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Molecular based test for detection of cyanotoxins in the domestic drinking water tanks in Baghdad

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Abstract: *This study was conducted to assess and analyze the common cyanobacteria in the domestic drinking water tanks in Baghdad and to see if they are potentially toxigenic.The study included isolating toxic blue green algae from domestic plastic water tanks .Samples were collected during September 2016 to March 2017. The microscopic diagnosis of the samples examined showed that the predominant blue-green algae which were (Synechococcus elongatus sp., Lyngbya sp.1, Anabena aequalis, Lyngbya sp.2).In this study, four sets of primers were used to detect the genetic material of blue-green algae and detect the toxins of mycrostin, cylindrospermopsin and saxitoxin. Molecular analysis showed that all isolates contained phycocyanin that shared by all cyanobacteria. The mycE gene was not detected in all isolated cyanobacteria and blooming samples, while the sxtA and aoaC genes were detected in isolated cyanobacteria and not detected in blooming samples. The aim of this study was to detect the cyanotoxin by using PCR. PCR was found to be a reliable and rapid method to test for the presence of potentially toxigenic cyanobacteria as early warning system to predict toxic cyanobacterial bloom in the domestic drinking water tanks in Baghdad*
Keywords : *cyanotoxin, phycocyanin, microcystin, cylindrospermopsin,saxitoxin, PCR.*

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I. Introduction

Cyanobacteria are successful bloom forming algae they grew well in high temperature , low light intensity , show resistance against grazing pressure and release allelochemicals to competition nutrient between organisms . They have gas vacuoles they facilitaes to migration in the water column to ensure enough light and nutrient availability. Cyanobacterial blooms adversely affect water quality, structure and composition of biological communities and a range of ecological services. Many of the bloom forming cyanobacteria produce toxins responsible for mass mortality of aquatic and exposed vertebrate populations [1]. Cyanotoxin have five classes of toxin :Hepatotoxin (Microcystin ,Nodularin) , Cytotoxin (Cylindrospermopsin) , Neurotoxin (Anatoxin , Saxitoxin) [2]. Microcystins are group of hepatotoxin produce by a number of genera (*Anabaena , Microcystis , Oscillatoria , Nostic*) that caused haemorrhaging of the liver [3]. Cylindrospermopsins and Saxitoxins are neurotoxins produce by avariety species of cyanobacteria (*Anabena , Oscillatoria ,Lyngbya, cylindrospermopsis Raciborskii*). CYN is a wide spread ,alkaloid that inhibition glutathione synthesis which lead to cell death and liver hemorrhage [4].Saxitoxin are commonly knowon as paralytic shellfish toxins that caused paralytic shellfish poisoning that associated with marin dinoflagellates [5]. Polymerase chain reaction (PCR) assay used this method due to specific , rapid, high sensitivity it is possible to detect toxic genotype before secretion of these toxins[6]. designed PCβF/PCαR primers are used to detect cyanobacteria based on specific DNA ,16S RNA ,from phycocyanin genes for the classification of fresh water cyanobacteria . The HEPF/HEPR primers are used to detect microcystin while Jungblut and Neilan [8] chose the aminotransferase domain to detect microcystin producing cyanobacteria. The CKc-F/R primers are used to detect aoaC gene that was specific to cylinderospermopsin producing cyanobacteria,SxtA-F/R primers were used to detected sxtA gene that specific to saxitoxi producing cyanobacteri [9]. In a several study by[10]. used real –time PCR assay to detect and quantify genes specific to cylindrospermopsin cyanobaceria, [11] used qPCR method to quantify saxitoxin producing cyanobacteria based on SYBR green. Therefore , the aim of this study utilize molecular method (PCR) for the early detection of toxigenic cyanobacteria using specific primers to detect cyanobacteria in the domestic drinking water tanks in Baghdad and to see if they are potentially toxigenic.

II. Material And Method

2.1 Study area and sampling collection

Between September 2016 and March 2017 water samples were collected between two months from two plastic water tanks in the Al- Karkh area of Baghdad city using 20µ mesh net. Samples were transported immediately to the lab and incubated under controlled conditions for algal growth (200) µE/m²/s and 26± 2 C˚.

2.2 Media and Culture Conditions

Different plating techniques as spread and streak method were carried out to purify the culture. A single colony formed on the surface of the agar plate was picked up and transferred to new plate. After several transfers, the single colony was inoculated into the liquid medium. For cultivation of cyanobacteria. 10 ml of water sample were inoculated in 50 ml sterilized standard BG-11 medium with and without nitrate nitrogen in 100 ml Erlenmeyer flasks in triplicates. The flasks were shaken well and incubated in growth room. Culturing was carried out with proper light (50 – 75 µE m-2 S-1) and incubation temperature (24ºC).

2.3 Morphological Studies

Pure culture was observed under microscope. The cell shape and size were observed, measured by micrometry and documented as microphotograph. Identification of specimens was carried out using the taxonomic publications [12].; [13].; [14]., [15].; [16].; [17].

2.4 Molecular analysis

2.4.1 DNA extraction

Genomic DNA was extracted from the cyanobacterial isolates using (G-spin DNA extraction kit , intron /Korea). Briefly , amount of fresh colony was placed in lysis buffer (200µl CL, 20µl proteinase K , 5µl RNase A) and mix vortexing vigorously. Incubated lysate at 56 ºC for 10-30 min. Add 200µl of BL and incubated the mixture at 70 ºC for 5 min to remove un lysed tissue particles . Transfer 350~ 400µl of supernatant into anew 1.5 ml tube .Add 200µl of absolute ethanol into lysate and mix well by pipetting .Carefully apply the mixture to the spin column and centrifuge at 13000 rpm for 1 min. Discard the filtrate and place the spin column in a 2ml collection tube .Add washing buffer (700µl of WA and WB)to spin column and centrifuge for 1 min at 13000 rpm .Discard the flow –through and place the column into a 2ml collection tube ,then again centrifuge for 1 min to dry the membrane . Place the spin column into anew 1.5ml tube and add 30-100µl of buffer CE ,incubated for 1 min at room temperature and centrifuge for 1min at 13000rpm to elute. The final solution was kept at -20 ºC until using as template for PCR.

2.4.2 Primers Selection

The first set of primers were PCβF (GGCTGCTTGTT TACGCGACA) and PCαR (CCAGTACCACCAGCA ACTAA) was used to detect the presence of cyanobacterial DNA and amplify *cpcB*-IGC-*cpcA* region in phycocyanin operon that produced a 650 bp gene fragment [18]. while the second set were HEPF (TTTGGGGTTAACTTTTTTGGGCATAGTC) and HEPR (AATTCTTGAGGCTGTAAATCGGGTTT) [8]. used to amplify *mcy*E gene of the microcystin synthetase that produced a 472bp fragment. The third set were Ckc-F: AATGATCGAAAACAGCAGTCGG and Ckc-R:TAGAACAATCATCCCACAACCT was used to amplify *aoa*C gene to detect the cylidrospemopsin that produce 325bp fragment [19]. the four set of primers sxtA-F: GATGACGGAGTATTTGAAGC and sxtA-R: CTGCATCTTCTGG ACGGTAA was used to amplify *aoa*C gene to detect the saxitoxin that produce 125bp fragment [11].

2.4.3 Polymerase chain reaction

PCR mixture was set up in total volume of 25µl included 5µl of PCR premix kit (intron /Korea) ,1µl of each primer and 1.5µl of template DNA were add then the rest volume was completed with sterile D.W. PCR reaction tubes were placed into thermocycler PCR instrument .PCR conditions consisted of an initial denaturation 94 ºC for 3min. to phycocyanin,microcystin, cylindrospermopsin and saxitoxin; 35 cycles of denaturation at 94 ºC for 45s. to phycocyanin and saxitoxin ,30s. for microcystin and cylindrospermopsin ,anneling for 45s. at 54 ºC to phycocyanin ,saxitoxin ,30s. to microcystin and 30 s. at 52 ºC to cylindrospermopsin, extension 45s. at 72 ºC to phycocyanin and saxitoxin ,30s. to microcystin and cylindrospermopsin , final extension for 10 min. at 72 ºC to phycocyanin and 7 min to microcystin ,cylindrospermopsin and saxitoxin.PCR product was separated in 2 % agarose gel electrophoresis stained with red stain staining (intron/Korea) and visualized on uv transilluminator.

III. Result And Desiccation

3.1 Morphological characterization

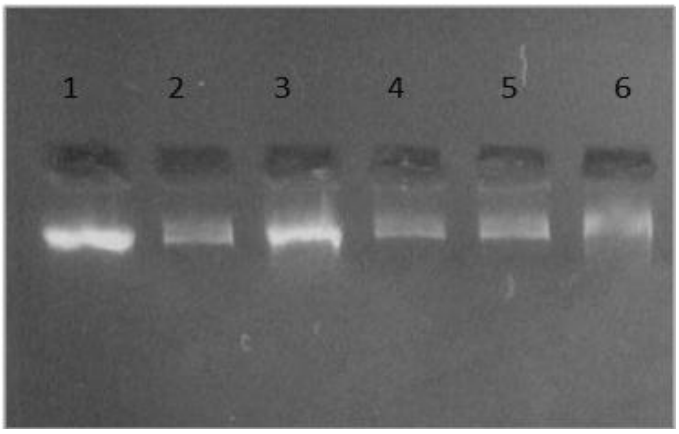
Four isolates of cyanobacteria were obtained from two plastic water tanks during September to March that collect between two months which were : *Synechococcus* elongatus , *Lyngbya* sp1, *Anabena* aequalis , *Lyngbya* sp2*).* which belonged to three cyanobacrial orders: Oscillatoriales , Nostocales , Chroococcales . *Lyngbya* sp. Characterstic are Filamentous, composed of a uniseriate, unbranched trichome of cells inclosed by a non-gelatinous, more or less firm sheath, planktonic and solitary, or aggregated, forming entangled masses on substrate or intermingled among other algae, some species spirally coiled; trichomes mostly cylindrical throughout and tapering very slightly, if at all, toward the apices, which are usually not capitates .

Synechococcus elongatus charecterstic are Cell oblong , cylindrical or ellipsoidal ,erect, seldom slightly bent with rounded apices , 1.4-2µ broad, 1-3 times as long as broad, single or in colonies of 2 , rarely in fours ,mucilage envelope absent or a very thin and narrow one present; division transverse ,contents homogeneous and light blue- green. *Anabena* aequalis characteristic are Trichomes straight , forming a small plant mass, or scattered among other algae; cells somewhat quadrate or barrel-shaped, (4.5)-5.5-(5.7)µ diameter, 7.5-8.5µ long; heterocysts ovate to subcylindric ,5.5 - 8µ in diameter, (10)-13-(15.2)µ long ; gonidia cylindrical ,remote from the heterocysts , the wall smooth and colorless , tychoplanktonic ;intermingled with other algae in shallow water.

3.2 Molecular analysis

3.2.1 Extraction of DNA from isolates cyanobacteria

Genomic DNA was successfully extracted from approximately 100 mg weight of cultured cells by using (G-spin DNA extraction kit, Intron/Korea) DNA bands were confirmed and analysed by gel electrophoresis.



Figure(1)Gel electrophoresis of genomic DNA extraction from cyanobacteria , 2% agarose gel at 5 vol /cm for 1:15 houre, stained with red safe stain and visualized on a UV transilluminator. Lane 1-6: Genomic DNA extracted from *(Synechococcus elongatus., Lyngbya* sp.1, *Anabena aequalis ., Lyngbya* sp.2, and two blooming samples).

3.2.2 Detection of isolated Cyanobacteria by PCR Technique

In this study identification of cyanobacteria were done using the polymerase chain reaction (PCR), based on the phycocyanin operon. A distinct amplicon patterns was produced from four samples, the DNA extracts with a size 650 bp when analyzed in gel electrophoresis (Figure 2), confirming the presence of cyanobacterial DNA from isolates collected from four samples that take from tanks in Al-Karekh Baghdad city,While the two blooming samples faild to produce PCR product. Other studies that used PCβ-PCα prime set for cyanobacterial detection and reported the same results [20] . But the negative results of two blooming samples that might be betong to inhibitors which found in blooming samples lead to inhibit the PCR reaction.

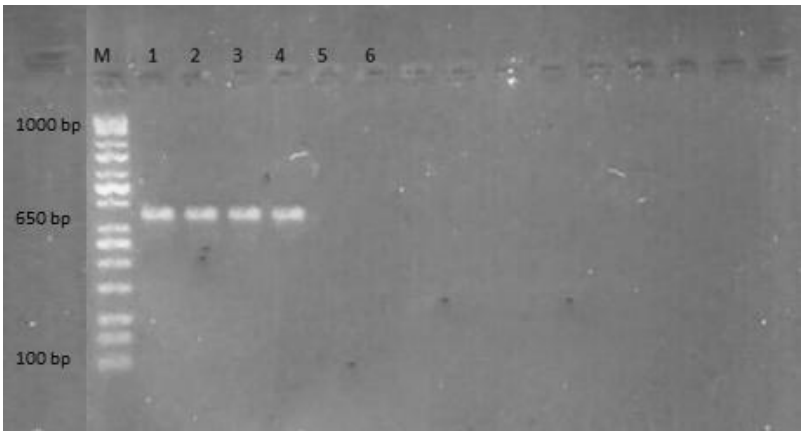


Figure (2) PCR product the band size 650 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hour, stained with red safe stain and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-4 represent (*Synechococcus elongatus., Lyngbya* sp.1, *Anabena aequalis., Lyngbya* sp.2) Lane 5,6 of blooming samples give negative.

3.2.3 Detection of cyanotoxin by PCR assay

3.2.3.1 Microcystin

The HEPF /HEPR primers are used to detection mcy gene were successfully amplified the 472bp fragment .they developed to identify potentially microcystin or nodularin-producing cyanobacterial blooms that posses the AMT domain of either mcy E or nda F, involved in the production of microcystin or nodularin [8]. The results showed no PCR products were obtained from all samples that show in figure(3) indicated that the mcy cluster is an ancient trait in cyanobacteria, and its current sporadic distribution in just a few genera is thought to be the result of repeated loss during evolution [21].

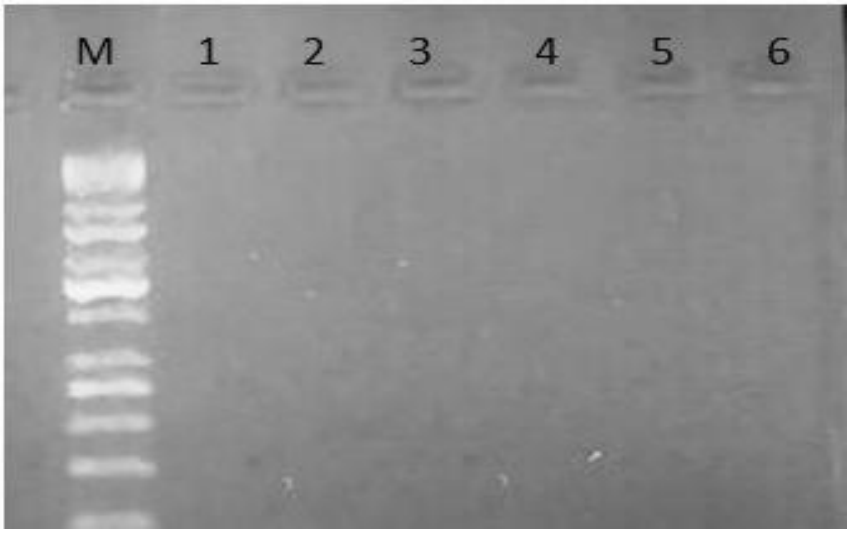


Figure (3) No PCR products. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hour, stained with red safe stain and visualized on a UV transilluminator.

These results agreed with the study of [22]. cyanobacterial blooms, which tested negative for microcystin or nodularin production, were not considered non-toxic due to the potential for production other harmful secondary metabolites, such as the hepatotoxic, cylindrospermopsin, saxitoxin . These negative results disagreed with the study of [23] , [24]. reported that the aminotransferase domain of *mcy*E which detect all potential microcystin cyanobacteria using HEP primers that PCR amplification .They chose aminotransferase domain because its essential fuction in the synthesis of all microsystin and nodularin that catalyzes the addition of D-glutamate to Adda [25].

3.2.3.2 Cylindrospermopsin

The primer (*CKc*-F/R) was used in this study to detect *aoa*C gene directly in samples that collected and isolated from plastic water tank .The results of current study revealed that *aoa*C gene found in genera isolated from the plastic water tanks included (***Synechococcus elongatus., Lyngbya* sp.1, and *Anabena aequalis, Lyngbya* sp.2),**While this gene not detected in both blooming samples that might be related to the inhibitors which found in blooming samples lead to inhibit the PCRreaction, that show in the figure(4). Many studies detected the cylindrospermopsin producers by PCR method because its speed ,specifity and sensitive. The positive results agreed with the study of [26]. have showed the applicability of the qPCR method for rapid on-site detection of *C. raciborskii* in reservoirs. In addition, , [19]. The results show the expected amplicons were only observed with toxic strains,cells were suitable as a source of purified DNA for the multiplex PCR; the assay could detect simultaneously 3 *aoa* and 3 *mcy* gene regions with mixed CYN^+ and MC^+ cyanobacteria cells.

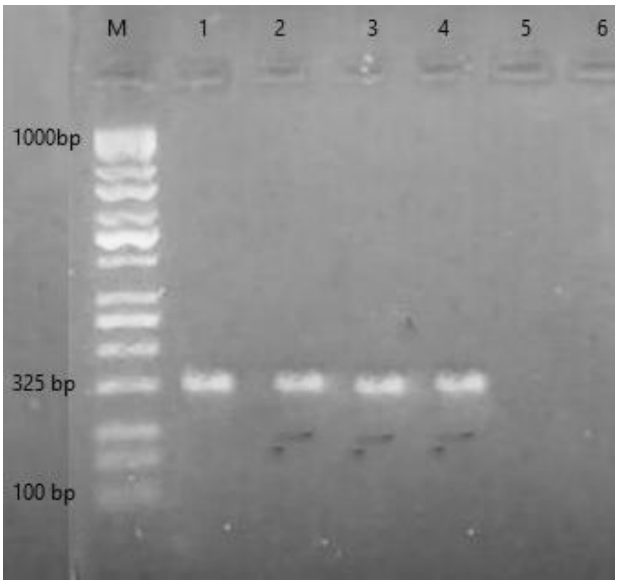


Figure (4) PCR product the band size 325 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hour, stained with red safe stain and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-4 represent (*Synechococcus elongatus*., *Lyngbya* sp.1, *Anabena aequalis*., *Lyngbya* sp.2) Lane 5,6 of blooming samples give negative.

3.2.3.3 Saxitoxin

The *sxtA*-F/R set primer was use to detected *sxtA* gene that specific to saxitoxin producing cyanobacteria that amplified 125bp .The results observed that *sxtA* gene was detected in isolated from plastic water tanks which genera (***Synechococcus elongatus* sp., *Lyngbya* sp.1, *Anabena aequalis*, *Lyngbya* sp.2)** whereas the gene was not detected in two blooming samples that might be related to the inhibitors which found in blooming samples lead to inhibit the PCRreaction, that show in the figure(5).

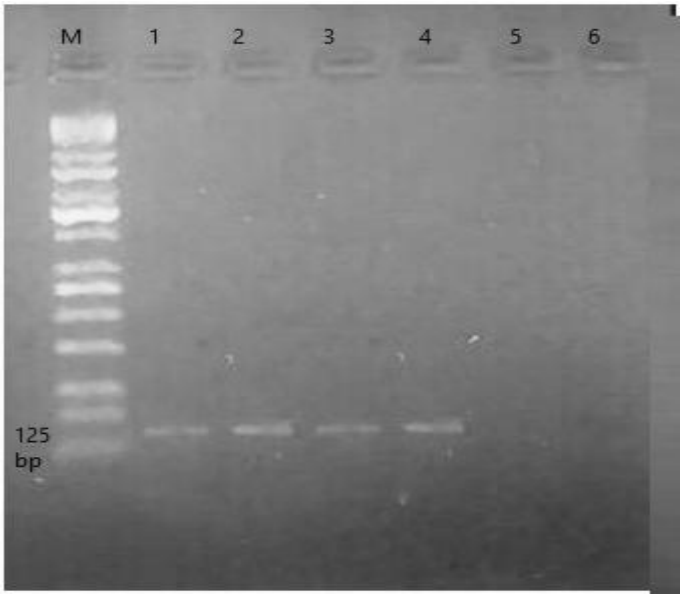


Figure (5) PCR product the band size 125bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours, stained with red safe stain and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-4 represent (*Synechococcus* sp., *Lyngbya* sp., *Anabena* sp., *Lyngbya* sp.) Lane 5,6 of blooming samples give negative.

The positive results agreed with several study [27]. utilizing SYBR green chemistry that targets the aminotransferase domain (*sxtA4*) of core *sxtA* gene in both *Alexandrium* and *Gymnodinium sp.* By qPCR assay.

IV. Conclusion

PCR was found to be a reliable and rapid method to test for the presence of potentially toxigenic cyanobacteria, However, PCR as a method to detect for the presence of toxigenic cyanobacteria and as an early monitoring system to warn for the build-up of toxigenic cyanobacteria in freshwaters should be supplemented with other laboratory assays that monitor the production of microcystin in water sources.

References

[1]. H.W. Paerl, R.S. Fulton III, P.H. Moisander, and J. Dyble. Harmful freshwater algal bloom, with an emphasis on cyanobacteria. The Scentific World Journal, 1, 2001, 76–113.

[2]. T.G. Otten, and H.W. Paerl (2015). Health Effects of Toxic Cyanobacteria in U.S. Drinking and Recreational Water : Our current understanding and proposed Direction. Curr. Enviro. Rpt., 2015, 75-84.

[3]. R.W. Zurawell , H. Chen , J.M. Burke , and Prepas. Hepatotoxic cyanobacterium ; A review of the Biological Importance of Microcystins in Fresh water Environments . Toxicol. Environ. Health , 8,1,2005, 1 -37.

[4]. L. Wormer , S. Cires , D. Carrasco, and Quesada. CYlindrospermopsin is not degraded by co –occuring natural bacterial communities during a 40 day .Harmful Algae , 7, 2008, 206-213.

[5]. M. Wiese, D' P.M. Agostino, T.K. Mihali, M.C. Moffitt, and B.A. Neilan. (2010) Neurotoxic alkaloids : saxitoxin and its analogs .Mar Drug , 2010, 2185-2211.

[6]. R. Kurmayer, and G. Christiansen. The Genetic Baisis of Toxin Production in Cyanobacteria. Freshwater Review; 2, 2009,31-50. B. A. Neilan , B. P. Burns , D. Relman ,and D. Lowe 2002. Molecular identification of cyanobacteria associated with Stromalolites from distinct geographical locations. Astrobiology , 2, 2002,271-280.

[7]. A. D. Jungblut , and B. A. Neilan 2006. Molecular Identification and Evolution of cyclic peptide Hepatotoxins , Microcystin and Nodularin ,synthetase Genes in Three order of cyanobacteria.Microbiology , 185 , 2006, 107-114.

[8]. I .J. Abed . PCR based test for early warning of both cylindrospermopsin and saxitoxin in Iraqi frehsh water.AL-Naahrain university, 18, 2015,109-114.

[9]. J.P. Rasmussen , S. Giglio , P.T. Monis, R.J. Campbell , and C.P. Saint. Development and field testing of a real time PCR assay for cylindrospermopsin -producing cyanoacteria . J Appl Microbiol, 104, 2008,1503-1515.

[10]. J. Al-Tebrineh , T. K. Mihali , F. Pomati ,and B. A. Neilan . Quantitative PCR detection of saxitoxin –producing cyanobacteria and *Anabaena circinalis* in environmental water blooms , Appl. Environ. Microbiol , 2010,4-10.

[11]. T. V. Desikachary. Cyanophyta. Indian Council of Agricultural Research, New Delhi, 1959, 686.

[12]. G.W. Prescott. The alga: A review. Houghton Mifflin comp, 1964.

[13]. G. W. Prescott . How to Know the Freshwater Algae. Wm. C. Brown, Dubuque, IA. 1978.

[14]. G.W . Prescott.Algae of the Western Great Lakes Area. Koenigstein, West Germany, 1982, 977.

[15]. F. Hustedt. Die Susswasser Flora Mitteleuropas. Heft 10. 2nd Edition. Bacillariophyta (Diatomeae). A. Pascher. Verlag von Gustav Fischer, Germany, 1930, 466.

[16]. F. Hustedt. The Pennate Diatoms. A Translation of Hustedt´SDie Kieslalgen. with Supplement by Norman G. Jensen. Printed in Germany By Strauss and Cramer Gmbh, 1959, 918.

[17]. B.A. Neilan, D. Jacobs and A. Goodmann. Genetic Diversity and Phylogeny of Toxic Cyanobacteria Determined by DNA Polymorphisms within the Pycocyanin Locus. Appl. Environ. Microbiol., 61, 1995,3875-3883.

[18]. A. Baron-Sola, Y. Ouahid, and F.F. delCampo. Detection of Potentially Producing Cylindrospermopsin and Microcystin Strains in Mixed Populations of Cyanobacteria by Simultaneous Amplification of Cylindrospermopsin and Microcystin Gene Regions. Ecotoxicology and Environmental Safety, 75, 2012,102-108.

[19]. M.L. Saker, M. Vale, D. Kramer and V.M. Vasconcelos. Molecular Techniques for the Early Warning of Toxic Cyanobacteria Blooms in Freshwater Lakes and Rivers. Appl Microbiol Biotechnol, 75, 2007,441-449.

[20]. A. Rantala, D.P. Fewer, M. Hisbergues, L. Rouhiainnen, J. Vaitomaa, T. Börner and K. Sivonen. Phylogenetic Evidence for the Early Evolution of Microcystin Synthesis. Proceedings of the National Academy of Sciences of the United States of America, 2004, 568-573.

[21]. M.L. Saker, B.A. Neilan and D.J. Griffiths. Two morphological forms of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolated from Solomon Dam, Palm Island, Queensland. J Phycol ,1999,599–606.

[22]. S.K. Ghosh , P.K. Das and S.N. Bagchi. PCR- Based Detection of Microcystin-Producing Cyanobacterial Blooms from Central India. Indian Journal of Experimental Biology; 46,2008, 66-70.

[23]. I.J. Abed , A.L.M. Jawad , G.A. Abdulhasan, A.A. Al-Hussieny and L.I. Moushib. Molecular detection of toxigenic new record cyanobacterium : Westiellopsis Prolifica in Tigris river .International Journal of scientific and Technology research, 2013,2277-8616.

[24]. J. Al-Tebrineh, M.M. Gehringer, M.M. AKcaelon, R. and B.A. Neilan, B.A.. A new Quantative PCR assay for the Detection of Hepatotoxigenic Cyanobacteria" Toxicon, 57, 2011,546-554.

[25]. Y.R. Marbun, H. Yen , T. Lin , H. Lin, and A. Michinaka. Rapid on –site monitoring of cylindrospermopsin –producers in reservoirs using quantitative PCR, Sustain. Environ. Res, 22 , 2012,143-151.

[26]. S.A. Murray, M. Wiese, A. Stuken, S. Brett, R. Kellmann, G. Hallegraeff and B.A. Neilan. *SxtA*-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters. Appl. Environ. Microbiol., 2011 ,7050–7057.

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