Morphological and Molecular Analyses of *Anabaena variabilis* and *Trichormus variabilis* (Cyanobacteria) from Korea

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Abstract − This study characterizes three *Anabaena* strains and 5 *Trichormus* strains isolated from Korean waters and 3 *Anabaena flos-aquae* strains procured from the UTEX based on morphological features and molecular analyses. The *Anabaena* and *Trichormus* isolates were morphologically assigned to *A. variabilis* Kützing and *T. variabilis* (Kützing ex Bornet et Flahault) Komárek et Anagnostidis, respectively. The *Anabaena* and *Trichormus* strains differed significantly in the mean length of their vegetative cells. The 16S rRNA genes from the *Anabaena* strains showed a 100% identity to that from *A. variabilis* ATCC 29413, while the 16S rRNA genes from the *Trichormus* strains showed a 99.9% identity to that from *T. variabilis* GREIFSWALD. The overall topology was in agreement for the 16S rRNA gene and *cpcBA*-IGS trees in the both tree-constructing methods. In a neighbor-joining tree based on the 16S rRNA gene, the 3 *Anabaena* strains were asso-ciated with *A. variabilis*, the 5 *Trichormus* strains with *T. variabilis*, and the 3 *Anabaena* (UTEX) strains were with *Nostoc*. To date, this is the first report on *A. variabilis* and *T. variabilis* strains originating from Korea.

Key words : 16S rRNA, *Anabaena*, *cpcBA*-IGS, cyanobacteria, morphological analysis, *Trichormus*

INTRODUCTION

Phylogenetic researches of cyanobacteria have established that phylogenetic relationships sometimes conflict with the morphological classification (Rajaniemi *et al.* 2005). The comparison of morphological and phylogenetical data is hindered by the lack of cultures of several cyanobacterial morphospecies and inadequate morphological properties of sequenced strains. Furthermore, it is not common for the names of cultured strains to be corrected or updated based on subsequent studies, or misidentifications of cultured strains to be corrected in collection list and databases. Phylogenetic analyses derived from molecular analyses of misidentified cultured strains thus may not reflect new results from morphological studies.

Anabaena and *Trichormus* belong to subsection IV. I based on a bacteriological classification (Rippka *et al.* 2001), and the order Nostocales and family Nostocaceae according to traditional classification (Komárek and Anagnostidis 1989), with *Anabaena variabilis* Kützing, *Anabaena flos-aquae* (Lyngb.) De Brébisson and *Trichormus variabilis* (Kützing ex Bornet et Flahault) Komárek et Anagnostidis as the type species, respectively (AlgaeBase 2007). The genus *Anabaena* is characterized by unbranched trichomes that possess intercalary heterocysts (Whitton 2002), and includes 34 species (NCBI 2011).

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To examine morphological variations among Korea *Anabaena* and *Trichormus* strains, we collected many samples from different localities throughout Korea, obtaining more than ten strains. This study focused on the combined molecular and morphological relationships of the two genera *Trichormus* and *Anabaena*. Five *Trichormus* and 3 *Anabaena* strains isolated from Korea and 3 *A. flos-aquae* strains from UTEX (Texas, USA) were identified based on their morphology and molecular analyses.

MATERIALS AND METHODS

1. Isolation and culture of *Trichormus* and *Anabaena* strains

Single trichomes of cyanobacteria were isolated from six surface-water samples from rivers and reservoirs in Korea (Table 1). The isolated strains were identified as *Trichormus* and *Anabaena* using a light microscope. Meanwhile, three *A. flos-aquae* strains were obtained from UTEX Culture Collection of Algae. The isolation and culturing of these strains were carried out using standard procedures. The purified unialgal filaments were plated on 1.2% (w/v) agarose (QA-Agarose TM, Qbiogene Inc., OH, USA) plates prepared in a BG-11 medium (Rippka 1988). All the strains were derived from a single trichome using a modified micromanipulator method (Oh and Rhee 1990). The purified filaments growing on the plate were then inoculated into a liquid BG-11 medium and cultured at 26 ± 1°C at a photosynthetic photon flux density of 100 μmol photons m^{-2} s^{-1} with cool white light fluorescent lamps (HYG-FPL36W, Heat Engineering Co., Korea) and continuous illumination.

2. Morphological analysis

Strains were indentified according to Komárek and Anagnostidis (1989). Morphological and morphometrical studies were measured based on 30 individuals per strain using a light microscope (Microphoto-FXA, Nikon Co., Japan). Micrographs were taken with a Nikon CCD camera system (Digital Sight DS-5Mc, Nikon Co., Japan). The parameters of isolated strains examined in this study were length and width of vegetative cells, heterocysts, and akinetes and shape of apices under the microscope using 400 times objective lenses.

3. DNA Extraction, PCR amplification, and sequencing

The cells were harvested in the late exponential phase by centrifugation for 10 min at 15,000 xg (4°C) and stored at −20°C. The DNA was extracted using an YGB100 Genomic DNA extraction kit (Real Biotech Co., Taiwan). The 16S rRNA gene was then amplified using the universal primer 27F (5′ AGA GTT TGA TCM TGG CTC AG) and 1522R (5′ AAG GAG GTG WTC CAR CC) (Cho and Giovannoni 2003), while the *cpcBA*-IGS was amplified using CPC1F (5′ GGC KGC YTG YYT RCG YGA CAT GGA 3′) and CPC1R (5′ AAR CGN CCT TGR GWA TCD GC 3′) (Kim *et al.* 2006). The amplification was carried out in 50 μL of a

Table 1. Cyanobacterial strains used for morphological and phylogenetic analyses in this study

Strain[a]	Origin/collection date	GenBank accession no.	
		16S rDNA	*cpcBA*-IGS
Trichormus variabilis			
KCTC AG10008	Yuseong paddy, Daejeon, Korea (1989)	DQ234829	DQ234812
KCTC AG10026	Daechung res., Chungbuk, Korea (1992)	DQ234830	DQ234813
KCTC AG10178	Hyundo-myun, Chongwon, Korea (1999)	DQ234831	DQ234814
KCTC AG10180	Hyundo-myun, Chongwon, Korea (1999)	DQ234832	DQ234815
KCTC AG10269	Bakgok res., Jincheon, Korea (2000)	DQ234833	DQ234816
Anabaena variabilis			
KCTC AG10059	NA[b]	DQ234826	DQ234820
KCTC AG10064	NA	DQ234827	DQ234821
KCTC AG10273	Jangsu-eup, Jeonbuk, Korea (2001)	DQ234828	DQ234822
Anabaena flos-aquae			
UTEX LB2557	Hebgen Lake, Montana, USA (1991)	DQ234825	DQ234819
UTEX LB2391	NA	DQ234824	DQ234818
UTEX LB2383	Burton Lake, Saskatchewan, Canada (1964)	DQ234823	DQ234817

[a]KCTC, Korea Collection for Type Cultures, Daejeon, Korea; UTEX, Culture Collection of Algae at the University of Texas at Austin, Austin, TX, USA.
[b]Not available

1x *n*Taq buffer, 0.2 mM of each dNTP, 10 pmol of each primer, 2.5 U of *n*Taq polymerase (Biostream Ltd., Korea), and 1 μL (ca. 20 ng) of the isolated DNA. The amplification parameters for the 16S rRNA gene were 94˚C for 4 min, followed by 30 cycles of 94˚C for 1 min, 55˚C for 1 min, and 72˚C for 1.5 min, plus an extension at 72˚C for 7 min. For the *cpcBA*-IGS, the amplification parameters were 94˚C for 4 min, followed by 30 cycles of 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 45 sec, plus an extension at 72˚C for 7 min. The presence of a PCR product was confirmed on a 0.8% LF agarose gel (VentechBio Ltd., Korea) run at 100 V with 2 μL of the PCR product and 100 ng of a 1 Kb ladder molecular size marker (Fermentor Ltd., Korea).

The PCR products were purified using a Hiyield Gel/PCR DNA fragment Extraction Kit (Real Biotech, Taiwan), then cloned with a p-Drive T-cloning vector (Qiagene Co., Japan). The plasmids were transferred to *Escherichia coli* DH-5α (Real Biotech Co., Taiwan) and isolated using a Hiyield plasmid mini kit (Real Biotech Co., Taiwan). The plasmids were then sequenced (Cosmogen Ltd., Korea) bidirectionally with SP6, T7, and internal sequencing primers 1100R (5′ GGG TTG CGC TCG TTG-3′) for the 16S rRNA gene sequences, and SP6 and T7 for the *cpcBA*-IGS sequences. The GenBank accession numbers for the 16S rDNA and *cpcBA*-IGS are as follows: DQ234812-DQ234833.

4. Sequence alignment and phylogenetic analyses

CLUSTAL-X was used to produce working alignments of the 16S rRNA gene and *cpcBA*-IGS sequences for the target strains. The final alignments were obtained by manual refinement, and ambiguous bases and hypervariable regions removed using PHYDIT 3.0 (Chun and Goodfellow 1995). As the highly variable intergenic spacer (IGS) region between the *cpcB* and *cpcA* genes did not allow a reliable alignment, these regions were excluded from the analysis. The alignments used in the phylogenetic analyses consisted of 1,302 bp (16S rRNA gene sequences) and 297 bp (*cpcBA*-IGS sequences). Phylogenetic trees were constructed using the neighbor-joining (NJ) and maximum-parsimony (MP) method from the TREECON software package (Van de Peer and De Wachter 1994). The statistical significance of the branches was estimated by a bootstrap analysis of the tree programs, involving the generation of 1,000 trees. The distances for the NJ tree were estimated using the algorithm of Kimura (Kimura 1980).

RESULTS

1. Morphological analysis

The trichomes and cells in the preserved samples exhibited typical morphological features of species of *Trichormus* and *Anabaena*, respectively (Fig. 1). According to traditional morphological criteria, among the examined 11 strains, 5 were benthic and identified as *Trichormus variabilis*, while 6 were planktonic and identified as *Anabaena variabilis* and *A. flos-aquae*. The trichomes of the isolated *Trichormus* strains were entangled and sank to the bottom of the culture flasks. Under the light microscope, the trichomes were observed as unsheathed, generally immobile, straight, and sometimes bent. The trichomes of the isolated *Anabaena* strains were solitary. Under the light microscope, the trichomes of the *A. variabilis* were single and straight formed. But the trichomes of the *A. flos-aquae* were coiled forming a solitary irregular spiral fashion.

The morphological characteristics of the strains are summarized in Table 2. The shapes of the terminal cells were rounded in the case of 8 strains, conical in 2 strains, and tapered in 1 strain. The cells of *T. variabilis* were 4.1 to 5.8 μm long and 3.5 to 5.6 wide, plus the cell length to width ratio varied from 0.85 to 1.19. The trichomes divided transversely and intercellularly, and were composed of cylindrical cells. The cells of *A. variabilis* were 5.5 to 6.0 μm long and 2.5 to 3.8 μm wide, plus the cell length to width ratio varied from 1.45 to 2.40. The trichomes divided transversely and intercellularly, and were composed of cylindrical cells. The apical cells were generally rounded or long-pointed after a trichome breakage and had no calyptra. Meanwhile, the cells of *A. flos-aquae* were 5.8 to 6.2 μm long and 4.1 to 4.2 μm wide, plus the cell length to width ratio varied from 1.38 to 1.48.

The *Trichormus* and *Anabaena* strains differed significantly in the length to width ratio of the vegetative cell and akinetes, where the *A. variabilis* strains showed wider vegetative cell and akinetes in comparison to the *T. variabilis* strains, indicating that the length of the heterocyst was a taxonomic character. Plus, the morphology of the end cells of the trichomes in the *Trichormus* strains was rounded, conical, and tapered, while that in the *Anabaena* strains was rounded.

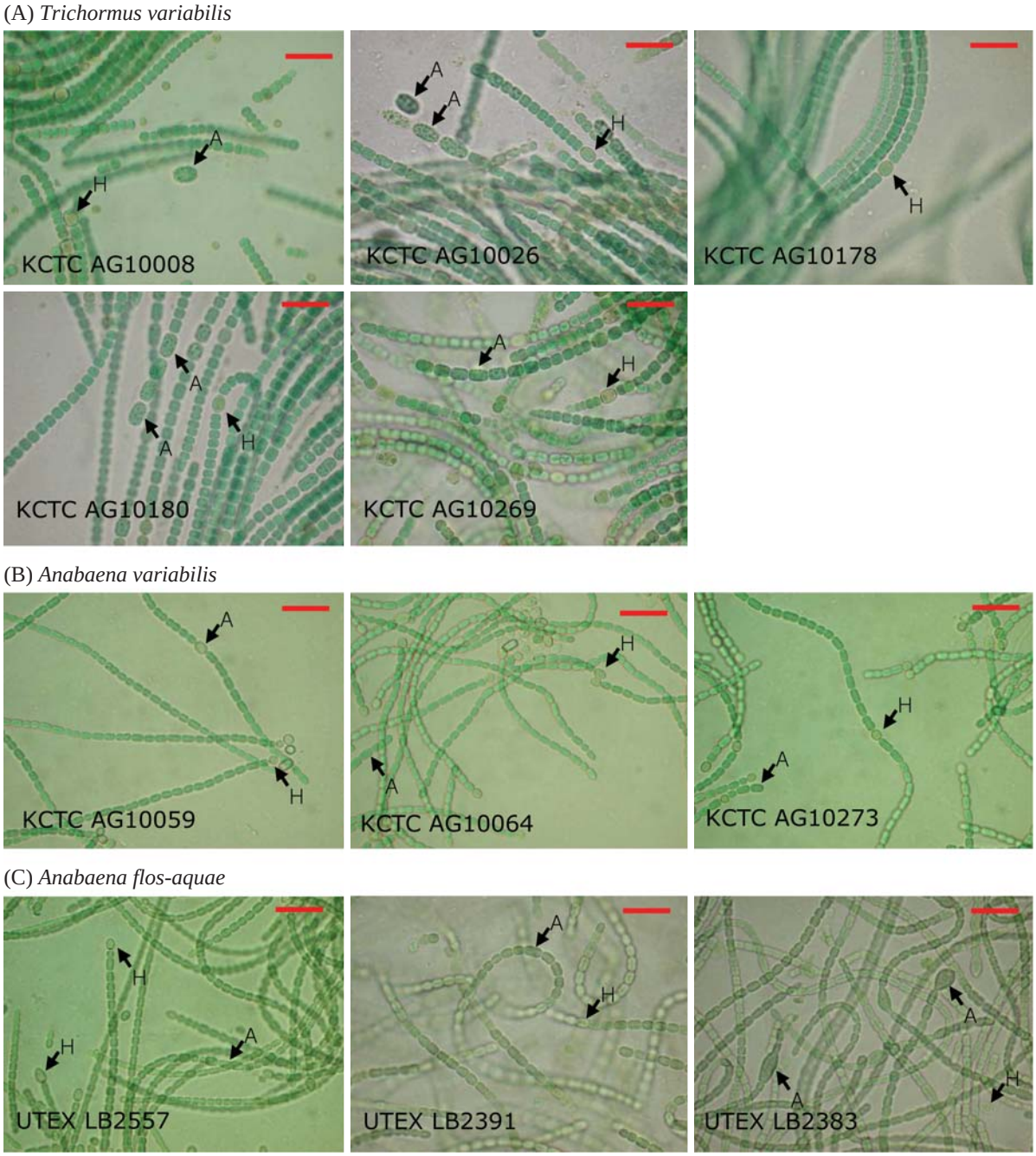

Fig. 1. Light microphotoimages of 5 *Trichormus variabilis* strains (A), 3 *Anabaena variabilis* strains (B), and 3 *Anabaena flos-aquae* strains (C). Magnification=400, Scale Bars=20 μm(A: akinete, H: heterocyst).

2. Sequence of the 16S rRNA and *cpcBA*-IGS

Near-full-length 16S rRNA gene sequences (1,444 bp) were determined for the 3 *A. variabilis* strains and 5 *T. variabilis* strains isolated from Korea and the 3 *A. flos-aquae* strains from UTEX. GenBank accession numbers are given in Table 1. The overall similarity of the 3 *A. variabilis* strains (KCTC AG10059, KCTC AG10064, and KCTC AG10273) was > 99.9%, and among these 3 strains, the similarity with *A. variabilis* ATCC 29413 was 99% (Table 3). Meanwhile, the 5 *T. variabilis* strains (KCTC AG10008, KCTC AG10026, KCTC AG10178, KCTC AG10180, and KCTC AG10269) shared high similarity values of at least 99.9%, and the similarity between 5 strains and *T. variabilis* GREIFSWALD was

Table 2. Selected morphological characteristics of cyanobacterial strains studied

Strain	Shape of terminal cell	Vegetative cell		Heterocysts		Akinetes		
		Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length/ Width
Trichormus variabilis								
KCTC AG10008	Rounded	4.1[a]	3.5	5.5	4.1	8.5	6.9	1.23
KCTC AG10026	Conical	5.0	4.7	8.2	5.1	10.2	7.6	1.34
KCTC AG10178	Conical	5.8	5.3	7.8	5.5	9.4	7.1	1.32
KCTC AG10180	Rounded	4.7	5.6	7.7	6.1	9.7	6.9	1.41
KCTC AG10269	Tapered	5.3	4.4	8.2	6.1	9.8	6.2	1.58
Anabaena variabilis								
KCTC AG10059	Rounded	6.0	2.5	5.5	3.9	–[b]	–	–
KCTC AG10064	Rounded	5.6	3.1	5.1	4.4	8.6	4.1	2.10
KCTC AG10273	Rounded	5.5	3.8	4.7	4.3	7.8	4.3	1.81
Anabaena flos-aquae								
UTEX LB2557	Rounded	5.8	4.2	5.7	4.8	7.1	4.8	1.48
UTEX LB2391	Rounded	6.2	4.2	5.8	4.6	7.8	5.1	1.37
UTEX LB2383	Rounded	6.0	4.1	6.8	5.2	13.7	8.6	1.59

[a]Numbers are means (n=30)
[b]Not found the heterocysts and akinetes

Table 3. Sequence identities of cyanobacterial strains in this study based on nucleotide-nucleotide BLAST (blastn) of the National Center for Biotechnology Information (NCBI)

Strain	GenBank accession no.			
	16S rRNA[a]		*cpcBA*-IGS[b]	
	Best match in NCBI database	Similarity (%)	Best match in NCBI database	Similarity (%)
Trichormus variabilis				
KCTC AG10008	*T. variabilis* GREIFSWALD	99	*A. compacta* 1403/24	94
KCTC AG10026	*T. variabilis* GREIFSWALD	99	*A. compacta* 1403/24	93
KCTC AG10178	*T. variabilis* GREIFSWALD	99	*A. compacta* 1403/24	94
KCTC AG10180	*T. variabilis* GREIFSWALD	99	*A. compacta* 1403/24	94
KCTC AG10269	*T. variabilis* GREIFSWALD	99	*A. compacta* 1403/24	93
Anabaena variabilis				
KCTC AG10059	*A. variabilis* ATCC 29413	99	*Nostoc* sp. PCC7120	99
KCTC AG10064	*A. variabilis* ATCC 29413	99	*Nostoc* sp. PCC7120	100
KCTC AG10273	*A. variabilis* ATCC 29413	99	*Nostoc* sp. PCC7120	100
Anabaena flos-aquae				
UTEX LB2557	*Nostoc entophytum* IAM M-267	99	*Aphanizomenon flos-aquae* CCAP 1401/1	97
UTEX LB2391	*Nostoc entophytum* IAM M-267	99	*Aphanizomenon flos-aquae* CCAP 1401/1	97
UTEX LB2383	*Nostoc entophytum* IAM M-267	99	*Aphanizomenon flos-aquae* CCAP 1401/1	97

[a]Analysis of 1444 bases of 16S rRNA gene sequences.
[b]Analysis of 384 bases of *cpcBA*-IGS sequences.

99% (Table 3). The 3 *A. flos-aquae* strains (UTEX LB2557, LB2391, and LB2383) shared similarity values of >99.7%, and the similarity with *Nostoc entophytum* IAM M-267 and *N. linckia* was 99% and 98%, respectively (Table 3).

The less conserved IGS between *cpcB* and *cpcA*, and corresponding flanking regions (henceforth referred to as *cpcBA* -IGS) were also sequenced for the strains examined in this study. The region covered the end of the *cpcB* gene (259 bp), an intergenic spacer (IGS, 90~104 bp), and the end of the *cpcA* gene (33 bp). The overall similarity of the 3 *A. variabilis* strains (KCTC AG10059, KCTC AG10064, and KCTC AG 10273) was up to >99.5%, and among these 3 strains, the similarity with *Nostoc* sp. PCC 7120 was 99% (Table 3). The 5 *T. variabilis* strains (KCTC AG10008, KCTC AG10026, KCTC AG10178, KCTC AG10180, and KCTC AG10269) shared high similarity values up to at least 99.5% and the similarity between 5 strains and *A. compacta* 1403/24 was 93~94% (Table 3). The 3 *A. flos-aquae* strains (UTEX LB 2557, LB2391, and LB2383) shared similarity values of > 99.0%, and the similarity with *Aphanizomenon flos-aquae*

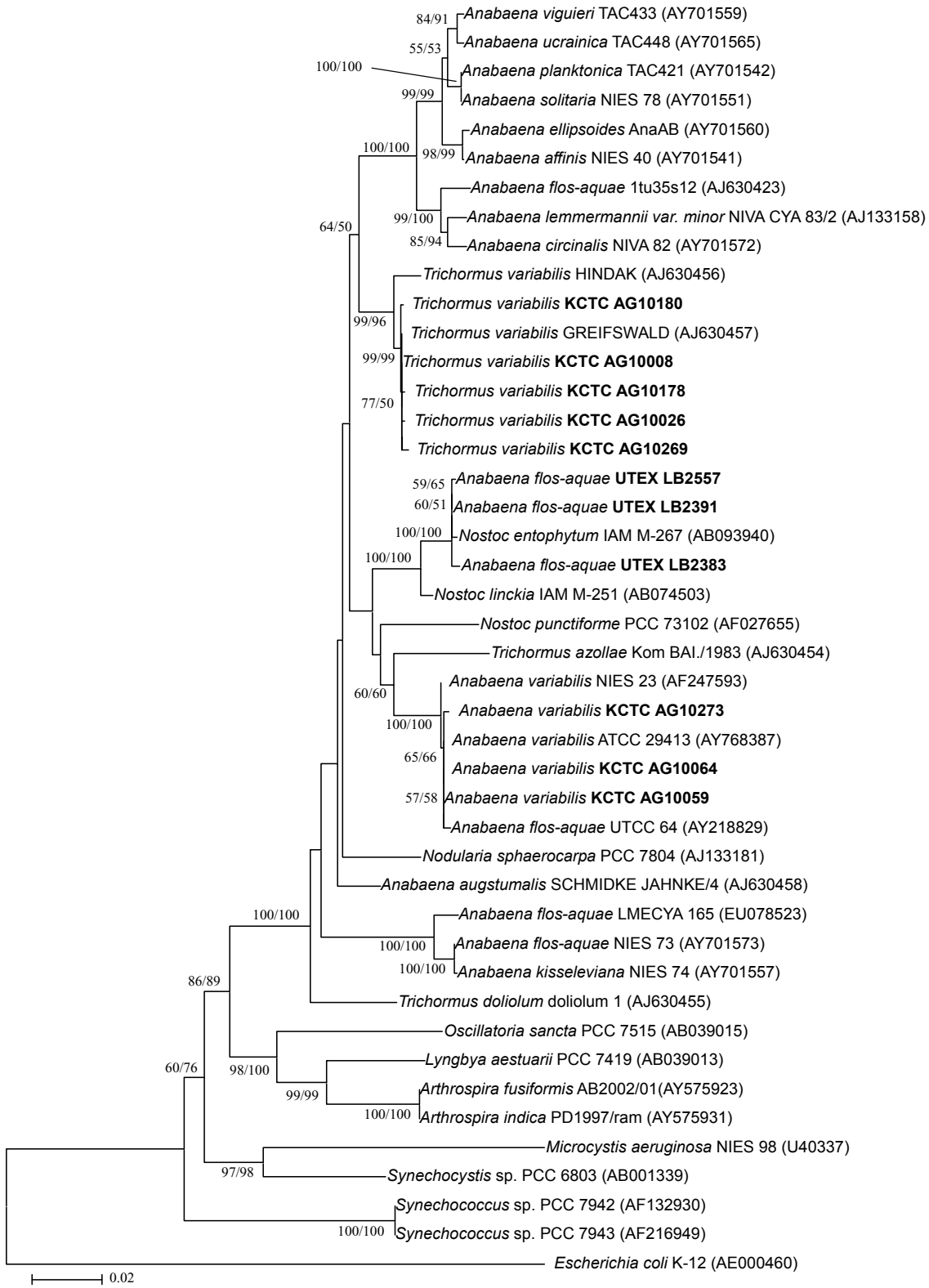

Fig. 2. Phylogenetic tree generated via the neighbor-joining method showing the phylogenetic relationships of the *Anabaena* and *Trichormus* strains based on the analysis of ca. 1300 bases of aligned 16S rRNA gene sequences. Sequences obtained during the present study are indicated in bold. Numbers near nodes indicate bootstrap values over or equal to 50% for NJ and MP analyses. Sequences from GenBank are indicated by accession numbers. Outgroup was *Escherichia coli* K-12 (AE000460). The scale bar represents 0.02 changes per nucleotide.

CCAP 1401/1 was 97% (Table 3).

3. Genetic relationships of the studied strains

Since the neighbor-joining (NJ) and maximum-parsimony (MP) trees were similar, only the NJ tree is presented to show the phylogenetic relationship of the cyanobacterial strains for each gene (Figs. 2 and 3). The GenBank accession numbers are shown Table 1. A phylogenetic 16S rRNA gene trees were reconstructed from an alignment of 43 sequences, in-

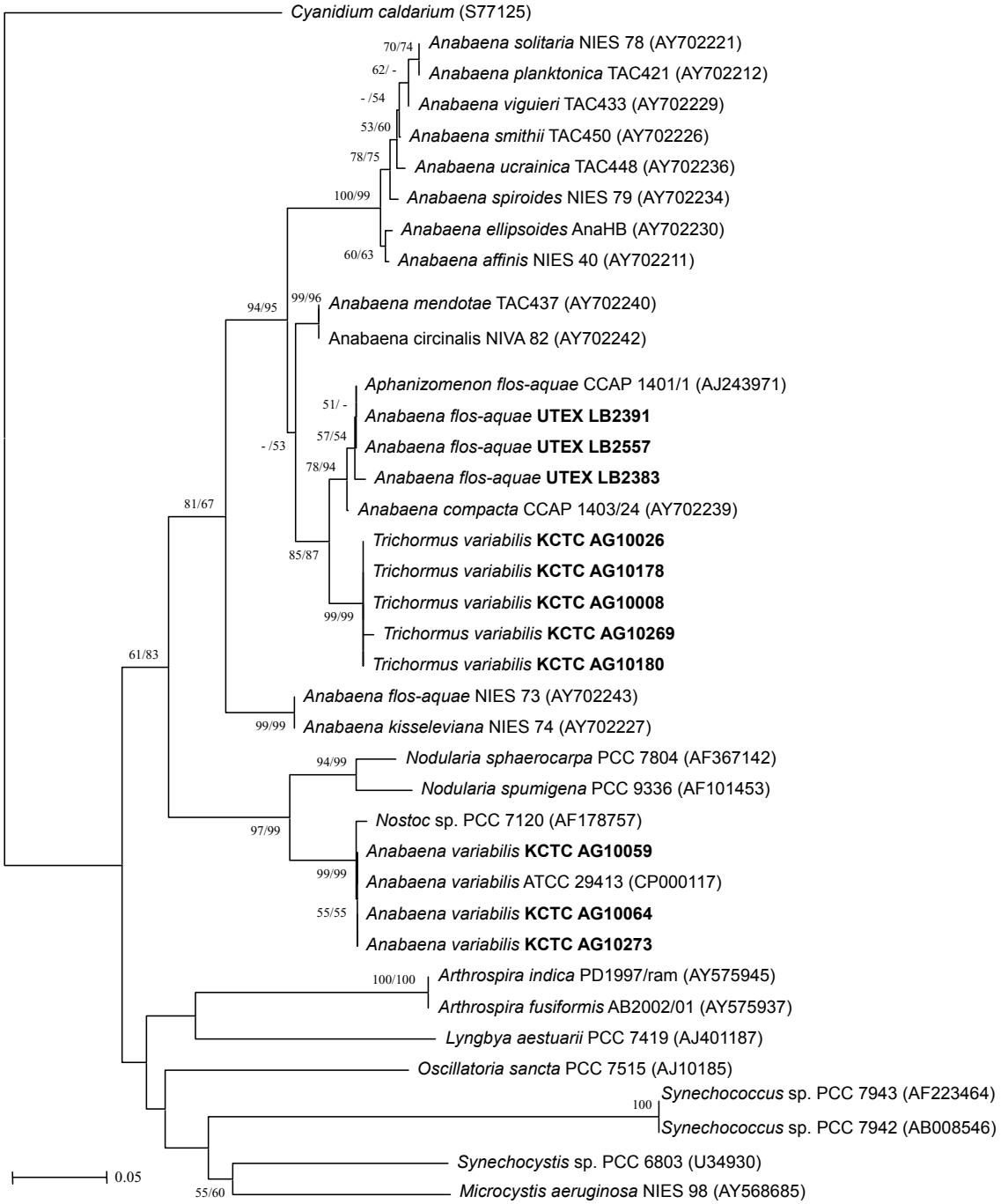

Fig. 3. Phylogenetic tree based on *cpcBA*-IGS sequences (297 bp), showing clustering of examined *Anabaena* and *Trichormus* strains. Sequences obtained during the present study are indicated in bold. Numbers near nodes indicate bootstrap values over or equal to 50% for NJ and MP analyses. Sequences from GenBank are indicated by accession numbers. Outgroup was chloroplast *Cyanidium caldarium* (S77125). The scale bar represents 0.05 changes per nucleotide.

cluding 6 *Anabaena* and 5 *Trichormus* isolates, representing the major lineage of cyanobacteria with *Escherichia coli* as the outgroup (Fig. 2). The cyanobacteria studied here were divided into three main groups, where the first consisted of filamentous cyanobacteria, *Anabaena* and *Nodularia*, capable of forming heterocysts and akinetes, the second contained non-heterocystous, filamentous *Oscillatoria, Lyngbya*, and *Arthrospira* strains, and the third contained unicellular *Microcystis, Synechocystis*, and *Synechococcus* strains. The 5 *T. variabilis* strains (KCTC AG10008, KCTC AG10026, KCTC AG10178, KCTC AG10180, and KCTC AG10269) clustered with *T. variabilis* GREIFSWALD. These six sequences shared greater than 99.8% sequence similarity and were supported by 99% bootstrap resamplings in both NJ and MP analysis. *Trichormus* strains, which were separated morphologically from *Anabaena* strains, were divided into 3 well-separated clusters and were not monophyletic. The 3 *A. variabilis* strains (KCTC AG10059, KCTC AG10064, and KCTC AG10273), sequenced in this study, clustered with *A. variabilis* ATCC 29413, *A. variabilis* NIES 23, and *A. flos-aquae* UTCC 64 and supported by 100% bootstrap resamplings in both analysis. The 3 *A. flos-aquae* strains (UTEX LB2557, LB2391, and LB2383), which classified with *A. flos-aquae* by morphological analysis, clustered with *N. entophytum* IAM M-267 with high bootstrap support and might actually belong to the genus *Nostoc* rather than to *Trichormus* or *Anabaena*. *A. flos-aquae* strains (1tu35s12, UTCC 64, and NIES 73) were dispersed in *Anabaena* subclusters.

The 37 sequences of *cpcBA*-IGS, including 6 *Anabaena* and 5 *Trichormus* isolates, were used in the both (NJ and MP) phylogenetic analysis (Fig. 3). To compare the congruence between the 16S rRNA gene and *cpcBA*-IGS sequences based on the phylogenies of *Nostocaceae*, 17 strains with both 16S rRNA and *cpcBA*-IGS sequences were selected from the NCBI database. Unfortunately, the *cpcBA*-IGS sequence of *T. variabilis* was not found in the NCBI database. A phylogenetic tree was constructed based on an unambiguous alignment of 297 bp (Fig. 3). Due to the highly variable nature of the IGS sequence and the resulting alignment difficulties, these 297 bp originated almost exclusively from within the coding regions of *cpcB* and *cpcA* flanking the IGS. The division of the more distantly related groups was in agreement with the previously determined 16S rRNA gene-based phylogeny. The *Synechococcus* strains were the deepest branching group in the 16S rRNA gene tree. Similarly, the *Arthrospira* strains associated with *Lyngbya aestuarii* PCC 7419 in both the *cpcBA*-IGS and 16S rRNA gene-based tree. The two sequences were supported by 100% bootstrap values in both NJ and MP analysis. Although *Oscillatoria sancta* PCC 7515 was associated with *L. aestuarii* PCC 7419 in the 16S rRNA gene tree, *O. sancta* PCC 7515 was interestingly associated with *M. aeruginosa* NIES 98 according to the *cpcBA*-IGS trees. The *A. flos-aquae* strains (UTEX LB2557, LB2391, and LB2383) were associated with the *A. variabilis* strains in the 16S rRNA gene tree. However, according to the *cpcBA*-IGS sequence tree, the *A. flos-aquae* strains were associated with the *T. variabilis* strains.

DISCUSSION

Analyses of the 16S rRNA gene and *cpcBA*-IGS sequences of the *Anabaena* and *Trichormus* strains showed a high similarity between the *A. variabilis* and *T. variabilis* strains and did not confirm the taxonomic validity of these two genera. Phylogenetic analyses based on 16S rDNA sequences have already been widely used. However, the 16S rRNA gene is often ineffective for resolving bacterial strains, due to its slow rate of evolution. In addition to analyzing the 16S rDNA sequences (Neilan *et al.* 1997; Otsuka *et al.* 1998; Honda *et al.* 1999), the genes encoding the major light-harvesting accessory pigment proteins, particularly the phycocyanin operon (*cpc*), including the intergenic spacer (IGS) between *cpcB* and *cpcA* and the corresponding flanking regions (*cpcBA*-IGS), have been targeted for phylogenetic studies of cyanobacteria (Barker *et al.* 1999). Phycocyanins are specific accessory pigments in *Cyanophyta, Rhodophyta, Glaucophyta*, and *Cryptophyta*. In a previous report (Teneva *et al.* 2005), the length of the IGS separating *cpcB* and *cpcA* was found to exhibit a strong relationship with the phylogenetic groups. However, there were generally lower in the *cpcBA*-IGS tree than in the 16S rRNA gene tree, due to a smaller number of *cpcBA*-IGS data bases.

Moreover, to define and delimit the genus *Anabaena* and *Trichormus*, this study also included other cyanobacteria, and a further goal was to identify specific molecular markers to fingerprint different strains of *Anabaena* and *Trichormus* and enable the development of tools for strain-specific identification. The phylogenetic analysis of the 16S rDNA sequences further reinforced the polyphyletic nature of this taxon.

The difference between the molecular and morphological results may have reflected the existence of species-variant populations or ecotypes adapted to different environmental conditions.

Recently, Baker *et al.* (2001, 2002) employed a PCR amplification method for the analysis of *cpcBA* from environmental samples using a primer set previously designed by Neilan *et al.* (1997) and found a limited cyanobacterial diversity. Although the primer set was originally designed with 6 *cpcBA* sequences to study the genetic diversity of several pure culture strains, *cpcBA* sequence information from various other cyanobacteria has also been deposited in public databases for potential enhanced primer design. Furthermore, since the IGS length of each group of cyanobacteria is different, the *cpcBA* gene amplification protocol could be directly coupled to length polymorphism analysis techniques, such as T-RFLP, to facilitate rapid monitoring of the cyanobacterial community.

In conclusion, *Trichormus* and *Anabaena* strains, which were isolated from Korean waters, were separated by morphological and phylogenetic analyses. However, the difference remained between morphology and phylogeny of *Trichormus* and *Anabaena* strains. The phylogenetic relationship of the studied strains did not follow the current taxonomic classification and therefore a revision of the taxonomy of these anabaenoid strains is needed.

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