°C and a final extension of 7 minutes at 72°C [14]. Primers pair of sxtaf and sxtar used to amplify saxitoxin (*sxtA*) gene cluster. PCR protocols was performed as followed: The first step was an initial denaturation step of 5 minutes at 94°C followed by 30 cycles of 10 s at 94°C, 20 s at 55°C and 60 s at 72°C and a final extension of 10 minutes at 72°C [2].

All PCR amplifications were performed with a Mastercycler® ep PCR System (Eppendorf, Germany). PCR reaction were carried out in 25 µL reaction mixtures containing 12.5 µL of 2X Taq Master Mix (Vivantis Technologies, Malaysia), 0.25 µL of each forward and reverse primers and 2 µl of DNA sample combined with sterile distilled water to make up a volume of 25 µL of total reaction. 2X Taq Master Mix consisted of 0.05 u/µl, 2X Vibuffer A, 0.4 mM dNTPs and 3.0 mM $MgCl_2$. All primers used in this study were synthesized by Integrated DNA Technologies, US.

After PCR amplification, the products were loaded on 1% agarose gel that was prepared by adding 0.25 g of agarose (Vivantis Technologies, Malaysia) to 25 mL of 1 x TBE buffer. 2.0 ul of gel stain (TransGen Biotech Co., Ltd, Beijing) added into the hot agar. 2ul of 6X loading dye (Vivantis Technologies, Malaysia) added into the PCR products. Gels were ran at 70 V for 40 minutes and viewed using a gel imaging system (Gel Doc^{TM} XR^{+} camera, Image Lab^{TM} software, BioRad) and the gel image was captured.

Purification of PCR product and sequencing

To purify DNA from the PCR product, a commercial kit; Ambiclean Kits – PCR & Gel (Vivantis Technologies, Malaysia) was used. The procedures were carried out according to handbook provided by the manufacturer. The purified DNA was stored at -20^{o}C before sent to Center for Chemical Biology, USM (CCB) for sequencing using the same primers used for PCR amplification. Sequence similarities between 16S rRNA gene sequences were compared with the available data from National Centre for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using nucleotide basic local alignment search tool (BLAST).

RESULTS

Identification of isolated cyanobacterial strains using molecular analysis

Total of forty-two strains were isolated from different locations in Malaysia. The isolated stains were unicyanobacterial but nonaxenic cultures. These strains were identified using molecular approaches of 16S rRNA gene sequences. Molecular identification was done due to limitation of morphological identification. In this study, a combination of primer pair, CYA106F, CYA781R(a) and CYA781R(b) were used to amplify a 654-699 bp of DNA fragment of isolated species. The PCR products were run by agarose gel electrophoresis and viewed under UV light to ensure that the gene had been amplified and produced a right size for cyanobacterial 16S rRNA gene. The PCR products were purified and sequenced. The similarities of the isolated species with the species available in NCBI GenBank was compared for the molecular identification.

Detection of toxin-encoding genes in isolated strains

All forty-two isolated strains had been identified using cyanobacterial 16S rRNA gene. Due to the presence of toxin-encoding gene in some environmental samples and some the strains have history of producing some cyanotoxins these strains were tested for the presence of the toxin-encoding genes. Results for detection of toxin-encoding genes in isolated strains are shown in Table 1.

Table 1: Summary results for detection of toxin-encoding genes in isolated strains.

No.	Cyanobacterial strains	Generic microcystin (*mcyE*) gene	Generic PKS gene	PKS specific-cylindro-spermopsin	PKS specific-anatoxin (ATX)	Generic PS gene	saxitoxin (*sxtA*) gene

				(CYN) gene	**gene**		
1.	*Synechococcus* sp. EO68	-	-	n.a	n.a	-	-
2.	*Synechococcus* sp. M1	-	-	n.a	n.a	-	-
3.	*Synechococcus elongatus* CENA126	-	-	n.a	n.a	-	-
4.	*Synechococcus lividus* C1	-	-	n.a	n.a	-	-
5.	Prochlorales cyanobacterium EV-7	-	-	n.a	n.a	-	-
6.	*Cyanobium* sp. CENA13	-	-	n.a	n.a	-	-
7.	*Synechocystis aquatilis* 1LT32S04	-	-	n.a	n.a	-	-
8.	*Synechocystis* sp. CCALA 700	-	-	n.a	n.a	-	-
9.	*Chroococcidiopsis thermalis* SAG 42.79	-	-	n.a	n.a	-	-
10.	*Leptolyngbya frigida* ANT.L52B.3	-	-	n.a	n.a	-	-
11.	*Leptolyngbya* sp. D1C10	-	+	-	+	+	-
12.	*Leptolyngbya* sp. S1C4	-	-	n.a	n.a	-	-
13.	*Leptolyngbya* sp. JS2	-	-	n.a	n.a	-	-
14.	*Leptolyngbya subtilissima* EcFYyyy700	-	-	n.a	n.a	-	-
15.	*Leptolyngbya* sp. Tir_cyanD	-	-	n.a	n.a	-	-
16.	*Leptolyngbya* sp. CENA520	-	-	n.a	n.a	-	-
17.	*Nodosilinea nodulosa* UTEX 2910	-	-	n.a	n.a	-	-
18.	*Nodosilinea* cf. *nodulosa* LEGE 10377	-	-	n.a	n.a	-	-
19.	*Nodosilinea* sp. IS-EAG2	-	-	n.a	n.a	-	-
20.	*Limnothrix redekei* BTA657	-	-	n.a	n.a	-	-
21.	*Limnothrix planktonica* CHAB763	-	+	-	-	-	-
22.	*Limnothrix* sp. B15	-	+	-	+	-	-
23.	*Limnothrix redekei* BTA65	-	-	n.a	n.a	-	-
24.	*Limnothrix* sp. PUPCCC 116.2	-	-	n.a	n.a	-	-
25.	*Pseudanabaena thermalis* PUPCCC 106.4	-	-	n.a	n.a	-	-
26.	*Pseudanabaena* sp. 40C_1_20	-	-	n.a	n.a	-	-
27.	*Pseudanabaena mucicola* PMC269.06	-	-	n.a	n.a	-	-
28.	*Pseudanabaena limnetica* PUPCCC 106.2	-	-	n.a	n.a	-	-
29.	*Cephalothrix lacustris* CCIBt 3261	-	-	n.a	n.a	-	-
30.	*Cephalothrix komarekiana* SAG 75.79	-	-	n.a	n.a	-	-
31.	*Aerosakkonema funiforme* strain Lao26	-	-	n.a	n.a	-	-
32.	*Oscillatoria* sp. OF9	-	-	n.a	n.a	-	-
33.	Oscillatoriales cyanobacterium OF9	-	-	n.a	n.a	-	-
34.	*Oscillatoria acuminata* PCC 6304	-	-	n.a	n.a	-	-
35.	*Alkalinema pantanalense* CENA531	-	-	n.a	n.a	+	-
36.	*Pantanalinema rosaneae* CENA539	-	-	n.a	n.a	-	-
37.	*Planktolyngbya circumcreta* CHAB5683	-	-	n.a	n.a	+	-
38.	*Scytonema sp.* U-3-3	-	-	n.a	n.a	-	-
39.	*Hapalosiphon welwitschii* M5	-	-	n.a	n.a	-	-
40.	*Westiellopsis* sp. NQAIF324	-	-	n.a	n.a	-	-
41.	*Mastigocladopsis* sp. CCG2	-	-	n.a	n.a	-	-
42.	*Nostoc* sp. TO1S01	-	-	n.a	n.a	+	-

'+' indicates the positive result while '–'indicates the negative result and 'n.a' indicates no reaction was carried out.

Detection of generic polyketide synthase (PKS) gene in isolated strains

All forty-two isolated strains were tested for the presence of generic polyketide synthase (PKS) gene using the same degenerate primer pair of DKF and DKR. Amplification of this gene generated a DNA fragment of 650 bp. Among forty-two isolated strains tested, only three strains positive to the generic PKS gene namely *Leptolyngbya* sp. D1C10, *Limnothrix planktonica* CHAB763 and *Limnothrix* sp. B15, while other strains show negative results (Table 1). Amplification of generic PKS gene in these isolated strains was shown in Figure 1.

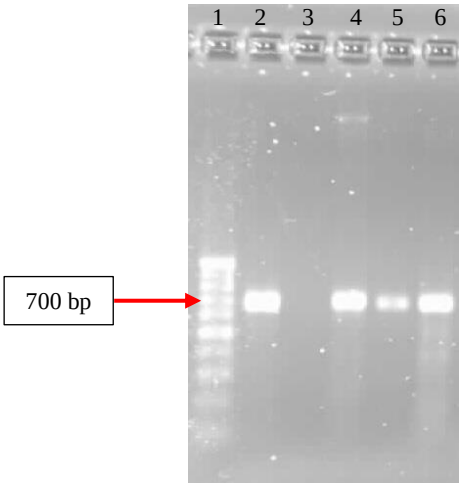

Figure 1. Agarose gel electrophoresis image of 650 bp of PCR product amplified using PKS-degenerate primer pair; DKF and DKR on DNA extracts from isolated cyanobacterial strains. 1 = Ladder (VC 100bp); 2 = Positive control; 3 = Negative control; 4 *Leptolyngbya* sp. D1C10; 5 = *Limnothrix* sp. B15 and 6 = *Limnothrix planktonica* CHAB763.

PCR product positive to generic PKS gene for both strains *Leptolyngbya* sp. D1C10 and *Limnothrix* sp. B15 were purified and sent for sequencing. Sequences obtained from the sequencing analysis were compared to the available database in NCBI using BLAST. Sequences with query length of 653 bp and 582 bp shows highest percentage similarity of 74% and 79% that identical to *Pseudanabaena* cf. *curta* LEGE 07160 clone 1 polyketide synthase gene and *Oscillatoria* sp. PCC 6506 anatoxin-a and homoanatoxin-a biosynthetic gene cluster (sequence data not shown).

Detection of PKS specific-anatoxin (ATX) and cylindrospermopsin (CYN) genes in isolated strains

Following the presence of generic PKS gene, *Leptolyngbya* sp. D1C10, *Limnothrix planktonica* CHAB763 and *Limnothrix* sp. B15 were tested to detect the presence of PKS specific-anatoxin (ATX) and cylindrospermopsin (CYN) genes. Primer pair of atxoaf and atxar used to amplify PKS specific-anatoxin (ATX) gene, while primer pair of M4 and M5 used for the amplification of PKS specific-cylindrospermopsin (CYN) gene. Amplification of both PKS specific gene generated DNA fragment of 434 bp and 650-725 bp for PKS specific to ATX and CYN genes, respectively. PKS specific-anatoxin (ATX) gene was detected in two strains out of three tested strains namely *Leptolyngbya* sp. D1C10 and *Limnothrix* sp. B15 (Figure 2). For PKS specific-cylindrospermopsin (CYN) gene, none of these strains was detected for the presence of this gene as DNA was not visible on the gel electrophoresis images (data not shown).

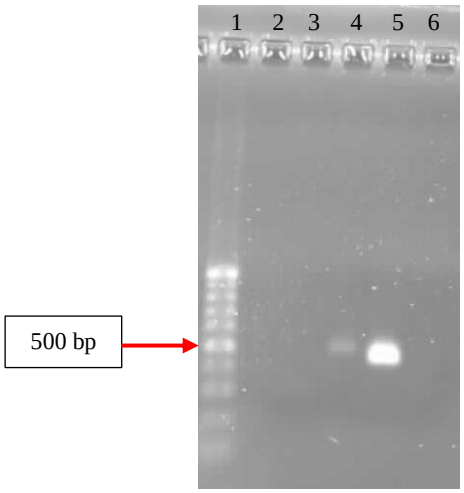

Figure 2. Agarose gel electrophoresis image of 434 bp of PCR product amplified using PKS-specific to anatoxin (ATX) gene primer pair; atxoaf and atxar on DNA extracts from isolated cyanobacterial strains. DNA was visible for *Leptolyngbya* sp. D1C10 (Lane 4) and *Limnothrix* sp. B15 (Lane 5). 1 = Ladder (VC 100bp); 2 = Empty; 3 = Negative control; 4 = *Leptolyngbya* sp. D1C10; 5 = *Limnothrix* sp. B15; 6 = *Limnothrix planktonica* CHAB763.

PCR product positive to PKS specific-anatoxin (ATX) gene for both strains *Leptolyngbya* sp. D1C10 and *Limnothrix* sp. B15 were purified and sequenced. Sequences obtained from the sequencing analysis were compared to the available database in NCBI using BLAST. Sequences with query length of 453 bp and 437 bp showed highest percentage similarity of 100% and 94% that identical to *Anabaena* sp. 37 anatoxin-a synthetase gene cluster and *Oscillatoria* sp. PCC 6506 anatoxin-a and homoanatoxin-a biosynthetic gene cluster (sequence data not shown).

Detection of generic peptide synthetase (PS) gene in isolated strains

All isolated strains was also tested for the presence of generic peptide synthetase (PS) gene. Amplification of generic PS gene generate a DNA fragment of 1150 bp. Among forty-two isolated strains, four of them was positive for the detection of generic PS gene (Figure 3). These strains namely *Leptolyngbya* sp. D1C10, *Alkalinema pantanalense* CENA531, *Planktolyngbya circumcreta* CHAB5683 and *Nostoc* sp. TO1S01. While other strains tested negative for the detection of generic PS gene. (Table 1). This positive result indicated the presence of the generic PS gene in the isolated strains.

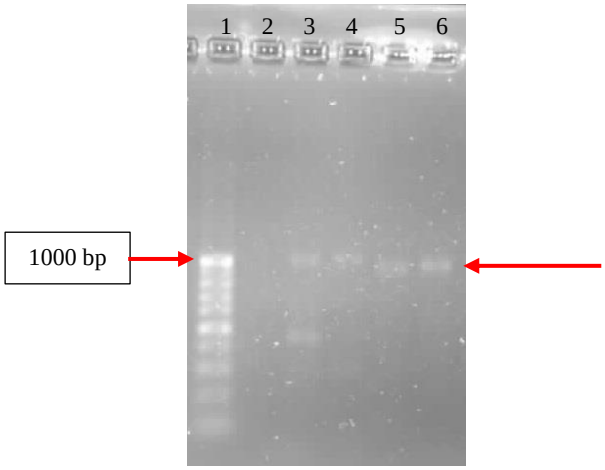

Figure 3. Agarose gel electrophoresis image of 1150 bp of PCR product amplified using PS degenerate primer pair; MTF2 and MTR primer; on DNA extracts from isolated cyanobacterial strains. 1 = Ladder (VC 100bp); 2 = Negative control; 3 = *Leptolyngbya* sp. D1C10; 4 = *Alkalinema pantanalense* CENA531; 5 = *Planktolyngbya circumcreta* CHAB5683; 6 = *Nostoc* sp. TO1S01.

Detection of microcystin (*mcyE)* gene and saxitoxin (*sxtA*) gene in isolated strains

All isolated strains were tested for the presence of generic microcystin (*mcyE*) gene and saxitoxin (*sxtA*) gene cluster. Amplification of *mcyE* gene using primer pair of mcyE-F2 and mcyE-R4 generate a DNA fragment of 809-812 bp, while amplification of saxitoxin (*sxtA*) gene cluster generated a DNA fragment of 650 bp using primer pairs of sxtaf with sxtar. None of them tested positive for microcystin (*mcyE*) and saxitoxin (*sxtA*) gene (Table 1). Thus, the negative results indicates that these genes does not present in the isolated strains.

DISCUSSION

Toxin producing and non-toxin producing cyanobacterial strains can be distinguished using the specific primers to amplify the presence of toxin-encoding genes. Some strains were reported to be non-toxic, but never been tested, thus this study was carried out to prove the that by testing the species and confirming the negative results. Many cyanobacterial strains have not been reported to produce toxin, but no studies were carried out to prove that the strains does not posses the gene producing the toxin. The status of toxin producing in some cyanobacterial can now be clarified through this study.

16S rRNA gene can be used to detect the presence cyanobacteria in a samples as well as to identify unknown cyanobacterial. However the method does not distinguish between toxic and non-toxic bacteria [7]. Toxin production in cyanobacterial is gene-specific rather than species-specific [3], thus identifying the species does not indicates the status of the toxin in the strains.

From the results out of forty-two isolated strains, anatoxin-a (ATX) gene was detected in two strains isolated from Malaysia. This gene was detected in two strains namely *Limnothrix* sp. B15 and *Leptolyngbya* sp. D1C10, while other strains shows the absence of toxin-encoding gene. This is the first times, anatoxin-a gene was detected in Malaysia. A PCR analysis was specifically developed for detection of a polyketide synthase (PKS) gene fragment of putative anatoxin biosynthesis gene clusters in both *Limnothrix* sp. B15 and *Leptolyngbya* sp. D1C10 strains.

Generic PKS gene was also detected in *Limnothrix planktonica* CHAB763, but tested negative for detection of PKS specific to anatoxin-a and cylindrospermopsin genes. From the sequence of the amplified PCR product of generic PKS gene, showed similarity of 85% *to Phormidium ectocarpi* SAG 60.90 clone 2 type I polyketide synthase gene. This result confirming the presence of the PKS gene in the samples, but none specific toxin were given, indicated that the gene might be new toxin-encoding gene which were not detected in this study. PS gene was also detected in four cyanobacterial strains namely *Leptolyngbya* sp. D1C10, *Alkalinema pantanalense* CENA531, *Planktolyngbya circumcreta* CHAB5683 and *Nostoc* sp. TO1S01. Both generic PKS and PS genes were detected in *Leptolyngbya* sp. D1C10. This result can implies that both PKS and PS cluster genes were responsible for the production of anatoxin-a (ATX) gene.

Several genera of *Anabaena, Anabaenopsis, Aphanocapsa, Arthrospira, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix, Snowella,* and *Woronichinia* has been described to produce hepatotoxic microcystin [15]. Although *Hapalosiphon, Nostoc,* and *Oscillatoria* were identified in the isolated cyanobacterial but these strains were described as non-toxic because detection of *mcyE* gene resulted a negative detection. *mcyE* gene was responsible for the biosynthesis of microcystin. *Anabaena, Aphanizomenon, Arthrospira, Cylindrospermum, Microcystis, Oscillatoria, Phormidium, Planktothrix* and *Raphidiopsis* were known to produce anatoxin-a [15]. Most of these anatoxin-a producers was absence in the study, except two strains were identified as *Oscillatoria* spp. by 16S rRNA gene sequence analysis, but detection of anatoxin-a gene showed negative result. Thus, this strain can be determined as non-toxic. Cylindrospermopsin production has been identified in several genera of *Anabaena, Aphanizomenon, Cylindrospermopsis, Raphidiopsis,* and

Umezakia [15]. But none of these toxic genera were identified and from the detection study no cylindrospermopsin gene was detected as well, thus it can conclude that no cylindrospermopsin gene was present in these isolated strains. Saxitoxin production has been reported in genera of *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Planktothrix* and *Cylindrospermopsis* [15]. Thus, according to negative result for the detection of this gene can proved as none of these genera were isolated and identified in this study. This results can be used to confirm to absence of these saxitoxin gene in other cyanobacterial genera.

The data presented for the detection of toxin-encoding gene can be proved that that production of toxin in cyanobacteria was gene-specific, not species-specific. Although some species or genera have histories of toxin production, but detection of toxin-encoding gene in these strains resulted negative outcomes due to the absence of these genes in the species.

In order to differentiate both toxic and non-toxic cyanobacteria, PCR amplification of specific gene sequence could be the best options. However, there are still some limitation within this approach. This conventional PCR amplification are not able to quantify the numbers of gene present the samples as well as the toxicity of the toxin-encoding gene. Presence of a toxin-encoding gene does not necessarily mean the existence of the toxin. Some strains might contain a certain toxin-encoding gene, but the toxin are not express due to some of the factors influencing the gene expression.

Toxicity of these gene can be tested using analytical or chemical analysis such as HPLC, GC-MS, immunology method, ELISA and high photodiode arrays detection in confirming and quantifying toxic content in environment [5]. However, most of these detection can only be applied to detect the toxin in the environment. Toxins can easily degraded in the environment and the half-life of these toxins is very short ranging from four to eight hours only, caused the analytic detection is rather difficult.

CONCLUSION

Toxin producing and non-toxin producing cyanobacterial strains can be distinguished using the molecular analysis by amplifying the toxin-encoding genes sequence. PKS specific to anatoxin-a gene was detected in two isolated strains by PCR amplification using specific primers. Potential ability of these strains to produce toxins also been determined using molecular analysis.

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