Environ Monit Assess (2011) 175:139–150
DOI 10.1007/s10661-010-1500-x

Molecular evaluation on the distribution, diversity, and toxicity of *Microcystis* (Cyanobacteria) species from Lake Ulungur—a mesotrophic brackish desert lake in Xinjiang, China

Shen Lin · Jianzhong Shen · Yang Liu · Xingqiang Wu · Qigen Liu · Renhui Li

Received: 18 October 2009 / Accepted: 6 May 2010 / Published online: 2 June 2010

Abstract The distribution and diversity of the waterbloom-forming cyanobacteria, mainly including *Microcystis* species, were investigated using molecular approaches in Lake Ulungur, an increasingly eutrophic and brackish lake located in Xinjiang, China. Real-time PCR analyses showed the abundance of *Microcystis* 16S rDNA gene copies in the Ulungur Lake is low, at 7.2×10^4 copies L^{-1} averagely and 4.5×10^5 copies L^{-1} at maximum. Two *Microcystis* species, *M. aeruginosa* (Kützing) Lemmermann and *Microcystis wesenbergii* (Komárek) Komárek were, for the first time, reported in this lake. The *mcyA* gene-specific PCR determination on the isolates of *Microcystis* showed that the *M. aeruginosa* strains are all *mcyA*-containing genotypes, while *M. wesenbergii* are non-*mcyA*-containing ones. The microcystin contents of the toxic *M. aeruginosa* strains were shown to be lower than those of the *Microcystis* strains isolated from other eutrophic lakes in China. Phylogenetic analyses based on 16S rRNA and *rpoC1 genes* showed that the *Microcystis* strains isolated from the Ulungur Lake were not genetically divergent from those isolated in the other freshwaters. Such an investigation would contribute to the knowledge on the bloom-forming cyanobacteria of the increasingly eutrophic and saline lakes in the desert area.

Keywords Lake Ulungur · *Microcystis* · Real-time PCR · HPLC · Microcystin · *Mcy* gene · Brackish lake

S. Lin · Y. Liu · X. Wu · R. Li (✉)
Key Laboratory of Aquatic Biodiversity and Conservation Biology, Institute of Hydrobiology, Chinese Academy of Sciences,
Wuhan, 430072, China
e-mail: reli@ihb.ac.cn

J. Shen
Fishery College, Huazhong Agricultural University,
Wuhan, 430070, China

Q. Liu
College of Fisheries and Life Sciences,
Shanghai Ocean University,
Shanghai 200090, China

S. Lin · Y. Liu · X. Wu
Graduate University of Chinese Academy of Sciences,
Beijing, 100039, China

Introduction

Cyanotoxins, mostly represented as hepatotoxic microcystins (MC) which are produced by a diverse of cyanobacterial groups, have posed a great environmental problem (Carmichael 1997). In China, it has recently been shown that many freshwater shallow lakes in subtropical areas have encountered problems caused by eutrophication and cyanobacterial blooms, with the worst

event of a heavy cyanobacterial bloom in Lake Taihu in 2007 (Guo 2007; Li et al. 2003; Geng et al. 2005). Therefore, water blooms, bloom-forming cyanobacteria and microcystins, from the eutrophic or hypereutrophic waters in Eastern and Southern China, have been studied extensively (Xie et al. 2003, 2005). Water eutrophication or hypereutrophication is usually regarded as evolving from oligotrophic and mesotrophic states, thus the research on the course of a lake from oligotrophic to eutrophic situations would help us to well understand the progress of the lake eutrophication and the development of water blooms. However, information about waterbloom-forming cyanobacteria occurring in increasingly eutrophic lakes is very scarce and detailed investigations on the distribution, molecular diversity, and toxins of cyanobacteria or *Microcystis* species at the early stage of eutrophic lakes were rarely reported. Therefore, it would be valuable to understand which conditions in nature favor the occurrence of microcystin-producing or non-microcystin-producing cyanobacterial strains.

The present study reports a survey on a large and deep lake—Lake Ulungur located in Xingjiang, northwest of China. The lake is characterized to be brackish, and had been in an oligotrophic state for a long time. Since 2005, it was reported that the lake was evaluated to be becoming increasingly eutrophic due to human activities (Li et al. 2005) and waterbloom-forming cyanobacteria such as species of *Aphanizomenon* and *Anabaena* have been observed in summer, and the abundance of these cyanobacteria reached the maximum at 17.7% of total phytoplankton, indicating the trend to become eutrophicated in this lake (Ye et al. 2004; Li et al. 2008). However, there has been no information about occurrence of *Microcystis* species in Lake Ulungur so far. During the survey performed in July 2007, large colonies of *Microcystis* spp., together with *Planktothrix agardhii* (Komárek and Anagnostidis) were found, implying the new trend for development of phytoplankton community in the lake. Thus, Lake Ulungur, with such newly emerging features, may become an ideal model for understanding the evolving course of waterbloom-forming cyanobacterial development in increasingly eutrophicated and brackish lakes. The main aims of the present study are: (1) to investigate general distributions of cyanobacteria, total *Microcystis* and toxic *Microcystis* around Lake Ulungur; (2) to detect the molecular diversity of *Microcystis* strains isolated from this lake; (3) to analyze intracellular microcystin content of the strains of *Microcystis* isolated from Lake Ulungur. We performed two independent real-time PCR assays, using primers for 16S rRNA gene and the *mcyB* (Kurmayer and Kutzenberger 2003) respectively, to specifically examine the distributions of total *Microcystis* and microcystin-producing *Microcystis* in the lake. Molecular diversity of *Microcystis* strains isolated from this lake was determined based on DNA sequences of 16S rRNA gene and *rpoC1* gene. Additionally, microcystin composition and intracellular content were measured by the HPLC analysis. Such a research on Lake Ulungur will also help us to form the basis of estimating the health risks associated with the occurrence of waterbloom-forming cyanobacteria.

Materials and methods

Source of samples Lake Ulungur, as one of the top ten largest freshwater lakes and an important fishery base in China, is located in the north of Junggar Basin, Xinjiang province (41°53′ N; 82°5′ E). The lake area, including Lake Buluntuo and Lake Jelekol as shown in Fig. 1, is about 917 km^2 in water surface size and has an average depth of 8 m. Lake Buluntuo is the downstream lake connecting Lake Jelekol and Irtysh River. The average salinity was described as 0.9%, and annually 185 million tons of water has been imported to complement for the loss by strong evaporation. The hydrochemistry environment has been investigated and recorded by Dong et al. (2008).

Samplings from the Lake Ulungur Samplings were performed at Lake Ulungur from July, 23 to July, 31 2007. As shown in Fig. 1, samplings covered 25 sites including 22 for the open Lake Buluntuo, the bays and the outlet into Lake Jelekol, and three in Lake Jelekol. The water

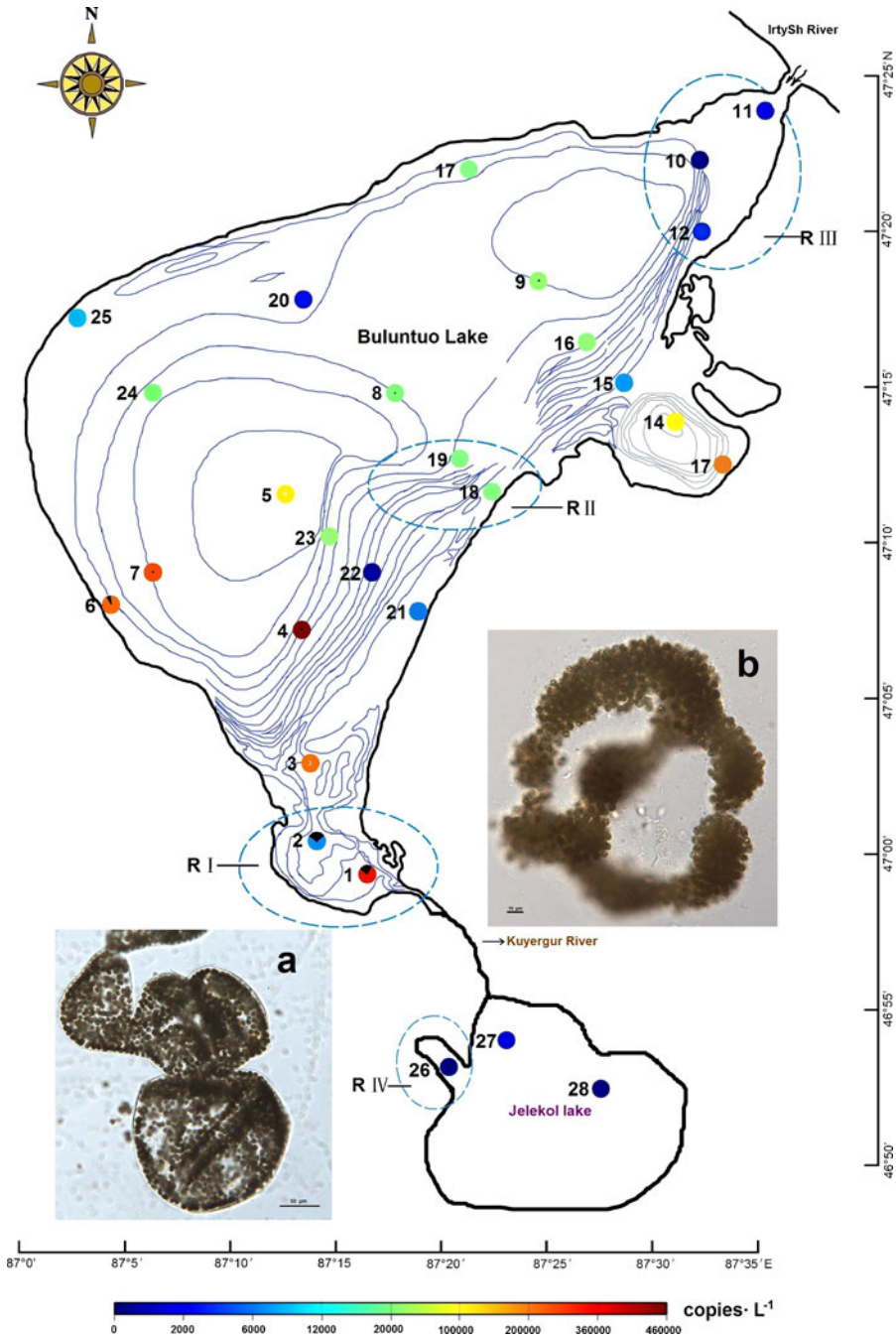

Fig. 1 Map of sampling sites in the study area. Abundance of *Microcystis*-specific *16S rDNA* gene copies and toxic *Microcystis* were quantified and shown at each site. The share marked in black represents the percentage of total toxic *Microcystis* accounting for the total *Microcystis* cells under the condition that all the *Microcystis* distribution in Lake Ulungur has the same copy number of 16S rDNA and mcyB gene. **a** Micrograph of *M. wesenbergii*, *1 bar* = 50 μm; **b** Micrograph of *M. aeruginosa 1 bar* = 10 μm

samples at 0.5 m depth below the water surface at each site were collected and partial water samples were preserved with a Lugol's solution for phytoplanton cell counting and kept dark in a refrigerator before microscopic examination; waters for DNA extraction were filtered through 0.22-μm polycarbonate membrane filters which were frozen and stored at −20°C before molecular analyses.

Strain isolation and culture conditions *Microcystis* colonies examined in Lake Ulungur were picked and purified as unialgal strains using the method of Pasteur micropipette, and the obtained

strains were then cultured in liquid CT medium (Ichimura 1979), under constant white light intensity as 25 μmol photons $m^{-2}s^{-1}$ (12: 12 L: cycle), at a temperature of 25±1°C.

DNA extraction DNA was extracted from the *Microcystis* strains isolated from the lake following the description by Li et al. (2008). Briefly, the harvested cell pellets were added to the lysis buffer (100 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 9.0) and lysozyme (10 mg ml^{-1}) and the samples were incubated in 37°C for 30 min. After 30 min, proteinase K (20 mg ml^{-1}) and 10% SDS were introduced to the samples to incubate in 55°C for 1–2 h. After incubation, the solutions were extracted using phenol–chloroform–isoamylol (25:24:1) and centrifuged at 12,000 rpm for 5 min. The DNA-containing supernatants were added in 10 M acetate sodium and equation volume ethanol to precipitation.

For DNA extraction from the lake samplings, the filtrated membranes were fragmentized by a sterilized scissors and extracted in 2 mL of the lysis buffer. The tubes containing extract solutions were frozen at −20°C for 12 h, and then abruptly dipped in 85°C hot water. The freezing and heating were repeated twice and 20 μL lysozyme with the final concentration as 10 mg ml^{-1} was added and the samples were incubated in 37°C for 30 min. Then 10 μL 20 mM proteinase K were added and the extraction solution mixture was incubated at 60°C for 2 h. DNAs were extracted with 2.5 mL phenol:chloroform:isoamyl alcohol (25:24:1), and the organic and aqueous phases were mixed 10 min thoroughly in each extraction and centrifuged at 12,000 rpm for 10 min. The upper aqueous phase was collected and added with 3 mL ice-cold ethanol and 250 μL ammonium acetate (10 M). The mixture was kept at −20°C for 2 h, subsequently the DNA was precipitated by micro-centrifugation at 12,000 rpm for 10 min. The supernatant was removed and the precipitated DNA was washed with cold 70% ethanol. The pellet of DNA was dried and resuspended in 50 μL deionized distilled water (ddH_2O) and stored at −20°C.

PCR and sequencing The genomic DNAs extracted from the *Microcystis* strains were used to detect the *mcyA* gene and amplify 16S rRNA gene and *rpoC1* regions by using series of oligonucleotide primers (Table 1). The reactions were carried out in a volume of 50 μL solution containing 5–10 ng total genomic DNA, 1 U Taq DNA polymerase (Takara, Japan), 1× PCR reaction buffer with 1.5 mM $MgCl_2$, 10 pmol of each primer, and 200 μM concentrations of each deoxyribonucleoside triphosphate (dNTPs). PCR was performed in a PTC-100 thermal cycler (MJ Research, USA), and the amplification program was set at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s; 55°C for 50 s, and 72°C for 2 min, followed by a final extension at 72°C for 5 min. The amplified products were cloned into the pMD18-T vector (Takara, China). The cloning procedures were performed as described (Sambrook and Russell 2001). Sequencing was carried by ABI 3730 Automated Sequencer (Perkin-Elmer Biosystems, USA). The sequences have been uploaded into a GenBank. The accession numbers are FJ595699–FJ595712 for *rpoC1* and FJ595684–FJ595698 for 16S rRNA gene.

Real-time PCR Real-time PCR reactions were run on an ABI PRISM 7000 Real-time PCR machine (Applied Biosystems). Each reaction was performed in a 25-μL mixture with 0.5 mM of both primers, 10 μL enzyme–nucleotide–dye mix (SYBR green mixture, TOYOBO, Japan) and template DNA. The primers used for amplification of cyanobacteria, total *Microcystis* and toxic *Microcystis* were listed in Table 1. The amplification program was set at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s; 60°C/60°C/53°C for 30 s (cyanobacteria/toxic *Microcystis*/*Microcystis*, respectively), and then 72°C for 30 s. Each measurement was performed in triplicate. Data was analyzed using ABI Prism 7000 SDS Software Version 1.1.

In this study, standard curve, relating gene copies concentrations to cycle threshold (C_T), were established as described by Koskenniemi et al. (2007). Exponentially growing *M. aeruginosa* PCC7806 was chosen as the standard strain for cyanobacteria and *Microcystis* quantification and tenfold serial dilutions of the samples were analyzed by real-time PCR. The standard curve for the copy number of cyanobacterial 16S rDNA

Table 1 Primers used in this study

Gene region and primer	Sequence	Product length (bp)	Reference
16S rRNA gene		1,375	
F1	TTGATCCTGGCTCAGGATGA		Otsuka et al. (1999)
R4	TACGGCTACCTTGTTACGAC		Neilan et al. (1997)
L646F	TTGGGAAGAACATCGGTGGC		This study
L786R	GGTCGATACAAGCCACGCCT		This study
rpoC1		576	Rantala et al. (2004)
rpoC1-RF	TGGGGHGAAAGNACAYTNCCTAA		
rpoC1-RR	GCAAANCGTCCNCCATCYAAYTGBA		
mcyA		297	Hisbergues et al. (2003)
mcyA-Cd1F	AAAATTAAAAGCCGTATCAAA		
mcyA-Cd1R	AAAAGTGTTTTATTAGCGGCTCAT		
Primers for qPCR			
Cyano 16S F	CGGACGGGTGAGTAACGCGTG	258	This study
Cyano 16S R	CCCATTGCGGAAAATTCCCC		
Micr 16S F	GCCGCRAGGTGAAAMCTAA	212	Neilan et al. (1997)
Micr 16S R	AATCCAAAGACCTTCCTCCC		
*mcy*B F	CCTACCGAGCGCTTGGG	78	Kurmayer and Kutzenberger (2003)
*mcy*B R	GAAAATCCCCTAAAGATTCCTGAGT		

Pairs of primers for real-time PCR including Cyano16S, Micr16S, and *mcyB* were used for the detection of cyanobacteria, *Microcystis*, and *mcyB*-containing *Microcystis*, respectively

5′ to 3′ orientation

is $y = 7 \times 10^{11} \times e^{(-0.8933 \times Ct)} (r^2 = 0.997)$, and the detection limit is 6 copies per reaction. The standard curve for the copy number of *Microcystis* 16S rDNA is $y = 6 \times 10^{11} \times e^{(-0.837 \times Ct)}$ ($r^2 = 0.996$), and the detection limit corresponded roughly to 15 copies per reaction. The standard curve for the copy number of mcyB gene corresponding to toxic *Microcystis* is $y = 6 \times 10^{12} \times e^{(-0.9273 \times Ct)}$ ($r^2 = 0.968$), and the detection limit is 3 copies per reaction.

Intracellular microcystin determination Intracellular microcystins of the *M. aeruginosa* strains were extracted with 75% methanol at 4°C in darkness (Fastner et al. 1998). Microcystin was determined using the HPLC method. The chromatographic system consisted of a Waters Alliance HPLC equipped with a 2695 separation module with an online degasser, Waters 2996 photo diode array detector and Waters Empower chromatography software (Waters). The concentrations of the peptide hepatotoxin microcystin-LR (MC-LR) and microcystin-RR (MC-RR) were determined by comparing the peak area with that of the standard and their separation were accomplished under a reversed-phase isocratic condition with a Hypersil base-deactived silica (BDS) C_{18} column (5 μm, 250 × 4.6 mm; Thermo Hypersil, Runcorn, Cheshire, UK) in a run with 68% solution A (100% methanol *v*/*v*) and 32% solution B (0.05% aqueous trifluoroacetic acid *v*/*v*) over 20 min. The flow rate was set at 1 ml min^{-1} and the column temperature was maintained at 30°C.

Phylogenetic analysis DNA sequences, both examined in this study and obtained from GenBank, were multiple-aligned using CLUSTAL X, version 2.0 (Larkin and Blackshields 2007). Genetic distances were calculated using the two-parameter method of Kimura (K2P). DNA sequences were assessed for the best-fit model to explain sequence evolution using Modeltest (Posada and Crandall 1998). The phylogenetic trees were constructed from the multiple-aligned data using the neighbor-joining (NJ) algorithmic Kimura's two-parameter as implemented within MEGA4 program package (Tamura and Dudley 2007). The maximum likelihood (ML) algorithms were constructed in PHYML version 3.5c (Guindon and Gascuel 2003). One hundred

bootstrap replicates were performed and only bootstrap values above 60% are indicated at the nodes of the trees. Clade support was estimated utilizing general time-reversible (HKY) model, the parameters of Ts/tv ratio and p-invar were set corresponding to the results output from modeltest (Posada and Crandall 1998). The program MrBayes was used to execute the Bayes algorithms. Parameters in MrBayes were set to five million generations and 50,000 trees, sampled every 100th generation, using the HKY model of DNA substitution, Nst = 2, rates = gamma, Burnin = 10,000 and the default random tree option was set to begin the analysis.

Results

The distribution of *Microcystis* in Lake Ulungur detected by real-time PCR

The general distribution of cyanobacteria, *Microcystis*, and potential toxic *Microcystis* in Lake Ulungur was investigated, with the emphasis on

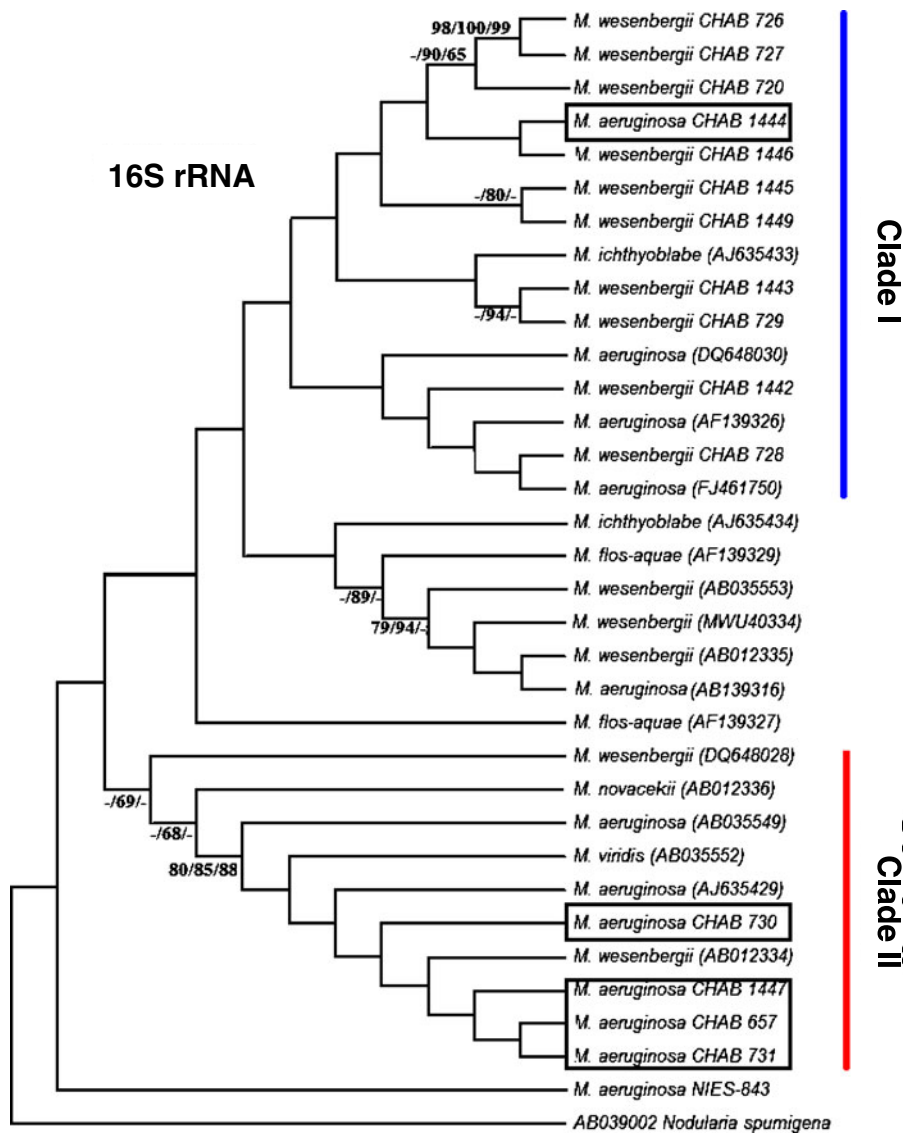

Fig. 2 Phylogenetic tree based on 16S rRNA gene region sequences of the fifteen *Microcystis* strains in this study. Bootstrap values greater than 60% with ML/Bayers/ NJ methods are indicated on the tree. *Nodularia spumigena* was used as the outgroup. The strains in box represents the toxic strains

the four regions of the lake including R I (Stations 1 and 2), R II (Stations 18 and 19), R III (Stations 10, 11, and 12), and R IV at Station 26 in Jelekol Lake (Fig. 1), and these four regions are closely related to human activities. Real-time PCR results showed that the average abundance of cyanobacterial 16S rDNA copies detected in Lake Buluntuo and Lake Jelekol were 1.56×10^7 copies L^{-1} and 4.6×10^7 copies L^{-1} respectively. The similar results were also obtained by the Chl *a* contents analyzed by Dong et al. (2008), indicating that Chl a content in Lake Jelekol was four to 50 times higher than that in Lake Buluntuo. The abundance of *Microcystis* 16S rDNA copies in Lake Ulungur was averagely 7.4×10^4 copies L^{-1}, with 4.6×10^5 copies L^{-1} at maximum. However, the *Microcystis* 16S rDNA copies in stations 10, 11, and 12 in the R III region were much lower than those of other stations in Lake Buluntuo. Analyses on microcystin synthesis gene fragment *mcyB* indicated that the *Microcystis* cells in most stations were not *mcyB*-containing genotypes, revealing that the majority of *Microcystis* in the lake were non-microcystin producers. The *mcyB*-containing *Microcystis* cells were detected at Region R I and stations around southwest part of Lake Ulungur. The abundance of *mcyB* gene copies in the two stations in the R I region were 1.40×10^4 copies L^{-1} and 2.12×10^4 copies L^{-1}, respectively.

The molecular diversity of *Microcystis* strains in Lake Ulungur

A total of fifteen strains of *Microcystis* were obtained from Lake Ulungur in this study, and they were examined using morphological and molecular approaches. According to the description by Komárek and Anagnostidis (1999) and Yu and Li (2007), these strains were morphologically assigned to two species as *Microcystis wesenbergii* and *M. aeruginosa.* Potential for microcystin

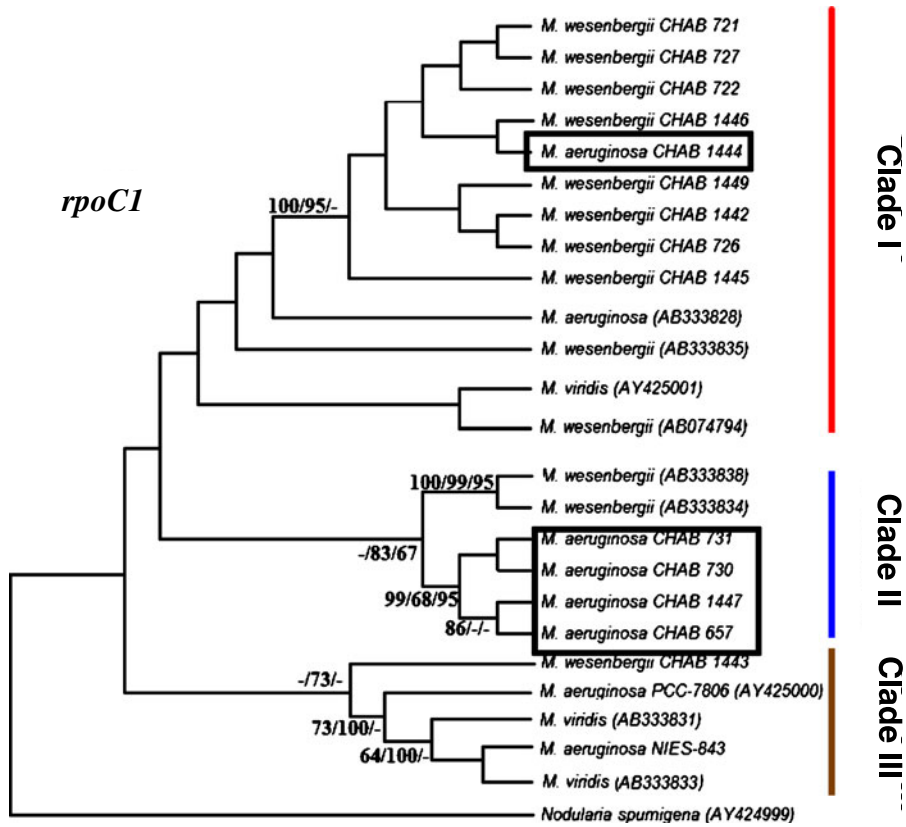

Fig. 3 Phylogenetic tree based on *rpoC1* region sequences of fifteen *Microcystis* strains in this study. Bootstrap values greater than 60% with ML/Bayers/NJ methods are indicated on the tree. *Nodularia* sp. was used as the outgroup. The strains in box represents the toxic strains

production of these fifteen *Microcystis* strains was examined by the molecular analysis for detecting the presence of *mcyA*, and five strains of *Microcystis aeruginosa* coded as CHAB730, CHAB731, CHAB1444, CHAB1447, and CHAB657 showed positive results.

16S rRNA gene regions of these fifteen strains were amplified and sequenced to evaluate their molecular diversity. The similarities of 16S rRNA gene sequences within the *Microcystis* strains were 98.9∼99.9%. The phylogenetic tree based on partial 16S rRNA gene sequences revealed that two clades of the *Microcystis* strains isolated from Lake Ulungur could be identified with a high support value. As shown in Fig. 2, the five strains with *mcyA* genotypes were clustered into two lineages with four strains (CHAB730, CHAB731, CHAB1447, and CHAB657) into one and CHAB1444 into the other together with the *M. wesenbergii* strains isolated from this lake.

As well, *rpoC1* gene region, encoding the γ-subunit of RNA polymerase and existing in cyanobacteria genome as a single copy (Palenik and Swift 1996), was sequenced for the fifteen strains (Fig. 3). The overall similarities of *rpoC1* sequences within the *Microcystis* strains were found to be 95.7∼100%. The phylogenetic relationship among these fifteen *Microcystis* strains based on *rpoC1* sequences showed three distant clades, basically similar with that of 16S rRNA gene, except for *M. wesenbergii* CHAB1443 clustered into an independent lineage, mixing with *M. aeruginosa* NIES843, *M. aeruginosa* PCC7806, and *Microcystis viridis*.

Microcystin analyses of *Microcystis* strains

The HPLC method was employed to chemically verify the intracellular microcystin content of the five *Microcystis* strains shown to contain *mcyA* fragment. In microcystin compositions of these strains (Fig. 4), with exception of strain CHAB1444 containing only MC-LR, the examined *Microcystis* strains were composed of both MC-LR and MC-RR. The intracellular contents of MC-LR and MC-RR of these five strains were 189.3∼306.9 μg g^{-1} and 188.3∼537.3 μg g^{-1}, respectively, and the strain CHAB731 was detected to have high contents of both microcystin types,

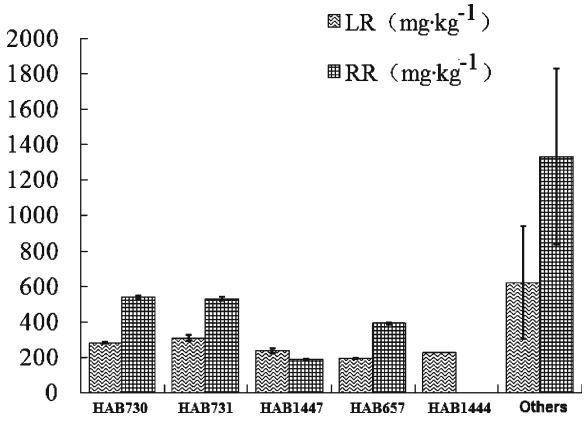

Fig. 4 Comparison of intracellular microcystin contents among five strains of toxic *M. aeruginosa*, 'others' represent the strains isolated from the other Chinese lakes

reaching 306.9 μg g^{-1} (MC-LR) and 527.8 μg g^{-1} (MC-RR). All analyses were performed in three replicates. Significance was analyzed with the software Microcal Origin (version 6.1, Microcal Software Inc.). Significant differences between the examined strains were determined by ANOVA. Differences were considered to be significant when $P < 0.05$.

Discussion

It is generally regarded that environmental changes such as accelerating anthropogenic nutrient loading, rising temperatures, and enhanced vertical stratification are likely to favor cyanobacterial dominance in a wide range of aquatic ecosystems (Paerl and Huisman 2009). It is notable that changes of water environment in Lake Ulungur during the recent decade accelerated the occurrence of waterbloom-forming cyanobacteria, mainly including *Aphanizomenon flos-aquae*, *Anabaena* spp., *Dactylococcopsis* spp., *Spirulina* spp., *P. agardhii*, and *Microcystis* spp. etc. The latter two are firstly reported to occur in a Chinese brackish lake, which reflects the trend of water deterioration in Lake Ulungur. As a deep lake in temperate zone of Northern China, Lake Ulungur is currently characterized to be increasingly eutrophic; however, the finding that the cyanobacterial components with waterbloom-causing

Microcystis species occurred in summer implied a potential threaten to the lake ecosystem. In contrast to the previous studies on this lake using the morphological examination that was not able to exactly detect the occurrence of *Microcystis*, the molecular detecting method provides an advantageous and powerful tool to monitor *Microcystis* and classify toxic and non-toxic ones. However, the abundance of *Microcystis* 16S rDNA copies, as shown in Table 2, were found to be much lower than cyanobacterial 16S rDNA copies, which suggested another possibility that *Microcystis* species were recently imported into this lake. Meanwhile, the distribution of cyanobacteria including *Microcystis* was found to differ considerably along Lake Ulungur. Compared to Lake Buluntuo, Lake Jelekol was detected by the real-time PCR method to have much higher abundance of cyanobacterial 16S rDNA copies. The similar results were also obtained by the Chl *a* contents analyzed by Dong et al. (2008), indicating that Chl *a* content in Lake Jelekol was four to 50 times more than that in Lake Buluntuo. Both results above may attribute to the higher nutrient levels in Lake Jelekol, as the described by Dong et al. (2008). Low abundances of

Table 2 The information of the staions we sampled and the distribution of cyanobacteria, *Microcystis* spp., and *mcyB*-containing *Microcystis* species

Locus	Longitude	Latitude	Microcystis (10^3 copies/L)	Microcystis mcyB (10^3 copies/L)	Cyanobacteria (10^6 copies/L)	Toxic Microcystis percentage (%)[a]	Microcystis percentage (%)[b]
1#	47°1.937′E	87°17.440′N	34.22	3.99	26.52	23.34	0.13
2#	47°2.115′E	87°15.999′N	6.8	1.16	19.52	34.2	0.13
3#	47°4.447′E	87°15.572′N	26.74	NK	8.32	NK	0.32
4#	47°8.429′E	87°13.044′N	454.42	0.52	12.34	0.23	3.68
5#	47°10.409′E	87°11.560′N	125.22	NK[c]	31.7	NK	0.40
6#	47°10.512′E	87°5.431′N	259.72	12.86	14.08	9.9	1.84
7#	47°10.665′E	87°7.375′N	304.62	1.04	19.8	0.68	1.54
8#	47°13.842′E	87°17.369′N	201.64	0.52	34.08	0.51	0.59
9#	47°20.664′E	87°28.100′N	52.98	0.13	13.7	0.48	0.39
10#	47°24.438′E	87°31.240′N	0.34	NK	4.82	NK	0.01
11#	47°24.655′E	87°33.146′N	2.74	NK	4.78	NK	0.06
12#	47°22.977′E	87°31.963′N	3.78	NK	9.06	NK	0.04
13#	47°14.228′E	87°31.986′N	201.88	NK	3.58	NK	5.65
14#	47°15.994′E	87°29.854′N	106.14	NK	9.36	NK	1.13
15#	47°17.528′E	87°27.528′N	7.64	NK	3.8	NK	0.20
16#	47°8.948′E	87°26.824′N	34.8	NK	9.76	NK	0.36
17#	47°21.746′E	87°18.672′N	27.14	NK	2.86	NK	0.95
18#	47°11.608′E	87°20.450′N	21.7	NK	1.28	NK	1.71
19#	47°12.285′E	87°19.227′N	21.76	NK	12.42	NK	0.17
20#	47°20.321′E	87°13.338′N	2.72	NK	6.98	NK	0.04
21#	47°9.057′E	87°18.431′N	6.14	NK	11.32	NK	0.05
22#	47°10.124′E	87°15.536′N	0.94	NK	5.9	NK	0.02
23#	47°108.25′E	87°13.955′N	33.18	NK	12.66	NK	0.26
24#	47°16.540′E	87°4.938′N	28.64	NK	12.22	NK	0.23
25#	47°18.153′E	87°3.459′N	10.8	NK	4.8	NK	0.23
26#	46°59.718′E	87°20.328′N	NK	NK	41.8	NK	NK
27#	46°59.383′E	87°23.985′N	1.66	NK	37.66	NK	NK
28#	46°58.699′E	87°27.982′N	0.54	NK	60.66	NK	NK

[a]Toxic *Microcystis* percentage represents the percentage of *mcyB*-containing *Microcystis* accounted for the total *Microcystis*

[b]*Microcystis* percentage shows the percentage of *Microcystis* accounted for the total cyanobacteria; Those estimations are based on the condition that all the Cyanobacteria and *Microcystis* distribution in Lake Ulungur have the same copy number of 16S rDNA and mcyB gene

[c]NK means undetected or under detected limit

Microcystis 16S rDNA copies were detected around the entire Lake Ulungur except for the western region such as stations 6, 7, and 1 (Fig. 1). The *mcyB* copies, representing the potential toxic *Microcystis* cells, were shown to have the similar distribution pattern as *Microcystis*, as being mainly found in the R I region. On the assumption that the 16S rRNA gene and the *mcyB* gene copy numbers in a *Microcystis* genome are the same for all *Microcystis* cells, the cell numbers of total *Microcystis* and toxic *Microcystis* could be estimated as previous reports (Kurmayer and Kutzenberger 2003). Therefore, the proportions of the *mcyB*-containing *Microcystis* in total *Microcystis* cells at stations 1 and 2 were calculated as 23.2% and 32.20%, respectively, while the proportions at the other southwest stations such as stations 4, 7, 8, and 9, were extremely low, even below 1%. The R I region is the main protection area for fishery seeding and reproduction, and characterized with a richness of macrophytes and higher TN (0.928 ± 0.222 mg L^{-1}), and the Chl *a* content in this area reached 5.16 ± 4.20 mg m^{-3} in average. However, the distribution of *Microcystis* in this region was uneven, and station 2 was detected to have much less *Microcystis* 16S rDNA gene copies than that in station 1. The R II region, near the public beach, is characterized by higher TN and lower phosphorus contents and with TN/ TP ratio as 48.7, and thus leading to the lower *Microcystis* abundance. The R III region is located at the inlet of River Irtysh into Lake Buluntuo, and the fast and strong flow from the upstream diluted the phytoplankton concentration in this region. Station 26, serving as a spawning ground, was detected to contain much higher TN (1.434 ± 0.614 mg L^{-1}) and TP (0.166 ± 0.220 mg L^{-1}), even reaching 0.42 mg L^{-1} of TP in summer. The large usage of chemical fertilizers for the purpose of aquaculture attributed to higher nutrient level. Lake Buluntuo is the downstream of Lake Jelekol linked by the Kuyergur River, and is brought into high TN and TP flows, which may contribute to the development and gathering of *Microcystis* downstream in the R I region and the regions close to the inlet.

Phylogenetic tree revealed that the fifteen strains isolated from the brackish Lake Ulungur were not genetically divergent from the strains isolated from freshwaters. Such a result can be explained by two possible assumptions: firstly, *Microcystis* species were newly introduced into this lake from the freshwater, so that not enough divergence occurred in such a short time; secondly, the 16S rRNA and *rpoC1* genes are not sensitive to such a low salinity which may not be the selective factor for achieving gene variances. These two genes distinctly distinguished toxin-producing *Microcystis* strains into two groups. However, similar to the findings by previous studies (Otsuka et al. 1999), the molecular boundary within the genus *Microcystis* at the species level is still unclear; which, as shown in the phylogenetic tree based on the sequences of 16S rRNA gene, the *M. wesenbergii* strains isolated in Lake Ulungur were mixed *Microcystis ichthyoblabe* and *M. aeruginosa* strains cited from GenBank, while the *M. aeruginosa* strains isolated from Lake Ulungur were mixed with *M. viridis* strains. Similarly, based on the sequences of *rpoC1* gene, *M. wesenbergii* strains isolated in Lake Ulungur were mixed with the strains of *M. aeruginosa*, while *M. aeruginosa* strains from this lake were mixed with strains of *M. wesenbergii* isolated from Lake Suwa, Japan. It was illustrated that these two gene loci were not proper for identifying *Microcystis* strains.

The intracellular microcystin content is another concern in the present study. Compared to the *Microcystis* strains isolated from other eutrophic waters in China (average 492.2 mg kg^{-1} and 1,331.2 mg kg^{-1} for MC-LR and MC-RR, respectively, Liu et al., unpublished), the five strains of *M. aeruginosa* isolated from this mesotrophic lake MC-LR and MC-RR showed significantly lower intracellular microcystin contents ($P < 0.05$, ANOVA; Fig. 4). In spite of the molecular basis that the microcystin biosynthesis via a multienzyme complex has been elucidated (Nishizawa et al. 2000; Tillett et al. 2000), the mechanism on the control of variation in the ability of microcystin production is still unknown, and possible recombination of the *mcy* gene occurring in *Microcystis* species revealed by Tanabe et al. (2004) further complicated the understanding the production of microcystins. It is noteworthy that occurrence of total *Microcystis* and toxic *Microcystis* strains was proven in Lake Ulungur from this study, and these newly emerging features in an

increasingly eutrophicated and brackish lake will increase the understanding for the development course of waterblooms in temperate and deep waters. To maintain the water quality of Lake Ulungur, frequent monitoring for waterbloom-forming cyanobacteria and toxic cyanobacteria need to be performed in this lake; and such monitorings will help us to form the basis for estimating the health risks associated with cyanobacterial occurrence in increasingly eutrophicated lakes.

Acknowledgements Thanks to You Dong from Shanghai Ocean University for his great support and contribution to the hydrochemistry analysis. Also, we are very grateful to Yu Liu, Hong Li (HZAU) and Mengling Zhu (IHB) and the staffs from Fuhai aquatic bureau, Xinjiang for their assistances in samples collection and data analysis. The comments from the anonymous reviewers contributed a lot and were greatly appreciated. This research was supported by the National Basic Research Program of China (973) (No.2008CB418002) and the Hundred Talent Scientist Program of Chinese Academy of Sciences (No. 082303-1-501).

References

Carmichael, W. W. (1997). The cyanotoxins. In J. Callow (Ed.), *Advances in botanical research* (Vol. 27, pp. 211–256). London: Academic.

Dong, Y., Jiang, M., Liu, Q., Huang, Y. Y., & Shen, J. Z. (2008). Research on water quality and trophic level in Wulungu Lake. *Journal of Shanghai Fisheries University, 17*(5), 564–569.

Fastner, J., Flieger, I., & Neumann, U. (1998). Optimised extraction of microcystins from field samples—A comparison of different solvents and procedures. *Water Research, 32*, 3177–3181.

Geng, H., Xie, P., Deng, D. G., & Zhou, Q. (2005). The rotifer assemblage in a shallow, eutrophic Chinese lake and its relationships with cyanobacterial blooms and crustacean zooplankton. *Journal of Freshwater Ecology, 20*, 93–100.

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *System Biology, 52*, 696–704.

Guo, L. (2007). Doing battle with the green monster of Taihu Lake. *Science, 317*, 1166.

Hisbergues, M., Christiansen, G., & Rouhiainen, L. (2003). PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Archives of Microbiology, 180*, 402–410.

Ichimura, T. (1979). Media for freshwater cyanobacteria. In K. Nishizawa, & M. Chihara (Eds.), *Methods in phycology* (pp. 295–296). Tokyo: Kyouritsu shuppan.

Komárek, J., & Anagnostidis, K. (1999). *Cyanoprokaryota. 1. Teil: Chroococcales. Süßwasserflora von Mitteleuropa, Band 19/1* (pp. 164–190). Jena: Spektrum Akademischer Verlag.

Koskenniemi, K., Lyra, C., Rajaniemi-Wacklin, P., Jokela, J., & Sivonen, K. (2007). Quantitative real-time PCR detection of toxic *Nodularia* Cyanobacteria in the Baltic Sea. *Applied and Environmental Microbiology, 73*, 2173–2179.

Kurmayer, R., & Kutzenberger, T. (2003). Application of real-time PCR for quantification of MC genotypes in a population of the toxic Cyanobacterium *Microcystis* sp. *Applied and Environmental Microbiology, 69*, 6723–6730.

Larkin, M. A., & Blackshields, G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*, 2947–2948.

Li, X., Dan, X. C., Li, H., & Gao, J. F. (2005). Eutrophic analysis and integrated water quality evaluation model for large lakes and reservoirs in Xinjiang. *Arid Land Geography, 28*(5), 588–591.

Li, Z., Jiang, Z., Ma, B., Huo, T. B., Guo, Y., & Cai, L. G. (2008). Cluster analysis and multidimensional scaling analysis in spring and autumn of phytoplankton community structure of Ulungur Lake, Xinjian. *Journal of Fishery Sciences of China, 15*, 984–991.

Li, X. Y., Liu, Y. D., Song, L. R., & Liu, J. T. (2003). Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon, 42*, 85–89.

Neilan, B. A., Jacobs, D., Therese, D. D., Blackall, L. L., Hawkins, P. R., Cox, P. T., et al. (1997). rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic and Evolutionary Microbiology, 47*, 693–697.

Nishizawa, T., Ueda, A., Asayama, M., Fujii, K., Harada, K., Ochi, K., et al. (2000). Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. *Journal of Biochemistry, 127*, 779–789.

Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S., et al. (1999). Phylogenetic relationship between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiology Letters, 172*, 15–21.

Paerl, H. W., & Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports, 1*, 27–37.

Palenik, B., & Swift, H. (1996). Cyanobacterial evolution and prochlorophyte diversity as seen in DNA-dependent RNA polymerase gene sequences. *Journal of Phycology, 32*, 638–646.

Posada, D., & Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics, 14*, 817–818.

Rantala, A., Fewer, D., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Börner, T., et al. (2004). Phylogenetic evidence for the early evolution of microcystin synthesis. *Proceedings of the National Academy of Sciences, 101*, 568–573.

Sambrook, J., & Russell, D. W. (2001). *Molecular coling: A laboratory manual* (3rd ed.). New York: Cold Spring Harbor Laboratory Press.

Tamura, K., & Dudley, J. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*, 1596–1599.

Tanabe, Y., Kaya, K. & Watanabe, M. M. (2004). Evidence for recombination in the microcystin synthetase (mcy) genes of toxic cyanobacteria *Microcystis* spp. *Journal of Molecular Evolution, 58*, 633–641.

Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T., & Neilan, B. A. (2000). Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: An integrated peptide. *Chemistry Biology, 7*, 753–764.

Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y., & Park, H. D. (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at differenttrophic levels from the eutrophic Lake Chaohu, China. *Environmental toxicology, 20*, 293–300.

Xie, L., Xie, P., & Tang, H. J. (2003). Enhancement of dissolved phosphorus release from sediment to lake water by *Microcystis* blooms—An enclosure experiment in a hyper-eutrophic, subtropical Chinese lake. *Environmental Pollution, 122*, 391–399.

Ye, S. M., Su, D. X., Liu, S., Chen, L., Liu, X. H., et al. (2004). The research on the bio-resource in the Lake Ulungur, Xinjiang. *Reservoir Fisheries, 24*(2), 51–54.

Yu, G., & Li, R. (2007). Three newly recorded species of *Microcystis* (Cyanophyta) from China. *Acta Phytotaxonomica Sinic, 45*(3), 353–358.