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Detection and Quantification of Toxin-Producing *Microcystis aeruginosa* Strain in Water by NanoGene Assay

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We demonstrated the quantitative detection of a toxin-producing *Microcystis aeruginosa* (*M. aeruginosa*) strain with the laboratory protocol of the NanoGene assay. The NanoGene assay was selected because its laboratory protocol is in the process of being transplanted into a portable system. The *mcyD* gene of *M. aeruginosa* was targeted and, as expected, its corresponding fluorescence signal was linearly proportional to the *mcyD* gene copy number. The sensitivity of the NanoGene assay for this purpose was validated using both dsDNA *mcyD* gene amplicons and genomic DNAs (gDNA). The limit of detection was determined to be 38 *mcyD* gene copies per reaction and 9 algal cells/ml water. The specificity of the assay was also demonstrated by the addition of gDNA extracted from environmental algae into the hybridization reaction. Detection of *M. aeruginosa* was performed in the environmental samples with environmentally relevant sensitivity (~10^5 algal cells/ml) and specificity. As expected, *M. aeruginosa* were not detected in nonspecific environmental algal gDNA over the range of 2×10^0 to 2×10^7 algal cells/ml.

Keywords: Algal bloom, cyanobacteria, *Microcystis aeruginosa*, microcystin, NanoGene assay

Introduction

Algal blooms are mass antagonists of fisheries stock and they rapidly suffocate aquatic life. Their recent carnage includes over 20 million Chilean salmons at a cost of ~800 million USD [1]. In 1972 and 2001, algal blooms wiped out 14 million yellowtails in Japan [2] and 1,100 tons of salmon in the North Seas [3]. An algal bloom is caused by the eutrophication of water bodies, which is fueled by excessive agricultural fertilizers and animal waste run-off. It can trigger a near full-scale trophic cascade, such as in Lake Erie, USA [4]. However, the more sinister side of an algal (cyanobacterial) bloom is the production of hepatotoxins such as microcystins. It has been known to accumulate in aquatic produce [5, 6] and eventually make its way to the human body. Excessive amount of microcystins can result in eventual liver failure [7, 8].

Microcystins are mainly produced by the genera *Microcystis*, *Anabaena*, and *Planktothrix* [9]. Particular strains of *Microcystis aeruginosa* (*M. aeruginosa*) are considered as dominant contributors of microcystin [10, 11]. *M. aeruginosa* belongs to freshwater cyanobacteria or blue-green algae. They are characterized by their microcystin synthetase (*mcy*) gene. This is because microcystins are produced by the enzyme complexes encoded in the *mcy* gene. Hence, toxin-producing *M. aeruginosa* strains can be specifically detected via the *mcy* genes [12–14]. Given the rate of growth of an algal bloom, it is in the interest of the water and public health authorities to identify toxin-producing *M. aeruginosa* strains as soon as an algal bloom seems likely. In this case, a portable detection system or a detection method that can be transplanted into a portable system will be useful.

Various genomic methods such as qPCR assay, DNA chip assay, and flow-based cantilever biosensor have so far been successful in detecting toxin-producing *M. aeruginosa* strains [12–18]. Furukawa *et al*. [15] performed qPCR assay and detected nine cyanobacterial cells per reaction. Rudi *et al*. [17] obtained a lower detection limit of 100 cyanobacterial cells/ml using a combination of competitive PCR and sequence-specific labeling of oligonucleotide probes. Johnson and Mutharasan [16] presented a cantilever-based

assay for the detection of toxin-producing *M. aeruginosa* and detected as low as 50 cells/ml. However, the possibility of utilizing these methods in a portable system is not evident.

In this study, we employed the NanoGene assay to detect and quantify the *mcy* gene of toxin-producing *M. aeruginosa.* The NanoGene assay was selected because its laboratory protocol is currently being transplanted into a portable system [19–21]. Together with a variety of portable lysis devices [19, 20], it is likely that we will be able to have a portable system for detecting toxin-producing *M. aeruginosa* in the near future. The NanoGene assay was first developed as an inhibitor-resistant gene quantification assay with high sensitivity and specificity [22, 23]. It has been shown previously to have similar sensitivity as PCR in a laboratory setting [24]. The NanoGene assay consists of two quantum dot nanoparticles (QD_{565} and QD_{655}) coupled with signaling and probe DNAs, respectively. Both signaling and probe DNAs hybridize with the target DNA. This allows the fluorescence measurement of QD_{655} (signaling DNA) to be normalized with that of QD_{565} (probe DNA). DNA hybridization has unparalleled specificity to recognize the target DNAs, as DNA hybridization takes place only when the complementary DNA sequence matches.

In this demonstration, we performed the detection and quantification of the microcystin synthetase D (*mcyD*) gene using the laboratory protocol of the NanoGene assay. The sensitivity of the NanoGene assay for the detection of *M. aeruginosa* was validated using both dsDNA *mcyD* gene amplicons and un-amplified genomic DNAs (gDNA). The assay specificity for *M. aeruginosa* was demonstrated with the use of nonspecific mixed environmental algae.

Materials and Methods

DNA Oligonucleotides

DNA oligonucleotides were constructed based on the sequences of the *mcyD* gene (accession numbers in GenBank: AB032549, AM778952, and JQ290094). The *mcyD* gene was selected because its specificity to *M. aeruginosa* has already been demonstrated in previous studies [13, 25]. The DNA sequences for the DNA probes used in this study are listed in Table 1 [26]. The probe DNA sequences were modified at the 5′ terminus with carbon spacers of various lengths ranging from 6 to 48 chains (*i.e.*, C_6, C_{12}, C_{24}, and C_{48}) to examine the effect of probe length. All oligonucleotides were commercially synthesized by Bioneer Corporation (Korea). Secondary structures of the DNA sequences were predicted using the Mfold server and it was confirmed that none of the DNA oligonucleotides retain the secondary structures [27].

Pure Culture of Algae

M. aeruginosa strain UTEX 2388 was purchased from the Culture Collection of Algae at the University of Texas, USA. The strain UTEX 2388 is well known as the producer of hepatotoxin [28]. *M. aeruginosa* was grown in modified Bold 3N medium [29], in which soil water was eliminated. The *M. aeruginosa* cells were cultured at ambient temperature with an agitation of 160 rpm under continuous illumination of 20,000 lux (30 W, SL230D; City E.L.G., Korea).

Standard Material Preparation: gDNA Extraction and *mcyD* Gene Amplification

In order to use both gDNA and PCR amplicons as the target standard materials for the gene quantification by the NanoGene assay, DNA extraction and PCR amplification were performed using pure *M. aeruginosa* culture. One milliliter of the pure *M. aeruginosa* culture was centrifuged at 14,550 ×*g* for 3 min. The pellet was subjected to gDNA extraction by a NucleoSpin Plant II Kit (Macherey-Nagel, Germany). Subsequently, the *mcyD* gene amplicons (297 bp) were prepared by PCR using the extracted gDNA and the primers of *mcyD*F2 and *mcyD*R2 (Table 1). The 50 µl reaction mixture contained 400 nmol/l of each primer, 1× Mg-free PCR buffer (Takara, Japan), 2 mmol/l $MgCl_2$, 200 nmol/l dNTPs, 2 U of *Taq* polymerase (Takara), and 20 ng of gDNA (20 ng/µl) template. The PCR was performed with an initial denaturation at 95ºC for 5 min, 50 cycles of 30 sec at 94ºC, 60 sec at

Table 1. Sequences of DNA oligonucleotides and PCR primers used in this study.

Oligonucleotides	Sequence (5′ to 3′)	Reference
Probe DNAs	NH_2-C_6 spacer- TTCGCCTGGTCAAAGTAATT	This study
	NH_2-C_{12} spacer- TTCGCCTGGTCAAAGTAATT	
	NH_2-C_{24} spacer- TTCGCCTGGTCAAAGTAATT	
	NH_2-C_{48} spacer- TTCGCCTGGTCAAAGTAATT	
Signaling DNA	**TGCTTTCATGGATGCTCTAA**-C_6-NH_2	This study
Target ssDNA	**TTAGAGCATCCATGAAAGCA**TTAGCTGCGGCATAATTACTTTGACCAGGC	This study
*mcyD*F2 primer	GGTTCGCCTGGTCAAAGTAA	[26]
*mcyD*R2 primer	CCTCGCTAAAGAAGGGTTGA	

The complementary sequences of the probe and signaling DNAs corresponding to the target ssDNA are represented by underline and boldface, respectively.

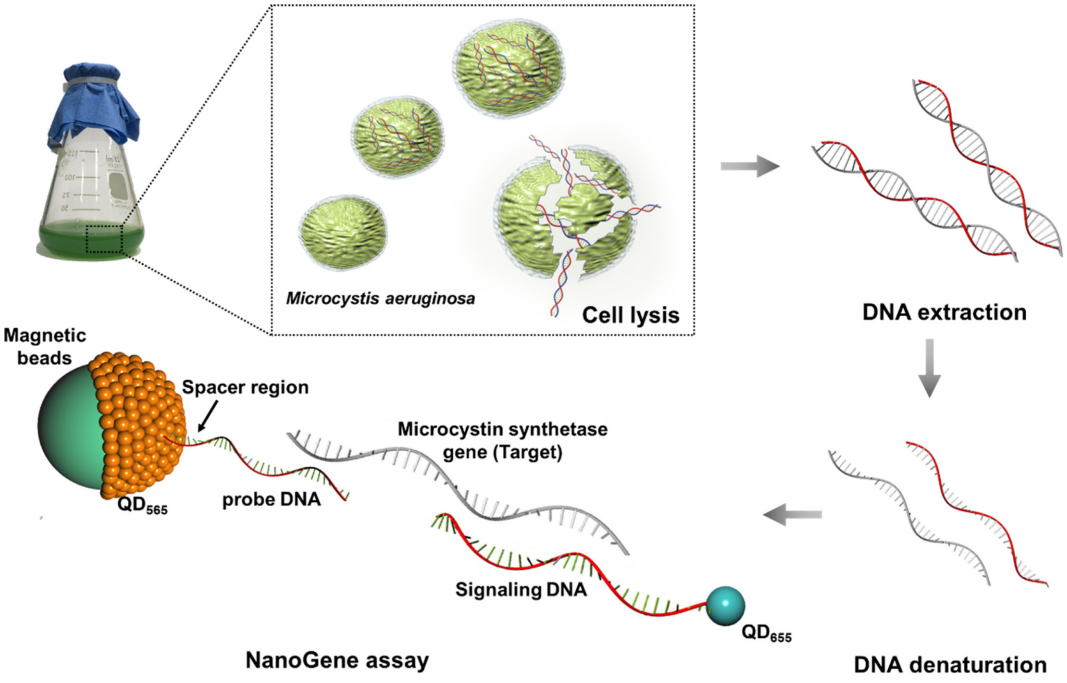

Fig. 1. Schematics of the NanoGene assay for the detection of the *Microcystis aeruginosa* strain.

56°C, and 30 sec at 72°C, and a final extension at 72°C for 15 min, using the StepOne real-time PCR system (Applied Biosystems, USA) [13]. The PCR product was then purified using the DNA Clean & Concentrator kit (Zymo, USA). The amplicons were visually checked through agarose gel electrophoresis. It was confirmed that no artefacts or undesirable products were generated from the PCR amplification. The quantities and purities of the extracted gDNA as well as the purified amplicons were measured with the NanoDrop 1000 spectrophotometer (Thermo Fischer Scientific, USA).

NanoGene Assay and Assay Sensitivity

The schematic diagram of the NanoGene assay for *M. aeruginosa* detection is depicted in Fig. 1. Aminated magnetic beads (MB; 2×10^7 beads/ml, Dynabead M270; Invitrogen, USA) were conjugated with carboxyl quantum dot nanoparticles (QD_{565}, 2 µmol/l, Invitrogen) by the formation of amide bonds mediated with ethylcarbodiimide (Sigma-Aldrich, USA) and *N*-hydroxysuccinimide (Sigma-Aldrich). The beads were employed for easy collection of the quantum dot nanoparticles via magnetic force. The aminated probe and signaling DNAs (100 µmol/l) were immobilized on the surface of MB-QD_{565} and QD_{665} (2 µmol/l; Invitrogen) via covalent bonds between the amine group of DNAs and the carboxylic group of QD, respectively. The detailed preparation procedure of MB-QD_{565}-probe DNA and QD_{655}-signaling DNA conjugates has been previously described [24]. The target DNAs (DNA amplicons or un-amplified gDNA) were hybridized with MB-QD_{565}-probe DNA and QD_{655}-signaling DNA in 300 µl of DIG Easy Hybridization buffer (Roche, Switzerland) at 37°C for 15 h under a gentile tilt rotation. As a negative control, ultrapure deionized water (DNase/RNase/Protease free; Intron Biotechnology, Korea) was added to the hybridization reaction instead of the target DNA. In order to characterize the NanoGene assay's sensitivity, both dsDNA amplicons and un-amplified gDNA were serially diluted. The target concentrations of dsDNA amplicons ranged from 6.5×10^1 to 6.5×10^8 gene copy number per reaction. The *mcyD* gene copy numbers were calculated from the Avogadro's number (*i.e.*, 6.022×10^{23} molecules/mole) and the DNA weight in daltons, with the assumption that the average weight of a base pair (bp) is 650 daltons. The target gDNA concentration of *M. aeruginosa* ranged from 2×10^0 to 2×10^7 algal cells/ml. Note that the cell density of *M. aeruginosa* was estimated from the relationship between absorbance at 750 nm and cell density (Fig. S1). Subsequently, the target amplicons or un-amplified gDNA were denatured at 95°C for 5 min prior to the hybridization with MB-QD_{565}-probe DNA and QD_{655}-signaling DNA complexes [30]. After washing off the unbound hybrids of target DNAs and QD_{655}-signaling DNA conjugates, which did not hybridize with MB-QD_{565}-probe DNA, using 0.1 mol/l phosphate buffer (0.022 mol/l of $NaH_2PO_4 \cdot H_2O$ and 0.077 mol/l of Na_2HPO_4, adjusted pH to 7.4) three times, the fluorescence intensity of QD_{565} and QD_{655} was measured on black 96-microplates (Thermo Fisher Scientific) using a SpectraMax M2 spectrofluorometer (Molecular Devices, USA) with emission wavelengths at 570 and 660 nm and excitation wavelength at 360 nm, respectively. The normalized fluorescence (*i.e.*, QD_{655}/QD_{565}) was used as an output of the gene quantities. All experiments were performed in biological and technical triplicates unless otherwise indicated.

Spacer Effect of Probe DNA

Various spacer lengths of the probe DNA were investigated in relation to the performance of DNA hybridization. MB-QD_{565}-probe DNA and QD_{655}-signaling DNA were conjugated as described above. Four probe DNAs labeled with different spacers (C_6, C_{12}, C_{24}, and C_{48}) were used when probe DNA was conjugated to the MB-QD_{565} complex via covalent bond. For clear comparison, the target DNA selected for this experiment was commercially synthesized as 50 bp single-stranded DNA (ssDNA). The detailed sequence of the target ssDNA is listed in Table 1. The concentrations of target ssDNAs for various spacers (C_6, C_{12}, C_{24}, and C_{48}) ranged from 10^{-11} to 10^{-18} mol/l. The procedures post DNA hybridization were the same as described above.

Validation with Mixed Environmental Algae

The specificity of the presented method was demonstrated by its ability to discriminate the toxin-producing *M. aeruginosa* strain from environmental algae. In the experiment, un-amplified gDNA of *M. aeruginosa* was used as a gene quantification target by the NanoGene assay. The gDNA of the environmental algae was added to each reaction. The environmental algae was obtained from Sum River in Wonju, Gangwondo in Korea by courtesy of Dr. Sang-Don Lee's group at Ewha Womans University in Korea. The water quality data of the algae containing river water are described in Table S1. Three milliliters of the environmental algae sample was centrifuged at 14,550 ×*g* for 3 min. The gDNA of the pelleted environmental algae was extracted in duplicate using the DNA extraction kit as described above and then combined together. Ten nanograms of the gDNA of the environmental algae was added to all the hybridization reactions to simulate the interference by environmental algae. As a negative control, the gDNAs of the environmental algae with concentration range of $2 \times 10^0 - 2 \times 10^7$ algal cells/ml were used for NanoGene assay, and the results were compared with that of *M. aeruginosa* only. The optical density of environmental algae was measured at 750 nm using a SpectraMax M2 spectrophotometer (Molecular Devices) and the absorbance ($OD_{750\ nm}$) was converted to algal cell concentration (algal cells/ml) based on the equation below.

$$\text{Algal cells/ml} = 1 \times 10^7 \times (OD_{750\ nm})$$

The above equation was derived from a report that evaluated freshwater algal cell densities (*Ankistrodesmus*, *Scenedesmus*, and *Selenastrum* spp.) in Australia [31]. Since *Ankistrodesmus*, *Scenedesmus*, and *Selenastrum* spp. were dominant in the environmental algal sample, the average conversion factor of 1×10^7 was used to estimate the algal cell concentration of the environmental algae.

Results and Discussion

Effect of the Length of the Spacers

Probe DNAs with various carbon spacers were used to examine the effect of the length of the probes on the gene quantification. The quantification result with C_6, C_{12}, C_{24}, and C_{48} spacers are presented in Figs. 2A–2D, respectively. The results in Fig. 2 indicated that the fluorescence values varied with the spacer length of the probe DNA. The slopes of the normalized fluorescence in the *y*-axis versus the target ssDNA concentrations in the *x*-axis decreased as the spacer length increased. The probe DNA labeled with C_6 spacer showed the greatest slope value of 0.90 (Fig. 2A), and the lowest slope value of 0.48 was achieved in the longest carbon spacer (C_{48}) (Fig. 2D). The slope was inversely proportional to the spacer length of the probe DNA.

Another observation pertaining to the spacer experiment is that the normalized fluorescence of negative controls (*i.e.*, background fluorescence or noise) increased with the length of the carbon spacer. The background fluorescence was proportional to the spacer length, meaning that the longer the spacers are, the higher the noise becomes. The shortest C_6 spacer showed the lowest value of noise (1.8 ± 0.4 RFU) as compared with those (3.4 ± 0.2 – 4.3 ± 1.9 RFU) of the $C_{12\text{-}48}$ spacer.

On the basis of the slope and noise values from the *mcyD* gene quantification, the C_6 spacer is the optimum length for the probe DNA of NanoGene assay, particularly for the detection of *mcyD* gene. This is not as we expected, since we hypothesized that the probe DNA with longer length spacer will enhance the hybridization reaction of the NanoGene assay by improving the accessibility with target DNA. This is probably due to highly extended configurations in the probe DNA with longer length spacer. Steel *et al*. [32] investigated the effect of DNA length on the immobilization of nucleic acids at a gold surface and reported that the surface coverage begins to decrease notably with probe lengths. They presented a model that would be applicable for probe lengths greater than 24 nucleotides. Longer probes are expected to exist as more flexible that coiling commences, presumably reflecting increasingly polymeric behavior. This probably can cause nonspecific binding between MB-QD_{565}-probe DNA and QD_{655}-signaling DNA, which resulted in increase of noise values with increasing probe length. Lim *et al*. [33] also reported that the oligo binding capacity of magnetic particles with long spacer probes of C_{54} was decreased, probably due to the formation of secondary structures on the spacer. These results are consistent with our observation. Therefore, it can be concluded that the reduced capability of the NanoGene assay for the gene quantification with longer spacers is probably caused by the steric and polymeric effects of the probe DNA with longer spacers. Therefore, we employed

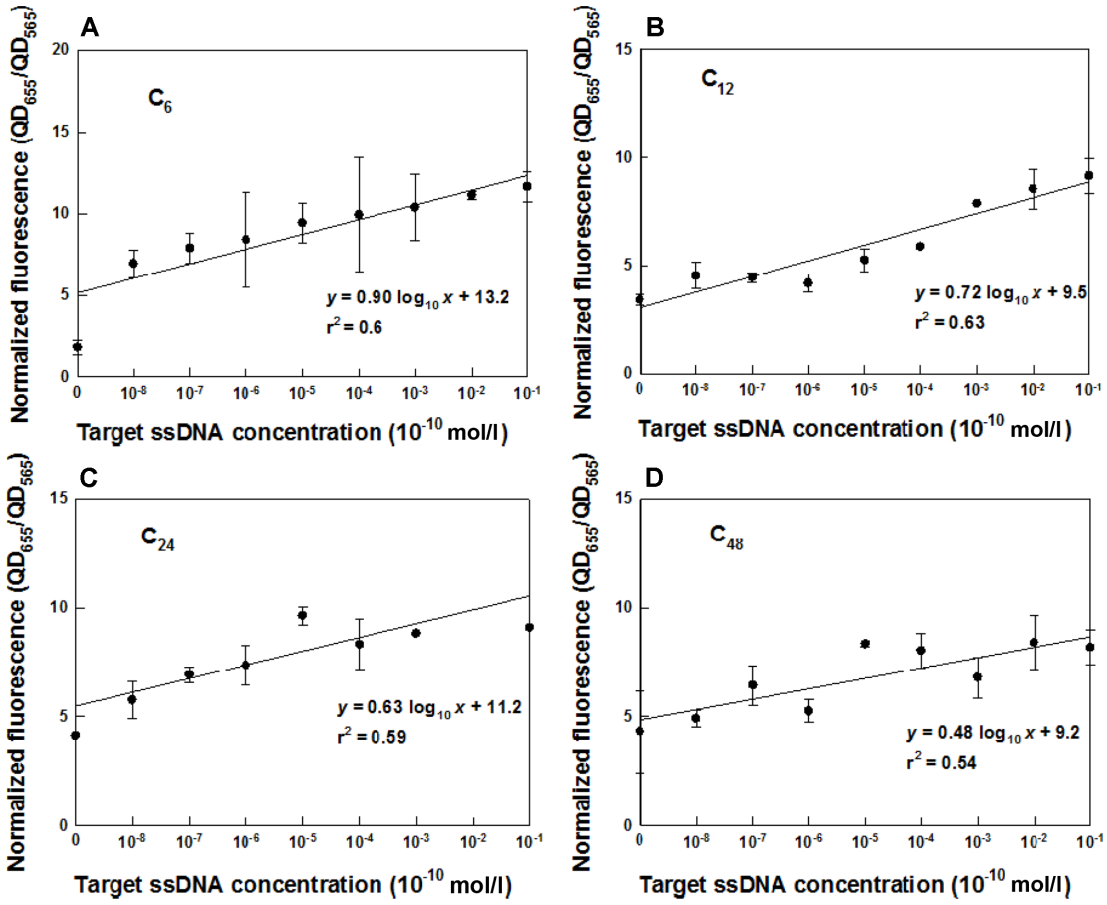

Fig. 2. Effects of probe DNA on the NanoGene assay by various spacers: (**A**) C_6 spacer, (**B**) C_{12} spacer, (**C**) C_{24} spacer, and (**D**) C_{48} spacer.

the C_6 spacer for the NanoGene assays for the following gene quantification.

Quantitative Detection of *Microcystis aeruginosa* with Amplified dsDNA

The sensitivity of the NanoGene assay for *mcyD* gene detection was validated using amplified dsDNA. The standard curve was established via the serial dilution of the *mcyD* gene fragments (*i.e.*, 297 bp of PCR amplicon). As shown in Fig. 3, the NanoGene assay successfully detected the *mcyD* gene of *M. aeruginosa*, ranging from 6.5×10^1 to 6.5×10^8 *mcyD* gene copy number per reaction. The LOD of the quantification was 38 *mcyD* gene copies per reaction as determined from the following equation:

$$LOD = X_b + 3S_b$$

where X_b is the mean normalized fluorescence of the blank sample and S_b is the standard deviation of the blank sample [34–37].

Rinta-Kanto *et al.* [13] reported a LOD of 25 *mcyD* gene copies per reaction via qPCR. Vaitomaa *et al.* [14] quantified *mcyE* gene at a range of 6.6×10^2 to 6.6×10^6 copies using qPCR and the LOD was 660 *mcyE* gene copies in a reaction mixture. Foulds *et al.* [38] were able to detect down to three copies of *mcyA* gene per sample using 5′-Nuclease PCR. The NanoGene assay demonstrated comparable sensitivity with the above-mentioned studies.

The normalized fluorescence was proportional ($y = 0.24 \log_{10} x + 1.12$, $r^2 = 0.50$) to the *mcyD* gene copies per reaction. The linearity is reasonable for gene quantification, but the slope (*i.e.*, 0.24) of the quantification is fairly low. It still requires more optimization and improvement for quantification efficiency.

Quantitative Detection of *Microcystis aeruginosa* with Un-Amplified gDNA in the Presence of Environmental Algae

Sensitivity. As shown in Fig. 4 (circles), the NanoGene

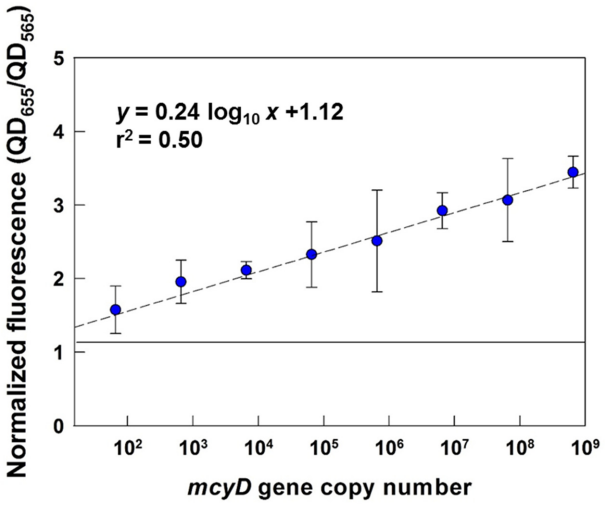

Fig. 3. Sensitivity validation of the developed NanoGene assay for the detection and quantification of the *mcyD* gene of the toxic *M. aeruginosa* strain.

The solid line depicts the normalized fluorescence value of no DNA template (negative control) for the NanoGene assay. Symbols and error bars indicate the mean and standard deviations of biological triplicates. The regression equation is $y = 0.24 \log_{10} x + 1.12$.

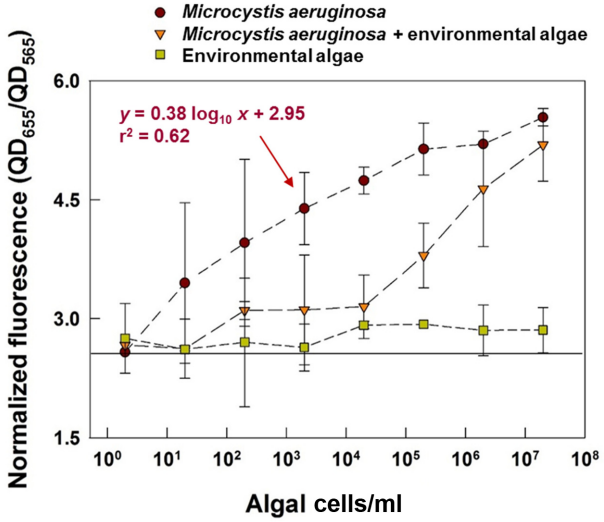

Fig. 4. Specificity validation of the developed NanoGene assay for the detection and quantification of the toxic *M. aeruginosa* strain from unamplified gDNA in the presence of environmental algae.

Solid line depicts the normalized fluorescence value of no gDNA template (negative control) for the NanoGene assay. Symbols and error bars indicate the mean and standard deviations of biological triplicates.

assay successfully quantified the cell numbers of *M. aeruginosa.* The normalized fluorescence was ($y = 0.38 \log_{10} x + 2.98$) proportional to the cell concentrations of *M. aeruginosa*. The NanoGene assay was able to detect toxin-producing *M. aeruginosa* strain in the range of $2 \times 10^0 - 2 \times 10^7$ algal cells/ml with the LOD of 9 algal cells/ml, which was estimated from the equation described above. However, as shown in Fig. 4 (circles), the results at lower concentrations ($2 \times 10^0 - 2 \times 10^2$ algal cells/ml) showed large standard deviations. It may be due to the use of untreated (*i.e.*, un-fragmented) algal gDNA. Previous study has shown that the fragmentation of gDNA improves the accuracy and sensitivity of NanoGene assay [30]. Owing to the large standard deviation in the lower concentrations, the quantification range of $2 \times 10^3 - 2 \times 10^7$ algal cells/ml will be more appropriate.

Specificity. gDNA (10 ng/μl) extracted from environmental algae in the environmental sample was added to validate the specificity of *M. aeruginosa* detection with gDNA. Based on microscopic observation, the majority of the environmental algae sample appears to consist of green algae, including *Ankistrodesmus* sp., which are phylogenetically far from microcystin-producing blue-green algae. The addition of the environmental algal gDNA interfered with the detection of *M. aeruginosa* by the NanoGene assay. As shown by markers in the form of inverted triangles in Fig. 4, the NanoGene assay was not able to detect *M. aeruginosa* at low concentration of $2 \times 10^0 - 2 \times 10^4$ algal cells/ml in the presence of environmental algal gDNA. The normalized fluorescence increased in the range of $2 \times 10^4 - 2 \times 10^7$ cells/ml. In particular, 2×10^7 cells/ml has shown a similar level (*i.e.*, 5.2 ± 0.8 RFU) of detection as the corresponding value (*i.e.*, 5.4 ± 0.2 RFU) in the absence of gDNA of environmental algae. The quantification signal was somehow interfered by co-existing nonspecific target and seems to be recovered by having more gDNA target template. The same inhibition pattern was observed in previous studies [22, 39] and the possible explanation is due to the nonspecific binding between gDNA target and interfering materials (or non-target genes), as described in Kim *et al.* [22]. Such interference can be improved by further purification and/or fragmentation for increasing hybridization proximity and buffer optimization, as partly described in the previous work [30, 40].

As expected, the NanoGene assay did not detect *M. aeruginosa* in environmental algal gDNA (*i.e.*, nonspecific target) over the range of 2×10^0 to 2×10^7 algal cells/ml (represented by markers in the form of squares in Fig. 4).

The WHO guideline for microcystin concentration in drinking water for human consumption is 1 μg/l [41]. The

study by Oh *et al*. [42] reported that 1 g dry cell weight (DCW) of *M. aeruginosa* produces 90 to 339 μg of microcystin for corresponding specific growth rates of 0.1 to 0.8/day. Long *et al*. [43] reported that for specific growth rates of 0.1 to 0.9/day, the DCW of *M. aeruginosa* varies from 18 to 43 picogram per algal cell. This means the average weight per single *M. aeruginosa* cell is ~30 picogram, and 1 g DCW of *M. aeruginosa* consists of 3.33×10^{10} algal cells. With reference to the study by Oh *et al*. [42] as mentioned above, 3.33×10^{10} algal cells produce 90 to 339 μg of microcystin for corresponding specific growth rates of 0.1 to 0.8/day. Inversely, 1 μg of microcystin is produced by 9.83×10^{7} – 3.70×10^{8} algal cells (depending on their specific growth rate), and 1 μg/l of microcystin is produced by 9.83×10^{7} – 3.70×10^{8} algal cells/l. This is equivalent to 9.83×10^{4} – 3.70×10^{5} algal cells/ml. If assuming that all microcystins are derived only from *M. aeruginosa*, the environmentally relevant *M. aeruginosa* concentration would be ~10^{5} algal cells/ml. The NanoGene assay was able to detect the toxin-producing *M. aeruginosa* strain at environmentally relevant concentrations.

One of the limitations of the NanoGene assay for *M. aeruginosa* detection in environmental samples lies in the DNA extraction process. This process is originally developed for other bioassays such as qPCR. It is not amenable for portable detection because it requires a number of purification steps. Therefore, a NanoGene assay-specific DNA extraction process will need to be developed and optimized in future studies. Algae containing environmental samples tend to be turbid. This means the washing step after hybridization needs to be more elaborate and optimized in order to minimize interference with the fluorescence measurement. A lower limit of detection (*i.e*., increased sensitivity) of *M. aeruginosa* or microcystin would be beneficial to ensure the safety of drinking water system.

The future perspective of the presented method is the portable system for algae detection. The first generation of system [21] consists of incubation with probe and target DNA followed by magnetic separation. The incubation, washing, and separation were implemented in the microfluidic chip with micro-pumps and a linear actuator controlled by a micro-controller. The second generation contains various pretreatment modules, such as a portable cell-wall lysis cartridge [19, 20]. The last generation contains the portable fluorescence detection with photodiodes and data logger (manuscript in preparation). Each generation presents a module that can be integrated or stand alone for the purpose.

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