Rapid on-site monitoring of cylindrospermopsin-producers in reservoirs using quantitative PCR

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ABSTRACT

Recently the cyanobacterium Cylindrospermopsis raciborskii has become a major concern in water quality monitoring and control perspective due to its production of an alkaloid hepatotoxin, called cylindrospermopsin, which may cause liver injury, in freshwater bodies. This cylindrospermopsin-producer may grow abundantly in lakes and reservoirs, particularly under conditions of eutrophication, potentially affecting the health of human and animals [1-4]. According to the monitoring result of water quality from 2000 to 2004 conducted by Taiwan Environmental Protection Administration, approximately half of Taiwan's 20 major reservoirs were categorized as eutrophic based on the Carlson's Trophic State Index [5]. These eutrophic reservoirs provide excellent environments for many cyanobacteria, such as Microcystis and Cylindrospermopsis, to grow. Yen et al. [6] analyzed water samples from 3 eutrophic lakes, 2 water treatment plants and 15 distri-
bution taps in Taiwan. They discovered that cylindrospermopsin was present with high concentrations in both reservoir water (maximum concentration = 36 μg L⁻¹) and tap water (8.6 μg L⁻¹). It clearly demonstrated that cylindrospermopsin and its potential producer, *C. raciborskii*, is an important issue for the management of water quality in Taiwan's reservoirs. Thus, a rapid and reliable method to detect the toxin producing *C. raciborskii* is urgently needed.

Traditionally microscopic methods are used to identify potential toxin producing cyanobacteria. However, the methods are not able to differentiate toxic from nontoxic cells. Recently, a molecular microbiology based method, quantitative polymerase chain reaction (qPCR), has been employed to quantify toxigenic cyanobacteria by detecting the concentrations of target genes involved in the synthesis of toxins [7-9]. A few genes relevant to the synthesis of cylindrospermopsin, including polyketide synthase (*pks*) and sulfotransferase (*cyrJ*) [7,10], have been selected as biomarkers for cylindrospermopsin producers.

In using the qPCR method, DNA extraction is often the time limiting step for quantifying the genes inside the target cyanobacteria. To quickly make intracellular DNA available for qPCR quantification, several DNA extraction methods have been developed, including microwave treatment, sonication treatment, thermocycling, beads beating, freezethaw method, and enzyme extraction [11,12]. These methods have been successfully tested in the field for DNA extraction [11,13]. Rasmussen et al. [11,13] concluded that microwave cell disruption coupled with a portable qPCR machine may quantify cylindrospermopsin-producing cyanobacteria in the field. Although the method had been successfully tested for several reservoir water matrix in Australia, it was only examined in the field for one reservoir. Therefore, more field tests may provide useful information for the evaluation of its applicability and for the improvement of the method. In addition, up to now, there is no report relevant to the quantification of *Cylindrospermopsis* using molecular methods in Taiwan.

Conventionally, when faced with cyanobacteria contamination problems, reservoir and water utility managers deliver water samples to a central laboratory for analysis of harmful cyanobacteria and metabolites. This usually requires long time for analysis. In addition, many of the reservoirs and water utilities may be far away from cities, delaying the availability of information due to long distance/time for sample transportation. Therefore, on-site analysis in the field may quickly provide information for decision-making regarding responses from the management of water supply facilities.

In this study, we attempted to test and apply the microwave-qPCR method for on-site tracking cylindrospermopsin-producing cyanobacteria in Taiwan's reservoirs. The method was first tested in laboratory and one artificial lake, and then applied to 10 reservoirs in Taiwan. The qPCR data were compared with cell count data and toxin concentrations sampled at the same time for evaluating the applicability of method.

**MATERIALS AND METHODS**

1. **Samples**

Toxigenic *C. raciborskii* strain CYP026J with 'straight' trichome from Australian Water Quality Centre culture collection was used as a standard strain in laboratory tests. The strain was cultured at 25 °C under constant light (1500-2500 Lx) in Jaworski Medium (JM) [14]. Environmental samples were collected from one artificial lake, Cheng Kung Lake (CKL), at National Cheng Kung University, Taiwan, and 10 reservoirs in Taiwan. Among the reservoirs, 6 locate in the main island of Taiwan, including Shih-Men Reservoir (SMR), Sin-Shan Reservoir (SSR), Ren-Yi-Yan Reservoir (RYTR), Lan-Tan Reservoir (LTR), Bao-Shan Reservoir No. 2 (BSR), Li-Yu-Tan Reservoir (LYTR), and 4 locate at the Kinmen Island in Taiwan, including Tai-Hu Reservoir (THR), Rong-Hu Reservoir (RHR), Yang-Min Reservoir (YMR), Lan-Hu Reservoir (LHR). Locations of the reservoirs and the sampling sites are shown in Fig. 1. Surface water samples (0-50 cm below surface) were taken in winter 2010 from SMR, in spring 2011 from SSR, RYTR, LTR, BSR, LYTR and THR, and in summer 2011 from RYTR, LTR, BSR, THR, RHR, YMR, and LHR. At each sampling point, 1 L of water was collected for the analysis of DNA and cylindrospermopsin. The samples were stored in PE bottles under 4 °C before analysis. For cell enumeration, 100 mL PE bottles were used for the samples.

2. **Cell Enumeration**

Cyanobacterial cell was enumerated according to Standard Method 10200 F [15]. The samples were fixed with Lugol's solution (1% by volume) right after sampling. In measuring the cells, 1 mL of the sample was placed to the Sedgwick Rafter chamber (Graticules Ltd, UK). The sample was allowed to settle for 30 min, and then enumerated at 200x magnification with a microscope (OlympusBX51, Olympus., Japan) and Analysis LS Research Software (Olympus Soft Imaging Solutions GMBH, Olympus., Japan). In measuring the cell number of the filamentous *C. raciborskii*, 30 filaments (trichome) were determined for their average cell number per unit length of trichome. Then, the cell numbers in the sample were calculated from total length of the trichomes observed and the average cell number per unit length of trichome.
3. DNA Extraction and Microwave Cell Disruption

Standard DNA was extracted from laboratory cultured *C. raciborskii* CYP026J using a FAST DNA kit for Soil (MP Biomedicals, CA). The extraction followed the instructions provided by the manufacturer. Concentration of DNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE) and then DNA copy numbers were calculated [16]. The DNA was used for qPCR as standard.

To extract DNA from the cyanobacteria-laden water samples collected in the field, microwave cell disruption was used. During the microwave treatment, 1 mL of cyanobacteria laden water sample was first centrifuged at 13,000 rpm for 10 min on a tabletop centrifuge (Hettich Mikro 20 microfuge w/24 place rotor, Biotech Equipment Sales, CA). Then, the supernatant was removed, and the remaining pellets were mixed with 50 µL detergent solution (0.5% TritonX-100, 5 mM DTT, 10 mM Tris-HCl, and 5 mM EDTA). The mixture was placed in a microwave oven (NN-S215; Panasonic, Japan) for breaking cyanobacteria cells and extracting genomic DNA. After treated with microwave at 750 W for 2 min, the sample was diluted 10 times with distilled water. Five µL of the treated sample were directly applied for qPCR reaction.

4. Quantitative PCR Conditions

Quantification of DNAs were performed on a portable qPCR device (Smart Cycler; Cepheid, CA). For detecting total *C. raciborskii*, the qPCR reaction targeted on DNA-dependent RNA polymerase gene (*rpoC1*) with the primer set cyl2/cyl4 [17]. This gene has been employed in molecular phylogenetic identification of cyanobacteria. Therefore, the data may be
used to compare with those from microscopic cell counting for *C. raciborskii*. To detect cylindrospermopsin producer, the reaction targeted on *pks* with the primer set k18/m4 [13]. The gene is located in *aoaC* which is responsible for production of cylindrospermopsin. Specificity of the two primers has been examined previously by Fergusson and Saint [7] and Rasmussen et al. [13] for more than 40 different cyanobacteria. The qPCR reaction used a PCR mixture consisting of 1x SYBR Premix Ex Taq™ (Perfect Real Time; Takara, Japan) and 0.2 µM of each primer on the portable qPCR device. The PCR mixture was pre-incubated at 95 °C for 10 s, with a PCR cycle of 5 s denaturation at 95 °C, 15 s annealing at 60 °C and 15 s extension at 72 °C. During the primer extension step of each amplification cycle, the fluorescent intensity of the amplified DNA was recorded using the SYBR Green I optic channel set at a wavelength of 495 nm. The threshold cycles (Ct values) were calculated by the second derivative maxima in Smart Cycler software (Smart Cycler; Cepheid, CA). Specificity was evaluated based on the melting curve analysis which was performed after amplification with the temperature program, 60-95 °C with a heating rate of 0.1 °C s⁻¹, continuous fluorescence measurements, and melting curve construction using Smart Cycler software (Cepheid, CA).

5. Detection of Cylindrospermopsin Concentration

Enzyme-Linked Immunosorbent Assay (ELISA) was employed to quantify cylindrospermopsin concentration in the water samples. The water sample was first sonicated (at 250 kW for 60 min) for rupturing the cell membrane to release components and 50 µL were used for the assay. The assay was carried out by a commercial ELISA kits (Cylindrospermopsin ELISA Kit PN 522011; Abraix LLC, PA), following the instructions provided by the manufacturer. The light absorbance was set at 450 nm using a microplate spectrophotometer (xMark; Bio-rad, CA) and analyzed by Microplate Manager ver. 6 software (Bio-rad, CA).

RESULTS AND DISCUSSION

1. Standard Curves of Total and Toxigenic *C. raciborskii* for qPCR Assay

Serial dilution samples, 10⁰ to 10⁷ copies of the DNA quantification standard, were prepared for standard calibration curves. The standard curve was generated as linear regression between Ct values and logarithmic copy numbers of the standard DNA. In Fig. 2, each datum point represents an average of 8 replicates, with standard deviation displayed as the error bars. The small error bars in the figures demonstrated the good reproducibility of the qPCR reactions performed in the experiments. Figure 2 also shows the linear regression line, with the correlation coefficient (R²) and the slope being 0.999 and -3.64 for the total *C. raciborskii* (*rpoC1*) and 0.996 and -3.43 for the toxigenic *C. raciborskii* (*pks*), respectively. The amplification efficiency estimated from the slope [18] was 90 and 95% for *rpoC1* and *pks*, respectively. The efficiencies obtained in the experiments fall within the typical range for qPCR, 90-110% [19], suggesting successful PCR amplification and permitting reasonable quantification. The minimum detection limit in this experiment was 1 x 10⁷ copies for each primer set.

2. Test of Rapid qPCR Approach

2.1. qPCR assay coupled with microwave pretreatment

To expedite the detection of *C. raciborskii* in water, the qPCR assay was coupled with microwave pretreatment for DNA extraction. Samples were prepared from the laboratory cultured *C. raciborskii* in different cell concentrations, and the DNA was extracted using microwave treatment. The data were then evaluated with the correlation between cell concentrations and Ct values of the qPCR. Figure 3 shows that
the correlation between the Ct values and the logarithmic cell concentrations was linear, with $R^2 = 0.999$ for both rpoC1 and pks. In the figure, each datum point represents an average of the duplicates, and the error bars were the standard deviations of the experiments. The small error bars shown in the figure also indicate good reproducibility of the reactions. The high $R^2$ values obtained explained that for both total and toxigenic C. raciborskii, the Ct value well correlated with cell concentrations in the samples. In addition, Fig. 3 also shows that the detection limit for both total and toxigenic C. raciborskii are in the order of 1,000 cells mL$^{-1}$.

2.2. Comparison between DNA copy number and cell number

Figure 4 shows the relationship between DNA copy number detected by qPCR and cell concentrations counted by microscope for the laboratory culture. The copy number correlated well with cell concentrations, with $R^2$ of 0.999 for rpoC1 and 0.998 for pks. From the slopes of the regression lines, it indicates that the numbers of C. raciborskii rpoC1 and pks target copies were similar in the laboratory culture. The results of microscopic cell counts and qPCR detection of DNA copies for the cultured C. raciborskii suggest that 1 cell of C. raciborskii strain CYP026J contains, on average, 2.67 and 2.25 copies of the rpoC1 and pks genes, respectively. Rasmussen et al. [13] reported that the number of copies of C. raciborskii rpoC1 was 5- to 7-fold higher than the number of cells counted in environmental samples, suggesting that C. raciborskii contains several rpoC1 genes per cell. The rpoC1 gene copies per cell between Rasmussen et al. [13] and the present study are different. In the present study, gene copies and cell numbers were determined from pure culture. Unlike ours, those in Rasmussen et al. [13] were obtained from environmental population, and different stains and different growth status for the targeted C. raciborskii are expected. This probably made their rpoC1 gene copies per cell different to the present study. Their results also showed that the number of pks and C. raciborskii rpoC1 target copies were approximately the same, similar to our observation. Al-Tebrineh et al. [20] reported that filamentous cyanobacteria are known to possess multiple copies (up to 10) of genome in each cell and cyanobacteria may have multiple replication origins. Although there are not enough data in the present study to ascertain the copy numbers of rpoC1 and pks genes per genome of C. raciborskii, based on the experimental results of the present study, the number of DNA copies per cell will be assumed to be 2.67 and 2.25 copies of the

![Fig. 3. Relationship between Ct values and cell numbers for rpoC1 primer (a) and pks primer (b). Each point plotted is an average of duplicated fluorescence values measured. Linear regression equations: $y = -3.1628x + 41.335$ for rpoC1 primer and $y = -2.7983x + 38.781$ for pks primer.](image1)

![Fig. 4. Comparison between copy number and cell number for rpoC1 primer (a) and pks primer (b). Linear regression equations: $y = -2.6653x$ for rpoC1 primer and $y = -2.2498x$ for pks primer.](image2)
rpoC1 and pks genes, respectively, for the environmental samples.

2.3. Impact of water matrix on qPCR analysis

In the rapid qPCR approach, the samples from microwave treatment were used in qPCR analysis without any purification. As PCR results may be compromised by the presence of inhibitors in environmental samples [21], this is needed to evaluate the impact of the water matrix on the analysis of qPCR. In the experiments, the same amount of pure cultured cells was spiked into both culture medium as control (JM) and reservoir water samples for microwave treatment and qPCR analysis. The reservoir water samples were taken from CKL, RYTR and THR, and were confirmed with no presence of C. raciborskii cells by microscope, PCR and qPCR.

Figure 5 presents the experimental results of the control and reservoir water samples for rpoC1 and pks primers. The figure clearly shows no significant difference between reservoir water samples and controls in Ct values (two way ANOVA, \( p = 0.004 \) for rpoC1, \( p = 0.008 \) for pks). Specificity of the two primer sets was also evaluated based on the melting curve analysis. In all the reservoir samples, only one melting peak was observed for each primer set (data not shown). The melting temperatures for all amplifications were around 81 °C for rpoC1 and 84 °C for pks, same as those obtained from the experiments for standard DNA. The melting curve analysis revealed no presence of primer-dimers.

3. Field Tests in Reservoirs

The quantification approach was applied to environmental samples in 1 artificial lake and 10 reservoirs in Taiwan (Table 1). The samples were first treated with microwave for cell disruption then directly tested by qPCR. In five of the field tests, including those in SMR, SSR, BSR, LYTR, and CKL, smart cycler and all devices associated with the analysis were moved to the sites, allowing for microwave treatment and qPCR analysis being conducted on-site. For the samples in 6 other reservoirs, the samples taken from these reservoirs were shipped back to laboratory for analysis. All the analysis, except the samples shipped from Kinmen Island, was completed within about 4 h after sampling. Based on the results of qPCR assay for total C. raciborskii (rpoC1), no target gene was detected in the samples taken from 6 reservoirs located in main-island of Taiwan, including SMR, SSR, RYTR, LTR, BSR, and LYTR. This is in accordance with the microscopic observation. Similarly, no cylindrospermopsin producer was detected by qPCR- pks assay. This is in consistency with the observation that all the cylindrospermopsin concentrations of the corresponding samples were below detection limit. To lower the detection limits for the target genes, the samples collected from the six reservoirs were concentrated 10 times by centrifuge. Again, no genes were detected for rpoC1 and pks, reflecting that both genes were below detection limits in the samples.

For the samples taken from the reservoirs located in Kinmen Island, the gene for total C. raciborskii (rpoC1) was detected in THR, RHR, YMR, and toxigenic C. raciborskii (pks) was detected in RHR and YMR. The melting temperatures for rpoC1 and pks were also confirmed to be similar to laboratory culture, suggesting that primers match the sequence of target template DNA. Total C. raciborskii cells determined with qPCR in the samples of THR, RHR and YMR were \( 1.2 \times 10^6 \), \( 2.3 \times 10^6 \) and \( 1.9 \times 10^6 \) cells mL\(^{-1}\), respectively. For that of LHR, the genes were lower than detection limit. Microscopic observation also showed the presence of C. raciborskii cells in the water samples of THR, RHR and YMR, but not in that of LHR (Table 1). The estimated cell concentration of qPCR-rpoC1 assay is close to the results of microscopic cell count, with the exception of one sample taken from YMR which was considered as an outlier.

Wu et al. [22] studied the C. raciborskii in Taiwan using phylogenetic analysis with 16S-23S rRNA internal transcribed spacer region during 2006 to 2008. The results suggested that the occurrence of C. raciborskii was dominated by 3 subpopulations, including 1 European and 2 Taiwan (TW) groups. The
TW sequevars accounted for greater than 87.5% of *C. raciborskii* in the reservoirs Taihu (same with THR in this study), Yangmin (YMR in this study), and Jinsha (RHR in this study). The *rpoC1* primer set used in this study has been successfully tested and detected for the Australian and Germany types of *C. raciborskii* [7,13]. Our observation for three of the four reservoirs/lakes, CKL, THR, and RHR, shows that the estimated cell concentrations between qPCR-*rpoC1* assay and microscopic enumeration are similar, suggesting that the qPCR-*rpoC1* assay may be able to capture most *C. raciborskii* subpopulations in the tested reservoirs. However, the estimated cell value by qPCR-*rpoC1* assay was only about 10% to microscopic results for YMR. Further examination of the primer set for all subgroups of *C. raciborskii* cells in Taiwan may be needed to improve the detection capability of the method.

Table 1 also shows that the toxigenic *C. raciborskii* cells determined with qPCR were 3.2 × 10^9 and 1.9 × 10^7 cells mL^-1 for RHR and YMR, respectively. The results were in accordance with the cylindrospermopsin concentrations detected for the two reservoirs, 0.67 and 0.47 µg L^-1 for RHR and YMR, respectively. According to microscopic inspection, *C. raciborskii* was the only dominated strain to produce cylindrospermopsin. Therefore, it is reasonable to assume that the cylindrospermopsin detected was produced by the toxigenic *C. raciborskii* cells determined with qPCR. Under this condition, cylindrospermopsin cell quota for the two reservoirs was estimated to be 0.00012 to 0.00025 pg cell^-1 for all *C. raciborskii* cells and 0.21 to 0.25 pg cell^-1 for toxigenic cells. These cell quotas are much lower than those estimated from the data reported by Rasmussen et al. [13], 0.0069 to 0.0289 pg cell^-1 for all *C. raciborskii* cells. McGregor and Fabbro [23] summarized cylindrospermopsin concentrations under various *C. raciborskii* cell concentrations in 15 reservoirs in Queensland, Australia. They concluded that cylindrospermopsin concentration was categorized as positive (~ 1 µg L^-1) only if *C. raciborskii* cell was >

### Table 1. The qPCR results for *rpoC1* and *pks* primers in different water sample

<table>
<thead>
<tr>
<th>Location</th>
<th>Reservoir</th>
<th>Date</th>
<th>Microscope cell count (cells mL^-1)</th>
<th>Rapid qPCR assay* cell number (cells mL^-1)</th>
<th>CYN** (µg L^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SMR</td>
<td>Jan. 25</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>SSR†</td>
<td>Mar. 7</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Main Island</td>
<td>RYTR</td>
<td>Apr. 10</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul. 7</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>LTR</td>
<td>Apr. 10</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul. 7</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>BSR‡</td>
<td>May 10</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul. 2</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>LYTR</td>
<td>May 10</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Kinmen Island</td>
<td>CKL</td>
<td>Site 1 May 2</td>
<td>2.9 E + 04</td>
<td>2.8 E + 04 (6.6%)</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site 2 May 2</td>
<td>2.4 E + 04</td>
<td>2.2 E + 04 (5.6%)</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>THR</td>
<td>Apr. 21</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul. 7</td>
<td>1.2 E + 06</td>
<td>1.2 E + 06 (6.0%)</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>RHR</td>
<td>Jul. 7</td>
<td>1.2 E + 06</td>
<td>2.3 E + 06 (1.8%)</td>
<td>3.2 E + 03 (2.2%)</td>
</tr>
<tr>
<td></td>
<td>YMR</td>
<td>Jul. 7</td>
<td>1.4 E + 07</td>
<td>1.9 E + 06 (1.1%)</td>
<td>1.9 E + 03 (1.1%)</td>
</tr>
<tr>
<td></td>
<td>LHR</td>
<td>Jul. 7</td>
<td>0</td>
<td>BDL</td>
<td>BDL</td>
</tr>
</tbody>
</table>

*Cell mL^-1* represented as a mean of duplicate with coefficient of variation following in brackets.

**CYN, cylindrospermopsin assayed by ELISA with 0.1 µg L^-1 detection limit, -: Not determined.

† Samples were taken from 7 sites. The same results were obtained in all samples.

‡ Samples were taken from 3 sites on May 2011, and 6 sites on July 2011. The same results were obtained in all samples.

BDL: Below detection limit.
Experimental results indicate that the Ct values obtained for total and toxigenic microwave pretreatment for the rapid on-site detection of total and toxigenic C. raciborskii cells in reservoir water samples collected, 11 were found to be lower than 0.07 to 0.3 pg cell⁻¹. These values were also much higher than our observation of 0.00012 to 0.00025 pg cell⁻¹ for all C. raciborskii cells. However, it is very close to the cell quota for toxigenic cells obtained in this study, 0.21 to 0.25 pg cell⁻¹. Although no differentiation between toxigenic and non-toxigenic cells was reported for the two Australia studies, it is speculated that the ratios of toxigenic C. raciborskii cells in the two studies were much higher than the present study. For the observations of the two Australian studies and the present study, it is also reasonable to say that the ratios of toxigenic and non-toxigenic cells in water samples may vary in a wide range. In such a case, microscopic enumeration might be under- or over-estimate the risk of cylindrospermopsin contamination. Therefore, to better characterize the potential risk associated with cylindrospermopsin contamination in source water, more reservoir dependent information, such as ratios of toxic species and cell quota for cylindrospermopsin, should be collected to support improved correlation between toxin producers and the toxin.

Table 1 also shows that in one of the THR samples cylindrospermopsin was detected as 0.14 µg L⁻¹. But with the current qPCR method toxigenic C. raciborskii cells were below detection limit. For the qPCR method employed, the detection limit for toxigenic C. raciborskii cells is about 1,000 cells mL⁻¹. Considering the cell quota obtained, the detection limit for cylindrospermopsin-producing cells is about 0.21-0.25 µg L⁻¹. Therefore, it is reasonable in this case that toxin was detected and toxigenic C. raciborskii cells were below detection limit. Luckily the detection limit of the qPCR method is still lower than the suggested guideline value (a health alert level for C. raciborskii of 15,000 cells mL⁻¹ and for cylindrospermopsin of 1 µg L⁻¹) for drinking water [23,24].

Yen et al. [6] discovered that cylindrospermopsin was present with high concentrations in YMR, THR, and RHR in Kinmen Island in July 2007. In the 16 reservoir water samples collected, 11 were found to have concentrations of 0.5-36 µg L⁻¹. Although in the present study the cylindrospermopsin concentrations detected were slightly lower (0.14-0.67 µg L⁻¹), the results suggest again that cylindrospermopsin is an important cyanotoxin in the reservoirs in Kinmen Island.

CONCLUSIONS

This study examined a qPCR method coupled with microwave pretreatment for the rapid on-site detection of total and toxigenic C. raciborskii in reservoir water. Experimental results indicate that the Ct values obtained are well correlated with total and toxigenic cell concentrations in both pure and reservoir waters. The quantification approach was further successfully applied in 10 reservoirs in Taiwan. The target gene concentrations quantified with the qPCR approach reasonably followed the microscopic data and cylindrospermopsin concentrations for total and toxigenic C. raciborskii, respectively. The on-site monitoring approach was able to quantify total and toxigenic C. raciborskii with detection limit of 1,000 cells mL⁻¹ in 2 h. This study successfully showed the applicability of the qPCR method for rapid on-site detection of C. raciborskii in reservoirs.

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REFERENCES


Discussions of this paper may appear in the discussion section of a future issue. All discussions should be submitted to the Editor-in-Chief within six months of publication.

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