
Short Communication

**Rapid DNA Extraction from a Bloom-forming
Cyanobacterium**RYUJI KONDO*¹, GO KAGIYA¹, SHINGO HIROISHI¹,
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(Received February 10, 1999—Accepted May 28, 1999)

A rapid small-scale DNA extraction method is described for the toxic and/or bloom-forming cyanobacterial genus *Microcystis*, producing enough genomic DNA for polymerase chain reaction (PCR) amplification. PCR templates from 43 *Microcystis* strains were extracted and analyzed by PCR amplification. Sonication was needed for some strains before extraction of DNA using InstaGene™ Matrix with heat treatment at 100°C. DNAs extracted from all strains used in this study by this method could be used as templates for PCR amplification. Depending on the apparatuses used, DNA extraction, PCR amplification and agarose gel electrophoresis analysis can be carried out on about 50 samples of *Microcystis* one day.

Key words: DNA extraction, PCR, *Microcystis*, cyanobacteria

The cyanobacterial (blue-green algal) genus *Microcystis* commonly forms water blooms in eutrophicated water systems during warmer seasons. Some members of the genus contain the cyclic heptapeptide hepatotoxin, microcystin. The blooms of this genus present a considerable public health threat and also cause death in native and domestic animals. Consequently, a rapid and reliable method for discriminating between species or toxic strains of *Microcystis* is needed for environmental management of this genus.

Current cyanobacterial identifications are based upon morphological features observed microscopically^{1,3}). Morphological characteristics of *Microcystis* species in selective culture conditions are

often considerably altered from the morphology in natural environments. In order to contribute to the improvement of *Microcystis* taxonomy, some genetic analyses such as 16S rRNA gene (16S rDNA) sequencing^{4,5,7,8,9,12}), RFLP of phycocyanin-intergenic spacer¹⁰), and RAPD^{6,11}) have been performed.

Isolation and purification of DNA molecules are required for genetic analysis techniques such as restriction endonuclease digestion, Southern hybridization, and PCR amplification. Most methods for extracting DNA from cyanobacteria are time-consuming and laborious, and further require large amounts of cell material. Easy DNA purification methods using paramagnetic beads or ion-chelating matrix have been developed for detection and quantification of cyanobacteria^{10,13,14}). In this study, we examined the usefulness of rapid, small-scale DNA extraction from

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the bloom-forming cyanobacterial isolates of *Microcystis* for use as a PCR template.

Microcystis strains used in this study were obtained from the National Institute for Environmental Studies (NIES), Environmental Agency, Japan, and Tsukuba Algal Collection (TAC), National Science Museum, Japan. Other strains, LMM and LMK, which were isolated from Lake Mikata, Fukui, Japan in 1995, were taken from our stock culture. All strains were cultured in MA medium²⁾ at 25°C under illumination of approximately 20 $\mu\text{E}/\text{m}^2/\text{sec}$ with a 12 h : 12 h light-dark cycle. Strains used in this study are outlined in Table 1.

A 1-ml aliquot of mid-exponential- to stationary-phase culture was centrifuged at 15,000 rpm for 5 min. Cell pellets were resuspended in 200 μl of 0.1% Triton X-100 (Sigma, USA) or InstaGeneTM Matrix (Bio-Rad Laboratories, USA). Suspensions were treated with or without sonication of 20 W for 20 sec by the use of a tip ultrasonic generator Model UR-20P (Tomy, Japan) on ice. Then the suspensions were heated at 100°C for 10 min and cell debris was pelleted by centrifugation at 15,000 rpm for 5 min. One μl or more of the supernatant was used as a template for PCR amplification.

PCR amplifications of a part of the 16S rDNA were performed using the set of MAF and MAR primers which are specific for the major *Microcystis* species³⁾. Primers (8F and 1512R) for conserved regions at the 5' and 3' ends of the prokaryotic 16S rDNA¹⁵⁾ were also used. The thermal cycling conditions were: Denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min, and primer extension at 72°C for 3 min, with a final elongation step of 7 min at 72°C. DNA was amplified using a DNA Thermal Cycler PJ2000 (Perkin-Elmer Co., USA) for a total of 25 cycles. After PCR amplification, the products were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide.

Figure 1 shows the results of PCR amplification using DNA templates extracted with 0.1% Triton X-100 or InstaGeneTM Matrix with or without sonication. The 16S rDNA from some strains was not amplified by either extraction method without sonication (Fig. 1 A and B). On the other hand, supernatants obtained

Table 1. List of *Microcystis* species used in this study.

Strain	Isolation locality	LN*
<i>Microcystis aeruginosa</i>		
NIES87	Lake Kasumigaura	1
NIES88	Lake Kawaguchi	2
NIES89	Lake Kawaguchi	3
NIES90	Lake Kawaguchi	4
NIES91	Lake Kasumigaura	5
NIES98	Lake Kasumigaura	14
NIES99	Lake Suwa	6
NIES100	Lake Suwa	7
NIES101	Lake Suwa	8
NIES298	Lake Kasumigaura	9
NIES299	Lake Kasumigaura	10
TAC157-2	Teganuma Pond	11
TAC169	Lake Okutama	12
TAC192	Lake Okutama	13
LMK9508-3	Lake Mikata	41
LMK9508-8	Lake Mikata	42
<i>Microcystis wesenbergii</i>		
NIES104	Chiyoda-ku	15
NIES105	Lake Kasumigaura	16
NIES106	Lake Kasumigaura	17
NIES108	Lake Suwa	18
NIES109	Lake Yogo	19
NIES110	Lake Kasumigaura	20
NIES111	Lake Kasumigaura	21
NIES112	Lake Suwa	22
NIES604	Lake Kasumigaura	23
TAC38	Lake Kasumigaura	26
TAC52-1	Lake Suwa	24
TAC57-1	Lake Suwa	25
LMM9509-al	Lake Mikata	39
<i>Microcystis viridis</i>		
NIES102	Lake Kasumigaura	27
TAC78	Lake Mikata	28
TAC92	Lake Barato	29
TAC140	Tameshowa-ike Pond	30
<i>Microcystis ichthyoblabe</i>		
TAC48-1	Lake Suwa	31
TAC51	Lake Suwa	32
TAC91	Lake Barato	33
TAC125	Lake Barato	34
TAC146	Kashima-Onuma	35
<i>Microcystis novacekii</i>		
TAC65-2	Chikazu-ike Pond	36
TAC66	Rokusuke-ike Pond	37
TAC75	Lake Yogo	38
LMK9508-2	Lake Mikata	40
<i>Microcystis</i> sp.		
LMK9508-v	Lake Mikata	43

* LN represents the lane number in agarose gel electrophoresis as shown in Fig. 1.

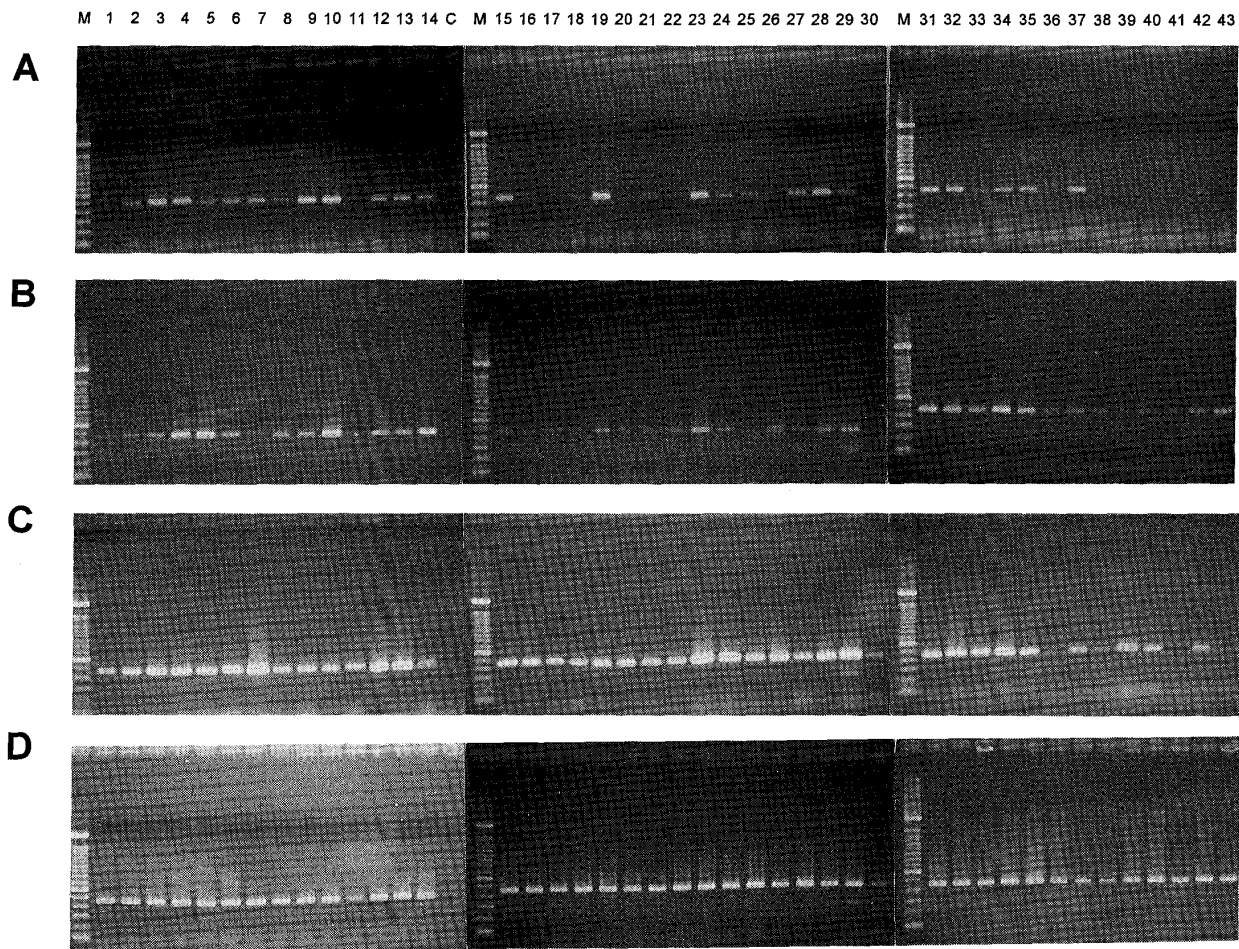


Fig. 1. Ethidium bromide-stained agarose gel of a 16S rDNA fragment from 43 strains of *Microcystis* using the set of MAF and MAR primers specific for the major *Microcystis* species. Ten percent of the amplification products (10 μ l) was loaded in each lane. DNAs were extracted with 0.1% Triton X-100 without sonication (A), InstaGene™ Matrix without sonication (B), 0.1% Triton X-100 with sonication (C), and InstaGene™ Matrix with sonication (D). Lane numbers are identified in Table 1, and lanes M and C show 100-base-pair ladder size marker and no-template control, respectively.

using 0.1% Triton X-100 and sonication before heating were found to contain sufficient DNA to act as templates for PCR amplification, except for that from *M. novacekii* strain LMK9508-2 (Fig. 1 C). Using InstaGene™ Matrix with sonication, the extracts from all strains tested in this study contained sufficient DNA for successful PCR amplification (Fig. 1 D). The PCR products from more strains were detectable on agarose gels stained with ethidium bromide using extraction with InstaGene™ Matrix than with 0.1% Triton X-100, as shown in the cases of *M. novacekii* and the Lake Mikata isolates. Based on these results, DNA extraction using InstaGene™ Matrix with sonication was used in the following experiments.

DNAs extracted from various strains using InstaGene™ Matrix with sonication were tested as PCR templates. Using the set of primers 8F and 1512R for the conserved regions at the 5' and 3' ends of the prokaryotic 16S rRNA gene resulted in successful PCR amplifications (Fig. 2). This means that regions at least 1500 nucleotides in length were amplified using PCR templates extracted by this method.

The relationship between the number of *M. aeruginosa* NIES87 cells and PCR amplification is shown in Fig. 3. A culture of *M. aeruginosa* NIES87 grown to 2.49×10^6 cells/ml was serially diluted with MA medium. PCR templates from 1-ml aliquots of the diluted culture were prepared by the InstaGene™

Matrix method and PCR was performed with 1 μ l of supernatant as PCR template. The PCR product was detected using the template from the 10^3 -fold dilution, in which the cell number was about 2×10^3 cells, on 2% agarose gels stained with ethidium bromide (Fig. 3, Lane 4), but not using the template from the 10^4 -fold

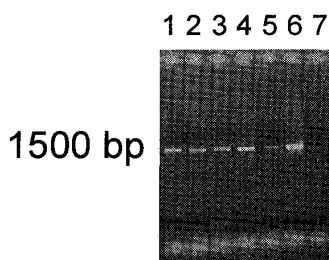


Fig. 2. Ethidium bromide-stained agarose gel of the almost-complete 16S rDNA from (1) *Microcystis aeruginosa* NIES100, (2) *M. aeruginosa* NIES98, (3) *M. wesenbergii* NIES105, (4) *M. viridis* NIES102, (5) *M. novacekii* TAC65-2, and (6) *M. ichthyoblabe* TAC91 after PCR amplification with the set of 8F and 1512R primers. Numbers in parentheses correspond to the lane numbers of the agarose gel. Lane 7 shows the no-template control.

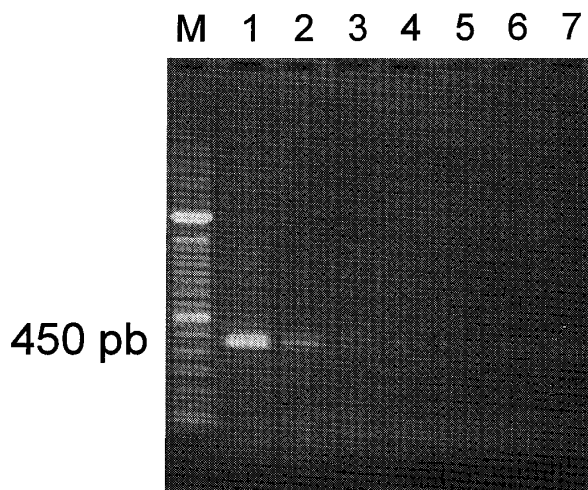


Fig. 3. Ethidium bromide-stained agarose gel of PCR-amplified 16S rDNA fragment from *Microcystis aeruginosa* NIES87 using the set of MAF and MAR primers. A culture of *M. aeruginosa* NIES87 grown to 2.49×10^6 cells/ml was serially diluted with MA medium through a dilution of 10^4 , and PCR templates from 1 ml of each dilution were prepared by the InstaGeneTM Matrix method. PCR was performed with 1 μ l (lanes 1–5) or 10 μ l (lanes 6 and 7) of supernatant as PCR templates. Lanes: 1, 10^0 -fold dilution; 2, 10^1 -fold dilution; 3, 10^2 -fold dilution; 4 and 6, 10^3 -fold dilution; 5 and 7, 10^4 -fold dilution.

dilution (Fig. 3, Lane 5). When 10 μ l of supernatant was used as the PCR template, however, the PCR product was visualized on the agarose gel in the sample from 10^4 -fold dilution (Fig. 3, Lane 7). This shows that at least 10^2 – 10^3 cells were needed for PCR amplification and agarose gel electrophoresis.

Figure 4 shows the growth curve of *M. aeruginosa* NIES87 and PCR amplification using template DNAs from cells at each stage of growth. PCR amplifications of the 16S rDNA were successful from the cells at all stages, indicating that the PCR template could be prepared from all growth stages of *Microcystis*.

A paramagnetic beads method suitable for purification of PCR-ready DNA from cyanobacteria, involving the detection and quantification of cyano-

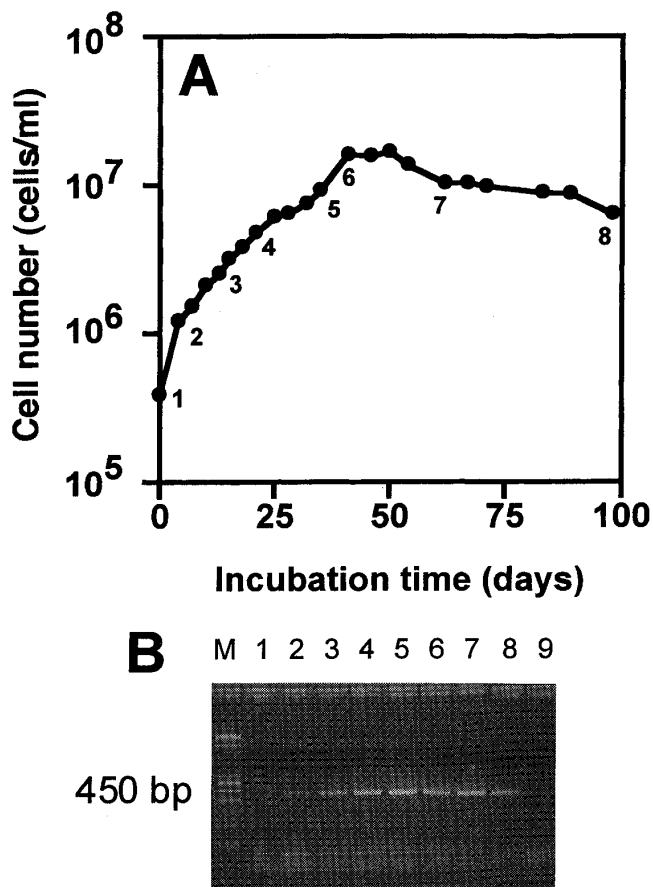


Fig. 4. Growth curve (A) of *Microcystis aeruginosa* NIES87 and PCR amplifications (B) of the 16S rDNA fragment from the cells at each growth stage using the MAF and MAR primers and analyzed by 2% agarose gel electrophoresis stained with ethidium bromide. Lane numbers correspond to the numbers in the growth curve of *M. aeruginosa* NIES87. Lane 9 is the no-template control.

bacteria in water has been reported by Rudi et al^{13,14}. Although the detection limits of our procedure were similar to or slightly less than those of their method, our DNA extraction method is thought to be more simple and rapid than the paramagnetic beads method because of the shorter handling time, and low number of reagents and apparatuses. Moreover, our procedure using InstaGene™ Matrix could be used for higher cell densities over 10⁶ cells/ml, at which concentration it is difficult to perform the paramagnetic beads method¹³. Thus, our method has the advantage of yielding enough DNA for PCR amplification in a short time. By this method, products at least 1,500 bp in length were amplified by PCR. DNA extraction, PCR amplification, and agarose gel electrophoresis analysis could be carried out on about 50 samples of *Microcystis* cultures one day, depending on the apparatuses used, i.e., the thermal cycler, centrifuge, electrophoresis system, and so on. The rapidity, simplicity and detection limits suggest that our method could be applied to the detection of cyanobacteria in natural environments.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. Further financial support from the Steel Industry Foundation for Advancement of Environmental Protection Technology is greatly appreciated.

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