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An optical biosensor-based quantification of microcystin synthetase A gene: Early warning of toxic cyanobacterial blooming

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ABSTRACT: Monitoring and controlling of toxic cyanobacterial strains, which can produce microcystins, is critical to protect human and ecological health. We herein reported an optical biosensor-based quantification of microcystin synthetase A (mcyA) gene so as to discriminate microcystins-producing from non-producing strains. In this assay, the mcyA-specific ssDNA probes were designed *in silico* with an online tool and then synthesized to be covalently immobilized on an optical fiber surface. Production of fluorescently modified target DNA fragment amplicons was accomplished through the use of Cy5-tagged deoxycytidine triphosphates (dCTPs) in the polymerase chain reaction (PCR) method, which resulted in copies with internally labelled multiple sites per DNA molecule and delivered the great sensitivity. With a facile surface-based hybridization process, the PCR amplicons were captured on the optical fiber surface, followed by fluorescence emission induced by an evanescent wave field. Under the optimum conditions, the detection limit was found to be 10 pM

(S/N ratio = 3) and equaled to 10^3 gene copies/mL. The assay was triumphantly demonstrated for PCR amplicons of mcyA detection, and showed satisfactory stability and reproducibility. Moreover, the sensing system exhibited excellent selectivity with quantitative spike recoveries from 87% to 102% for *M. aeruginosa* species in the mixed samples. There results confirmed that the method would serve as an accurate, cost-effective and in-field testing for rapid detection of toxic *Microcystis* sp. in water, giving early information for water quality monitoring against microcystins-producing cyanobacteria.

INTRODUCTION

The accelerated eutrophication of surface water sources and climate change have led to an annual occurrence of cyanobacterial blooms in many drinking water resources.[1] Blooms caused by cyanobacteria (blue-green algae) are continually formed by heterogeneous populations of toxin-producing and non-producing strains and exhibit significant threat to human health and ecological system.[2] The cyanotoxins released by cyanobacterial blooms in surface waters are usually grouped in hepatotoxins, neurotoxins, cytotoxins, irritants, and gastrointestinal toxins according to their mode of action.[2,3] One of the well-known hepatotoxins named microcystins (MCs), produced by a diverse range of unicellular and filamentous genera: *Microcystis, Anabaena, Nostoc,* and *Planktothrix* (*Oscillatoria*), are the most plentiful, varied, and ubiquitous cyanotoxins.[4-6]

The cyclic heptapeptide MCs possess variable amino acids at positions 2 and 4, and their toxicity is mainly grounded on the inhibition of protein phosphatases of types 1 and 2 A.[7-9] MCs can cause various side effects in humans and even fatal damage to the liver such as hepatocellular carcinoma when long-term exposure to the MCs-contaminated water.[10-12] Microcystin-LR (MC-LR) is one of the most toxic MCs. In the light of the widespread contamination and its strong toxicity, the residue of MC-LR in drinking water and surface water is under strict control, for example <1.0 μg/L set by World Health Organization (WHO) and Chinese government.[13] MCs are produced by the microcystin synthetase enzyme complex containing a non-ribosomal peptide synthetase (NRPS) and a polyketide synthase (PKS). The biosynthetic enzymes are codified by the mcy genes cluster via a thio-template mechanism.[5,14-16] The mcy genes are present in microcystin-producing cyanobacteria but usually absent in their nontoxic counterparts. Therefore, detection of toxin biosynthetic genes in water samples is a general approach to identify potential hepatotoxin-producing cyanobacteria from heterogeneous populations of strains, which is especially critical for any mitigation measures.

Roles of various mcy genes in the microcystin biosynthesis have been clarified by genome sequencing and characterization.[5,15] For example, the microcystin synthetase (mcy) genes cluster in *Microcystis aeruginosa* spans 55 kb and contains 10 genes organized in two large bidirectionally transcribed operons, mcyA-C and mcyD-J.[14,15,17] Among them, the genes mcyA and mcyB have proven to be the most popular targets for identification of microcystin-producing genotypes of different cyanobacterial

genera.[14] Polymerase chain reaction (PCR) is a common approach to amplify the target DNA and then followed with various quantitative technologies, such as fluorescent detection system named as quantitative real-time PCR (qPCR)[18-23] and oligonucleotide microarray chip assay.[24,25] However, the requirement of absolute quantifying DNA target based on the above-mentioned technologies often means a large investment in equipment, which have prevented their widespread utilization in the area of toxin-producing cyanobacterial diagnostics.[14] Besides, one approach in qPCR assays relies on construction of an accurate standard curve using appropriate external standards of known concentration, which is time-consuming, usually taking three days.[26]

Biosensors are analytical devices incorporating a biological sensing element, which harness the exquisite sensitivity and specificity of biology in conjunction with transducers to deliver measurements with simple, easy-to-use formats.[27] Among these, optical fiber-based evanescent wave fluorescent biosensors have attracted intensive attention because of their potential for easy miniaturization, high selectivity and sensitivity because of the narrowed sensing scale and the use of fluorescein labeling.[28] Over the past two decades, various fiber-based fluorescent biosensors for variety of environmental contaminants using different biological recognition elements have been developed, including antibodies,[29-31] aptamers,[32,33] DNAzymes[34] and other functional DNA strands.[35,36] Moreover, nucleic acids are used as receptors in such a biosensor for the determination of complementary nucleic acid strands.[37,38] These biosensors require simple sensing instrument and less professional personnel, serving as a beneficial

supplement to traditional instrumental techniques. However, to our best knowledge, researches on exploration of such a biosensor for cyanobacteria species-specific genes detection, even an extension to other cells, are rare, although the PCR-assisted DNA sensor systems have been reported via electrochemical transducers.[39,40] The technical challenges may arise from: 1) it suffers from limited sensitivities if circumventing PCR amplification; 2) fluorescein labeling is required to enable the sensing process readable.

In order to cope with these two challenges, we herein described a PCR-assisted optical fiber-based evanescent wave fluorescent biosensor for early warning of the presence of toxic cyanobacterial blooming. The fluorescein labeling of DNA targets was accomplished by using Cy5-tagged deoxycytidine triphosphates (dCTPs) during the PCR amplification. To our best knowledge, this report is the first on a PCR-assisted fluorescent biosensor for identification of hepatotoxin-producing cyanobacteria, providing an easy-to-use, sensitive, and specific method for rapid detection of microcystin synthetase A (mcyA) genes. More importantly, this study can serve as a good reference and provide important implications in sensing other specific genes for diverse potential applications in environmental monitoring, clinical diagnostics and other hygiene.

EXPERIMENTAL SECTION

Materials, Reagents and Apparatus

See Supporting Information (Section 1) for materials, reagents, buffers and DNA sequences used in this work. The description of independent-developed optical

fiber-based evanescent wave fluorescent biosensor used in this work can be found in Supporting Information (Section 2) with its schematic diagram as shown in **Figure S1**. Briefly speaking, fluorescently labeled DNA target amplicons were pumped into the flow cell of the fluorescent biosensor, where the fluorophores would be excited within the evanescent field around the optical fiber. And then the fluorescent emission signals were subsequently coupled to the optical fiber and could be quantitatively recorded.

Biological Material and Culturing Conditions

Unialgal cultures of toxic *M. aeruginosa* FACHB-905 was purchased from the Culture Collections of the Freshwater Algae of the Institute of Hydrobiology (FACHB; Wuhan, China), which was grown in BG-11 medium at pH 7. Two strains of *Chlorella* species (ZTY4 and ZTY5) were provided by Prof. Hongying Hu in our school, and both cultures were grown in msBG-11 medium at pH 7.[41] All cultures were maintained under controlled culture conditions (25 °C ± 1 °C, 1500 Lux and 12:12 h of light/dark cycle).

***in silico* Primer and Probe Design**

The DNA probe in length of 20 nt specific to *M. aeruginosa* mcyA (the GenBank database sequence accession: No.AB019578.2) was designed *in silico* with an online tool, UPS Unique Probe Selector (http://array.iis.sinica.edu.tw/ups/), the advantages of which have been reviewed.[42] The UPS algorithm considered the GC content, the secondary structure, the melting temperature (T_m), the stability of the probe-target duplex estimated by the thermodynamic model, sequence complexity, similarity of probes to non-target sequences, and other empirical parameters used in the laboratory

when selecting probes.[43] The mcyA-specific primers as shown in Section 1 of Supporting Information, **Table S1**, were designed using an online tool, Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/ primer-blast). This tool combined BLAST with a global alignment algorithm to ensure a complete primer-target alignment and was sensitive enough to detect targets which had a remarkable number of mismatches to primers.[44] By inputting the desired amplicon size of 300 bp and the starting position of forward primer and the end position of reverse primer which covered the mcyA-specific probe sequence, a preferred specific region of mcyA (219 bp in length) was outputted by Primer-BLAST. The 219 bp sequence was confirmed with 100% homology to mcyA sequence of *M. aeruginosa* by comparison with the GenBank database. The selected probe candidates in **Table S1** were subjected to a further specificity test checked against the nucleotide collection (nr/nt) database using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The complete probes set chosen for further experiments consisted of forward primer and reverse primer (probes numbered 5−8 and 9−12) and UPS-generated best probe (probes numbered 1−4) as shown in Supporting Information, **Table S2**. When immobilizing the DNA probe onto solid surface, a spacer is usually introduced between the surface and the capture probe, which helps to reduce possible steric hindrances during hybridization between the immobilized capture probe and DNA targets in samples, and hence improves the hybridization efficiency on sensing surface.[32,45,46] Therefore, each of the probes was designed with a 10-adenine spacer and amino modification at either the 5′ or 3′ end.

Algae DNA Extraction and PCR

Algae cell cultures were subjected to several freeze-thaw cycles and gDNA was extracted with TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa) as per the manufacturer's instructions. The Kit included a proprietary lysis buffer and spin column for binding and purifying gDNA. DNA quality and quantity were evaluated by using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Purified DNA was preserved at -20°C until further analysis.

The specific region of mcyA (219 bp) covering the investigated probe sequences was amplifying by using the PCR technique. Production of fluorescently labelled DNA fragment targets was accomplished directly through the use of fluorescently modified deoxycytidine triphosphate (dCTP). The approach resulted in amplicons that were internally labelled at multiple sites per molecule and delivered the great sensitivity. Details can be found as follows.

PCR amplification of the target DNA was carried out using 200 μL PCR tubes with a reaction mixture of 25 μL. Each of the reaction mixtures contained 1×KCl PCR buffer, 0.2 mM each of deoxyadenosine triphosphates (dATPs), deoxyguanosine triphosphates (dGTPs), deoxythymidine triphosphates (dTTPs) in combination with 0.16 mM dCTPs and 0.04 mM Cy5-labeled dCTPs, 1.5 mM $MgCl_2$, 1.25 U of Taq DNA polymerase, 0.4 μM of each primer, 300 ng gDNA and sterile purified water up to 25 μL. The PCR instrument (Bio-Rad, USA) was standardized with an initial denaturation step 95 °C for 3 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, and a final extension step at 72 °C for 10 min. The reaction without the template served as a non-template control. Four parallel replications for the

non-template control and template were conducted in this work during the PCR amplification process. 5 μl of each PCR amplicons was electrophoresed on an agarose gel (1.5% w/v) containing 1×GelRed (Biotium, USA) in 1×TAE buffer at 100 V for 1.5 h. Gel photographs were taken in a Gel Documentation System (Bio-Rad, USA). Upon completion of the above reaction, the PCR amplicons were purified using the PureLink® PCR Purification Kit (Invitrogen-Life Technologies) following manufacturer's instructions. Purified PCR amplicons were preserved at -20°C for usage.

Preparation of ssDNA-Modified Optical Fiber

The combination tapered optical fiber for sensing in evanescent wave was fabricated by the tube-etching method as depicted by our previous study.[31] The ssDNA immobilization on the optical fiber surface was accomplished as similar as that reported before.[45] Briefly speaking, the combination tapered optical fiber was soaked in freshly prepared piranha solution to introduce hydroxyl groups, followed by immersing in 1% (v/v) (3-Aminopropyl) triethoxysilane (APTS) toluene solution to form amino groups on the fiber surface, and then thoroughly washed with dry toluene, dried with nitrogen, fixed by heating in an oven. Afterwards the fiber was immersed in 1% v/v glutaraldehyde (GA) aqueous solution and then in 300 nM NH_2-DNA and 100 nM NH_2-A_{10} solution (NH_2-A_{10} was used as a short strand mixer). After that, glycine aqueous solution was used to remove excess aldehyde groups. Last, the fiber was reduced with $NaCNBH_3$ aqueous solution. After above-mentioned steps, the fiber was kept in the isoelectric bovine serum albumin (BSA) solution and preserved at 4°C for

usage. The schematic diagram can be found in Section 3 of Supporting Information, **Figure S2**.

Recovery in *Chlorella* sp. Contained Samples

To assess the practical applicability of this assay, the PCR amplicons of mcyA gene from cultured *M. aeruginosa* mixed with *Chlorella* sp. (ZTY4 and ZTY5) samples were detected and evaluated by using this PCR-assisted optical biosensing assay. Firstly, 1mL *Chlorella* sp. sample (ZTY4 and ZTY5, the concentration were 2×10^8, 5×10^6 cells/mL respectively) was mixed with various concentrations (3×10^4, 1×10^5, and 3×10^5 cells/mL) of unialgal cultures of *M. aeruginosa*. Subsequently, the gDNA of the mixture was extracted followed by PCR amplification with conditions identical to those for the sample containing *M. aeruginosa*. Lastly, the PCR amplicons were purified and diluted with binding buffer. The dilutions in series concentrations were pumped into the optical biosensor for measurement.

RESULTS AND DISCUSSION

Sensing Mechanism

Figure 1 illustrates the sensing mechanism of the PCR-assisted evanescent wave biosensor for detection of mcyA gene of microcystins-producing *M. aeruginosa*. In this assay, on the one hand, the mcyA-specific single-stranded DNA (ssDNA) probes were designed *in silico* and then synthesized for optical fiber surface modification. The fiber was activated by APTS/GA for covalent immobilization of ssDNA probes, which was then blocked by isoelectric BSA solution. On the other hand, gDNA of the cultured algae cells was extracted and isolated for PCR amplification. Production of

fluorescently modified target DNA fragment amplicons was accomplished through the use of Cy5-tagged dCTPs in the PCR method, which resulted in copies with internally labelled multiple sites per DNA molecule, thereby correlating with higher fluorescence signals. The purified PCR amplicons were thermally denatured by heating at 95°C for 3 min and immediately reannealing in ice bath for 1 min, to generate the ssDNA for biosensing.[40,46,47] With a facile surface-based hybridization process after the accomplishment of above-mentioned steps, the PCR amplicons were captured on the optical fiber surface, which was detected by evanescent wave-induced fluorescence emission and directly related to the concentrations of mcyA genes and microcystin-producing *Microcystis* sp. in samples. In the end, the fiber surface was regenerated with wash buffer, leaving it ready for a new test cycle.

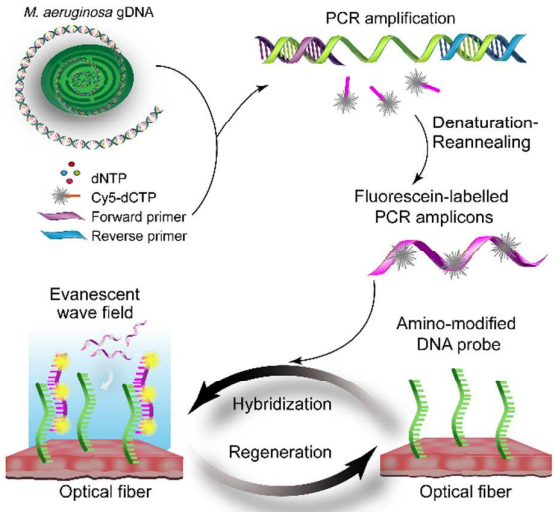

Figure 1 Schematic diagram of PCR-assisted optical fiber-based evanescent wave fluorescent biosensing platform for detection of mcyA gene

Optimization of McyA-specific DNA Probe Sequence

In order to obtain the DNA probe sequences specific and sensitive towards the target mcyA sequence, this was initiated by *in silico* probe design and then validated on the optical fiber biosensor platform by using the fluorescently labeled mcyA-specific PCR amplicons. The investigation considered the difference between sense and antisense hybridization as well as the potential variability that might be caused by immobilizing the probes at either the 5′ or 3′ end of the ssDNA probe, which were proven critical for the surface-based hybridization efficiency.[40,46] To address the last issue, the DNA probes were modified with the amino group at the 5′ or 3′ end of the sequence, separately, and immobilized on the fiber surface in both orientations. Moreover, sense (the strand corresponding to mRNA sequence) and antisense (the strand complementary to the mRNA sequence) sequences were investigated considering the possible variability in hybridization efficiency in this regard. So it doubled the numbers of the probe set from three (primers and probe deduced through the selection process) to twelve and numbered as shown in **Table S2**.

The hybridization of fluorescence-labeled PCR amplicons, which were prepared in binding buffer (0.1 M PBS, 0.15 M NaCl, pH 7.2) with final concentration of 5 nM, resulted in the fluorescence intensities as shown in **Figure 2**. In spite of being selected by *in silico* analysis, most of probe sequences proved to be inefficient to hybridize with the mcyA PCR amplicons. Results demonstrated that the probe orientation and relative probe/target position had strong effects on the hybridization efficiency of long fragment PCR amplicons (219 bp) with surface-immobilized probes, consistent with the previous results reported by other researchers.[40,46,48,49] Among them, the 6AS5′

probe, *i.e.* the antisense strand corresponding to the forward primer sequence with the 5′ end immobilized on the optical fiber, showed the highest fluorescent signal, therefore it was consequently chosen for further bioassays. These results reveal that experimental validation of *in silico*-designed probes is indispensable for the authentication of probes that bind efficiently to long DNA targets of interest before their immobilization on the fiber surface.

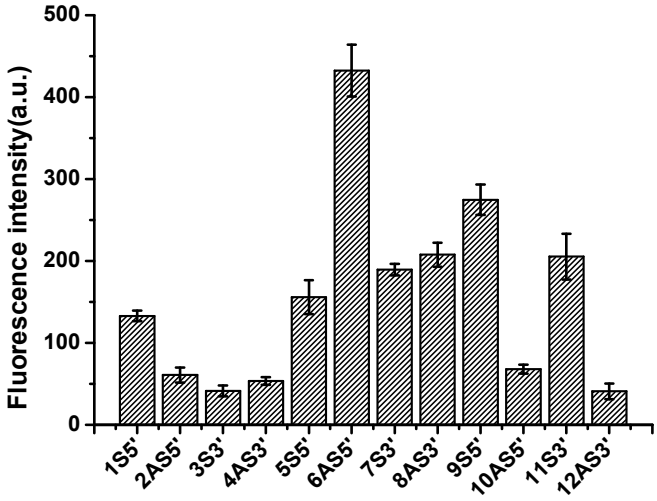

Figure 2 Fluorescence intensity for each probe developed *in silico* upon hybridization of 5 nM Cy5-labeled mcyA PCR amplicons (error bars: SD, n = 3). The nomenclature signifies the probe number followed by sense (S)/antisense (AS) sequence output by UPS software, with either the 5′ or 3′ end immobilized on the optical fiber

Optimization of Binding Buffer

We tested the effect of buffer compositions and ionic strengths on the surface-based hybridization efficiency, judging from the fluorescent signals of biosensor. The PCR amplicons at 5 nM in various binding buffers (please see Supporting Information,

Section 1) were pumped into the flow cell of fiber biosensor and hybridized with the immobilized 6AS5′ DNA probe on the surface. The relationship between the different buffer solutions and corresponding fluorescent signals is shown in **Figure 3** by plotting the fluorescence intensity versus the binding buffer type. The optimum binding buffer under the investigated conditions was 2×Saline-sodium Citrate (SSC). It also concluded that the hybridization signals increased with the stronger ionic strength in some range. At the low salt concentration status employed, these ions might be insufficient to alleviate the electrostatic repulsion between two ssDNA molecules,[50] thereby hindering the efficient surface-based hybridization, eventually leading to lower biosensor signals. In the end, 2 × SSC buffer was chosen and employed in the following experiment.

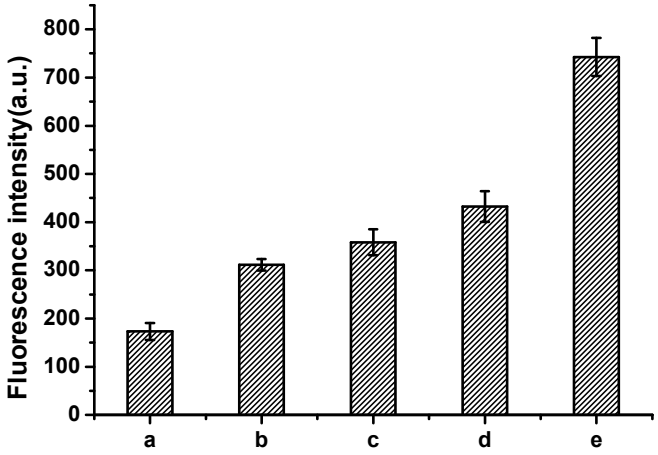

Figure 3 Response signals of the ionic strength with different types was established by plotting the fluorescence intensity versus the binding buffer type: (a) 0.01 M PBS, pH 7.2; (b) 0.1 M PBS, pH 7.2; (c) 0.01 M PBS + 0.15 M NaCl, pH 7.2; (d) 0.1 M PBS + 0.15 M NaCl, pH 7.2; (e) 2 × SSC (300 mM NaCl + 30 mM Na-citrate), pH 7.2

Detection of PCR Amplicons of mcyA Gene

In order to detect the toxic *M. aeruginosa*, the PCR amplicons of 219 bp which covers investigated probe sequences were utilized to be sensed by the immobilized ssDNA probe on the optical fiber surface. Under the optimized experimental conditions, the fluorescently labeled amplicons at different concentrations were pumped into the flow cell of biosensor at a constant flow rate of 2 mL min^{-1} for 15 s and hybridized with the immobilized ssDNA probe for 5 min to form fluorophore-labeled dsDNA. Once the reaction equilibrium state was reached, the peak value of fluorescence intensity was recorded and directly proportional to the target DNA concentration. In the end, a regeneration process was performed by rinsing the fiber probe with wash buffer at a constant flow rate of 2 mL min^{-1} for 60 s and binding buffer for another 60 s, leaving it ready for a new test cycle.

Figure 4A shows the original fluorescence signal traces of the optical fiber evanescent wave biosensor platform observed in the flow of PCR amplicons solution at various concentrations (0~10 nM). It revealed that the dynamic process of fluorescent signals, which increased with more hybridization of PCR amplicons target with the immobilized probe. The calibration curve of PCR amplicons target was plotted in **Figure 4B**. Higher logarithm concentrations of PCR amplicons resulted in higher fluorescent intensities. A proportional relationship was observed between amplicon concentration and the fluorescence intensity in a linear range from 0.05 to 5 nM ($F = 151.3$ c/nM + 24.1, $R^2 = 0.98$) with the LOD calculated to be 10 pM at an S/N ratio of 3. Compared with the sensing performances of several DNA based

optical fiber sensors,[51-54] this platform showed more sensitive in DNA target detection. We attributed the improved sensitivity to the internally labelled multiple sites per DNA molecule in PCR amplification. Moreover, its sensitivity was comparable with those of other reported PCR-assisted electrochemical DNA sensors.[39,40] The mcyA PCR amplicons of 10 pM were generated from 10^3 cells/mL, as determined by microscopic counting. Therefore, such a biosensor showed the LOD of 10^3 gene copies/mL based on the theory that one cell contains one microcystin biosynthetic gene cluster.[55]

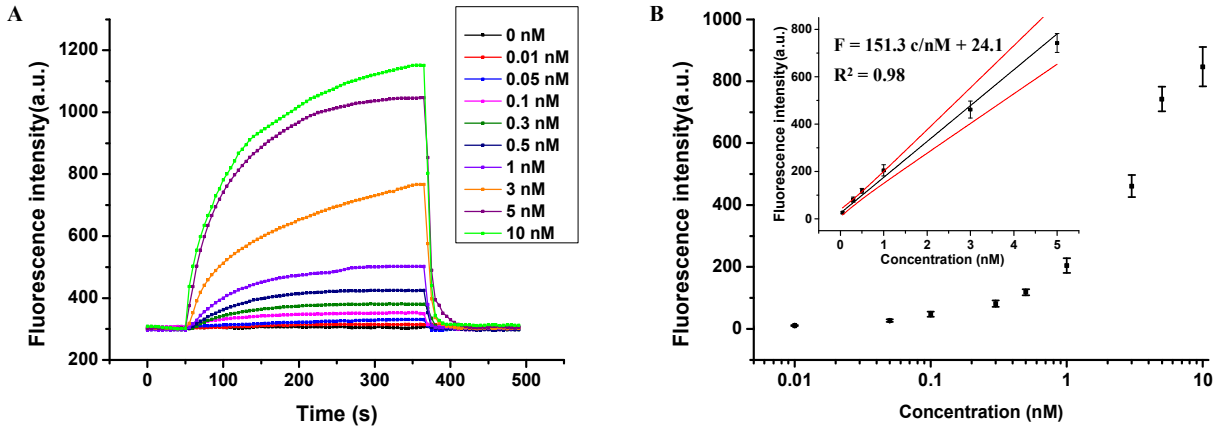

Figure 4 (A) Real-time fluorescence responses versus different concentrations of PCR amplicons of mcyA gene; (B) calibration curve for PCR amplicons detection by using the proposed optical fiber-based evanescent wave fluorescent biosensor. Inset: the linear range of PCR amplicons in the range of 0.05~5 nM with a 95% confidence band (shown in red)

For comparison, we also tested the synthetic short ssDNA target in length of 20 nt labeled with Cy5 at 5′ end (**Table S1**, fully complementary to the fiber surface-immobilized probe) by using the present optical fiber evanescent wave

DNAsensor. Details can be found in Section 4 of Supporting Information. As shown in **Figure S3**, a linear relationship between the hybridization signal and synthetic short ssDNA target concentrations could be observed in the range of 0.1-5 nM (F = 117.2 c/nM + 12.9, R^2 = 0.99), with the LOD value of 30 pM. Compared with PCR amplicons (**Figure 4**), the sensitivity was overshadowed might due to that per short synthetic DNA target molecule was tagged with only one Cy5 fluorophore.

Reusability

The optical fiber surface of such a biosensor was reversible by using wash buffer without destruction of the biosensing properties. Three representative regenerated signal traces were plotted in **Figure 5**. It was found that the fluorescence signal decreased 2 % after the 100^{th} usage and 10% after the 150^{th} usage. This indicated that the optical fiber sustained its sensing ability after being used at least 150 times with the less signal loss. The excellent reproducibility of evanescent wave fluorescent biosensing platform were beneficial to achieve cheap, automatic, on-line and facile detection.[35]

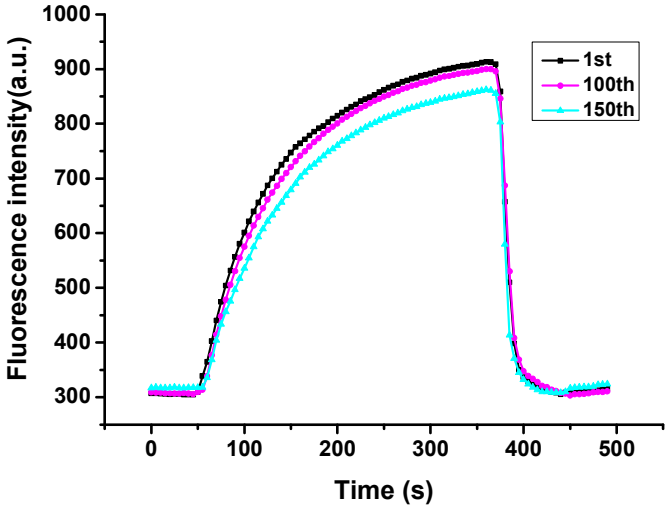

Figure 5 Real-time fluorescence responses for the optical fiber-based evanescent wave fluorescent biosensor experiencing different cycles of measurements during the assay

Recovery Evaluation with *Chlorella* sp. Contained Samples

In the recovery experiment, PCR amplicons from gDNAs of *M. aeruginosa*, *Chlorella* sp. (mixtures of ZTY4 and ZTY5) and mixture of *M. aeruginosa* with *Chlorella* sp. were analyzed by agarose gel electrophoresis. The expected PCR amplicons were produced only by *M. aeruginosa* containing the target mcyA gene (Section 5 of Supporting Information, **Figure S4**), demonstrating that the designed primers were specific to mcyA gene. The detection performance of this method for the spiked *Chlorella* sp. (mixtures of ZTY4 and ZTY5) samples with various concentrations of *M. aeruginosa* (Sample 1, 2 and 3) is presented in **Table 1**. The average percent recoveries ranged from 87% to 102%, with the acceptable variations that the relative standard deviation was no more than 9% (n=3). These results confirm the satisfactory accuracy of the developed evanescent wave fluorescent biosensing platform for

microcystin synthetase gene (mcyA) and mcyA-producing species detection in real microalgae samples.

Table 1 Recovery in *Chlorella* sp. contained samples

Samples[a]	*Microcystis aeruginosa* cells		Recovery (%)	Relative SD (%)
	Spiked (copies·mL^{-1})	Measured mean[b]±SD[c] (copies·mL^{-1})		
1	3×10^4	$(2.63\pm0.236)\times10^4$	87	9.0
2	1×10^5	$(9.42\pm0.176)\times10^4$	94	8.3
3	3×10^5	$(3.07\pm0.185)\times10^5$	102	6.0

[a] 1~3 represent the *Chlorella sp.* samples with various spiked concentrations of *M. aeruginosa* cells

[b] Mean value of three individual determinations

[c] Standard deviation

CONCLUSIONS

In summary, a PCR-assisted optical fiber-based evanescent wave fluorescent biosensor was built to enable the facile detection of mcyA from microcystin-producing *M. aeruginosa* with high sensitivity and selectivity. To the best of our knowledge, this report is the first on a sensitive, selective, facile, robust and cheap biosensor-based assay of and early warning system for toxic cyanobacterial blooming, possible to provide new insight into the production mechanism of MCs and prevention of cyanobacterial blooms. It can also be expected, by combination with the widely-reported miniaturization of PCR devices and their analogs, the simple and sensitive sensing platform endows its potential for on-site, real-time identification ability of toxic cyanobacteria during the blooming process. More importantly,

considering the diverse applications of nucleic acids detection, the proposed assay system has great potential to be expanded into new areas and thus increase the power of this new tool to answer many monitoring or research interests questions.

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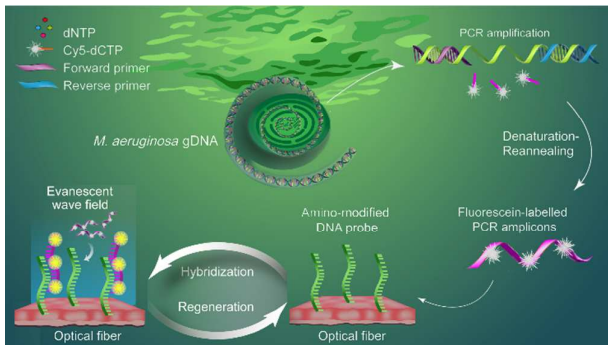
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Cy5-dCTP
Forward primer
Reverse primer
PCR amplification
M. aeruginosa gDNA
Denaturation-
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Evanescent
wave field
Amino-modified
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