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Detection of Toxigenic Cyanobacteria in Aquatic Environments by Quantitative PCR

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Waterbloom in eutrophic lakes may be caused by toxigenic cyanobacteria which produce toxin such as microcystin. Microcystin is synthesized by multifunctional enzyme complexes known as peptide synthetases encoded by the *mcy* (microcystin synthetase) gene cluster. It is serious problems due to the toxicity to human and wild lives. Several methods such as HPLC, ELISA and mouse bioassay have been used to detect microcystin in water. However, those methods require laborious work and take a long time to detect the toxic compound.

In this study, quantitative competitive PCR (QC-PCR) was applied for the efficient detection of microcystin-producing cyanobacteria in aquatic environments. In order to evaluate primers reported previously, seventeen *Misrocystis* strains were examined by PCR with seven sets of primers. Some microcystin-producing cyanobacteria were not detected with FAA-RAA, TOX4F-TOX4R and FP-RP primers. NSZW1-NSZW2 and TOX1P-TOX1F primers failed in amplification of toxin-producing strains. Two primer sets of MSF-MSR and TOX2P-TOX2F amplified the fragments of *mcyA* and *mcyB* genes from microcystin-producing cyanobacteria, respectively (Table 1). The water samples taken from eight lakes in Korea were analyzed by PCR using each of the primers. In all the water samples, cyanobacteria capable of producing microcystin were detected by the PCR with MSF-MSR and TOX2P-TOX2F primers. These results indicate that MSF-MSR and TOX2P-TOX2F primer sets are better than other primers for detection of microcystin-producing cyanobacteria in Korea.

The competitor for QC-PCR with TOX2P-TOX2F primers was constructed from the PCR product of *M. aeruginosa* NIER10010. The amplified product of 355 bp was cloned into the pGEM-T vector to create plasmid pTP1. The competitor (pTD1) was constructed from pTP1 by deletion of 30bp.

To quantify the number of *mcyB* gene copy, the genomic DNA of *M. aeruginosa* and a serial dilution of the competitor of a known copy number were coamplified in a series of PCR tubes. The sample and competitor amplicons, differing by 30 bases in length, were separated on a 1.5% agarose gel and stained with ethidium bromide. Band intensities of amplicons were measured by the image analysis software. Progressive competition between the competitor and sample DNA was observed on the gel. A regression line for each plot was generated, and the amount of sample DNA could be calculated (Fig. 1). The amount of template DNA of *M. aeruginosa* was plotted against the number of *mcyB* gene estimated by QC-PCR. This result showed a strong positive correlation between the number of *mcyB* gene and the amount of genomic DNA (Fig. 2).

DNA samples extracted from the cyanobacteria in Korean lakes was analyzed by the QC-PCR. Microcystin-producing cyanobacteria were detected in all lakes. The copy number of *mcyB* gene was ranged between 7.7×109 and 1.2×1013copies/ng of genomic DNA. The maximum copy number of *mcyB* gene was observed in Lake Keumma (Table 2).

This study suggests that QC-PCR is a useful technique for detection of the toxigenic cyanobacteria in aquatic environments.

Table 1. PCR results of 17 cyanobacterial strains amplified with 7 primer sets

Strain	Source	Microcystin production	PCR products amplified with primers						
			mcyA		*mcyB*				*mcyC*
			NSZW1[1] NSZW2	MSF[2] MSR	TOX1P[3] TOX1F	TOX2P[4] TOX2F	TOX4F[5] TOX4R	FAA[6] RAA	FP[7] RP
Microcystis aeruginosa Kangwon	Korea	+	-	+	-	+	+	+	+
Microcystis aeruginosa NIER 10001			-	+	-	+	+	+	+
Microcystis aeruginosa NIER10010			-	+	-	+	-	-	+
Microcystis aeruginosa NIER10038			-	+	-	+	+	+	+
Microcystis aeruginosa NIER 10039			-	+	-	+	+	+	-
Microcystis aeruginosa PUCC1117			-	+	-	+	+	+	+
Microcystis aeruginosa TAC12	Japan		-	+	-	+	ND	+	+
Microcystis aeruginosa PAC17			-	+	-	+	ND	+	+
Microcystis aeruginosa PAC18			-	+	-	+	ND	+	+
Microcystis viridis TAC17			-	+	-	+	ND	-	+
Microcystis viridis TAC18			-	+	-	+	+	+	+
Anabaena mactospora NIER1016	Korea	-	-	-	-	-	-	-	-
Microcystis wesenbergii PUCC1113			-	-	-	-	-	-	-
Microcystis ichthyoblabe NIER10045			-	-	-	-	-	-	-
Microcystis nocacekii NIER10029			-	-	-	-	-	-	-
Microcystis viridis PUCC1002			-	-	-	-	-	-	-
Microcystis ichthyoblabe TAC125	Japan		-	-	-	-	ND	-	-

[1]Nishizawa T. *et. al.*, 1999, Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in *Microcystis spp., J. Biochem.*, 126, 520-529.

[2]Tillett D. *et. al.*, 2001, Detection of toxigenicity by a probe for the microcystin synthetase A gene(*mcyA*) of the cyanobacterial genus *Microcystis*: Comparison of toxicities with 16S rRNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies, *Appl. Environ. Microbiol.*, 67, 2810-2818.

[3]Pan H. *et. al.*, Investigation of target genes by PCR from intact cyanobacterial cells, Ecotechnology in environmental protection and fresh water lake management, 59-66.

[4]Pan H. *et. al.*, Investigation of target genes by PCR from intact cyanobacterial cells, Ecotechnology in environmental protection and fresh water lake management, 59-66.

[5]Kurmayer R. *et. al.*, 2002, Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis* spp. in lake wannsee(Berlin, Germany), 43, 107-18.

[6]Neilan BA. *et. al.*, 1999. Nonribosomal peptide synthesis and toxigenicity of cyanobacteria, *J. Bacteriol.*, 181, 4089-4097.

[7]Nishizawa T. *et. al.*, 1999, Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in Microcystis spp., *J. Biochem.*, 126, 520-529.

Table 2. Detection of microcystin-producing cyanobacteria in Korean lakes by QC-PCR

Lake	Sampling date	*mcyB* gene* (Copies/ng of DNA)	Chl-a ($\mu g/l$)
Keummam	2001.07.16	1.2×10^{13}	5120
Namil	2001.07.14	2.5×10^{11}	12540
Dunpo	2001.07.16	2.5×10^{10}	146960
Daecheongho	2001.07.14	5.0×10^{11}	1729
Yanggu	2001.08.31	1.0×10^{11}	2261
Chungjuho	2001.09.01	1.3×10^{11}	377
Paroho	2001.08.31	7.7×10^{9}	2197
Paldang	2001.07.15	8.3×10^{12}	ND

* The copy number of *mcyB* gene was estimated by QC-PCR with TOX2P-TOX2F primers.

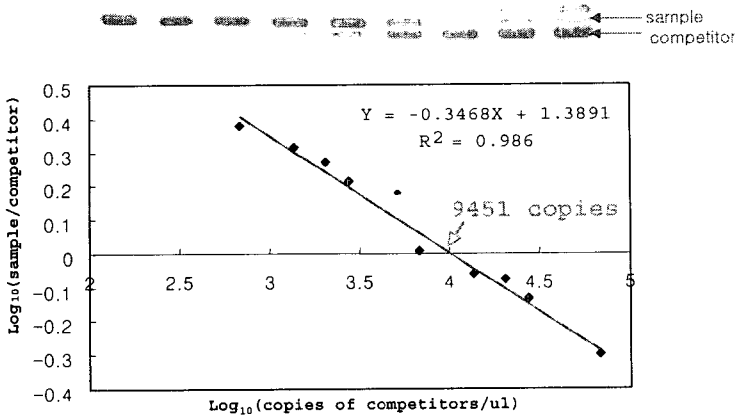

Fig. 1. Quantitative competitive-PCR. Genomic DNA of *Microcystis aeruginosa* was coamplified with serially diluted competitors, the ratio of the intensities of the sample to the competitor was plotted against the concentration of the competitor on a log scale.

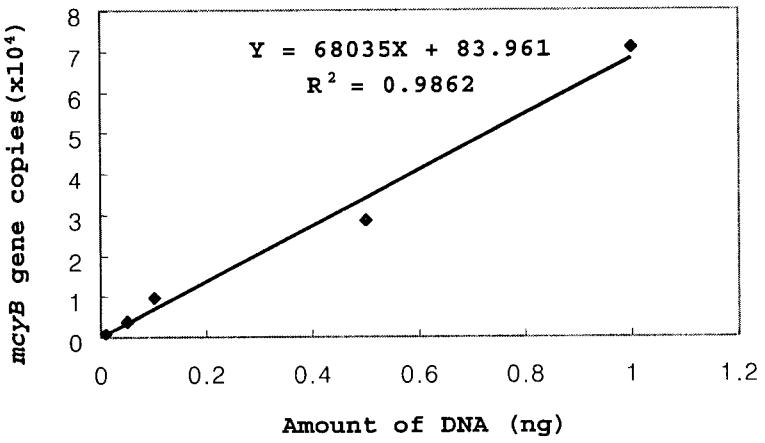

Fig. 2. Relationship between the amount of genomic DNA of *Microcystis aeruginosa* and the number of *mcyB* gene estimated by QC-PCR.