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Decrease of NH_4^+-N by bacterioplankton accelerated the removal of cyanobacterial blooms in aerated aquatic ecosystem

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Abstract

We used aerated systems to assess the influence of the bacterioplankton community on cyanobacterial blooms in algae/post-bloom of Lake Taihu, China. Bacterioplankton community diversity was evaluated by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting. Chemical analysis and nitrogen dynamic changes illustrated that NH_4^+-N was nitrified to NO_2^--N and NO_3^--N by bacterioplankton. Finally, NH_4^+-N was exhausted and NO_3^--N was denitrified to NO_2^--N, while the accumulation of NO_2^--N indicated that bacterioplankton with completely aerobic denitrification ability were lacking in the water samples collected from Lake Taihu. We suggested that adding completely aerobic denitrification bacteria (to denitrify NO_2^--N to N_2) would improve the water quality. PCR-DGGE and sequencing results showed that more than 1/3 of the bacterial species were associated with the removal of nitrogen, and *Acidovorax temperans* was the dominant one. PCR-DGGE, variation of nitrogen, removal efficiencies of chlorophyll-*a* and canonical correspondence analysis indicated that the bacterioplankton significantly influenced the physiological and biochemical changes of cyanobacteria. Additionally, the unweighted pair-group method with arithmetic means revealed there was no obvious harm to the microecosystem from aeration. The present study demonstrated that bacterioplankton can play crucial roles in aerated ecosystems, which could control the impact of cyanobacterial blooms in eutrophicated fresh water systems.

Key words: cyanobacterial blooms; PCR-DGGE; bacterioplankton community; aerated ecosystem

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Introduction

Eutrophication is a worldwide environmental problem that has tremendous impacts on the sustainable development of society and the economy (Jin et al., 2005). Due to pollution and eutrophication, cyanobacterial blooms, especially *Microcystis* blooms, are becoming a widespread problem in the aquatic environment (Paerl et al., 2001; Lehman et al., 2010).

Lake Taihu is the third largest shallow eutrophic lake in the eastern part of China (30°55′40″N–31°32′58″N and 119°52′32″E–120°36′10″E). Recently, cyanobacterial blooms have extended their coverage in summer, which affected the function of Lake Taihu as a drinking water supply (Yang et al., 2008). Cyanobacterial blooms have resulted in widespread deterioration of the quality of surface water and may create low oxygen conditions (Oliver and Ganf, 2002). During this process, large quantities of *Microcystis* will accumulate and decompose (Cole et al., 1982), resulting in the decrease of pH and dissolved oxygen (DO) (Chen et al., 2010). DO is rapidly exhausted below the surface of the blooms, and the micro environment quickly becomes anoxic (Wang et al., 2006). These physiochemical changes may have strong impacts on other aquatic organisms (Zhang et al., 2009).

Heterotrophic bacteria play crucial roles in biogeochemical cycling and energy flux (Azam et al., 1983), and the changes of bacterial community composition (BCC) may also decrease the water quality (Paerl et al., 2003; Masango et al., 2008). Numerous investigations have demonstrated that the seasonal dynamics of BCC is closely related to the decomposition of *Microcystis* blooms (Wu et al., 2007; Xing and Kong, 2007). Bacteria play an important role in the release of phosphorus accompanying the decomposition of *Microcystis* (Wang and Chen, 2008), while little is known about the influence of microbes on the nitrogen cycle, and their community dynamics during the process

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needs further investigation.

Therefore, we conducted mesocosm experiments using a *Microcystis* slurry that encompasses the natural range of *Microcystis* biomass from Lake Taihu, and the anaerobic/aerobic degradation process was allowed to proceed in glass aquariums for approximately 240 hr. The objective of this study was to characterize the BCC in water columns with different DO concentrations, to document *Microcystis* changes following the BCC and to identify physiochemical parameters of Lake Taihu.

1 Materials and methods

1.1 Experimental design

Experiments were conducted to simulate natural lake environments, with 2 L cyanobacterial slurry and 50 L water in six transparent glass aquariums (75 cm length × 55 cm width × 45 cm height). Two systems were applied to six aquariums (controls: No. 1, 2, 3, without aeration; treatments: No. 4, 5, 6, simulation of aerobic conditions). Water used in the ecological simulation experiment was collected from Gonghu Bay of Lake Taihu during cyanobacterial blooms. When the prepared water was transferred into the experimental aquariums, aerated treatment and light/dark cycling (12 hr/12 hr) were applied to simulate natural environments for 24 hr continuously, and this experiment was carried out for 240 hr. DO was maintained at 6 mg/L by stirring in the aerated systems. Triplicate samples of each treatment were analyzed at each sampling point.

1.2 Chemical analysis and chlorophyll-*a* determination

Chemical parameters, including total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), NH_4^+-N, NO_2^--N, NO_3^--N, and chlorophyll-*a* (Chl-*a*) were determined according to standard methods (Yan et al., 2009) .

1.3 DNA extraction and PCR-DGGE

Water samples were collected every 48 hr and filtrated through a 0.22-μm filter membrane (Millipore). The membranes were kept at –20°C for DNA extraction.

DNA was extracted with a DNA extraction kit (Omega). The primer sets and GC clamp (underlined) used were described by Muyzer et al. (1993) and Ovreås et al. (1997). PCR was carried out with primers 341f (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3') and 518r (5'-ATTACCGCGGCTGCTGG-3') to amplify the V3 regions of bacterial 16S rDNA (Liu et al., 2009). Each reaction mixture (25 μL) contained 2.5 μL of 10× PCR buffer (Fermentas), 0.2 mmol/L each of deoxyribonucleotide triphosphates (dNTPs), 0.5 μmol/L of each primer, 1 U of Ex Taq DNA polymerase (TaKaRa) and 5 ng of template DNA. Touchdown PCR was performed on a thermocycler (BioRad) according to the program: 94°C for 5 min, 94°C for 1 min, annealing (at 65–55°C for 1 min, decreasing 1°C each cycle) and extension (at 72°C for 1 min); 10 standard cycles of denaturation (at 94°C for 1 min), annealing (at 55°C for 1 min) and extension (at 72°C for 1 min), and a final extension at 72°C for 10 min. PCR products were verified and quantified by 1% agarose gel electrophoresis.

DGGE was performed with the D-Code Universal Mutation Detection System (BioRad) using 8% polyacrylamide (*W*/*V*). PCR products containing approximately equal amounts of DNA with similar sizes were separated on a gel containing a linear gradient of denaturants (urea and formamide). The concentration of denaturants increased from 40% at the top to 60% at the bottom of the gels (100% denaturant was defined as 7 mol/L urea and 40% formamide). Electrophoresis was performed at 56°C with 75 V for 15 hr. After that, gels were stained in 1× TAE buffer containing Gel Red (Biotium).

The gel was crushed in 40 μL sterile deionised water and allowed to equilibrate overnight at 4°C, and 3 μL of the DNA extract was used as the template in PCR for amplification as described above, except that the primer was 341f without the GC clamp. The PCR products were purified and further cloned into the pMD18-T cloning vector (TaKaRa) according to the manufacturer's instructions, and DNA sequencing was finished by Majorbio (Shanghai, China). All the sequences of the 16S rDNA were submitted to the NCBI.

1.4 Statistical analysis

Bands from the different lanes of each gel were identified, and a pairwise calculation was made using Bray-Curtis's similarity coefficient. The unweighted pair-group method with arithmetic means (UPGMA) clustering was used to investigate the similarity among samples. The removal efficiencies of Chl-*a* were compared using analysis of variance (ANOVA) followed by Tukey post hoc tests to identify significant differences between control and treatment. The statistically significant level was set at 0.01. To reveal relationships between BCC and environmental variables (including the physical and chemical parameters and biomasses of Chl-*a*), canonical correspondence analysis (CCA) was carried out with CANOCO (version 4.5).

2 Results and discussion

2.1 Variations of chemical parameters and removal efficiencies of Chl-*a*

The main physiochemical parameters are summarized in **Fig. 1**. The average water temperature ranged from 31 to 34°C. Concentrations of TN, TP, COD and pH fluctuated slightly during the whole experimental process. In unaerated aquariums, NH_4^+-N average concentrations remained at 4.32 mg/L levels before 96 hr, and even increased during the experimental process after 96 hr. It

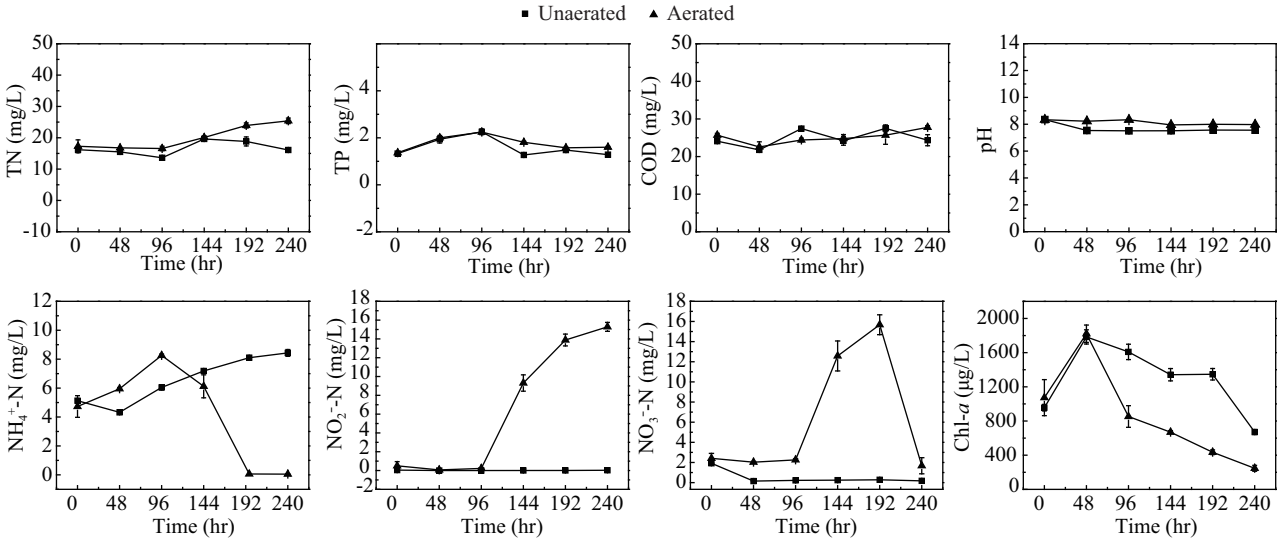

Fig. 1 Variation of TN, TP, COD, pH, NH_4^+-N, NO_2^--N, NO_3^--N and Chl-*a* between unaerated/aerated systems.

should be explained that nitrification was lacking under the anaerobic conditions and NH_4^+-N was accumulated. Meanwhile, the NO_2^--N and NO_3^--N concentrations approached zero. However, from 96 hr onward, NH_4^+-N concentrations decreased notably and the NO_2^--N and NO_3^--N concentrations increased sharply at the same time point in aerated aquariums.

Chl-*a* concentrations decreased drastically after 48 hr of incubation in all aquariums from 1784.11 to 670.91 μg/L (unaerated) and 1821.04 to 246.67 μg/L (aerated). Removal efficiencies of Chl-*a* between unaerated and aerated aquariums from 96 hr had significant differences ($p < 0.01$) and the reduction rate reached 69.6% and 98.4% at 240 hr, respectively (**Fig. 2**). Cyanobacteria began to decline naturally after 48 hr (**Fig. 1**), while cyanobacterial blooms were more effectively removed in aerated aquariums. We supposed that the decrease of NH_4^+-N at 96 hr aggravated the death of cyanobacteria.

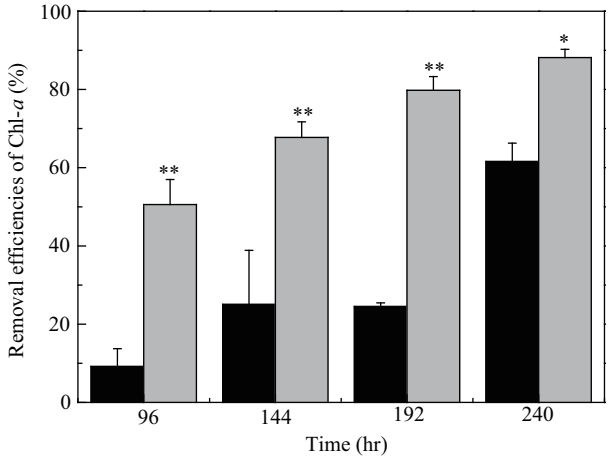

Fig. 2 Removal efficiencies of Chl-*a* between unaerated/aerated boxes (univariate ANOVA, $p < 0.01$).

2.2 PCR-DGGE fingerprinting and statistical analysis

The PCR-DGGE profiles of bacterial DNA extracted from the unaerated/aerated aquariums showed different patterns after 96 hr of incubation (**Fig. 3**). All the bands were marked according to the different locations (bands with intensity smaller than 0.05 were excluded from the analysis). Finally, thirty-five sequences are listed in **Table 1**. Band 17 became the dominant species after the incubation of 96 hr (**Fig. 3**). It belonged to *Acidovorax temperans*, which has been a consistent member of the general activated sludge microbial community, and widely used in wastewater treatment (Schloe et al., 2000). *A. temperans* have beneficial metabolic pathways to degrade biodegradable plastics and other organic contaminants, including nitrobenzene, nitrophenols, halobenzoates, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons, and for removal of nutrients by denitrification (Chen et al., 2009). After 192 hr, bands 6, 12, 17, 25, 26, 27 and 28 were the dominant

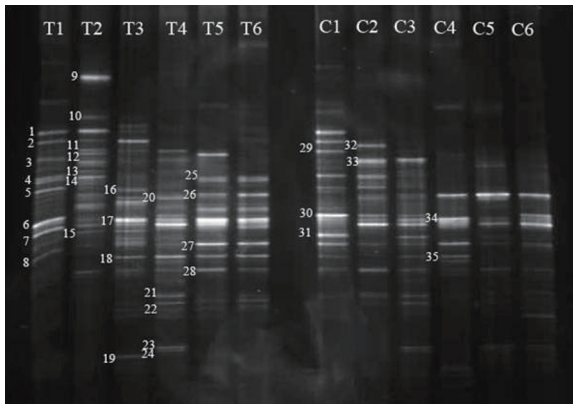

Fig. 3 DGGE band patterns obtained from both aerated and unaerated water samples. T: the boxes were aerated; C: the boxes were unaerated; 1, 2, 3, 4, 5, 6: the samples were collected at 0, 48, 96, 144, 192 and 240 hr, respectively. The numbers show the bands whose sequences were determined and correspond to the locations of bands.

Table 1 Information of 16S rDNA sequences of PCR-DGGE

Bands in **Fig. 3**	Length (bp)	Accession number	Species
Band 1	194	HQ691850	*Runella* sp.
Band 2	194	HQ691851	*Acinetobacter lwoffii*
Band 3	194	HQ691856	Gamma proteobacterium
Band 4	169	HQ691857	*Flavobacterium* sp.
Band 5	169	HQ691859	*Bacteriovorax* sp.
Band 6	195	HQ691863	*Brevundimonas intermedia*
Band 7	189	HQ691866	Uncultured actinobacterium
Band 8	189	HQ691868	Uncultured delta proteobacterium
Band 9	195	HQ691869	Uncultured beta proteobacterium
Band 10	194	HQ691845	*Stenotrophomonas maltophilia*
Band 11	194	HQ691848	*Bdellovibrio* sp.
Band 12	174	HQ691854	Uncultured actinobacterium
Band 13	194	HQ691855	Beta proteobacterium
Band 14	194	HQ691858	Uncultured Bacteroidetes bacterium
Band 15	189	HQ691860	*Bacteriovorax* sp.
Band 16	189	HQ691862	*Flavobacterium hercynium*
Band 17	195	HQ691872	*Acidovorax temperans*
Band 18	195	HQ691849	*Flavobacterium* sp.
Band 19	174	HQ691865	Gamma proteobacterium
Band 20	189	HQ691870	*Pseudomonas viridiflava*
Band 21	169	HQ691878	Alpha proteobacterium
Band 22	194	HQ691869	Uncultured beta proteobacterium
Band 23	194	HQ691853	*Legionella pneumophila*
Band 24	174	HQ691864	*Cellvibrio* sp.
Band 25	169	HQ691868	Uncultured delta proteobacterium
Band 26	194	HQ691852	Uncultured comamonadaceae
Band 27	194	HQ691871	Alpha proteobacterium
Band 28	194	HQ691873	*Hydrogenophaga defluvii*
Band 29	169	HQ691875	*Arcobacter cryaerophilus*
Band 30	194	HQ691878	*Sphingomonas* sp.
Band 31	194	HQ691880	*Runella* sp.
Band 32	194	HQ691881	*Acinetobacter lwoffii*
Band 33	194	HQ691882	*Candidatus rhodoluna*
Band 34	194	HQ691883	Uncultured alpha proteobacterium
Band 35	169	HQ691884	Uncultured beta proteobacterium

species, which belonged to proteobacteria (**Table 1**), and it is well known that members of the gamma and alpha proteobacterium bacterial groups can be responsible for nitrogen and phosphorus removal (Chen et al., 2011). Bands 7, 12, 14 and 26 could not be matched unequivocally to species from BLAST analysis.

Additionally, microbial communities in unaerated and aerated aquariums were further assessed using UPGMA (dendrogram) based on the time and intensity of the bacterioplanktonic bands. The similarity of bacterioplankton communities, as shown by the DGGE bands of 16S rDNA, indicated that the samples derived at 192 and 240 hr were the same as those at 0 hr in aerated aquariums (**Fig. 4b**). However, bacterial communities in unaerated groups were highly diverse with incubation time (**Fig. 4a**). UPGMA results of cluster analysis revealed that the bacterioplankton community was not significantly affected by the aerated treatment. Therefore, aeration had no obvious harmful effects on the water microecosystem in our experiment, which was consistent with Yan et al. (2009).

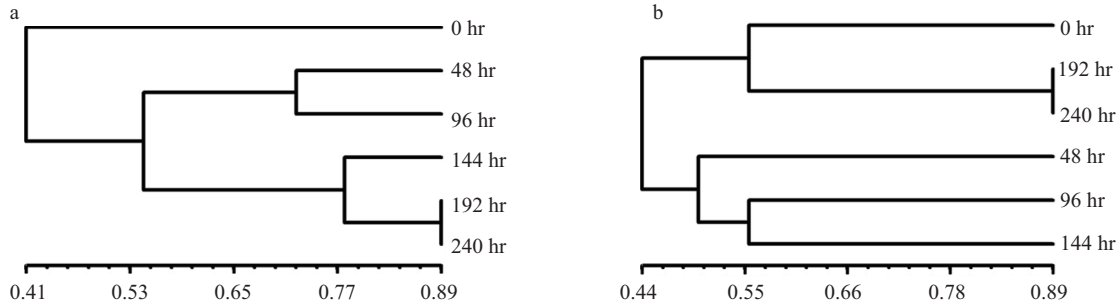

Fig. 4 Dendrograms displaying results of cluster analysis using the Bray-Curtis measure of similarity among samples from unaerated aquariums (a) and aerated aquariums (b).

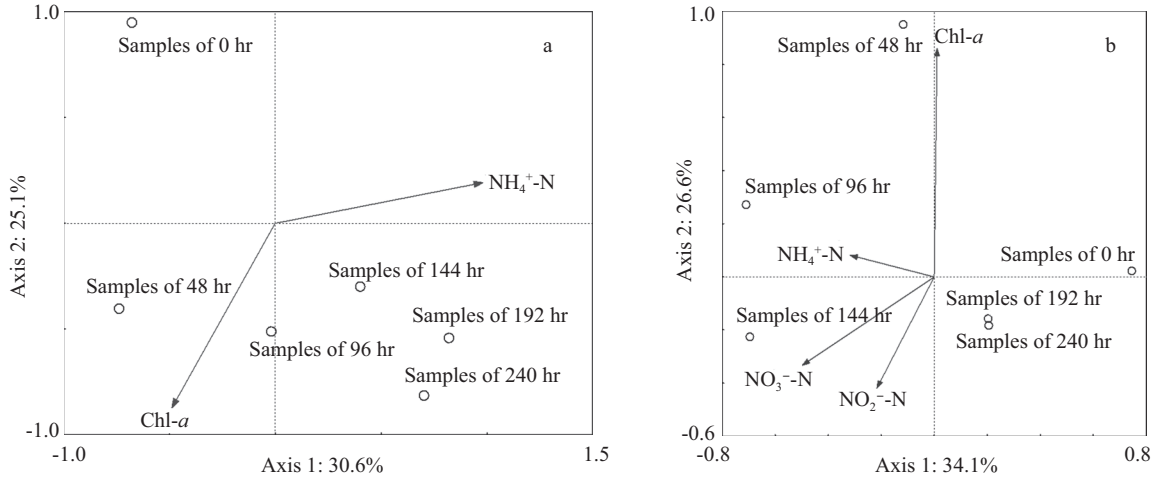

Fig. 5 CCA biplot based on DGGE data and environmental variables of unaerated (a) and aerated aquariums (b).

2.3 Effects of bacterioplankton on variations in the nitrogen cycle and Chl-*a*

The PCR-DGGE profiles showed different patterns after 96 hr of incubation. Interestingly, changes in NH_4^+-N, NO_2^--N, NO_3^--N and Chl-*a* concentrations coincided with changes in microorganism diversities (**Figs. 1**, **2**, and **3**). Nitrification and denitrification are important steps in the global nitrogen cycle (Zhang et al., 2011), and several bacteria are involved in the main biochemical reactions during aeration (Ollivier et al., 2011). It was reported that cyanobacteria are related to phosphorus and nitrogen cycles during eutrophication of lakes, rivers and estuaries (Chen et al., 2010), which could be influenced by the nitrification and denitrification effects of bacteria in this process. Denitrification could also occur under fully aerobic conditions (Robertson and Kuenen, 1984; Chen et al., 2003), and these phenomena indicated that aeration increased the abundance of the nitrifying and denitrifying bacteria in our experiment, resulting in higher mortality rates of cyanobacteria in aerated aquariums. NH_4^+-N was nitrified to NO_2^--N and NO_3^--N after 96 hr. As it was exhausted at the end of 192 hr, NO_3^--N was denitrified and caused the further accumulation of NO_2^--N. This demonstrated that bacteria with completely aerobic denitrifying ability were lacking in the water samples collected from Lake Taihu. High concentrations of NO_2^--N are harmful to the environment, aquatic and human health, and bacteria with completely aerobic denitrification ability would be able to transform NO_2^--N to N_2 to decrease water hazard (Chang et al., 2011).

According to the CCA results for unaerated/aerated aquariums (**Fig. 5a**), differences in BCC were related to the two most important environmental variables (concentrations of NH_4^+-N and Chl-*a*, $p < 0.05$) in unaerated aquariums. The two axes explained 55.7% of the observed variation in BCC. Similarly, the statistical results of CCA (**Fig. 5b**) illustrated that the differences in BCC were related to the four most important environmental variables (concentrations of NH_4^+-N, NO_3^--N, NO_2^--N and Chl-*a*) in aerated aquariums. The two axes explained 60.7% of the observed variation in BCC. Bacterioplankton played cruial roles in the nitrogen cycle and Chl-*a* variations in this process.

3 Conclusions

In the present study, bacterioplankton influenced the nitrogen cycle in an aerobic environment by nitrification/denitrification, resulting in physiological and biochemical changes to cyanobacteria. Bacterial accumulation and NH_4^+-N exhaustion were coincident with the removal of the cyanobacterial bloom. Therefore, we concluded that bacteria have an influence on the physiological changes of cyanobacteria. The relationship between bacterioplankton and cyanobacteria was complicated, and further studies would give a more clear explanation in the future.

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