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Determination of Cyanobacterial Diversity during Algal Blooms in Daechung Reservoir, Korea, on the Basis of *cpcBA* Intergenic Spacer Region Analysis

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The detection and prevention of cyanobacterial blooms are important issues in water quality management. As such, the diversity and community dynamics of cyanobacteria during cyanobacterial bloom in the Daechung Reservoir, Korea, were studied by analyzing the intergenic spacer (IGS) region between phycocyanin subunit genes *cpcB* and *cpcA* (*cpcBA* IGS). To amplify the *cpcBA* IGS from environmental samples, new PCR primers that could cover a wider range of cyanobacteria than previously known primers were designed. In the samples taken around the bloom peak (2 September 2003), seven groups of *cpcBA* IGS sequences were detected, and none of the amplified *cpcBA* IGSs was closely related to the *cpcBA* IGS from chloroplasts. Apart from the *Microcystis*-, *Aphanizomenon* (*Anabaena*)-, *Pseudanabaena*-, and *Planktothrix* (*Oscillatoria*)-like groups, the three other groups of *cpcBA* IGS sequences were only distantly related to previously reported sequences (<85% similarity to their closest relatives). The most prominent changes during the bloom were the gradual decrease and eventual disappearance of the *Aphanizomenon* (*Anabaena*)-like group before the bloom peak and the gradual increase and sudden disappearance of *Planktothrix* (*Oscillatoria*)-like groups right after the bloom peak. The community succession profile obtained based on the *cpcBA* IGS analysis was also supported by a PCR-denaturing gradient gel electrophoresis analysis of the 16S rRNA genes.

Bloom-forming cyanobacteria in freshwater are a serious problem for the management of drinking water, since they produce a wide range of toxic compounds, including neurotoxins and hepatotoxins (5). Thus, proper environmental management of water supplies relies on prior knowledge of cyanobacterial ecology. However, the study of cyanobacterial diversity relies largely on the use of microscopic techniques and is very labor intensive. Moreover, since the isolation of cyanobacteria from samples is not always successful, rapid identification of cyanobacteria without cultivation is important. Molecular technologies based on 16S rRNA gene amplification are already widely employed for the analysis of environmental samples (9, 19). Yet, the 16S rRNA gene is often restricting in regard to resolving bacterial strains due to its slow evolution. Furthermore, when other bacteria are present in samples, selective identification of cyanobacteria can be severely hindered. Indeed, diverse noncyanobacterial prokaryotes are associated with cyanobacterial blooms (9).

Of the functional genes used for the taxonomic study of cyanobacterial strains, including the *cpcBA* intergenic spacer (IGS) (7, 15, 17, 20, 22), *nifH* (7), *rpoC1* (24), and *gyrB* (24), the *cpcBA* IGS is specific to cyanobacteria and has been widely used for the phylogenetic analysis of pure cyanobacterial culture strains. Baker et al. (1, 2) recently employed a PCR amplification method to analyze the *cpcBA* IGSs from environmental samples, using a primer set previously designed by Neilan et al. (20), and found a limited cyanobacterial diversity. Although the primer set was originally designed with six *cpcBA* IGSs to study the genetic diversity of several pure culture strains, *cpcBA* IGS sequence information from various other cyanobacteria has also been deposited in public databases for potential enhanced primer design.

Cyanobacterial blooms capable of producing microcystins are a seasonal problem every summer in the Daechung Reservoir, which is a representative large eutrophic lake in Korea (21). Accordingly, to further elucidate the composition and dynamics of cyanobacteria during bloom, this study investigated the *cpcBA* IGS diversity in addition to physicochemical and biological factors. To analyze the *cpcBA* IGS diversity, new degenerate primers were designed based on more than 300 *cpcBA* IGS sequences that are currently available in public databases. Finally, the cyanobacterial diversity derived from the *cpcBA* IGS analysis was compared with that determined by 16S rRNA gene PCR-denaturing gradient gel electrophoresis (DGGE).

MATERIALS AND METHODS

Sampling and field survey. The Daechung Reservoir in Korea is an artificial lake created by the construction of a dam in 1980, and its water is used for drinking, as well as agricultural and industrial uses. The reservoir is a large branch-type lake with a 72-m-high dam and a gross storage capacity of 1,490

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Mm³. The water sampling was conducted weekly from a floating wharf about 20 m off shore near the Daechung dam from 15 July to 14 October 2003. Surface water above a depth of 20 cm was collected after some mixing, and then the samples were stored in 20-liter polyethylene bottles at 4°C in the dark and the laboratory analysis performed within 24 h.

Water quality analysis. The water temperature, pH, and conductivity were all measured in situ, using a YSI meter (63/100 FT; YSI Inc., Yellow Springs, OH), while the dissolved oxygen (DO) and turbidity were measured with a DO meter (95/100 FT; YSI Inc., Yellow Springs, OH) and a turbidimeter (DRT-15CE; HF Scientific Inc., Fort Meyers, FL), respectively. The Secchi depth was measured using a Secchi disk. The total N (TN) and P (TP) were determined after persulfate oxidation to nitrate (6) and orthophosphate (18), respectively. The resulting nitrate was then determined by a second-derivative method (4), while the orthophosphate was determined using an ascorbic acid method (8). The total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were determined after filtering the water sample through a GF/C filter (Whatman Ltd., Maidstone, United Kingdom) and persulfate oxidation. The total particulate nitrogen (TPN) and total particulate phosphorus (TPP) were obtained by subtracting the TDN from the TN and the TDP from the TP, respectively.

The samples used for plankton identification and enumeration were preserved in Lugol's solution and enumerated with a hemocytometer (Fuchs-Rosenthal; Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany) under phase-contrast microscopy (Microphot-FXA; Nikon Corp., Tokyo, Japan). The chlorophyll *a* was extracted using a chloroform-methanol mixture (2:1 [vol/vol]) and measured with a fluorometer (Turner 450; Barnstead/Thermolyne, Dubuque, IA) (29). To determine the picocyanobacterial chlorophyll *a*, the large cyanobacteria were removed using a filter with a 3-μm pore size, and only the fraction that remained on a 0.2-μm filter was used (10).

Primer design for PCR amplification of *cpcBA* IGS. To monitor the diversity and community composition of the cyanobacteria based on the *cpcBA* IGS, a PCR primer set was specifically designed for cyanobacterial *cpcBA* IGS amplification. Among all known *cpcBA* IGS sequences (more than 300 sequences), 24 representative *cpcBA* IGSs from diverse cyanobacteria (*Cylindrospermopsis raciborskii*, GenBank accession number AF426795; *Cylindrospermopsis raciborskii*, AF426792; *Arthrospira* sp. strain Maxima, AJ401168; *Microcystis aeruginosa* EAWAG175, AJ003181; *Fischerella* sp., M75599; *Nostoc* sp. strain PCC 7120, X05239; *Anabaena lemmermannii* BC Ana 0018, AY886908; *Nodularia spumigena* nsb105, AF101444; *Synechococcus elongatus*, D13173; *Synechocystis* sp. strain PCC 9413, AF068771; *Pseudanabaena* sp., M99426; *Calothrix* sp., M36276; *Fremyella diplosiphon*, X07012; *Synechocystis* sp. strain PCC 6803, U34930; *Chroococcus dispersus*, AJ003184; *Agmenellum quadruplicatum*, K02660; *Synechococcus* sp. strain WH7803, X59809; *Synechococcus* sp. strain PCC 7942, AB008546; *Synechococcus elongatus* PCC 6301, AP008231; *Anacystis nidulans*, M94218; *Oscillatoria* sp. strain PCC 6304, AJ401186; *Planktothrix rubescens* BC-Pla 9307, AJ131820; *Planktothrix* sp. strain FP1, AF212923; *Gloeobacter violaceus* PCC 7421, BA000045; *Microcystis* sp. strain KLL MG-K, AY524850; *Microcystis* sp. strain KLL MB-J, AY524849; *Microcystis* sp. strain KLL MB-K, AY524848; *Aphanizomenon* sp. Norman Lake isolate, AJ243969; and *Aphanizomenon flos-aquae*, AJ243971) were aligned by using Clustal X (27). In addition, two conserved regions of *cpcB* and *cpcA* were chosen to design the primer set (named CPC1F and CPC1R, respectively) used in this study.

The genomic DNAs from these bacteria were extracted as described previously (13), and the PCR was performed with a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) using a 20-μl (total volume) reaction mixture containing 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, pH 9.0), four deoxyribonucleoside triphosphates at concentrations of 1 mM, 1.5 mM $MgCl_2$, each primer at a concentration of 1 μM, 4 μg of bovine serum albumin (Roche Diagnostics Corp., Indianapolis, IN), and 2.5 U of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, CT). The amplification conditions were 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 7 min.

***cpcBA* IGS and 16S rRNA gene analysis of bloom samples.** For genomic DNA extraction from the bloom samples, about 500 ml of the water samples was filtered using 0.22-μm membrane filter (Millipore System, Bedford, MA) and the dozens of resulting filter papers were stored at −65°C until required. The bulk community DNA was directly extracted from the filter by grinding the frozen samples and treating them with sodium dodecyl sulfate for cell lysis (13). The conditions used for *cpcBA* IGS amplification from the environmental samples were the same as those used in the PCR primer evaluation described above. The amplified PCR products were directly ligated into a pCR II vector obtained from Invitrogen (San Diego, CA). The ligation and transformation were carried out as described previously (28). A total of 100 clones per sample were screened, and 50 positive clones per sample were randomly selected and sequenced using the primers CPC1F and CPC1R. The diversity coverage by the clone libraries was analyzed using a rarefaction method, as previously described (25).

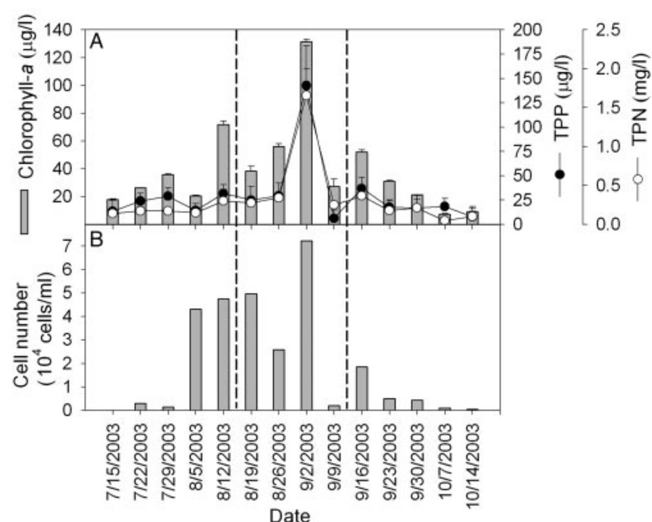

FIG. 1. Changes in chlorophyll *a*, TPP, and TPN concentrations (A) and number of total phytoplankton (B) in Daechung Reservoir. The sampling was carried out at 1-week intervals. The samples between the dashed lines were used for further molecular analysis. The error bars indicate standard deviations ($n = 3$).

The *cpcBA* IGS sequences obtained in this study were aligned with all those available in current databases by using the Clustal X program (27) and then edited using BioEdit (12). The initial phylogenetic trees were based on all available sequences and constructed using the neighbor-joining DNA distance program (23) in MEGA 2 (16), with bootstrap values based on 1,000 replications (11). Based on the initial phylogenetic results, appropriate subsets of *cpcBA* IGS sequences were selected and subjected to a final phylogenetic analysis.

Bacterial diversity analysis based on 16S rRNA gene PCR-DGGE was performed as described by Ishii and Fukui (14) and Muyzer et al. (19). A 560-bp (bp 341 to 907 in *Escherichia coli* numbering) fragment of the 16S rRNA gene from four samples used in the *cpcBA* IGS analysis was amplified and analyzed using DGGE. The bands were excised from the DGGE gel and incubated in distilled water for 24 h at 4°C. The eluent was then reamplified for sequencing with the primers without a GC clamp.

Nucleotide sequence accession numbers. The GenBank accession numbers for the clones are as follows: P1-5R, AY942919; P1-30, AY942910; P2-20, AY942916; P2-31, AY942914; P2-48, AY942917; P2-52, AY942907; P3-26, AY942908; P3-36, AY942918; P3-51, AY942909; P3-59, AY942915; P4-9, AY942906; P4-21, AY942905; P4-25, AY942912; P4-29, AY942913; P4-30, AY942911; P4-56, AY942920; 16S-1, AY942899; 16S-2, AY942896; 16S-3, AY942902; 16S-4, AY942903; 16S-5, AY942894; 16S-6, AY942895; 16S-7, AY942897; 16S-8, AY942900; 16S-9, AY942904; 16S-10, AY942901; and 16S-11, AY942898.

RESULTS AND DISCUSSION

Physicochemical and biological characteristics of blooming water body. The chlorophyll *a* concentration, an indicator of a trophic state, revealed that the Daechung Reservoir was in a eutrophic state that became more severe in summer and early autumn (Fig. 1A). The cell count profile, water turbidity, Secchi depth, and chlorophyll *a* (Fig. 1B and Table 1) collectively indicated that the most significant bloom occurred around 2 September 2003. Four samples out of a total of 14 were collected before and after this bloom peak to analyze the cyanobacterial community. In the bloom sample taken on 2 Sep-

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TABLE 1. Biogeochemical characteristics of bloom water

Property (unit)	Value on sampling date (mo/day):			
	8/19	8/26	9/2	9/9
Turbidity (NTU[a])	8.33	14.33	33.33	12.00
Secchi depth (m)	1.33	1.10	1.00	1.20
Chlorophyll *a* (μg/liter)	38.4	56.1	131.5	27.3
pH	9.06	9.73	9.26	8.79
Water temp (°C)	25.2	26.5	24.8	25.5
Conductivity (μS/cm)	88.3	97.0	88.3	91.0
DO (mg/liter)	8.50	11.63	9.60	12.43
Nutrient concn[b]				
TN (mg/liter)	1.21 (0.06)	1.10 (0.03)	2.59 (0.34)	0.91 (0.02)
TDN (mg/liter)	0.93 (0.02)	0.76 (0.03)	0.93 (0.04)	0.66 (0.03)
TPN (mg/liter)	0.28 (0.06)	0.34 (0.04)	1.66 (0.34)	0.25 (0.03)
TP (μg/liter)	49.4 (7.0)	42.0 (9.6)	154.3 (41.0)	17.0 (8.0)
TDP (μg/liter)	24.2 (12.2)	12.6 (8.6)	11.6 (2.3)	10.9 (2.7)
TPP (μg/liter)	24.9 (14.1)	29.4 (12.9)	142.7 (41.1)	6.2 (8.4)
Cell count (% or no./ml)[c]				
Cyanobacteria	98.6	95.2	95.8	74.2
Phormidium	0	0	0	375
Planktothrix	40,250	18,313	44,125	750
Microcystis	9,375	7,500	28,125	406
Anabaena	0	0	83	113
Chroococcus	0	0	0	306
Green algae	1.2	3.8	4.1	19.9
Chlamydomonas	0	400	0	0
Scenedesmus	63	125	0	25
Chlorella	344	463	3,125	500
Staurastrum	219	50	0	0
Diatoms	0.1	0.9	0.0	5.7
Synedra	63	38	0	13
Aulacoseira	0	0	0	25
Fragilaria	0	225	0	113
Melosira	0	0	0	31
Surirella	0	13	0	0
Asterionella	0	0	0	56
Nitzschia	0	0	0	6

[a] NTU, nephelometric turbidity units.
[b] The nutrient concentrations were measured three times, and the averages and standard deviations (in parentheses) are shown.
[c] The relative values of the cell counts for cyanobacteria, green algae, and diatoms are indicated as percentages; counts for individual groups are indicated as number of cells per milliliter.

tember 2003, several characteristics of the water body were significantly different from those taken at different times. Most notably, the peak of the bloom was associated with the highest concentrations of TP and TN. It has already been reported that increased concentrations of N and P after rainfall influence cyanobacterial growth in Daechung Reservoir (21). As such, the apparent peak of cyanobacterial bloom that occurred on 2 September 2003 is an annual phenomenon, which is generally observed at the transition point between summer and autumn.

By microscopic counting, the cyanobacterial counts were found to remain high (accounting for 74 to 99%) during the bloom season (Table 1), thereby directly contributing to the bloom peak on 2 September 2003 and the higher chlorophyll *a* concentration observed at this time. However, the most significant change at the bloom peak, as determined by the microscopic analysis, was a prominent increase in the mass of *Planktothrix* and *Microcystis*. After the bloom peak, there was a decrease in both the relative and absolute numbers of cyanobacteria in the total algae (Table 1). Nonetheless, the eukaryotic algae (green algae and diatoms) did not significantly affect the bloom, and there was no significant difference in any other physicochemical properties, such as the pH, water temperature, DO, TDN, and TDP, during the bloom.

Design of primers for PCR amplification of the *cpcBA* IGS. Although the reported *cpcB* sequences are quite diverse (nucleotide similarity of 266 bp at the 3′ end of the *cpcB* gene, 72.3 ± 7.4% [mean ± standard deviation]; range of pairwise similarity, 56 to 98%), several highly conserved regions were useful for the primer design. The priming site of PCbF (20), positioned at nucleotide 250 according to the numbering for *Microcystis* sp. strain KLL MB-J (accession no. AY524849) *cpcB*, was selected for the design of the new forward primer CPC1F (5′-GGCKGCYTGYYTRCGYGACATGGA-3′). Since several mismatches were found in the PCbF primer, degeneracy was applied to increase the coverage of CPC1F. In addition, a new priming site near the 5′ end of *cpcA* (nucleotide 43 according to the numbering for *Microcystis* sp. strain KLL MB-J [accession no. AY524849] *cpcA*) was explored for the

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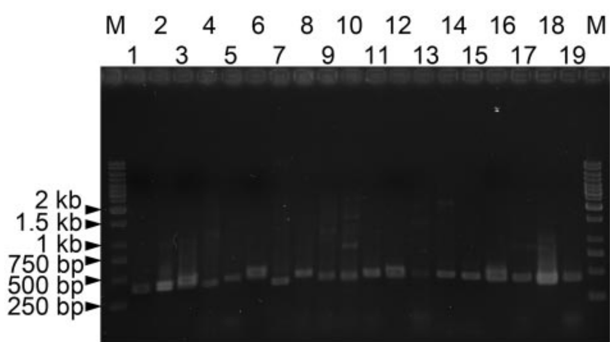

FIG. 2. Photographs of ethidium bromide-stained gels showing amplification products of *cpcBA* IGSs from reference cyanobacteria. The strains used were as follows: 1, *Aphanothece nidulans* KCTC AG10041; 2, *Aphanothece naegelii* KCTC AG10042; 3, *Microcystis aeruginosa* UTEX 2385; 4, *Microcystis* sp. strain PCC 7806; 5, *Synechococcus* sp. strain PCC 7002; 6, *Synechocystis* sp. strain PCC 6803; 7, *Merismopedia tenuissima* NIES 230; 8, *Arthrospira maxima* SAG 49.88; 9, *Arthrospira platensis* NIES 39; 10, *Oscillatoria tenuis* UTEX 1566; 11, *Oscillatoria tenuis* NIES 33; 12, *Planktothrix agardhii* NIES 204; 13, *Anabaena flos-aquae* UTEX 2517; 14, *Anabaena affinis* KCTC AG10008; 15, *Anabaena* sp. strain KCTC AG10059; 16, *Aphanizomenon flos-aquae* NIES 81; 17, *Nodularia spumigena* UTEX 2092; 18, *Nostoc* sp. strain PCC 7120; 19, *Chlorogloeopsis* sp. strain PCC 9212. Lanes M, markers (1-kb ladder). The numbers on the left indicate the sizes of the markers after electrophoresis.

reverse primer PC1R (5′-GCHGATWCYCAAGGNCGYT T-3′). Among the primer sets previously reported (3, 20) and those designed in this study, the CPC1F-CPC1R pair produced the most successful amplification of representative test strains from the cyanobacterial collections of PCC, UTEX, NIES, SAG, and the Korean Collection for Type Cultures (KCTC) (data not shown). As shown in Fig. 2, *cpcBA* IGSs were amplified from diverse cyanobacteria. As expected, due to size variations in the IGS region, the amplicon size ranged between 400 and 500 bp, indicating that the newly designed primer set was able to cover *cpcBA* IGSs from diverse cyanobacteria.

***cpcBA* IGS diversity among cyanobacterial bloom samples.** *cpcBA* IGSs were successfully amplified within 25 cycles from all four bloom samples. For the screened clones, all the sequences were related to the *cpcBA* IGS, and the rarefaction analysis showed that the number of clones reached a hypothetical saturation value (data not shown), where 50 clones covered most of the *cpcBA* IGS diversity within each sample. Based on the phylogenetic tree, seven groups of *cpcBA* IGS sequences were found from the libraries. The *cpcB* gene part of the PCR product was also selected for further phylogenetic analysis, and although the seven distinct groups of *cpcB* genes in the phylogenetic tree were widely distributed (Fig. 3), there was little variation in the sequences within each group. Furthermore, since the IGS part did not vary among subgroups, it is possible that each group of sequences was from a genetically identical ecotype. Only the *Microcystis*-like group (group F) exhibited some variations in the *cpcBA* IGS sequence, with a >95% intragroup sequence similarity.

With the exception of the *Microcystis* (group F, >95% similarity to closest relatives)-, *Aphanizomenon* (*Anabaena*) (group E, 90% similarity)-, *Pseudanabaena* (group B, 89% similarity)-, and *Planktothrix* (*Oscillatoria*) (group D, 100% similarity)-like groups, all the remaining groups of *cpcBA* IGS sequences were only distantly related to previously reported sequences (about <85% similarity to closest relatives). None of the amplified *cpcBA* IGSs was closely related to *cpcBA* IGSs from chloroplasts. However, the amplification of chloroplast *cpcBA* IGSs should be excluded, since the primer sites had several mismatches in the chloroplasts (1- to 5-bp mismatches in the forward and reverse primers) (data not shown). Consequently, this suggests that the present protocol could be useful for the specific analysis of cyanobacteria in the presence of other bacteria and eukaryotic algae in environmental samples.

Dynamics of the cyanobacterial community during bloom. To monitor the changes in the cyanobacterial community during cyanobacterial bloom, the relative composition of the *cpcBA* IGS sequences in each sample was analyzed. Groups D, E, and F were the major components of the cyanobacterial community on 19 August 2003, as shown in Fig. 4. The two most significant changes around the bloom peak on 2 September 2003 were the gradual decrease to extinction of group E and gradual increase and sudden disappearance of group D on 9 September. Meanwhile, group F steadily increased and finally predominated on 9 September. Interestingly, after the bloom peak, the cyanobacterial community profile did not return to that observed before the peak, as group F accounted for up to 85% of all the clones. However, the total cyanobacterial count, including *Microcystis*, decreased (down to 30% of that at the bloom peak), as shown in Table 1.

This relative increase of *Microcystis*-like cyanobacteria correlates well with the increased microcystin concentration measured after the bloom peak. Oh et al. (21) also observed that the particulate microcystin concentration in the Daechung Reservoir started to increase from early September after the bloom period. This phenomenon could be explained by an increase of *Microcystis* in the water body, as observed in this study. Thus, the *Aphanizomenon* (*Anabaena*)-like cyanobacteria were not involved in toxin production. The complete disappearance of group D also seemed to be an important marker for the transition of the cyanobacterial community from summer to autumn. The other groups (A, B, C, and G) had only a minor presence during the bloom period. Thus, the present *cpcBA* IGS-based approach revealed that the changes in the cyanobacterial community in response to environmental stimuli were more dynamic than those observed by microscopy.

Comparison of *cpcBA* IGS and 16S rRNA gene analyses for cyanobacterial bloom study. The *cpcBA* IGS-based method was compared with a 16S rRNA gene-based method using 16S rRNA gene PCR-DGGE. The best matches with the 16S rRNA gene sequences from the DGGE bands were identified using BLAST (Table 2). As expected, 16S rRNA genes for bacteria and chloroplasts were amplified together with those for cyanobacteria. Dynamic changes in the bacterial community were observed during the bloom period in the DGGE profile (Fig. 5), and based on a comparison of the phylogenies of the 16S rRNA and *cpcBA* IGS and their succession patterns, tentative correlations were found between the *cpcBA* IGS sequence groups and cyanobacterial DGGE bands. For example, the sequences of DGGE band 10 and *cpcBA* IGS group F, which closely matched those of *Microcystis* (>98% and 100%

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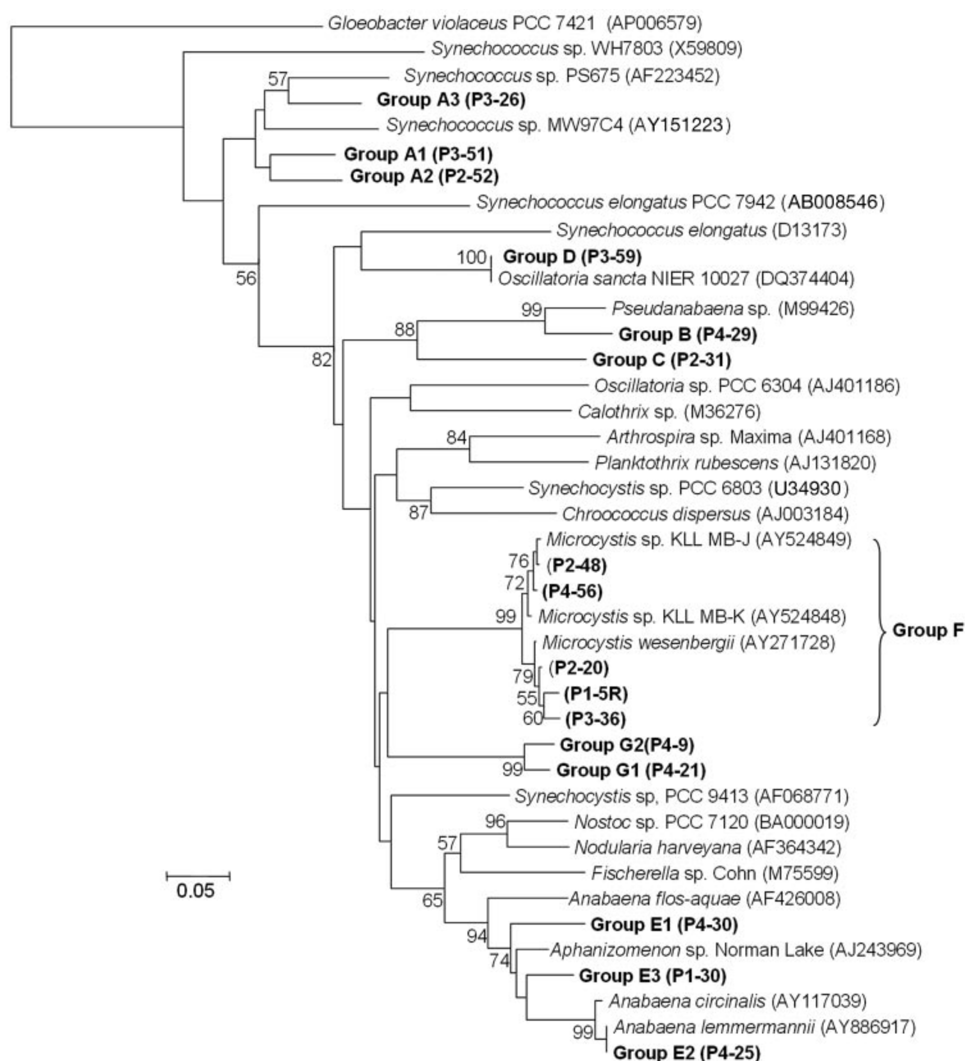

FIG. 3. Phylogenetic relationships of *cpcB* genes cloned from Daechung Reservoir. The reference sequences were from GenBank. The nucleotide sequences (267 bp from the 3′ end) from *cpcB* were used for the phylogenetic analysis. *cpcB* genes with a similarity of more than 95% are grouped. A representative clone from each group is indicated in parentheses. The bar indicates 0.05 substitution per nucleotide position. The local bootstrap probabilities are indicated at the nodes if they are larger than 50%.

similarities, respectively), were present throughout the bloom period, while the sequences of DGGE band 8 and *cpcBA* IGS group E, which closely matched those of *Aphanizomenon* and *Anabaena*, occupied a major proportion on 19 August 2003. Group E and band 8 were present as a minor component in the sample taken from 26 August to 9 September 2003. However, no *Aphanizomenon*- or *Anabaena*-like microorganisms were observed by microscopy on 19 August 2003 (Table 1). Considering that *Aphanizomenon* and *Anabaena* were observed in over 10% of cyanobacteria on 12 August 2003, *Aphanizomenon* and *Anabaena* were presumed to have existed on 19 August, even at low densities, but were not observed by microscopic enumeration. These results indicate that molecular techniques are more sensitive than microscopic observation for community study. Another reason could be the different sample volumes for each analysis, because about a 10-times-greater volume was used for molecular methods than for microscopy. Small flock formation by rarely occurring *Aphanizomenon* and *Anabaena* and their heterogeneous distribution could have amplified such a discrepancy.

The pattern of DGGE band 11 and profile of *cpcBA* IGS group D were highly related to those of *Planktothrix* (*Oscillatoria*)-like cyanobacteria identified in the microscopic analysis (Table 1). The sequences of DGGE band 11 and *cpcBA* IGS group D (P3-59) perfectly matched those of *Oscillatoria sancta* NIER 10027, which was isolated in Daechung Reservoir (Table 2 and Fig. 3). Because most members of *Oscillatoria* were taxonomically revised as members of *Planktothrix* and *O. sancta* NIER 10027 showed the characteristics of *Planktothrix mougeotii* (26), *O. sancta* NIER 10027 could be regarded as *Planktothrix*. Group D and band 11 were absent in the sample taken on 9 September 2003. The DGGE band 3, 4, and 9 sequences were from uncultured bacteria, whereas the DGGE band 2 and 7 sequences were from eukaryotic algal chloroplasts. Therefore, these comparison data indicate that the *cpcBA* IGS analysis correlated well with the 16S rRNA gene analysis. The

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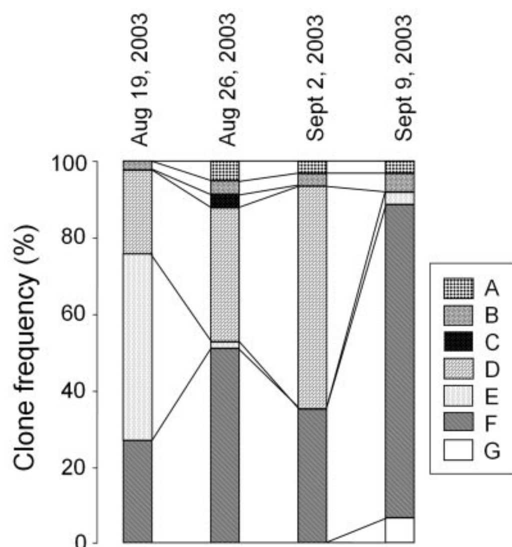

FIG. 4. Distribution dynamics of the *cpcBA* IGS sequence group during cyanobacterial bloom in Daechung Reservoir. The frequency distribution was calculated based on 50 clones analyzed from each sample. The dates of sampling are indicated.

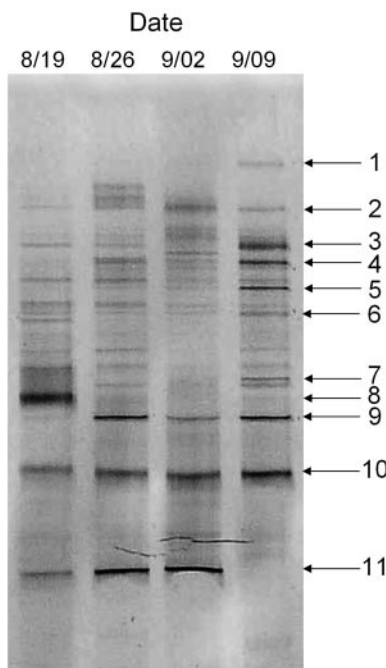

FIG. 5. 16S rRNA gene-DGGE profile during cyanobacterial bloom in Daechung Reservoir. The reproducibility of the DGGE profile was confirmed by three replications of the PCR-DGGE of the genomic DNA. The dates of sampling are indicated.

small discrepancies observed between the molecular and microscopic analyses may have reflected the presence of picocyanobacteria that were missed during the microscopic counting. The presence of picocyanobacteria (fewer than 5% of the clones) was also indirectly supported based on the chlorophyll *a* fraction of picocyanobacteria, which was at 4.90% ± 0.72% and 5.09% ± 0.44% on 24 August and 31 August 2005, respectively.

Improvement of water quality management depends on the development of proper methods to analyze cyanobacterial diversity and determine the cause of toxin production from blooms. In the present study, a molecular analysis of the *cpcBA* IGS was shown to be efficient for analyzing cyanobacterial diversity, not least because it can exclude other bacteria. Thus, the present protocol could complement conventional tools, such as morphology-based microscopic analyses, the direct determination of toxins, and 16S rRNA gene analyses. Furthermore, since the IGS length of each group of cyanobacteria is different, the proposed *cpcBA* IGS amplification protocol could also be directly coupled to length polymorphism analysis techniques, such as terminal restriction fragment length polymorphism, to facilitate rapid monitoring of the cyanobacterial community.

TABLE 2. Identities of bands obtained from DGGE analysis of bloom samples[a]

Band no.	Closest relative	GenBank accession no.	Similarity (%)
1	*Oscillatoria sancta*[b]	AY074801	99
2	*Aulacoseira ambigua* chloroplast	AJ536463	97
3	CFB group bacterium[c]	AF236016	95
4	Uncultured *Bacteroidetes*	AJ583816	97
5	Uncultured phytoplankton ESR 3	AF268287	96
6	Uncultured phytoplankton ESR 3	AF268287	97
7	*Chlamydomonas reinhardtii* chloroplast	BK000554	85
8	*Aphanizomenon flos-aquae*	AJ630443	100
9	Uncultured *Fibrobacteres* bacterium	AY509521	99
10	*Microcystis flos-aquae*	AF139327	100
11	*Oscillatoria sancta*[b]	AY074801	100

[a] See Fig. 5.
[b] This isolate was deposited under the name *Oscillatoria sancta* (unpublished), which was verified as a *Planktothrix*-like cyanobacterium (see the text for details).
[c] CFB, *Cytophaga-Flavobacterium-Bacteroides.*

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