Molecular Detection of Toxogenic Cyanobacteria Isolated from Tigris River in Baghdad City –Iraq

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Abstract

Algae and their contamination are being increasingly reported worldwide that cause a serious hazard to environmental and human health. Cyanotoxin was the most algal toxin reported to be produce by several orders of cyanobacteria. In 2017 cyanobacteria were isolated from fresh water of Tigris River and identified by light compound microscope as well as conventional PCR. Five isolates of cyanobacteria which successfully amplified a gene fragment from the phycocyanin shared by all cyanobacteria and only four isolates successfully amplified a gene fragment from the myc E belonged to microcystin. Our results concluded that PCR assay can be used for early detection of microcystin producing algae in fresh water that useful to stations responsible for the preparation of drinking water.

Keywords: *Algae, Cyanotoxin, phycocyanin and microcystin.*

Introduction

Cyanobacteria, cyanophytes or blue green algae are widely distributed in natural environments and are considered a major component of microbial populations in terrestrial and aquatic habitats worldwide, Harmful algal blooms have been identified in fresh water, Estuarine, and marine system. In fresh water some cyanobacterial may produce dermal toxins, neurotoxins and hepatotoxins which including nodularins and microcystins (1).

They are also an interesting functional food source (2) . These microbes have also been reported to be rich sources of healthy nutrients such as proteins, carbohydrates, vitamins, minerals amino acids, and fatty acids.

Among all the cyanotoxins, microcystins are the most frequently studied because of their wide distribution and high toxicity. Up to now, more than 80 different structural variants have been identified, among which microcystin-LR is the most common and potent variant, followed by microcystin-RR and microcystin-YR (3).

Monitoring systems are needed to prevent water users from these toxins. Good methods, such as ELISA and high performance liquid chromatography have been recorded for most cyanotoxins, but they extremely use laborious sample preparation protocols as well as priced machinery and purified toxin standards that are often difficult to obtain. Nevertheless, molecular detection techniques such as conventional PCR, quantitative real-time PCR and microarrays/DNA chips that are rapid, extremely sensitive and specific for detecting toxic cyanobacteria in water supplies (4; 5).

The delivery of phycocyanin in the cyanobacterial makes the study of phycocyanin genes good idea for the classification of cyanobacteria (6). Phycocyanin operon contains genes coding for two bilin subunits and three linker polypeptides. The intergenic spacer (IGS) between the two bilin subunit genes, designated as b (*cpcB*) and a (*cpcA*) showed variations in their sequences which are useful of differentiating genotypes below the generic level make it capable for the identification of cyanobacteria via PCR (7).

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Conventional PCR could be usful to detect microcystin producers and several primers are available [8]. *mcy*E primers were successfully used to get PCR product from all known microcystin and nodularin producers [9].

Baghdad city have two main sources of drinking water for Iraq, Tigris River is the most important once in serving population approximately seven million people settled in this city, this river usually affected by industrial eutrification as well as the sewage effluent and agricultural which provide enhancement of cyanobacteria growth and potential mycrocystin production. Therefore, this study was aimed to use Molecular PCR technique for identification of cyanobacteria and mycrocystin producing isolates from Tigris River for early detection of toxic species that could be useful to companies responsible for the provider of drinking water to this city.

Material and Method

Water samples were collected from the higher superficial layer of Tigris River from a depth of 20-30 cm monthly in April 2017 by using 20µ mesh net. Sampling site located in civil region in AL-Greaat region near AL-Greaat bridge , located on longitude 44°20'37.52"E and latitude 33°25'3.49"N.Samples were transported to the laboratory on ice. 10 ml of water sample was added to chu-10 culture medium and incubated at 28ºC for two weeks with continuous illumination of 50 µE/m²/s. one ml of growth inoculated on agar plates containing BG-11 and incubated in the same condition for one week to isolate unialgal [10]. Microscopic examination were performed to ensure the culture were unialgal.

Extraction of DNA from chlorophyta and cyanbacterial isolates

Genomic DNA was extracted from the chlorophyta isolates for specificity test using CTAB method [11]. While the Genomic DNA was extracted from the cyanobacterial isolates using the genomic DNA mini Kit(plant)

Polymerase chain reaction test

Polymerase chain reaction was performed with two sets of primers. PCβF (GGCTGCTTGTTTACGCGACA) and PCαR (CCAGTACCACCAGCA ACTAA) [12] to amplify *cpcB*-IGC-*cpcA* region in phycocyanin operon while the HEPF (TTTGGGGTTAACTTTTTTGGG CATAGTC) and HEPR (AATTCTTGAGGCTGTAAATCGGGTTT) [13] used to amplify *mcy*E gene of the microcystin synthetase. PCR protpcols involved an initial denaturation for 2 min at 95ºC; 35 cycles of denaturation for 90 sec at 95ºC, annealing for 30 sec at 52ºC (PCβ-PCα primer set) and for 90 sec at 95ºC(HEP primer set), extension for 1min at 72ºC and final extension for 8 min at 72ºC. 10µl of PCR product was separate in 1.5% agarose gel electrophoresis stained with ethidum bromide and visualized on a UV transilluminator, the size of amplified products were compared with the 100pb DNA ladder to determine the exact size of these products.

Results

Isolation and identification of algae from water samples five isolates of cyanobacteria were obtained from the Tigris River included, *Westellopsis sp , Oscillatoria sp* , *Spirulina sp , Chroococcus sp and Lyngbya sp* . Which belonged to four cyanobacterial orders: Oscillatoriales, Chroococales, Stigonematales and Nostocales as well as one isolate of chlorophyceae included *Cladophora glomereta* (Macro algae) where used as negative control test.

Molecular detection of cyanobacteria by PCR test

The gene fragment of the phycocyanin operon containing the IGS (*cpcBA*-IGC) from cyanobacteria was amplified. A distinct amplicon patterns was produced from all of the DNA extracts with a size of 650 bp when analyzed in gel electrophoresis (Fig. 1), confirming the presence of cyanobacterial DNA from isolates collected from fresh water of Tigris River in Baghdad. While lysates of a green alga *Cladophora glomereta* does not possess pycocyanin operon, gave no PCR product suggested the highly specificity of used primers.

Detection of Microcystin by PCR assay

In this study, conventional PCR used as a tool to identify potentially microcystin producing cyanobacteria possess aminotransferase enzyme. The HEP primers were successfully amplified the 472 bp fragments of *mcy* E gene from all microcystin-producing cyanobacterial isolates except *spirulina sp.* (Fig. 2). The specificity of HEP primers appeared to be highly specific for isolates producing microcystin since there was no DNA amplified from chlorophyta used in this study.

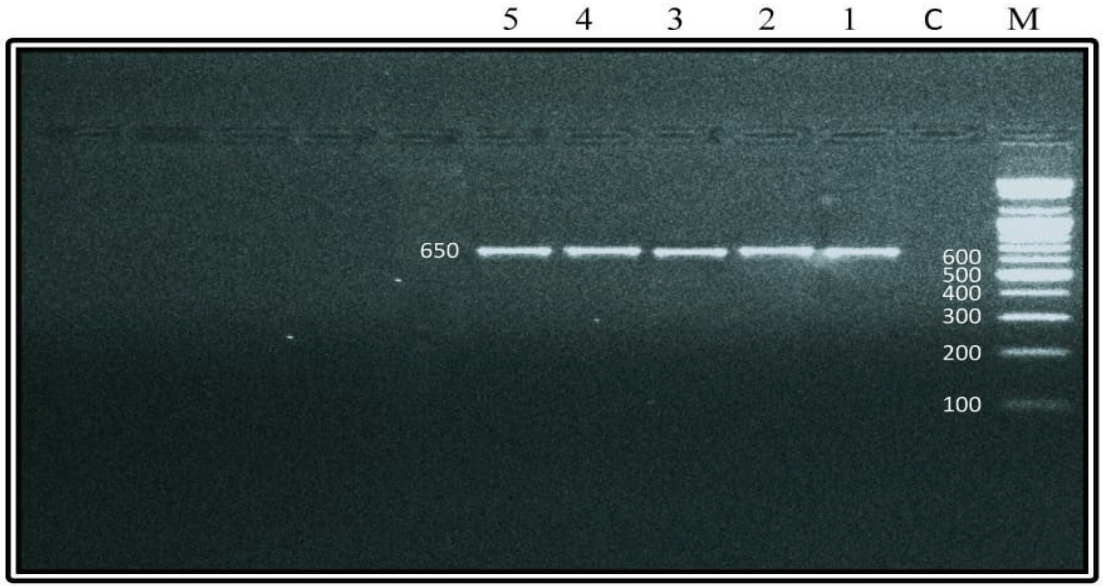

Fig. 1. Gel electrophoresis of amplified *cpcBA-IGC* (650bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidum bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5 . *Westellopsis sp* , *Oscillatoria sp* , *Spirulina sp* , *Chroococcus sp and Lyngbya sp* . Lane C. *Cladophora glomereta*. M.Marker.

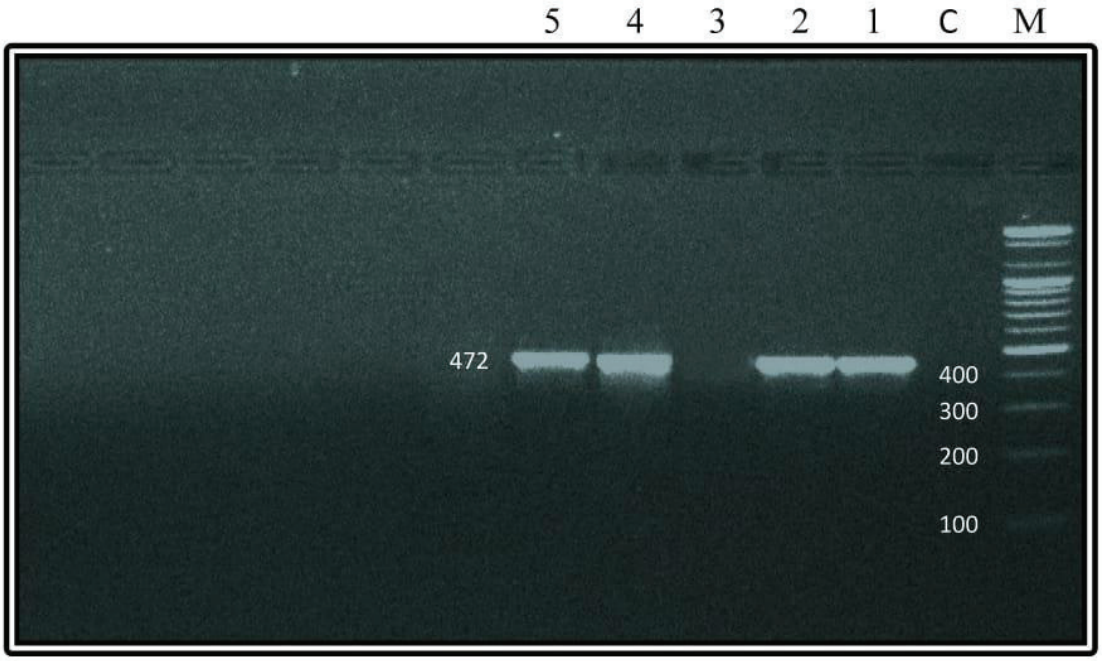

Fig. 2. Gel electrophoresis of amplified *mycE* (472bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidum bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5 . Westellopsis sp , Oscillatoria sp , Spirulina sp , Chroococcus sp and Lyngbya sp .Lane 6. Cladophora glomereta. 7.Negative control.

Discussion

The microscopic results revealed that all species of cyanobacteria that isolated from Tigris River related to toxic dominant genera which produce microcystins except *spirulina platent*. This might be related to the capability of these species to highly competition to remain dominant utilizing all environmental conditions such as high temperature, optical density and abundance of nutrients , all these factors allowed to form the blooming and can increase microcystin production rates (14).

Morphological identification is time consuming and it requires high expertise. In fact, morphological features used for the identification of species such as

colonial form, mucilage patterns and cell arrangement in the colony is frequently variable and dependent on the environment [15]. Furthermore, the co-occurrence of toxin producing and non-producing cells that are morphologically indistinguishable [16; 17]. Therefore, the development of a molecular method for the identification of cyanobacteria is essential for the rapid and accurate analysis members of cyanobacterial population [6]. Several investigators used PCβ-PCα primer set for cyanobacterial detection and showed the same results revealed in this study [12; 18]. Except in [6] study, he was reported that phycocyanin gene fragments from *Nostoc commune* and *Nostocpunctiforme* were unable to be amplified using these primers while strains of all of the cyanobacterial genera were successfully amplified. In recent research, found that *Nostoc punctiforme* had short sequence and incomplete of *cpcBA*-IGC region resulting in high variability in these genes and cause heterogeneity of genus *Nostoc*[19].

The detection of cyclic peptide hepatotoxin genes by using HEPF and HEPR primers was developed to identify potentially microcystin or nodularin-producing cyanobacterial blooms that possess the aminotransferase domain of either *mcy E* or *nda F*, involved in the production of microcystin or nodularin from four order of cyanobacteria included *Oscillatoriales, Chroococales, Stigonematales* and *Nostocales* [13].

The aminotransferase domain was chosen as the target sequence because of its essential function in the synthesis of all microcystins as well as nodularins that catalyzes the addition of D- glutamate to Adda, an essential step in the synthesis of both microcystin and nodularin [20]. Thus, can use these described primers to amplify a 472 bp PCR product from the aminotransferase domains of all tested hepatotoxic species and bloom samples. In adittion, these primers can be used for distinguished between toxic and non-toxic populations of cyanobacteria that coexist simultaneously in a single ecosystem and are indistinguishable by microscopy [13].

Tigris River usually affected by agricultural and industrial eutrification as well as the sewage effluent, high turbidity, river discharge or by agricultural runoff which provide protected mesocosms of cyanobacteria growth and potential microcystins production. The results by [21] and [22], suggested that eutrophication increased the co-occurrence of potentially microcystins producing cyanobacterial genera, raising the risk of toxic-bloom formation.

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