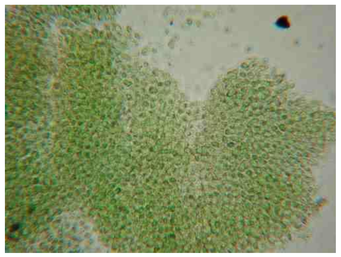

Gene identification and Screening for toxin genes in cyanobacteria from Lake Trafford

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Abstract

Cyanobacteria, common name blue-green algae, are small single cell organism that have in recent years become an important topic of discussion in Florida. This is largely due to their ability to produce a class of toxins called cyanotoxins which include potent neurotoxins, hepatotoxins, cytotoxins, and endotoxins. To characterize the threat to human health we worked on two aspects of understanding and characterizing the nature of freshwater cyanobacteria blooms and we believe that early detection of toxin genes could be used in conjunction with control methods before a bloom becomes too difficult to treat. In addition to getting morphical data we aimed to identify the gene complexes responsible for producing specific cyanotoxins and use Molecular detection methods to test for the specific genes in the samples, even though the sample may not have been currently producing the toxin at time of collection. This is due to toxins being released during cell lysis. To achieve this, we tested samples from three different points in lake Trafford monthly over the span of a year and used qPCR to test for the presence or absence of the gene complexes responsible for four different cyanotoxins; Microsystin (mcyE gene) Saxitoxin (sxtA gene), Anatoxin (anaC gene), Cylindrospermopsin (cyrA gene).

Introduction

Human health risks from cyanobacterial blooms are primarily related to cyanotoxins that some cyanobacteria produce. Not all species of cyanobacteria can produce toxins and those that do, often do not produce toxins at levels harmful to human health. Due to these factors, we hypothesized that there is no pattern between the cyanotoxin gene presence and the presence of toxins in the water. Monitoring programs that are currently in use can overestimate risk and lead to unnecessary health advisories. To characterize the true threat to human health we focused on two aspects of understanding and characterizing the nature of freshwater cyanobacteria blooms and we believe that early molecular detection of toxin genes could be used in conjunction with control methods before a bloom becomes too difficult to treat.

Materials and Methods

Approximately 500 ml of water were collected in sterile polyethylene bottles from each of the three locations around lake Trafford, they were collected at approximately one foot deep in the water column, collections were conducted by collier county once a month over the span of one year. The samples were filtered through 0.45-micron cellulose filter and once all of the cells transferred over to the filter membrane they were frozen at -80°C until they were ready to be processed. To extract DNA from cyanobacterial cells on the filters, they were then processed by starting with three rounds of the freeze-thaw process that was performed using the -80d°C freezer and a 65°C heating block for the lysis of cyanobacterial cells then followed the protocol provided by the manufacturer. (DNeasy PowerLyzer PowerSoil Kit) the extracted DNA was tested using qPCR (AriaMx Real-Time PCR System) to test for the presence of the gene complexes responsible for four different cyanotoxins. Each of the developed qPCR systems was performed individually and separately with a qPCR device. For the Microcystis system, primer set MicrF/MicrR was used to target the 16S rRNA region unique to Microcystis cells, while mcyF/mcyR were used to specifically target the microcystin synthetase (mcyE) region unique to potentially toxic Microcystis cells. Samples were also tested individually and separately for Saxitoxin (sxtA gene), Anatoxin (anaC gene), and Cylindrospermopsin (cyrA gene). All Primers and TaqMan probes used in this study were from IDT and the information and sequences can be found in [Table 1]. The qPCR cycling protocol for each primer pair was applied as previously described by the authors.

Target Gene	Primer/Probe Sequence	Tm (°C)	Size (BP)	References
mcyE	mcyF – AAT TCT AAA GCC CAA AGA CG mcyR – GAA ATT TGT GTA GAA GGT GC	50	128	J. Al-Tebrineh et al., 2012
sxtA	SxtF – GGA GTG GAT TTC AAC ACC AGA A SxtR – GTT TCC CAG ACT CGT TTC AGG SxtP – AGA AGA AAG TAT CCT CTC AG	60	148	J. Al-Tebrineh et al., 2012
cyrA	CyrF – GTC TGC CCA CGT GAT GTT ATG AT CyrR – CGT GAC CGC CGT GAC A CyrP – ACG AAA TTC TCG AAG CAA CT	58	71	J. Al-Tebrineh et al., 2012
anaC	AnaF – TCT GGT ATT CAG TMC CCT CYA T AnaR – CCC AAT ARC CTG TCA TCA A	52	366	John, Nijoy et al., 2019

[Table 1]

Sample Data	Microsystin (mcyE gene)	Saxatoxin (sxtA gene)
LT1 (11/12/20)	(+)	(+)
LT2 (11/12/20)	-	(+)
LT3 (11/12/20)	-	(+)
LT1 (12/15/20)	-	+
LT2 (12/15/20)	+	+
LT3 (12/15/20)	+	(+)
LT1 (1/11/21)	+	+
LT2 (1/11/21)	-	+
LT3 (1/11/21)	-	+
LT1 (2/22/21)	+	+
LT2 (2/22/21)	(+)	(+)
LT3 (2/22/21)	(+)	+
LT1 (3/9/21)	-	+
LT2 (3/9/21)	-	(+)
LT3 (3/9/21)	-	+
LT1 (4/13/21)	+	+
LT2 (4/13/21)	+	(+)
LT3 (4/13/21)	+	(+)
LT1 (5/4/21)	+	+
LT2 (5/4/21)	+	+
LT3 (5/4/21)	+	(+)
LT1 (6/15/21)	+	-
LT2 (6/15/21)	+	-
LT3 (6/15/21)	+	(+)
LT1 (7/20/21)	+	-
LT2 (7/20/21)	+	-
LT3 (7/20/21)	+	-
LT1 (8/10/21)		(+)
LT2 (8/10/21)		(+)
LT3 (8/10/21)		+
LT1 (9/28/21)		(+)
LT2 (9/28/21)		+
LT3 (9/28/21)		+
LT1 (10/19/21)		(+)
LT2 (10/19/21)		+
LT3 (10/19/21)		+

Figure 1: + represents amplification of target gene; (+) represents weak amplification; - represents no amplification

Results

The data was tested in order of how samples were received from November 2020 – October 2021, and we can see in the figure to the right that the presence of these gene complexes do not follow a specific pattern or trend, but we can see presence of at least one toxin gene in every sample throughout the year. I was unable to analyze every sample for all four gene complexes as I originally intended, this was due to time constraints as well as challenges throughout this research, one of which trying to optimize all of the qPCR methods. This took time, research, and some trial and error.

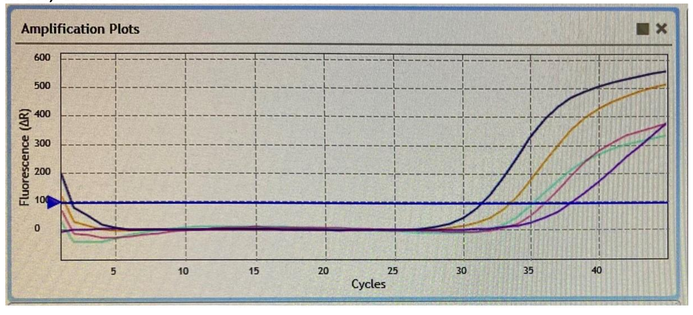

Figure 2: Example of amplification plot used to determine presence of gene complex

Discussion/Conclusions

The qPCR systems were successfully optimized and developed for quantifying commonly observed harmful cyanobacteria in this study; both the MICR and mcyE primers and protocols established in the literature [J. Al-Tebrineh et al., 2012] proved successful. For the sxtA primers it was difficult to find a positive control that we already knew was producing the saxitoxins, one the positive control was found we still had trouble getting specific binding of the primers, so we switched to using the primers and probe (sxtP) which proved to be much more successful. A lot of time was also spent trying to establish a positive control for anatoxin and optimize the protocols using the anaC primer but was unable to achieve consistent results before I ran out of time. While this information is important to understanding and characterizing the nature of freshwater cyanobacteria blooms, it only focuses on the molecular presence of toxin genes, the research needs to be continued specifically with the toxin analysis testing to see if the samples that were positive for the gene complex are currently producing the toxins at time of collection. Testing for known toxin producers in combination with toxin analysis testing can improve our understanding of the effect they have as well as our response to HABs. Overall, the results support our hypothesis and by analyzing our data we see no observable pattern or statistical trend present.

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References cited

John, Nijoy et al. “First report of anatoxin-a producing cyanobacteria in Australia illustrates need to regularly up-date monitoring strategies in a shifting global distribution.” Scientific reports vol. 9,1 10894. 26 Jul. 2019, doi:10.1038/s41598-019-46945-8

Du, Xingde et al. “The Diversity of Cyanobacterial Toxins on Structural Characterization, Distribution and Identification: A Systematic Review.” Toxins vol. 11,9 530. 12 Sep. 2019, doi:10.3390/toxins11090530

Moreira, Cristiana, et al. "Methods to Detect Cyanobacteria and their Toxins in the Environment." Applied Microbiology and Biotechnology, vol. 98, no. 19, 2014, pp. 8073-82. ProQuest, https://www.proquest.com/scholarly-journals/methods-detect-cyanobacteria-their-toxins/docview/1561820088/se-2?accountid=10919, doi:http://dx.doi.org/10.1007/s00253-014-5951-9.

Ribeiro, Matheus S. F., et al. "Detection of Cyanotoxin-Producing Genes in a Eutrophic Reservoir (Billings Reservoir, São Paulo, Brazil)." Water, vol. 12, no. 3, 2020, pp. 903. ProQuest, https://www.proquest.com/scholarly-journals/detection-cyanotoxin-producing-genes-eutrophic/docview/2383745915/se-2, doi:http://dx.doi.org/10.3390/w12030903.

Yi-Ting, Chiu, et al. "A qPCR-Based Tool to Diagnose the Presence of Harmful Cyanobacteria and Cyanotoxins in Drinking Water Sources." International Journal of Environmental Research and Public Health, vol. 14, no. 5, 2017, pp. 547. ProQuest, https://www.proquest.com/scholarly-journals/qpcr-based-tool-diagnose-presence-harmful/docview/1910604922/se-2, doi:http://dx.doi.org/10.3390/ijerph14050547.