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Changes in abundance and community structure of bacteria associated with buoyant *Microcystis* colonies during the decline of cyanobacterial bloom (autumn–winter transition)

Limei Shi, Yuanfeng Cai, Fanxiang Kong* and Yang Yu

State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, P.R. China

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Abstract – The structure and composition of the bacterial community associated with buoyant *Microcystis* colonies were monitored during the decline of a cyanobacterial bloom (from October 13, 2009 to January 27, 2010). When temperature decreased, the ratio between the colony-associated bacteria and the *Microcystis* gradually decreased as estimated by a quantitative real-time polymerase chain reaction (qRT-PCR)-based approach. Diversity of bacterial communities was determined through denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments. Cluster analysis of the DGGE profiles showed that most of the bacterial communities associated with *Microcystis* colonies collected on the nearby dates were clustered together. The bacterial clones from four clone libraries in different months were classified into 5, 12, 6 and 12 operational taxonomic units, most of which were affiliated with *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes*. Shift in dominance from pathogenic *Aeromonas* sp. to *Shewanella* sp. capable of remineralization of many organic materials was observed, and both species seemed to be associated with *Microcystis* colonies along with the bloom decline. These results indicated that the potential harmful effects of the *Microcystis* bloom on the safety of lake water during the decline period should be taken into account.

Key words: *Microcystis* / associated bacteria / abundance / diversity / decline

Introduction

Microcystis is a common and ubiquitous bloom-forming cyanobacterial genus in eutrophic freshwater lakes. *Microcystis* often dominates the phytoplankton during summer, declines in late autumn when the temperature is low, then begins recovering the next spring (Reynolds *et al.*, 1981). Many temperate lakes annually suffer from overabundant cyanobacterial blooms.

Microcystis colonies often live in association with numerous bacteria (Pearl, 1988; Bostroëm *et al.*, 1989). Substances released from algal cells are trapped in the mucilage, which mainly consists of polysaccharide, because of the high viscosity of the mucilage (Amemiya *et al.*, 1990). The colony sheath of *Microcystis* is assumed to be a hot spot compared to the surrounding water (Worm and Søndergaard, 1998). The contribution of *Microcystis*-associated bacteria constitutes 19–40% of the total bacterial abundance (Brunberg, 1999). The bacteria colonize *Microcystis* in old, as well as in young and healthy colonies. Symbiotic interactions may occur in the mucilage (Steppe *et al.*, 1996). The exudates of *Microcystis* provide a source of substrates for attracting heterotrophic bacteria, and have an antibiotic effect on non-co-occurring bacteria (Casamatta and Wickstrom, 2000). On the other hand, bacteria might be able to provide beneficial metabolites to cyanobacteria through remineralized nutrients or lysing the cyanobacteria by excreting algicidal substances (Salomon *et al.*, 2003).

Bacterial community composition changes along with the development of phytoplankton blooms (Riemann and Winding, 2001). Shifts in the species composition of the phytoplankton and attached bacterial communities have been linked in a coastal marine environment (Rooney-Varga *et al.*, 2005). Several studies have reported that bacteria play a significant role in aquatic ecosystems as decomposers of phytoplankton (Sigee, 2005; Reynolds, 2006), and may terminate freshwater blooms (Manage *et al.*, 2000; Rashidan and Bird, 2001). Significant changes in the bacterial community after viral-induced mass lysis of cyanobacterial blooms have also been reported (Van Hannen *et al.*, 1999). Dissimilar bacterial communities

*Corresponding author: fxkong@niglas.ac.cn

may be favored by different cyanobacterial blooms even under similar environmental conditions (Eiler and Bertilsson, 2004). The specific association between *Microcystis* and bacteria has been documented by Shi *et al.* (2009). Interactions between *Microcystis* species and their associated bacteria in colonies, where bacterial–algal associations are more dense and abundant than free-living bacteria in the water column (Bell and Mitchell, 1972), may be a key to understanding their role in the cycling of organic and inorganic compounds and their ecological impact on bloom development. Understanding the bacterial communities associated with *Microcystis* and how they change during the bloom decline may help establish an understanding of the physiological status of *Microcystis*. Shifts in the bacterial community composition may affect the health of *Microcystis* and its susceptibility to decay.

The diverse presence of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteriodetes* and *Actinobacteria* in the water column in Lake Taihu during the *Microcystis* bloom event has been documented (Wu *et al.*, 2007). The presence of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*, *Deinococcus–Thermus* and *Gemmatimonadetes* associated with *Microcystis* colonies during the vigorous period of the bloom has been studied by Shi *et al.* (2010). However, little is known about the identity of bacteria that inhabit *Microcystis* colonies during the decline period, even though these organisms are involved in significant biodegradation processes and carbon dynamics (Burnberg, 1999). Thus, in the present study, the community structure of bacteria associated with *Microcystis* colonies collected during bloom decline is investigated for the first time. Their ecological role in the decline of cyanobacterial bloom is also discussed.

Materials and methods

Sample collection

During the bloom decline period (from October 13, 2009 to January 27, 2010), cyanobacterial samples were collected with plankton net (mesh size: 25–50 μm) from the surface water in Meiliang Bay (31°43'N, 120°18'E) in the northern part of Lake Taihu in China, which is hypertrophic with annual *Microcystis* bloom. Once a month, dissolved oxygen (DO) and pH were measured *in situ* using a Yellow Springs Instrument water quality sonde (YSI 6600, USA). Total nitrogen (TN) and total phosphorus (TP) were analyzed according to standard methods (Jin and Tu, 1990), and phycocyanin was measured according to Asai *et al.* (2001). To separate these collected *Microcystis* colonies from other particles, samples were allowed to stand until most of the *Microcystis* colonies floated to the water surface. Only the colonies that floated to the water surface were collected by micropipetting. These colonies were then checked under a binocular microscope. To remove free-living bacteria, the samples were resuspended and washed 10 times on autoclaved 20 μm pore size nylon screen with autoclaved phosphate-buffered saline (pH 7.2). About 10 mg wet weight of the *Microcystis* colonies were transferred to 1.5 mL sterile Eppendorf tubes, and were then stored at −20 °C.

DNA extraction

Community DNA extraction was conducted using the potassium xanthogenate sodium dodecyl sulfate (xs procedure) method, as described by Tillett and Neilan (2000).

Quantitative real-time polymerase chain reaction (qRT-PCR)

A qRT-PCR-based approach was used to estimate the ratios between the colony-associated bacteria and *Microcystis*. qRT-PCR was conducted with an Mastercycler Ep Realplex (Eppendorf, Germany) using SYBR Green PCR Master Mix kit (Qiagen, Valencia, CA). All reactions were carried out in a total volume of 25 μL and amplified in triplicate. The universal bacterial primer pair Bac341F (5'-CCTACGGGAGGCAGCA-3')/Bac518R (5'-ATTACCGCGGCTGCTG-3') was used to amplify the bacterial 16S rRNA gene under the following conditions: 95 °C for 10 min, followed by 45 cycles of 95 °C for 1 min, 55 °C for 30 s and 72 °C for 1 min. Using the primer pair Micr 184F (5'-GCCGCRAGGTGAAAMCTA-3') and Micr 431R (5'-AATCCAAARACCTTCCTCCC-3') (Rinta-Kanto *et al.*, 2005), the amplification of *Microcystis*-specific 16S rRNA gene was conducted under the following conditions: 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s. The PCR products were gel-purified and cloned into the pGEM-T vector (Promega) following the manufacturer's instructions. Inserts in the clones were confirmed through PCR using the specific primers above and subsequently electrophoresed. One positive plasmid containing each target gene was used as the qRT-PCR standard. The plasmid DNA was diluted 10 times to yield the standard curve. The concentrations of the diluted plasmid DNA ranged from 10^8 to 10^3 copies per microliter, the log of which has a linear relationship with the cycles of threshold (CT value). Melting curve analysis of the PCR products was conducted to assess reaction specificity and to determine the melting point of the amplification products. The target copy numbers of each sample were calculated from the standard curves, and the ratios between the colony-associated bacteria and *Microcystis* were calculated from the copy numbers.

PCR–denaturing gradient gel electrophoresis (DGGE)

PCR amplifications were performed with the primers 341f (5'-CCTACGGGAGGCAGCA-3') with a 40 bp GC-clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) attached to its 5' end and 518r

Table 1. The monthly DO, pH and concentrations of TN, TP and phycocyanin in the surface water during the decline of *Microcystis* bloom in Meiliang bay of Lake Taihu.

	October 2009	November 2009	December 2009	January 2010
DO ($mg.L^{-1}$)	8.4	11.06	13.22	12.59
pH	8.47	8.34	8.31	8.51
TN ($mg.L^{-1}$)	1.35	2.21	2.64	3.73
TP ($mg.L^{-1}$)	0.05	0.11	0.08	0.11
Phycocyanin ($\mu g.L^{-1}$)	57.89	25.40	11.65	2.13

(5'-ATTACCGCGGCTGCTG-3') (Muyzer *et al.*, 1993). The 50 μL PCR mixture contained 0.2 μM of each primer, 1 × PCR buffer (without Mg^{2+}), 2.25 mM of $MgCl_2$, 0.8 mM of deoxynucleotide triphosphates, 2 U of DNA Taq polymerase (Takara, Japan) and 1 μL of template DNA, which was similar to that analyzed with quantitative PCR. The amplification program consisted of an initial denaturation step at 94 °C for 5 min, followed by 20 cycles of 1 min at 94 °C, 1 min at 65–56 °C (decreasing by 0.5 °C every cycle) and 1 min at 72 °C, then 10 additional cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. The tubes were then incubated for 10 min at 72 °C. PCRs were performed in Bio-Rad thermal cyclers, and the PCR products were inspected on 1.2% (w/v) agarose gels followed by goldview staining.

DGGE was performed with the DGGE-2001 system (CBS Scientific). The PCR products were directly applied onto 8% (w/v) polyacrylamide gels containing a denaturing gradient from 40 to 70% (100% denaturant corresponded to 7 M urea and 40% (v/v) formamide). Electrophoresis was performed in 1 × TAE (20 mM Tris-acetate, pH 7.4, 10 mM acetate and 0.5 mM disodium EDTA) at 60 °C for 16 h at 75 V. After electrophoresis, the gels were stained in SYBR Green I (1:10 000 dilution; Molecular Probes) for 30 min and photographed using Bio-Rad gel explorer. The DGGE band position and intensity were determined using Quantity One software version 4.6 (Bio-Rad) and manually modified. The pairwise similarity values were performed based on the Jaccard's coefficient that was calculated as follows: $J = N_{AB}/(N_A + N_B - N_{AB})$ where N_{AB} is the number of bands present in lanes A and B, and N_A and N_B are the total number of bands in lanes A and B, respectively. Cluster analysis of DGGE fingerprints was conducted by the unweighted pair group with mathematical averages (UPGMA) using the program MVSP version 3.1 (Kovach Computing Services).

Clone library

One representative sample of every month was selected to estimate changes in the diversity of the associated bacteria. In these lanes, except for the most dominant bands derived from *Microcystis* (indicated with an arrow), which were sequenced separately, other bands in the same lane were excised from the gel using a sterile scalpel, collected together and resuspended in 500 μL of sterile Milli-Q water overnight at 4 °C. Excised DNA was then re-amplified using primers 341f and 518r, as previously described. The PCR products were ligated into the pGEM-T vector (Promega, Germany) and transformed into competent DH5α cells. Approximately 30 positive clones in each clone library were selected and sequenced using the an ABI Bigdye 3.1 Terminator cycle sequencing kit (PerkinElmer Inc.) and an ABI 3730xl DNA Analyzer with T7 primer at the Shanghai Sangon Biological Engineering Technology Limited Company, China. Sequences with 97% sequence similarity were grouped in operational taxonomic units (OTUs). One representative sequence from each group was selected for phylogenetic analysis. These sequences were then compared with 16S rRNA sequences available in the National Center for Biotechnology Information database using BLAST search. In addition, the taxonomic affiliation of the partial 16S rRNA sequences was analyzed using the RDP classifier tool (http://rdp.cme.msu.edu/classifier) to confirm the taxonomic assignments.

The partial sequences of 16S rRNA genes obtained in this study were deposited in GenBank with accession numbers: HQ902591–HQ902629.

Results

Physicochemical variables and relative abundance of bacteria associated with *Microcystis*

During the sampling period, the DO and TN concentrations increased, whereas the pH and TP fluctuated. An obvious decline in phycocyanin concentration was observed (Tab. 1). The water temperature decreased gradually, from 20 °C to approximately 4 °C (Fig. 1). Results of the qRT-PCR analysis of the samples during the decline period of *Microcystis* bloom are presented in Figure 1. The quotients between copy numbers of colony-associated bacteria and that of *Microcystis* varied from 5.3 ± 1.6 to 0.1 ± 0.1. The maximum bacterial quotient was observed at the beginning of the decline period and then gradually decreased along with bloom decline, despite slight variations.

Community structure based on DGGE profile analysis

Different DGGE fingerprint patterns reflected changes in the associated bacterial community compositions (Fig. 2). Cluster analysis of the DGGE profiles grouped

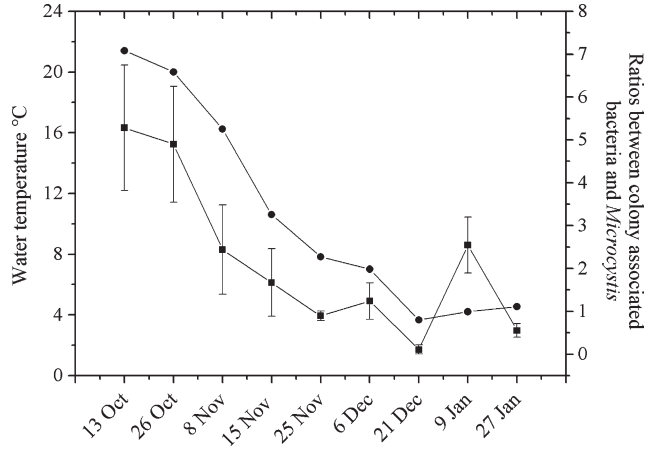

Fig. 1. Water temperature (●) on the sampling date, and the ratios between copy numbers of colony-associated bacteria and that of *Microcystis* (■) in each sample estimated using the quantitative PCR assays. Error bars are the standard errors calculated from the mean of the three replicates.

the bacterial communities into two major clusters (Fig. 3). Cluster 1 was composed of bacterial communities associated with the *Microcystis* colonies collected before 6 December at a water temperature of 7 °C, whereas Cluster 2 was composed of those collected after 21 December at a water temperature of 4 °C. Most of the bacterial communities associated with *Microcystis* colonies collected on the nearby dates were clustered together.

Clone analyses of bacterial communities

The most dominant bands (arrows in Fig. 2) in lanes 13 Oct, 8 Nov, 21 Dec and 27 Jan were excised and sequenced. Sequence analysis revealed that they originated from cyanobacteria. Other bands in these four lanes were excised, mixed and used to construct four clone libraries. Approximately 20–30 clones in each clone library were sequenced. Based on sequence similarity, they were sorted into 5, 12, 6 and 12 OTUs. The sequencing analysis is listed in Table 2. Most sequences have high similarities (98–100%) to the partial bacterial 16S rRNA gene sequences retrieved from GenBank. The taxonomic assignment according to BLAST search was consistent with that analyzed using an RDP II classifier. All these sequences were related to members of *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* phyla. *Gammaproteobacteria* and *Bacteroidetes* were the most abundant. Additionally, several cyanobacterial sequences were detected. The clones from the clone library in October 2009 belonged to five OTUs. Among the OTUs, one (Oct1, affiliated with *Aeromonas* sp.) occurred with the highest frequency (19/30). OTU Oct4, which was affiliated with *Rhodobacter* sp., occurred at a frequency of 3/30, whereas Oct2 and Oct5 belonged to *Shewanella* sp. and *Aeromonas* sp. and were dominant in this clone library.

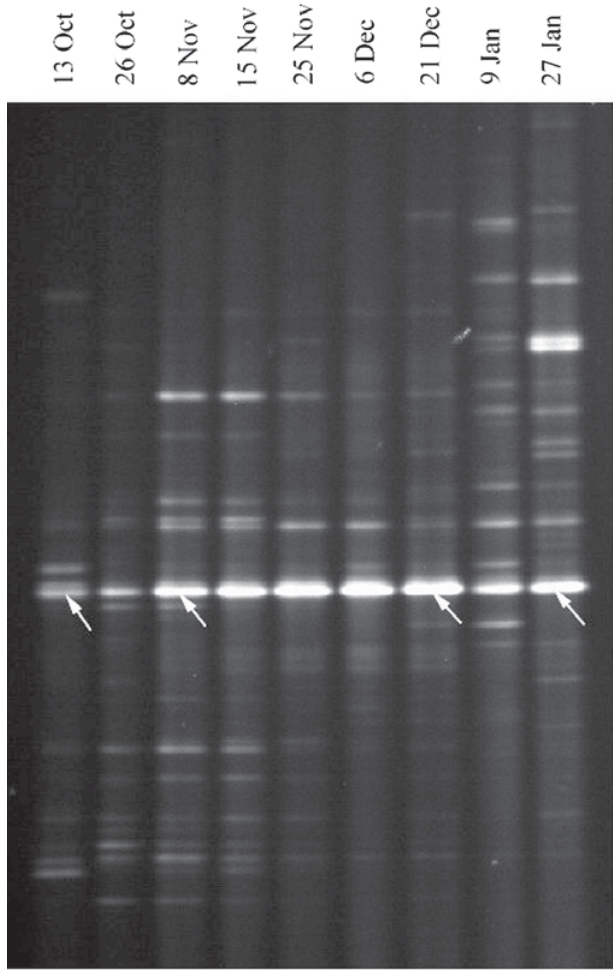

Fig. 2. DGGE profiles of 16S rRNA gene fragments of bacterial communities associated with *Microcystis* colonies.

For the clones obtained from the clone library in November 2009, OTUs Nov5, which included 6 of 25 clones, and Nov8 belonged to *Aeromonas* sp. Four OTUs, Nov6 (3 clones), Nov4 (2 clones), Nov10 (1 clone) and Nov9 (1 clone), were inferred to be members of the group of *Bacteroidetes*. OTU Nov3 (4 clones) was related to *Gammaproteobacteria*, whereas OTU Nov1 including 5 of 25 clones, was related to *Shewanella* sp. OTUs Nov11, Nov2 and Nov7 were related to *Phenylobacterium* sp., *Pseudanabaena* sp. and *Aquamonas* sp., respectively.

Regarding the clones in December 2009, six OTUs were observed. OTU Dec1 was related to *Rhodobacter* sp. (2 clones), and OTUs Dec2 and Dec6 were related to *Aeromonas veronii* (5 clones). OTU Dec3 belonged to cyanobacteria. OTU Dec4 was grouped with *Shewanella* sp. (7 clones), whereas OTUs Dec5 (1 clones) and Dec6 (4 clones) were grouped within *Erythrobacter* sp. and *Bacteroidetes*, respectively.

The clones from the clone library in January 2010 were also phylogenetically diverse. Eighteen clones belonging to five OTUs (Jan1, Jan2, Jan6, Jan11 and Jan12) were affiliated with *Shewanella* sp. Ten clones belonging to two OTUs (Jan4 and Jan7) were related to *Pseudomonas* sp.

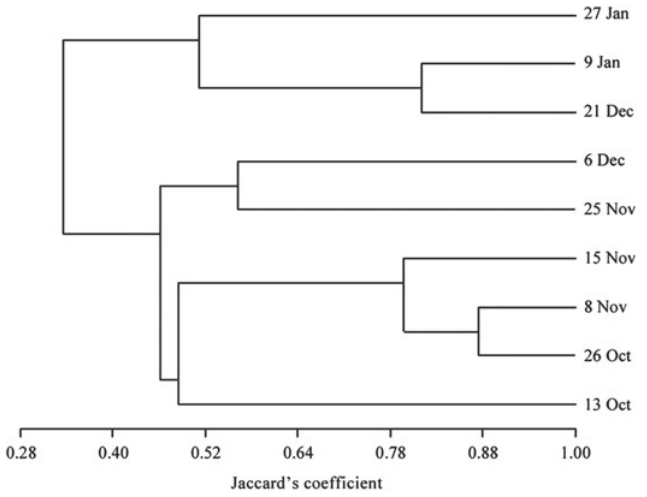

Fig. 3. Dendrogram constructed by UPGMA cluster analysis of bacterial communities associated with *Microcystis* colonies based on the DGGE profile.

Two OTUs (Jan3 and Jan10) belonged to *Bacteroidetes*. Three OTUs, Jan5, Jan8 and Jan9, belonged to *Sporocytophaga* sp., *Aeromonas* sp. and *Bradyrhizobium* sp., respectively. *Shewanella* sp. was dominant in this clone library.

Clones related to *Aeromonas* sp. and *Shewanella* sp. were detected in all four clone libraries. A change in the dominant species from *Aeromonas* sp. in October 2009 to *Shewanella* sp. in January 2010 was observed.

Discussion

The bacteria associated with *Microcystis* are sensitive to changes in the biochemical characteristics of their host. Changes in the bacterial community reflect the changes in physicochemical conditions within the colonies. To reveal the decline of *Microcystis*, the study therefore focused on the bacteria associated with *Microcystis* colonies.

The obvious decline in phycocyanin concentration coincide with the decrease of temperature, indicated the decline of *Microcystis* bloom. The DO and TN concentrations were influenced by this process. A decreasing trend in the ratios between colony-associated bacteria and *Microcystis* was observed (Fig. 1). Largely, the buoyant *Microcystis* colonies collected were the surviving colonies of the water population when the temperature decreased, indicating that the remaining integrated *Microcystis* colonies in the pelagic water at low temperature were more resistant to colonization. Brunberg (1995) demonstrated that colonies with different pre-histories and ages have different resistances to bacterial attack and decomposition. In addition, low temperature may be another reason for the declining number of mucilage bacteria, and would be propitious to the survival of *Microcystis* through the cold winter.

Cluster analysis indicates that most of the *Microcystis* colonies collected showed similar bacterial community structures (Fig. 3). Samples were grouped according to their sampling date. During the decline of cyanobacterial bloom, the structure of the associated bacterial assemblage was influenced by water temperature, which is an important environmental factor for the decline of *Microcystis* colonies. The algae-derived organic matters were influenced by temperature (Van der Westhuizen *et al.*, 1986; Davis *et al.*, 2009), which may inversely influence the associated bacterial community.

To reveal the diversity of the colony-associated bacteria, PCR product amplified using the primers 27f and 1492r was used to construct clone libraries for sequencing analysis. However, because of the low proportions of the associated bacteria in the colonies, more than 80% of the sequences originated from *Microcystis*, which could also be amplified using the two primers. To eliminate the interference of *Microcystis*, previously described methods were used. DGGE analysis shows that re-amplification of the mixed bands did not influence the band patterns of the bacterial community (data not shown).

The dominance of *Gammaproteobacteria* and *Bacteroidetes* associated with declining *Microcystis* colonies was observed in this study. This is different from the diverse bacteria groups, such as *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes*, which are associated with *Microcystis* colonies during the vigorous period according to a previous study (Shi *et al.*, 2010). The study performed by Wu *et al.* (2007) on the bacterial community in the water column during the course of the cyanobacterial bloom suggested that species richness slightly decreased from July to September. *Bacteroidetes*, which are chemo-organotrophic bacteria abundant in cyanobacterial blooms (Eiler and Bertilsson, 2004), can efficiently degrade a variety of high-molecular-mass fraction DOM and other compounds, such as protein, cellulose, pectin and chitin (Kirchman, 2002). *Bacteroidetes* can dominate the microbial community after viral lysis of the filamentous cyanobacteria (Van Hannen *et al.*, 1999). These populations may be responsible for the microbe-mediated decomposition of *Microcystis* colonies during the decline period.

Majority of the phylotypes of the colony-associated bacteria belonged to *Gammaproteobacteria*. An increase in the proportion of *Gammaproteobacteria* in the decline of the bloom was also observed in Xuanwu Lake (Zheng *et al.*, 2008). The dominant bacteria were *Aeromonas* spp., which are opportunistic animal and human pathogens, and *Shewanella* spp., which are capable of remineralization of many organic materials. Both micro-organisms were not observed to inhabit *Microcystis* colonies in summer during the bloom (Shi *et al.*, 2010). *Aeromonas* has been found in wastewater, chlorinated water supplies, surface water and ground water, as well as estuarine and marine environments (Borrell *et al.*, 1998; Pettibone, 1998; Bianucci *et al.*, 2001). Some bacterial strains of these species are cold-adapted (Singh *et al.*, 2000). In addition, the *Aeromonas* genera, which may cause adverse effects to the health of humans and animals, were found to be

Table 2. BLAST analysis of the *Microcystis* bloom-derived 16S rDNA fragments retrieved from the DGGE gel. The Oct-M, Nov-M, Dec-M and Jan-M were sequences from bands that were indicated with arrows in Figure 2. In addition, the RDP classifier tool was also used to confirm the taxonomic assignments.

Clone library and band	OTU	Accession number	Closest relative (accession number)	Similarity (%)	Taxon assignment
October 2009	Oct1	HQ902591	*Aeromonas* sp. A254 (HQ413137)	100	Gammaproteobacteria
	Oct2	HQ902592	*Shewanella xiamenensis* strain CAIM 1494 (HM584096)	100	Gammaproteobacteria
	Oct3	HQ902593	*Aeromonas* sp. 5A12S1 (HQ246266)	100	Gammaproteobacteria
	Oct4	HQ902594	*Rhodobacter* sp. RCRI19 (HQ392507)	100	Alphaproteobacteria
	Oct5	HQ902595	*Shewanella* sp. BAM79 (AB300600)	100	Gammaproteobacteria
November 2009	Nov1	HQ902596	*Shewanella* sp. POL1 (FN870751)	100	Gammaproteobacteria
	Nov2	HQ902597	*Pseudanabaena* sp. 0tu30s18 (AM259268)	100	Cyanobacteria
	Nov3	HQ902598	Uncultured gammaproteobacterium clone MEsu06cnp11C8 (FJ828374)	100	Gammaproteobacteria
	Nov4	HQ902599	Uncultured bacteroidetes bacterium clone hsh-8–46 (GU323652)	98	Bacteroidetes
	Nov5	HQ902600	*Aeromonas veronii* strain CL0901 (HM240294)	100	Gammaproteobacteria
	Nov6	HQ902601	Uncultured bacteroidetes bacterium clone hsh-8–46 (GU323652)	100	Bacteroidetes
	Nov7	HQ902602	Uncultured *Aquamonas* sp. clone IG1bG02 (FJ718009)	97	Betaproteobacteria
	Nov8	HQ902603	*Aeromonas* sp. AKB-2008-HE40 (AM989223)	100	Gammaproteobacteria
	Nov9	HQ902604	Uncultured bacteroidetes bacterium clone MEsu06cnp11H9 (FJ828426)	98	Bacteroidetes
	Nov10	HQ902605	Uncultured flexibacteraceae bacterium clone Nitri_2_a2 (HM193113)	94	Bacteroidetes
	Nov11	HQ902606	Uncultured *Phenylobacterium* sp. clone XZXXH71 (EU703427)	100	Alphaproteobacteria
	Nov12	HQ902607	Uncultured bacterium clone GBII-64 (GQ441331)	98	unknown
December 2009	Dec1	HQ902608	*Rhodobacter* sp. INCT285 (AB546235)	99	Alphaproteobacteria
	Dec2	HQ902609	*A. veronii* strain CL0901 (HM240294)	100	Gammaproteobacteria
	Dec3	HQ902610	*Anabaena mendotae* TAC584 (AB551482)	99	Cyanobacteria
	Dec4	HQ902611	*Shewanella* sp. S7 (FN994185)	100	Gammaproteobacteria
	Dec5	HQ902612	*Erythrobacter* sp. H301 (HQ622544)	100	Alphaproteobacteria
	Dec6	HQ902613	*A. veronii* strain NS01(GQ141742)	98	Gammaproteobacteria
January 2010	Jan1	HQ902614	*Shewanella* sp. S7 (FN994185)	98	Gammaproteobacteria
	Jan2	HQ902615	Uncultured *Shewanella* sp. partial 16S rRNA gene, clone OuchyA-69 (FN679137)	100	Gammaproteobacteria
	Jan3	HQ902616	Uncultured *Flavobacterium* sp. clone L6B-142 (GU000476)	100	Bacteroidetes
	Jan4	HQ902617	*Pseudomonas* sp. PsS31 (HM627624)	100	Gammaproteobacteria
	Jan5	HQ902618	Uncultured *Sporocytophaga* sp. clone Cat004C_H10 (EU572368)	100	Bacteroidetes
	Jan6	HQ902619	*Shewanella* sp. IPC4 (HQ108170)	100	Gammaproteobacteria
	Jan7	HQ902620	*Pseudomonas* sp. FD6 (HM768205)	100	Gammaproteobacteria
	Jan8	HQ902621	*Aeromonas sobria* partial 16S rRNA gene, isolate LE 76 (FN908438)	100	Gammaproteobacteria
	Jan9	HQ902622	*Bradyrhizobium* sp. Z2-YC6857 (GQ369125)	100	Alphaproteobacteria
	Jan10	HQ902623	Uncultured bacteroidetes bacterium clone MEsp06cnp11H3 (FJ828248)	95	Bacteroidetes
	Jan11	HQ902624	*S. xiamenensis* strain H3 (HQ418493)	100	Gammaproteobacteria
	Jan12	HQ902625	*Shewanella baltica* (AJ000216)	100	Gammaproteobacteria
Bands	Oct-M	HQ902626	*Microcystis aeruginosa* UADFM7 (HM854736)	99	Cyanobacteria
	Nov-M	HQ902627	*M. aeruginosa* UADFM7 (HM854736)	98	Cyanobacteria
	Dec-M	HQ902628	*M. aeruginosa* UADFM7 (HM854736)	100	Cyanobacteria
	Jan-M	HQ902629	*M. aeruginosa* UADFM7 (HM854736)	100	Cyanobacteria

associated with cyanobacterial blooms (Berg *et al.*, 2009; Kormas *et al.*, 2010). Evidence for *Aeromonas* chemotaxis to cyanobacteria (*Aphanizomenon flos-aquae*) was found by Kangatharalingam *et al.* (1991). The crude microcystin extracted from *Microcystis* could improve the change of *Aeromonas* from viable but non-culturable state to culturable state (Pan *et al.*, 2008). The presence of *Aeromonas* sp. may prevent *Microcystis* colonies from becoming prey

to zooplankton suggesting a potential increased risk for public health caused by cyanobacterial blooms. *Shewanella* sp. is a common aquatic Gram-negative bacterium with a worldwide distribution. It is capable of thriving at low temperatures and plays an important role in the re-mineralization of organic material in water column (Brettar and Höfle, 1993). In addition, *Shewanella putrefaciens* displays positive chemotaxis to most electron acceptors utilized for anaerobic growth (Nealson *et al.*, 1995). A positive effect of *Shewanella* sp. was found on the growth of *Nodularia spumigena*, with which it coexists in the Baltic Sea (Salomon *et al.*, 2003). The ability of *Shewanella* sp. to re-mineralize organic materials may be helpful in prolonging *Microcystis* blooms.

Other phylotypes are affiliated with genera, such as *Sphingomonas*, *Rhodobacter* and *Bradyrhizobium* (*Alphaproteobacteria*), *Flavobacterium* (*Bacteroidetes*), or *Pseudomonas* (*Gammaproteobacteria*). These genera are also associated with *Microcystis* spp. during the vigorous period of bloom (Shi *et al.*, 2010). Bacteria related to *Sphingomonas* or *Flavobacterium* are capable of degrading cyanobacterial toxins or other problematic organic complex compounds (Valeria *et al.*, 2006; Berg *et al.*, 2009).

Although bacterial community structure changed along with the bloom decline, persistent associations of *Aeromonas* spp. and *Shewanella* spp. with *Microcystis* colonies were observed. A possible explanation for the appearance of these distinct bacteria is the release of different extracellular compounds from the phytoplankton cell during the period of the bloom decline. For example, transparent exopolymer particles (TEP), composed of extracellular polysaccharide, dead algal cells and so on, are frequently formed during the decline of diatom and cyanobacterial bloom (Schuster and Herndl, 1995; Grossart and Simon, 1997), and many TEP are considered as excellent substrates for bacteria (Berman and Viner-Mozzini, 2001). However, the extracellular compounds from buoyant *Microcystis* cells during the bloom decline period are not very clear and need to be studied in the future. The ability of these bacteria to adapt to low temperatures could be another reason for their dominance. However, the mechanisms of these associations still need further study.

In conclusion, buoyant *Microcystis* colonies on the water surface harbor distinct bacterial communities. To our knowledge, this is the first report on microbial communities associated with buoyant *Microcystis* colonies, which would take an important role during the next summer bloom (Verspagen *et al.*, 2005), during the decline period of annual freshwater bloom. qRT-PCR revealed a gradual decrease in the ratio of bacteria associated with *Microcystis* colonies, indicating that *Microcystis* colonies remaining buoyant on the water surface are resistant to bacterial colonization. The colony-associated bacteria are dominated by *Gammaproteobacteria* and *Bacteroidetes*. Potential pathogens, such as *Aeromonas* sp. and *Shewanella* sp., are also present. These bacteria may facilitate the survival of the *Microcystis* colonies. They also highlight the potential ecological importance of the decline period of cyanobacterial blooms to the safety of lake water.

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