Rapid, Portable, and Multiplexed Detection of Freshwater HAB-forming Genera

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection and management technologies through collaboration, partnership and cutting-edge science

Lead PI: George S. Bullerjahn, Bowling Green State University

PROBLEM: Cyanobacterial Harmful Algal Blooms (CHABs) and their management have a costly impact in freshwater resources of the United States. Detection, treatment, and mitigation of blooms is a large task, especially since blooms rapidly change composition seasonally. CHABs can alter composition in short time frames in which some management efforts cannot match. Consequently, both scheduled and opportunistic monitoring is challenging if results are unavailable for several days. Laboratory methods such as microscopy and qPCR used for detection and analysis of CHABs can also be cost prohibitive.

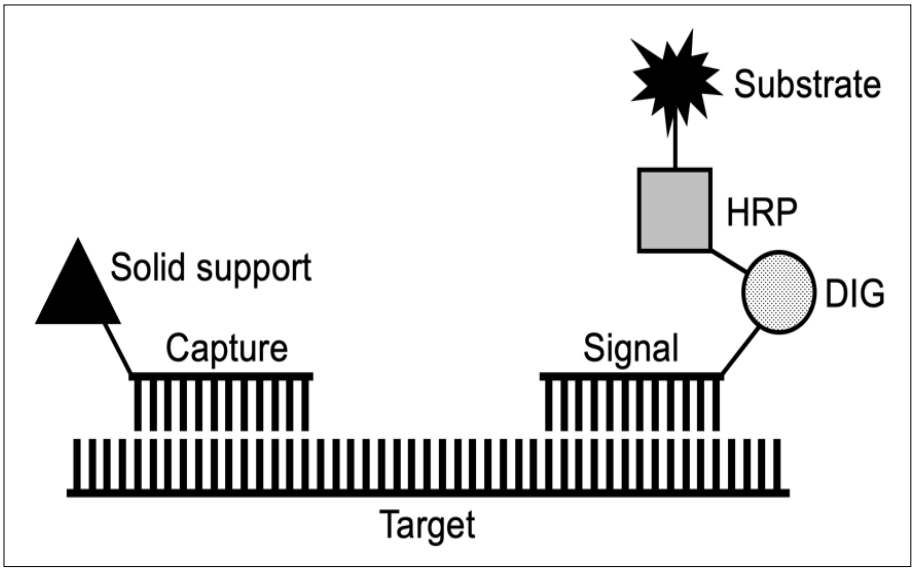

Figure 1: *The principle of SHA. The capture and DIG-labeled signal probes hybridize to reverse complement regions of the target sequence. Then, the HRP antibody binds to the DIG and substrate, producing a measurable color change.*

APPROACH: The use of new molecular techniques has led to faster, easier, and more reliable CHAB quantification. The sandwich hybridization assay (SHA) is one of these techniques, having already been utilized for marine phytoplankton. SHA is a tool that incorporates reverse complementary hybridization of two oligonucleotide probes; One of which is specific to a desired taxonomic rank, the 'capture' probe, and the other being more general to a broader group of organisms, the 'signal' probe. The capture probe is carefully designed to hybridize to 16S rRNA of the target organism(s) and discriminate against outgroups. The signal probe contains a detection method which is often a digoxygenin label recognized by an antibody linked to horseradish peroxidase, thereby yielding a chromogenic signal.

The past year, we have developed and successfully tested oligonucleotide probes specific for species of the following toxic, bloom-forming genera: *Raphidiopsis (Cylindrospermopsis)*, the ADA clade *(Anabaena/Dolichospermum/Aphanizomenon)* and *Planktothrix* (Table 1). SHAs with our culture collection have shown that each probe successfully hybridizes only to lysates of the appropriate target genus. In development of a multiplexed detection method, these probes are being used in concert with the previously available *Microcystis* probe developed by Dianne Greenfield's group.

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Table 1: *Capture and signal probes developed this year.*

Probe	Target	Sequence	Reference
CYL186	*Cylindrospermopsis* spp.	5'-biotin-[C9x3]-ACGCCTTGGTGGTCCCTTA-3'	This study
PLX594	*Planktothrix* spp.	5'-biotin-[C9x3]-GTTATAGCCCAGCAGAGCG-3'	This study
EUB338	All bacteria	5'-DIG-[C9]-GCWGCCWCCCGTAGGWGT-[C9]-DIG-3'	Daims et al. 1999
ADA724	ADA clade	5'-biotin-[C9x3]-CACAGCTCGGGTCGATACGA-3'	This study
CYA762	All cyanobacteria	5'-DIG-[C9]-CGCTCCCCTAGCTTTCGTC-[C9]-DIG-3'	Schönhuber et al. 1999

ASSESSING SHA PROBE PERFORMANCE: Standard curves yielded an environmentally relevant dynamic range for CHAB biovolumes, as seen using the ADA probe against homogenates of *Dolichospermum circinale* CS-541/06 (Figure 2). Similar results were obtained using the other probes developed thus far.

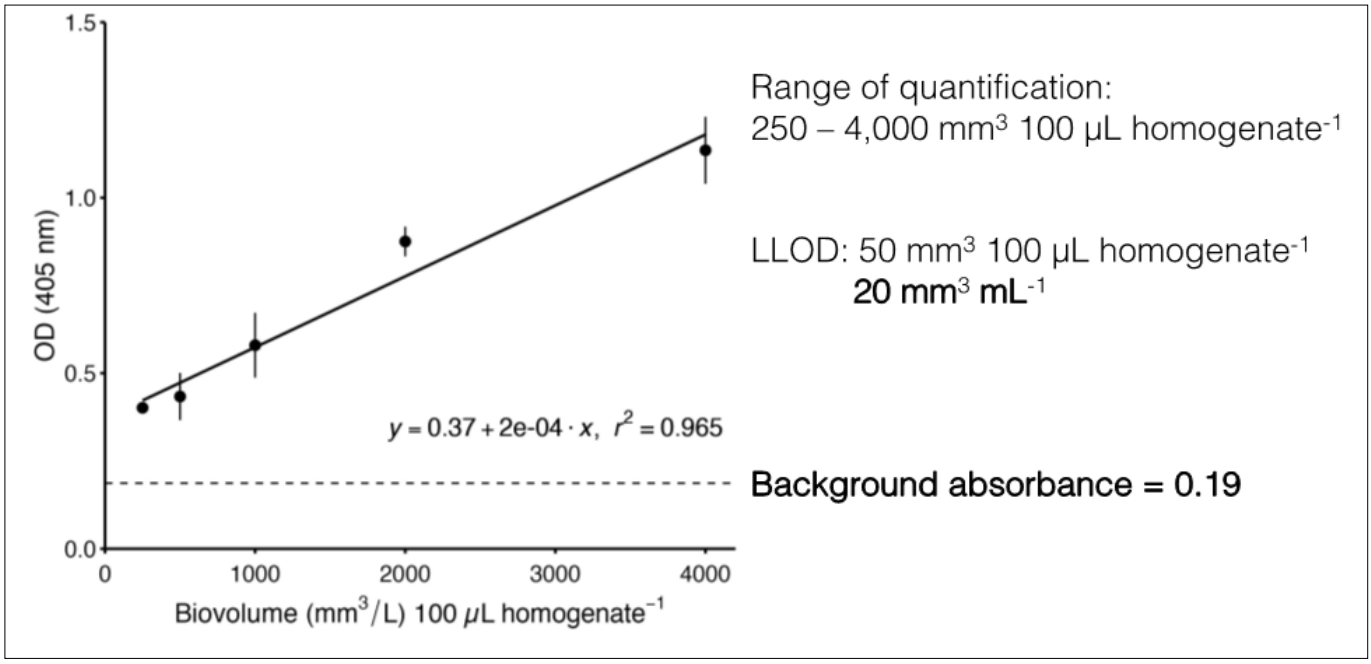

Figure 2: *Linear response of **ADA724** and D. circinale CS-541/06*

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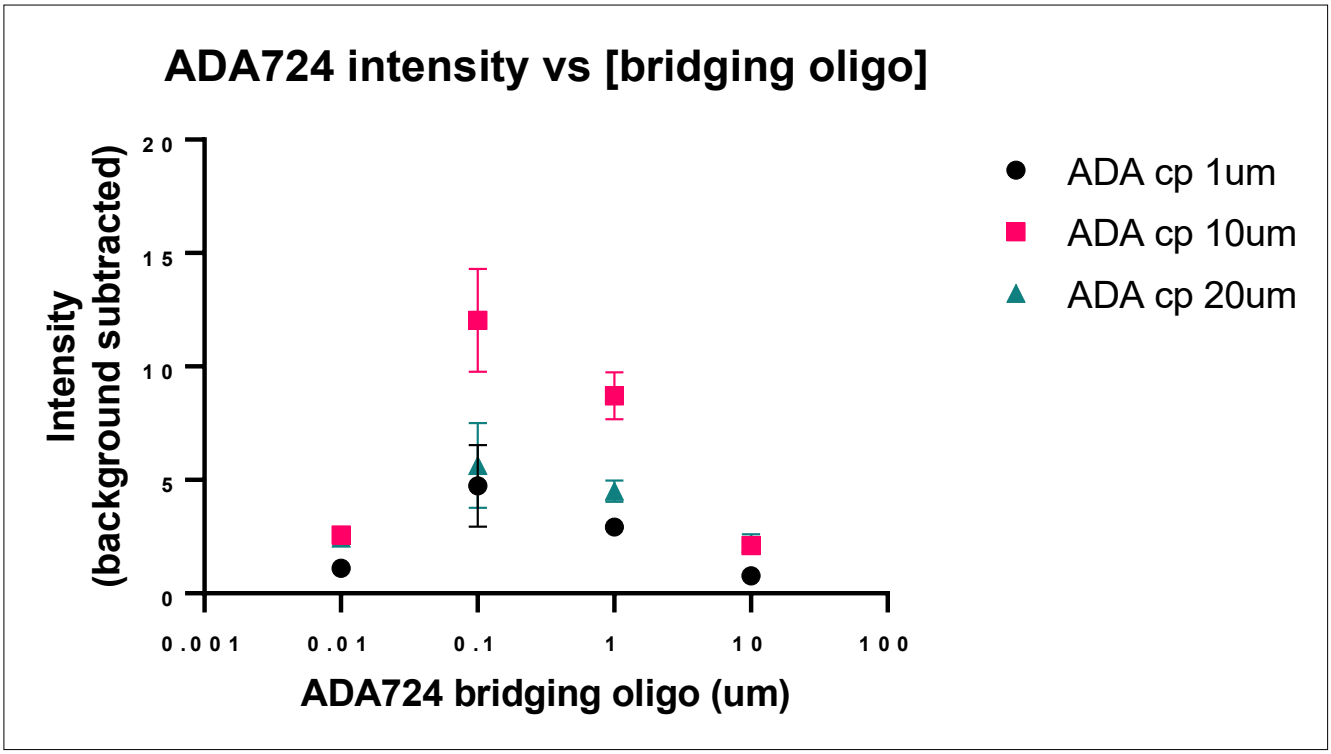

Figure 3: Fluorescence signal from the printed ADA capture probe hybridized with a complementary Cy5 fluorescent signal probe

ADAPTATION TO THE LIGHTDECK PLATFORM: The current SHA is a plate-based assay, yielding a colorimetric signal following a reaction with an HRP-conjugated digoxygenin antibody. For a rapid field assay, modifying the protocol to the fluorescence-based LightDeck system affords a means to develop a multiplexed SHA, employing cartridges printed with multiple genus-specific capture probes. Pilot experiments with cartridges printed with the ADA724 probe demonstrates hybridization and fluorescence detection from the signal probe (Figure 3).

These data are a proof of concept that the LightDeck platform can be adapted to the SHA, using the capture probes developed in this study.

FUTURE GOALS: The next steps include testing the SHA response with cultures at different growth stages, testing the specificity of the probes in mixed cultures, and finally field testing of the SHA on bloom biomass obtained from the field. Kowing that the technology is now working, additional probes targeting benthic strains known to produce neurotoxins (e.g. Microcoleus spp.) are also a high priority.