Development of a Near Real-Time Field Test Kit for the Rapid, Simultaneous Detection and Quantitation of High Priority Toxic Cyanobacteria

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USACE Harmful Algal Bloom

Delivering scalable freshwater HAB prevention, detection and management technologies through collaboration, partnership and

Problem Many cyanobacterial species produce a wide variety of cyanotoxins that can sicken humans and kill fish and other freshwater zooplankton. There is a lack of field-deployable and portable tools capable of simultaneous detection and quantification of multiple cyanotoxin-producing genera.

Objective Develop and validate a multiplexed test kit to rapidly identify and quantify the seven most common cyanotoxin-producing cyanobacterial genera. The test kit includes real-time quantitative PCR (qPCR) primer sets and Sandwich Hybridization Assay (SHA) capture/signal probes that are compatible with the MBio® system.

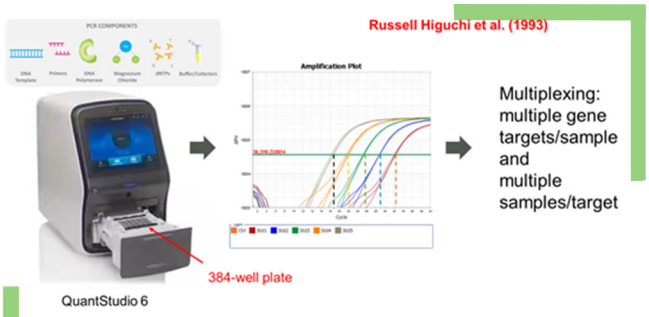

Figure 1: *Scheme of real-time quantitative polymerase chain reaction (qPCR) allowing detection of multiple cyanobacterial genera by developing genus-specific primers.*

Approach In collaboration with Bowling Green State University (BGSU), we employ a bioinformatics approach (e.g., multiple sequence alignment, phylogenetic tree construction and entropy calculation) to design genus-specific qPCR primers and SHA capture probes that can distinguish up to seven cyanobacterial genera (*Microcystis*, *Microcoleus*, *Lyngbya*, *Planktothrix*, *Cylindrospermopsis*, *Synechocystis* and the ADA clade (*Aphanizomenon* & *Dolichospermum*)) from each other and from other non-target species/genera. The SHA capture probes, zip code tags/anti-tags, signaling probes (designed by BGSU team) and qPCR primers (designed by ERDC team) are both screened and validated using pure cultures and environmental samples. Validated probes will be transformed to fit to MBio® system by LightDeck Diagnosis Inc.

Figure 2: *The anticipated final product of a sandwich hybridization assay (SHA)-based sensor cartridge that can produce readout in a portable MBio® LightDeckTM diagnostic platform (collaborative work with BGSU*

Table 1: *Testing results of genus-specific and cyanobacterial universal qPCR primers*

Primer set	Lowest C_t for nontarget species	Single-peak melt curve?	Primer efficiency (mean ± SD)	R^2 value > 0.99?
Genus-specific	n = no. of strains	Target genus/clan (n = no. of strains × repeats)		
ADA⁺	28 (n=8)	Yes	97 ± 8 (n=2×2)	Yes
Microcoleus #1⁺	29* (n=9)	Yes	88 ± 6 (n=1×2)	Yes
Microcystis #2⁺	26 (n=7)	Yes	91 ± 4 (n=2×1+1×2)	Yes
Synechocystis #2⁺	30** (n=9)	Yes	112 ± 23 (n=1×2)	Yes
ADA⁺⁺	31 (n=8)	Yes	88 ± 1 (n=1×2)	Yes
Microcystis⁺⁺	31 (n=7)	Yes	86 (n =1×1)	Yes
Planktothrix⁺⁺	15 (n=9)	No	67 (n=1)	No
Synechocystis⁺⁺	28 (n=9)	No	93 (n=1)	Yes
Cyanobacterial universal		Cyanobacterial strains (n = 10)		
LF1/LR1⁺	26	Yes	97 ± 4	Yes
LF2/LR2⁺	18	Yes	98 ± 5	Yes
JF2/JR3⁺	28	Yes	97 ± 4	Yes
BD16SF1/R1⁺⁺	18	No	103 ± 17	No
Commercial set⁺⁺⁺	8	No	91 ± 30	No

⁺Designed in Present Study; ⁺⁺Literature reported; ⁺⁺⁺Commercially available; **Lyngbya* amplified at 18 cycles; ***Microcystis* amplified at 19 cycles

Results (1) Four sets of de novo designed qPCR primers specifically targeting 16S rRNA in ADA clade, *Microcystis*, *Microcoleus*, and *Synechocystis* not only met the performance evaluation criteria but outperformed their literature-reported counterparts. (2) Two sets of universal qPCR primers (LF1/LR1 and JF2/JR3) demonstrated better performance than literature-reported and commercial primers. (3) We also developed good-quality genus-specific primer sets for *Planktothrix* and *Cylindrospermopsis*. (4) We independently validated the four SHA capture probes. (5) We will use qPCR assays to validate SHA results obtained by BGSU team.

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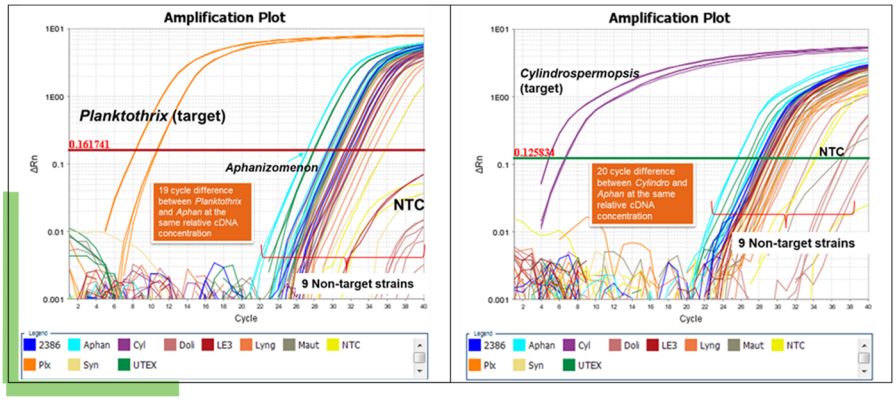

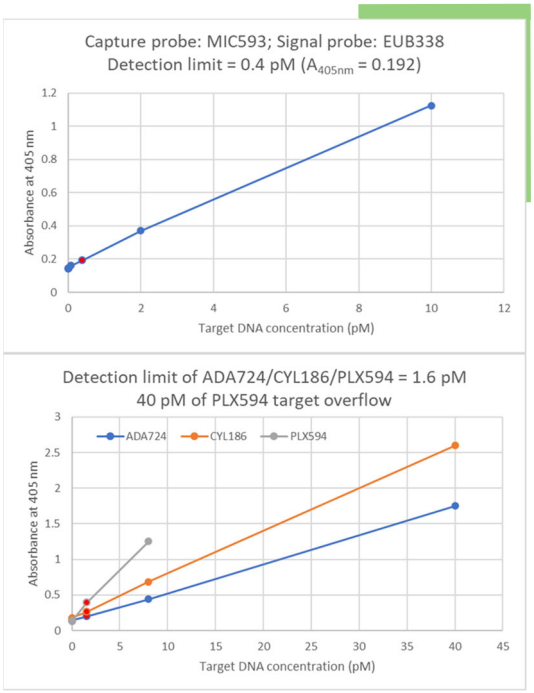

Figure 3 (Up): Testing results for two new good-quality qPCR primer sets targeting *Planktothrix* and *Cylindrospermopsis,* showing separation of their respective target strain from non-target cyanobacterial strains

Figure 4 (Right): ERDC team's independent validation of four SHA capture probes MIC593, ADA724, CYL186 and PLX594 developed by BGSU team targeting 4 genera/ clade: *Microcystis*, ADA clade, *Cylindrospermopsis* and *Planktothrix*, respectively.

Major Milestones

Date	Milestones
FY21, Q1	Literature review and *de novo* design of genus-specific capture probes
FY21, Q2	Review and design signal probes and zip code tags
FY21, Q3	Screening the probes using lab cultures belonging to 7 target genera
FY21, Q3	RNA extraction method optimization
FY21, Q3	Field collection of HAB samples
FY21, Q4	Development of genus-specific qPCR assays for cell number calibration
FY21, Q4	Data analysis and reporting
FY22, Q1	Revise and submit the review article on SHA
FY22, Q2-3	Test and validate genus-specific qPCR primers
FY22, Q3	Design and test new genus-specific capture probes and signal probes
FY22, Q4	Optimize SHA protocol
FY23, Q1-2	Validate the SHA probes and qPCR primers
Costs	**FY21:**$223k **FY22:**$149K **FY23:**$50K **TOTAL:$**422K

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units including "Small regulatory ribonucleic acids for the control of harmful algal blooms", and "Rapid, portable and multiplexed detection of freshwater harmful algal bloom-forming genera".