Molecular Detection of Some Toxigenic Cyanobacteria in Tigris River in Baghdad–Iraq

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Abstract

Cyanobacteria and their emissions are becoming more widely reported around the world, posing a serious threat to both the environment and human health. Several orders of cyanobacteria have been identified to make cyanotoxin, the most common algal toxin. The aim of this research was to develop a method for detecting cylindrosprmopsin and saxitoxin biosynthesis genes in rivers .In November, December 2019 and January2020. Cyanobacteria were isolated from Tigris River freshwater and detected using a compound microscope as well as traditional PCR .All cyanobacteria isolates contained phycocyanin gene fragment. Five isolates of cyanobacteria in these study was successfully amplified a phycocyanin gene *(Microcystis flosaquae, Microcystis sp, anabaena circinalis ,nostoc commune and westiellopsis prolifica)* and all isolates successfully amplified aoaC gene to detecting the cylidrospemopsin and the saxitoxin. Our findings show that a PCR assay can be used to detect cylidrospemopsin and saxitoxin-producing cyanobacteria in river water, which is useful for stations that prepare drinking water for the public.

Introduction

Cyanobacteria (blue-green algae) are a prokaryotic group of microorganisms with gram-negative bacteria-like characteristics. They are the only prokaryotes that can perform oxygenic photosynthesis, which makes them exceptional among prokaryotes. Furthermore, within the same organism, certain cyanobacteria have the ability to fix atmospheric nitrogen. Cyanobacteria are the best bacteria because of these characteristics (1) .Cyanobacteria accumulations can degrade water quality by lowering transparency, loss of biological diversity, generating taste and odor-causing compounds, and, most importantly, creating cyanotoxins, which are dangerous to humans (2).

In fresh water, most cyanobacteria may make dermal toxins, neurotoxins, and hepatotoxins like nodularins and microcystins. (3)

They're also an intriguing source of food (4). Good nutrients such as proteins, carbohydrates, vitamins, minerals, amino acids, and fatty acids have also been found in these bacteria.

Microcystins are the most studied of all the cyanotoxins Because of their broad distribution and high toxicity, they are extremely dangerous. There are currently over 80 structural variants of microcystin, with microcystin-LR being the most widespread and potent, followed by microcystin-RR and microcystin-YR. (5).

To protect water users from these poisons, monitoring systems are needed. For most cyanotoxins, effective methods such as Enzyme - linked immunosorbent assay ELISA and high-performance liquid chromatography have been confirmed, they requires time-consuming sample preparation techniques, expensive equipment, and purified toxin standards, which are often hard to obtain.(6)

The delivery of phycocyanin in cyanobacteria makes studying phycocyanin genes a good idea for cyanobacterial classification (7). The phycocyanin operon codes for two bilin subunits and three linker

polypeptides. Between the two bilins is an intergenic spacer (IGS). The b (cpcB) and a (cpcA) subunit genes revealed sequence differences that are useful for diffraction genotypes below the generic level, making it capable of cyanobacteria identification through PCR (8).

Saxitoxins (STXs) and Cylindrospermopsins (CYNs) are cyanobacterial neurotoxins generated from a variety of species. CYN is a widely spread, stabilized alkaloid with a broad toxicity spectrum. This toxin is rapidly becoming known as one of the most important freshwater algal toxins. The spreading of CYN-producing bacteria into temperate areas has sparked concerns that the toxin could pose serious environmental and health risks (9). STX as well as its 57 analogs are a diverse group of naturally occurring neurotoxic alkaloids that cause paralytic shellfish poisoning and are primarily found in marine dinoflagellates (eukaryotes) (10, 11).

The Tigris River is the most important source of drinking water for Iraq, It serves a population of about seven million people. This river is usually affected by industrial eutrification, sewage industrial waste, and agricultural runoff, all of which encourage cyanobacteria growing and the production of cyanotoxins such as Cylindrospermopsins (CYN) and saxitoxins (STXs). As a result, the aim of this research was to use Molecular PCR to detect cyanobacteria and Cylindrospermopsins (CYN) and saxitoxins (STXs) producing isolates from the Tigris River in order to detect toxic species early, which could be useful to companies that provide drinking water to the region.

Materials And Methods

Collection of samples

Surface water samples were collected weekly between November 2019 to January 2020 from one sampling stations site at Tigris river of Baghdad city. (Samples were collected in sterile bottles) and stored for later analysis. Cyanobacterial culturing by Tow Culture Medium were used in these study BG-11 culture medium and Chu-10media. The cyanobacteria in the liquid medium spread over the entire surface of the solid medium, causing separation in many areas. On the surface of agar, 0.1 ml of diluted cyanobacterial solution was inoculated and spreader (12). The cultures were incubated at 28±2°C under continuous illumination of 54-67 50 μE/m²/s light intensity. To ensure that the culture was unialgal, a microscopic examination was performed.

Genomic DNA extraction and PCR amplification

The DNA extractions were performed by using Easy Pure® Genomic DNA Kit according to the Gram-negative bacterial procedure as directed by the manufacturer. Extracted DNA was quantified and stored at -80 degrees Celsius until needed. Four different primer sets were used to analyze each sample. The sequences of the oligonucleotide primers used for PCR are listed in (Table1) with final concentrations 25 μl. All the PCRs were carried out 5μl of the extracted DNA were mixed with PCR mixture that composed of 12.5μl of Easy Taq PCR Super Mix, 1μl from each of primers (Forward and Reverse /10poml) and 5.5 μl of nuclease free water was added to complete volume to 25μl. PCR reaction tubes were mixed by vortex

and finally placed into thermocycler PCR instrument, the PCR conditions for these primers were involved as follows: an initial denaturation for 5 minutes at 95 degrees Celsius; 36 cycles of denaturation for 1 minute at 94 degrees Celsius, annealing for 1 minute at 62 degrees Celsius ((PCβ-PCα primer set) and 1 minute at 56 degrees Celsius (Ckc and sxtA primer set), extension for 1 minute at 72 degrees Celsius, and ultimate extension for 1 minute at 72 degrees Celsius. After the reaction, 5 µl of amplified DNA was separated on 1.5% agarose gels (Promega, USA), The size of amplified products was compared to the 100 bp DNA ladder, which was treated with ethidium bromide and visualized on a UV transilluminator, to determine the precise size of these products.

Table (1) : The sequence of Primers used in PCR amplification genes

Target	Primer	Sequence (5-3)	C	bp	Reference
cpcB-IGC-cpcA region to detect the presence of cyanobacterial	PCβF	(GGCTGCTTGTTTACGCGACA)	62	650	(15)
	PCαR	(CCAGTACCACCAGCAACTAA)	62		
aoaC gene to detect the cylidrospemopsin	Ckc-F	AATGATCGAAAACAGCAGTCGG	56	325	(16)
	Ckc-R	TAGAACAATCATCCCACAACCT	56		
aoaC gene to detect the saxitoxin	sxtA-F	GATGACGGAGTATTTGAAGC	56	125	(16)
	sxtAR	CTGCATCTTCTGG ACGGTAA	56		

Results And Discussion

Isolation and identification of algae

Five cyanobacterial isolates were derived from the Tigris River, including Microcystis flosaquae, Microcystis sp, anabaena circinalis, nostoc commune, and westiellopsis prolifica, which contributed to four cyanobacterial orders: Chroococales, Stigonematales, and Nostocales, as well as one chlorophyceae isolate, Chlorella vulgaris where used as negative control test.

Extraction of DNA algae

Genomic DNA was successfully extracted from samples(algae) by Easy Pure® Genomic DNA Kit , Gel electrophoresis was used to validate and analyze the DNA bands. as in (Fig. 1)

Detection of cyanobacteria by PCR test

A phycocyanin operon gene fragment from cyanobacteria containing the IGS (cpcBA-IGC) was amplified. When all of the DNA extracts with a size of 650 bp were analyzed in gel electrophoresis (Fig. 2), separate amplicon patterns were generated, confirming the presence of cyanobacterial DNA from samples obtained from fresh water of the Tigris River in Baghdad. Whereas the lane of a green alga, Chlorella

vulgaris, lacks the Pycocyanin operon, no PCR product was obtained, indicating the high specificity of the primers used. The findings confirmed the presence of cyanobacterial DNA in isolates taken from the Tigris river in Baghdad city and successfully amplified a fragment usingPCβ-PCα primes set for cyanobacterial detection, and the same results were reported in study of (13).

Five different cyanobacterial species were identified, and these potentially cyanotoxin-producing species were found in Tigris river samples, such as *Microcystis flosaquae, Microcystis sp, Anabaena circinalis, Nostoc commune and Westiellopsis prolifica.* The common cytotoxin that blocks protein synthesis, with renal and liver failure as the first clinical symptoms, has a biologically active alkaloid found in several cyanotoxin producing species.

Detection of Cylidrospemopsin by PCR assay

in this study, Traditional PCR has been used to identify cyanobacteria that produce cylidrospemopsin and possess an aminotransferase enzyme. The (CKc-F/R) primers successfully amplified the 325bp fragments of the aoaC gene from all microcystin-producing cyanobacterial isolates. With the exception of Chlorella vulgaris, (Fig. 3). The specificity of 325bp primers showed to be highly specific for samples forming cylidrospemopsin ,since there was no DNA amplified from chlorophyta used in this study., The findings corroborated the findings of (14).

PCR methods were used in several experiments to find the cylindrospermopsin producers in freshwater. While bio-molecular detection methods have gained popularity only a few studies have focused on using PCR methods to rapidly track cylindrospermopsin producers in river water due to their specificity and speed. Marbun et al., (15) have shown that the qPCR approach can be used to detect C. raciborskii in reservoirs quickly and on-site. Furthermore, the findings indicate that cylindrospermopsin is a major cyanotoxin in Kinmen Island's reservoirs.

Detection of Saxitoxin by PCR assay

The sxtA-F/R set primers were used to detect the sxtA gene, which is unique to cyanobacteria that produce saxitoxin and amplified 125bp. the results showed that the sxtA gene was found in all samples isolated from the Tigris River Since no DNA from chlorophyta was amplified (figure 4). The findings were in line with those of many other studies. (16,17).

Using traditional PCR, the sxtA-F/R set primer designed by (18) was seen to be specific to saxitoxin-producing Anabaena circinalis. The sxtAF/R primers also amplified the sxtA gene from other cyanobacteria that produce saxitoxin. Saxitoxins (STXs) cause neurotoxicity by blocking sodium ion channels in nerve axon membranes; STXs are also known to block calcium channels. STXs also affect heart muscle cells by prolonging potassium channel gating, which can result in changes in ion influx into the cell. (19,20). To generate fresh portable water, most existing drinking water treatment plantes (WTPs) use traditional treatment methods such as coagulation-flocculation, sedimentation, sand filtration, and disinfection (21).

The increased incidence of cyanobacterial blooms in freshwater bodies in several decades has prompted water management agencies to pay closer attention to the dangers posed by toxic cyanobacteria. While reports of neurotoxins are becoming more common, toxic events are still mainly diagnosed using microcystins. (22).

Conclusions

Based on the results presented in this paper, it is possible to estimate the toxigenicity of cyanobacterial in river water by using a PCR assay to detect the sxtA and aoaC genes. This approach would benefit managers because it would allow them to track the development and progression of cylidrospemopsin and saxitoxin in Iraqi river water. The ability of the PCR technique to simultaneously identify cyanotoxin-producing genes and potentially cyanotoxin-producing species shows its efficiency as a monitoring tool, especially when the number of target organisms in the freshwater sample is low.

Declarations

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Conflicts of Interest: The authors declare no conflict of interest.

Ethical approval: the Ethics Committee of the Mustansiriyah University approved and oversaw this study.

Research involving human participants: No human sample was used.

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Figures

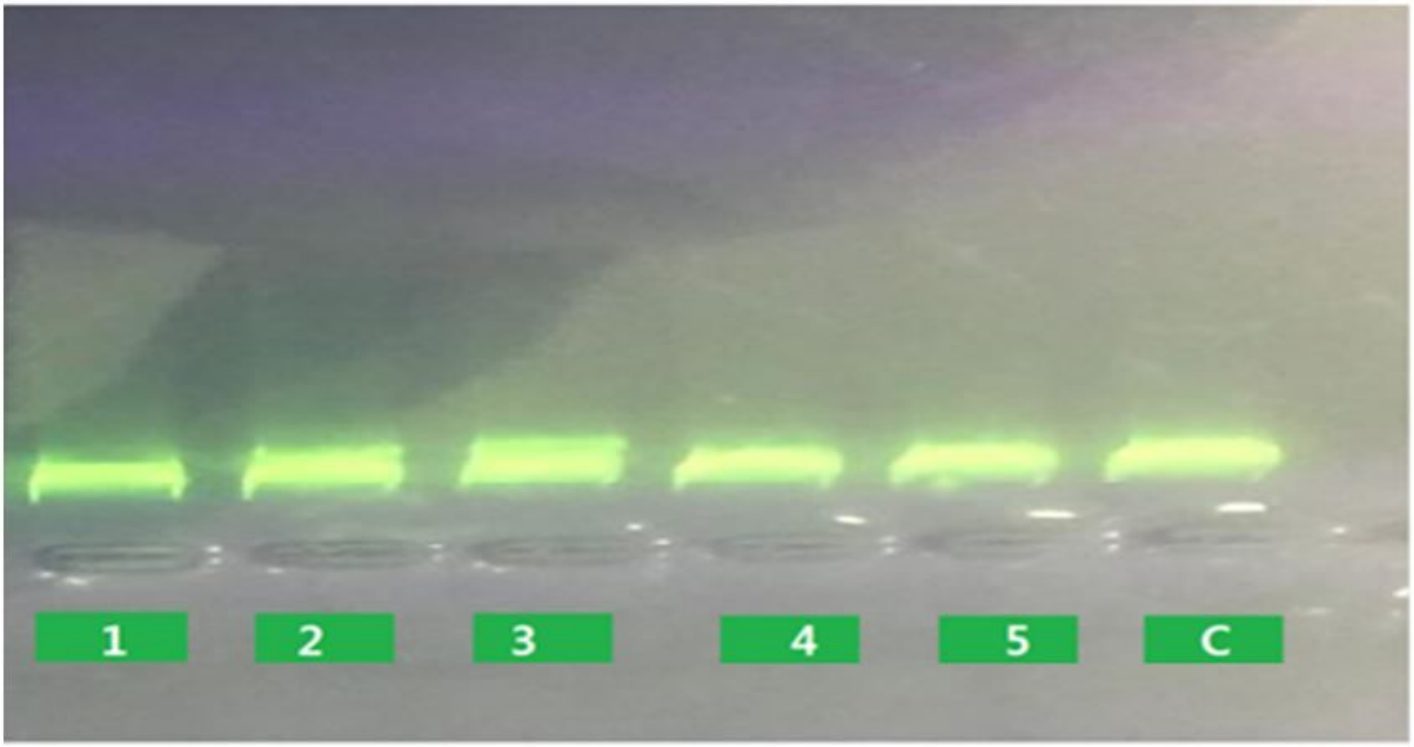

Figure 1

Gel electrophoresis of genomic DNA extraction from cyanobacteria and green algae as control in Agarose (1.5%), 5 V/cm for 2 hours after staining with ethidum bromide and visualizing with a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5 . , Microcystis flosaquae, Microcystis sp , anabaena circinalis ,nostoc commune and westiellopsis prolifica Lane C. Chlorella vulgaris. L.Marker.

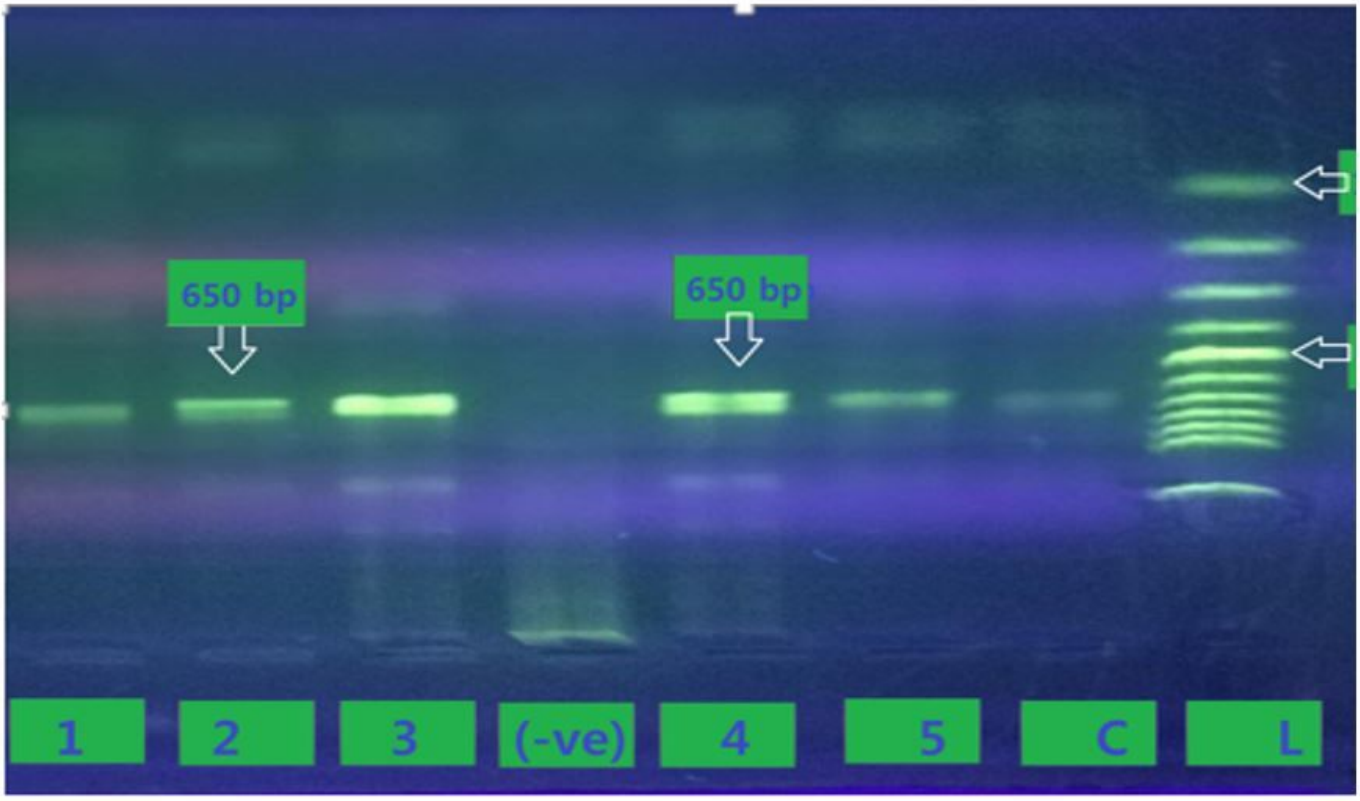

Figure 2

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 . , Microcystis flosaquae, Microcystis sp , anabaena circinalis ,nostoc commune and westiellopsis prolifica Lane C. Chlorella vulgaris. M.Marker.

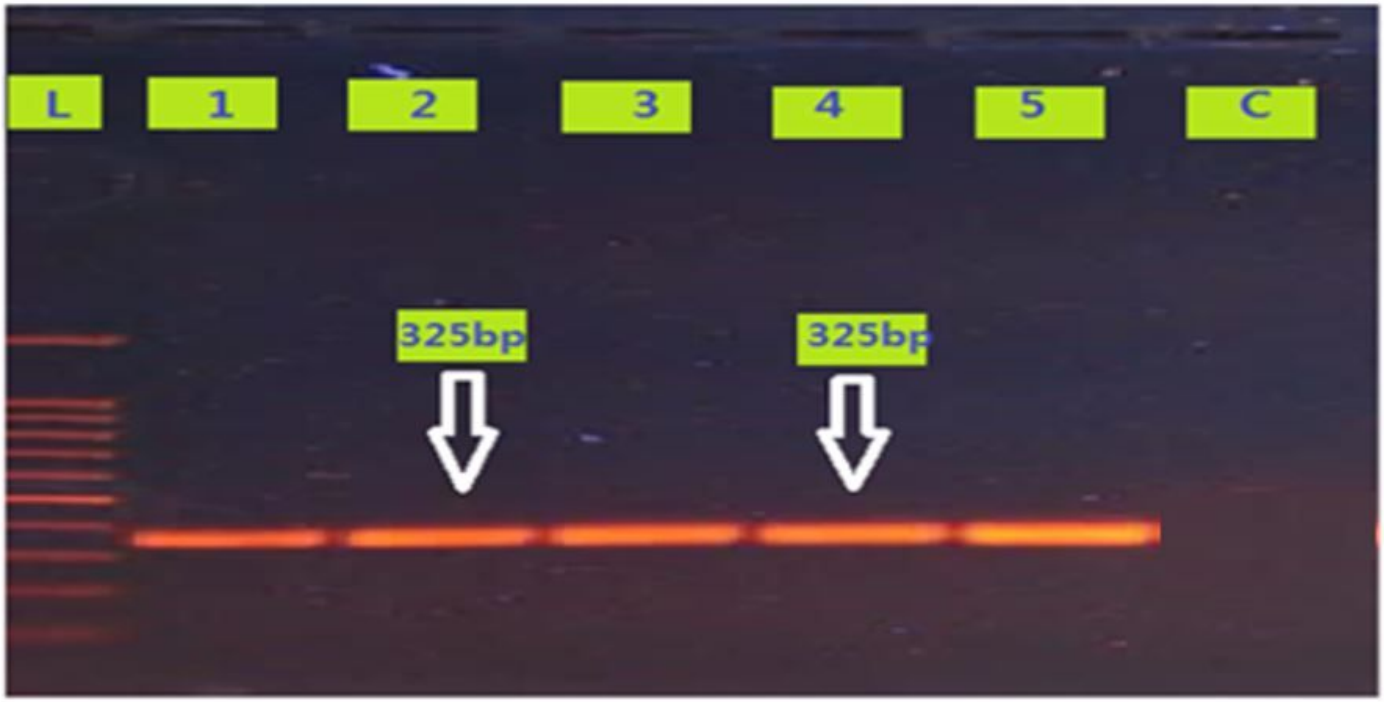

Figure 3

PCR amplification of the aoaC gene (325bp) 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 . , Microcystis flosaquae, Microcystis sp , anabaena circinalis ,nostoc commune and westiellopsis prolifica Lane C. Chlorella vulgaris. L.Marker.

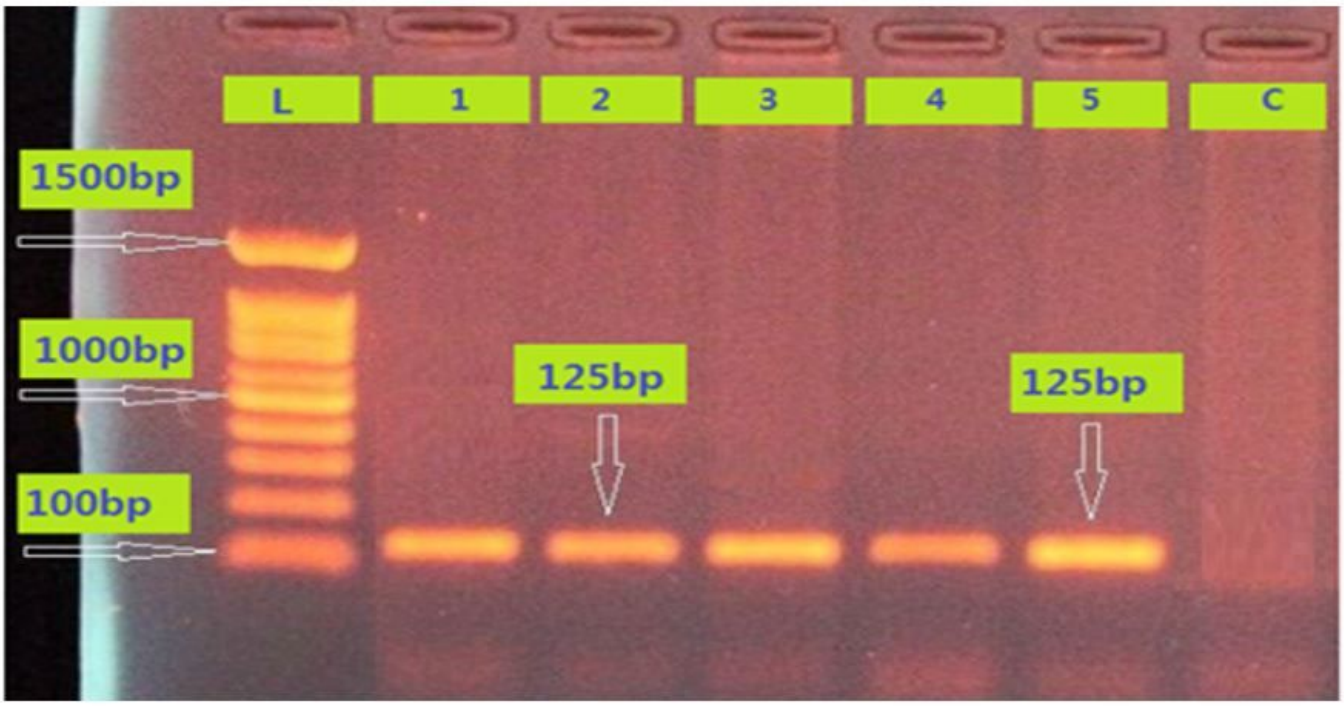

Figure 4

PCR amplification of the sxtA gene (125bp) 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 . , Microcystis flosaquae, Microcystis sp , anabaena circinalis ,nostoc commune and westiellopsis prolifica Lane C. Chlorella vulgaris. L .Marker.