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Full Length Research Paper

Effects of lake water chemistry on bacterioplankton community structures of three lakes

Dayong Zhao[1,2*], Ting Ma[1], Jin Zeng[3], Wenming Yan[2], Jianqun Wang[1], Rui Huang[1] and Meng Wang[1]

[1]College of Hydrology and Water Resources, Hohai University, Nanjing, 210098, China.

[2]State Key Laboratory of Hydrology-Water Resources and Hydraulic Engineering, Hohai University, Nanjing, 210098, China.

[3]State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China.

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To investigate the effects of lake water chemistry on bacterioplankton community composition, three different sized lakes (Lake Taihu, Lake Zixia and Lake Pipa) were studied. Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA, followed by clone library analysis was used to explore the bacterial community structure in lake water. Cluster analysis of DGGE patterns indicated that bacterioplankton community compositions within one lake were similar. Canonical correspondence analysis (CCA) was carried out to interpret the response of the bacterial community structure to water chemistry. Sampling lakes and total phosphorus were two variables found to significantly correlate with DGGE patterns. Clone library analysis results indicated that the bacterioplankton community composition of three lakes differed markedly, even at the phylum and subphylum levels. The results of the homologous and heterologous coverage curves analysis based on the LIBSHUFF program also showed significant differences among the three clone libraries.

Key words: Bacterioplankton community structures, denaturing gradient gel electrophoresis (DGGE), multivariate analysis and water chemistry.

INTRODUCTION

In recent years, Lake eutrophication has become one of the most serious environmental problems worldwide. Excess inputs of nitrogen and phosphorus in freshwater ecosystems are the main reasons for Lake eutrophication. Bacteria are the dominant participants of nutrients biogeochemical cycling and are also the key factor to maintain the virtuous circle of the lake ecosystem (Pace, 1997). Therefore, it is especially important to investigate the bacterial community structures and diversities for comprehensively understanding the compound biogeochemical process in the lake ecosystem (Tamaki et al., 2005). Traditional techniques such as the culture-dependent and microscope have been used to investigate the microbial ecology in various environments for very long time. In recent years, a series of culture-independent methods based on the bacterial 16S rRNA such as denaturing gradient gel electrophoresis (DGGE) and restriction fragment length polymorphism (RFLP) have been widely employed to investigate the microbial communities in environmental samples, which have been greatly broadened the knowledge of microbial diversities (Wise et al., 1997; Eiler and Bertilsson, 2004; Schwarz et al., 2007; Wu et al., 2007; Yang et al., 2011).

Bacterioplankton is the prokaryotic group living in the water column of the lake ecosystem. More and more attentions have been paid on the community structure of bacterioplankton and its response to environmental

*Corresponding author. E-mail: dyzhao@hhu.edu.cn. Tel: +86 25 83787891. Fax: +86 25 83787891.

variables in the lake ecosystem (Raymond and Bauer, 2000). Community structure of bacterioplankton is closely related to the physicochemical characteristics of lake water (Wu et al., 2007).

However, only few studies explain the dynamics of microbial community structures based on water chemistry (Sapp et al., 2007). Several multivariable analysis methods such as principal com-ponent analysis (PCA), multidimensional scaling (MDS) and canonical correspondence analysis (CCA) have been proved more effective in elucidating the relationship between microbial community composition and environmental factors (Iwamoto et al., 2000; Salles et al., 2006). Based on these techniques, nitrogen, phosphorus, organic matters and pH have been considered as the key factors in influencing the microbial community structure (Rooney-Varga et al., 2005; Haukka et al., 2006).

Lake Taihu is a large, shallow lake located in the Middle and Lower Yangtze River regions (area: 2338 km^2; average depth: 1.9 m). Water input amount of Lake Taihu is about 8×10^9 m^3 every year and the hydraulic retention time is about 5 months (Qin et al., 2007). Lake Taihu was given oligotrophic status before the 1950s, however, large amounts of nutrients input has led to eutrophication (Cai and Gao, 1995; Chen et al., 2003). Lake Zixia (area: 2.2×10^5 m^2; maximum depth: 8.6 m) is a small, urban lake located in the scenic spot of Sun Yat-sen's Mausoleum of Nanjing, China (Bian et al., 2010). Lake Zixia is in mesotrophic status in 2006 and it became eutrophic in 2009. Lake Pipa (area: 5.46×10^4 m^2) is an urban mini lake located in zhongshan scenic spot of Nanjing, China. Domestic wastewater from a hotel near Lake Pipa has led to sever eutrophication. The hotel was demolished in October of 2005 and water quality is expected to recover slowly.

In this study, DGGE and clone library techniques were employed to investigate the differences of the bacterioplankton community structures in three different scale lakes. The acquired data were analyzed and combined with water characteristics using multivariate analysis to demonstrate the microbial responses to water chemistry.

MATERIALS AND METHODS

Water sample collection

In October of 2010, water samples were collected from three different scale lakes (Lake Taihu, Lake Zixia and Lake Pipa) for bacterioplankton community structures and water chemistry analysis. Five (Lake Taihu), four (Lake Zixia) and two (Lake Pipa) sampling sites were chosen from three lakes and the GPS positions were shown in Table 1.

Water sample collection followed the methods described by Jin and Tu (1990), water samples were collected at 0.5 m depth of each site. Three replicate samples at each station were combined equally and stored in sterile plastic bottles. All the samples were transported to the laboratory within 1 h for further analysis.

Water chemistry analysis

pH was analyzed *in situ* with specific electrodes (PHB-4, REX, China). Other parameters were analyzed immediately after samples were transported to the laboratory. Concentrations of total nitrogen (TN), total phosphorus (TP), ammonia (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) were analyzed with continuous flow analyzer (San++, SKALAR, Netherlands).

DNA extraction

Water samples were passed through 5 µm filters to remove the phytoplankton and large suspended particles. The obtained filtrate was then passed through 0.22 µm cellulose acetate filters to concentrate bacterioplankton cells. DNA was extracted following the in situ lysis method of (Gillan, 2004) with some modifications. The filters were added with 0.3 ml sterile H_2O, 70 µl Tris-HCl buffer (pH 8.0), 70 µl 0.5 M EDTA and 150 µl 10% SDS. The tubes were maintained at 65°C for 60 min with gentle end-over-end inversions every 15 min. Samples were then centrifuged at 10000 rpm for 10 min. Supernatant was transferred to another tube and added with 90 µl 5 M NaCl, 70 µl CTAB (10%) and 10 µl proteinase K (20 mg/ml). The mixture was incubated at 65°C for 10 min and added with 0.5 ml chloroform–isoamyl alcohol (24:1). After 5 min centrifugation at 3000 rpm, aqueous upper phase was transferred into a clean tube and 75 µl NaAc (3 M) and 450 µl isopropyl alcohol were added. To precipitate the DNA, the tubes were incubated for 2 h at -20°C and centrifuged for 20 min at 12000 rpm (4°C). After elimination of the supernatant, the DNA pellet was washed with 1 ml 75% ethanol and centrifuged for 10 min to remove ethanol. The pellet was air-dried and dissolved in 50 µl of TE buffer.

PCR-DGGE analysis

PCR amplification was carried out using the *Bacteria*-specific primers 341f (5′- CCTACGGGAGGCAGCAG-3′) with a 40 bp GC-clamp and 926r (5′-CCGTCAATT CCTTTGAGTTT-3′) (Muyzer et al., 1993). The 50 µl PCR reaction mixture contained 5 µl of 10 × buffer (ExTaq, Takara, Japan), 2 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTPs (Takara), 1.25 µl of each primer (10 mM) (Invitrogen, Shanghai Branch, China), 2 U Taq DNA polymerase (Takara) and 1 µl of DNA template. The amplification program was 5 min at 95°C; 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C; and finally 10 min at 72°C. The amplification products were detected by 1.5% agarose gel electrophoresis.

PCR products were loaded on a 6% polyacrylamide gel with a 30 to 60% DNA-denaturant gradient using the DGGE-2001 system (CBS, USA). The gel was run at 100 V for 16 h at 60°C in 1 × TAE buffer. Afterwards, the gel was stained with SYBR Green I for 30 min and documented using a Gel Doc system (Bio-Rad Laboratories, Hercules, CA). The acquired DGGE pattern was processed using the Gelcompar II software package (Applied Maths, Inc., USA) with the presence and absence model. After normalization, all bands with relative peak intensities greater than 2% were included for analysis.

Cluster analysis of DGGE profiles was performed using the Gelcompar II package. A dendrogram was constructed using the unweighted pair group method (UPGMA) based on the Dice similarity coefficient calculated from the complete set of densitometric curves.

Table 1. Sampling station locations and water characteristics of Lake Pipa, Lake Zixia and Lake Taihu.

Sampling stations	GPS	pH	TN (mg/L)	TP (mg/l)	NO_3^-(mg/L)	NH_4^+ (mg/L)	NO_2^- (mg/L)
P1	32º03′20.37″N, 118º48′59.60″E	7.56	1.02±0.01	0.08±0.008	0.12±0.003	0.19±0.08	0.030±0.003
P2	32º03′18.25″N, 118º49′04.19″E	7.47	0.58±0.02	0.06±0.008	0.11±0.000	0.07±0.00	0.021±0.003
Z1	32º03′42.64″N, 118º50′17.68″E	7.90	4.08±0.08	0.19±0.018	1.02±0.020	0.31±0.04	0.032±0.001
Z2	32º03′41.95″N, 118º50′20.79″E	7.61	2.09±0.06	0.16±0.001	1.12±0.003	0.08±0.03	0.021±0.003
Z3	32º03′43.57″N, 118º50′22.96″E	7.35	1.98±0.00	0.14±0.004	0.21±0.028	0.04±0.03	0.061±0.002
Z4	32º03′45.56″N, 118º50′20.49″E	7.12	2.70±0.03	0.11±0.003	1.15±0.040	0.08±0.01	0.032±0.002
T1	31º00′27.35″N, 120º27′31.23″E	6.98	0.96±0.10	0.05±0.017	0.42±0.004	0.25±0.02	0.002±0.001
T2	31º06′15.05″N, 120º29′00.64″E	7.24	0.78±0.03	0.02±0.006	0.24±0.017	0.20±0.01	0.003±0.000
T3	31º12′32.41″N, 120º26′45.44″E	7.38	0.55±0.09	0.01±0.002	0.16±0.021	0.16±0.01	0.011±0.004
T4	31º26′41.52″N, 120º18′42.18″E	7.23	0.55±0.05	0.01±0.003	0.16±0.024	0.15±0.01	0.009±0.002
T5	31º06′01.24″N, 120º18′27.52″E	7.34	0.82±0.03	0.02±0.002	0.23±0.002	0.21±0.01	0.011±0.004

Cloning, sequencing and phylogenetic analysis

Three clone libraries were constructed with samples amplified from Lake Pipa, Lake Zixia and Lake Taihu. For amplification equal amounts of samples, DNA extracted from sites P1 and P2 (Lake Pipa), Z1, Z2, Z3, Z4 and Z5 (Lake Zixia) and T1, T2, T3, T4 and T5 (Lake Taihu) were mixed, respectively. PCR amplification of bacterial 16S rRNA genes was performed using the primer set Bac27f/Bac1492r as described by Crump and Koch (2008). PCR products were purified and ligated into the pGEM-T vector (Promega, Madison, WI, USA) following the manufacturer's instructions. Plasmids were transformed into *Escherichia coli* cells (DH5a, Takara, Japan), and the positive clones were randomly selected for sequencing at Shanghai Majorbio Bio-technology Co., Ltd.

The Mallard software package was used to identify the chimeric sequences and all suspicious sequences were excluded from further analysis (Ashelford et al., 2006). The remaining sequences were compared with GenBank entries using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The Ribosomal Database Project classifier was applied to assign the acquired sequences to the taxonomical hierarchy (Wang et al., 2007). Then the sequences obtained in this work were aligned separately using Clustal
X 1.8 (Thompson et al., 1997). A neighbor-joining tree with Jukes–Cantordistancecorrection(Kuhnerand Felsenstein, 1994) was built using MEGA 4.0 software package (Tamura et al., 2007).

Nucleotide sequence accession numbers

The nucleotide sequences of the 16S rRNA genes obtained in this study has been deposited in the GenBank database under accession numbers JF811458–JF811578.

Statistical analysis

Principal component analysis (PCA) was performed to investigate the water characteristics collected from three different lakes using the MVSP 3.1 software package (Kovach, UK). Multivariate analysis was performed to reveal the relationship between bacterioplankton community structures and environmental factors with the CANOCO 4.53 software package (Biometris, Netherlands). The detrended correspondence analysis (DCA) results indicated the suitability of CCA to explain the data set. Environmental factors that best described the most influential gradients were identified by forward selection (Ter Braak, 1987). Species data were acquired from the output matrix (showing the presence and absence of DGGE bands) of the Gelcompar II package. Both of the environmental data and specie data were not trans-formed for analysis. The significance of the environmental factors was tested using Monte Carlo permutation tests (499 unrestricted permutations, $P < 0.05$) (Lepš and Šmilauer, 2003). To determine if any significant differences existed between two clone libraries, variation between homologous coverage curves and heterologous coverage curves were calculated and compared statistically with the LIBSHUFF program (Singleton et al., 2001).

RESULTS

Lake water characteristics

Results of the lake water characteristics collected from three different scale lakes are shown in Table 1. The pH values of the lake water collected from Lake Pipa and Lake Zixia were higher than those of Lake Taihu. The total nitrogen (TN) concentrations in the four sampling stations of Lake Zixia were closely to or higher than 2 mg/l, which were significantly higher than those of the other lakes. Water collected from Lake Taihu maintained the lowest concentrations of TN

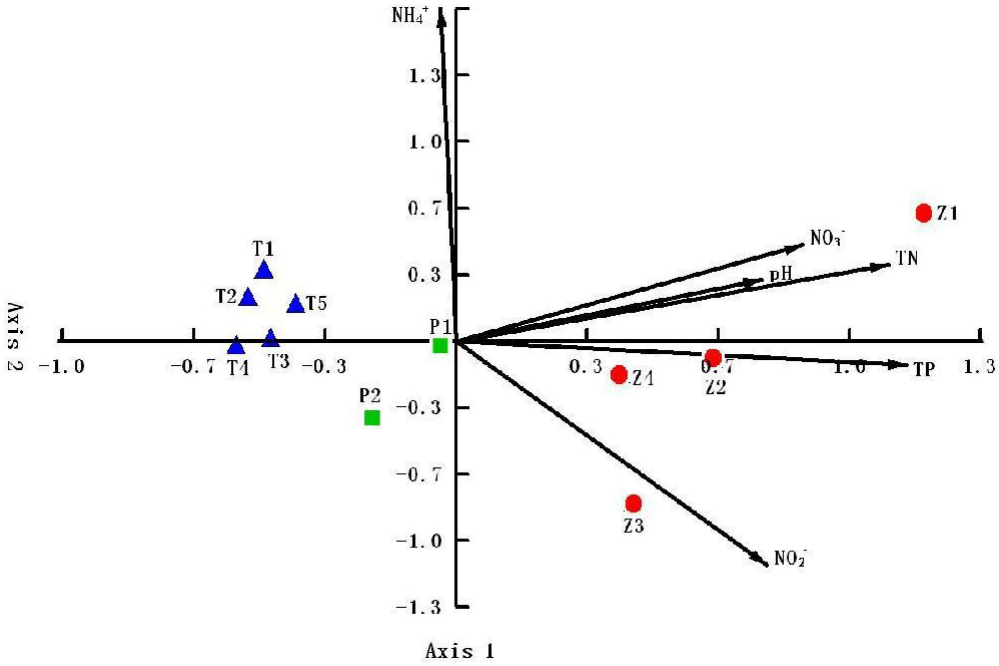


Figure 1. Principal component analysis of the water chemistry factors from three different lakes. Blue symbols indicate samples from Lake Taihu. Red and yellow symbols indicate samples from Lake Zixia and Lake pipa.

(average value: 0.73 mg/L). The total phosphorus (TP) concentrations in Lake Zixia were higher than 0.1 mg/L, which were the highest among all three lakes. TP concentrations in Lake Taihu were all less than 0.05 mg/l. Small variations of nitrite concentrations were observed in different lakes with all the values were below 0.1 mg/L.

To investigate the differences of water characteristics among different lakes, principal component analysis (PCA) was carried out and the result was shown in Figure 1. In Figure 1, the first axis explained a large proportion of the total variance (55.28%), and the second axis explained 23.23%. TN and TP were better correlated with the first axis. Environmental factors contributing more to the second axis were NO_2^- and NH_4^+. Sampling stations (T1 to T5) in Lake Taihu formed an individual cluster, indicating similar water characteristics in these stations. Sampling stations in Lake Pipa and Lake Taihu distributed on the left of the second axis, suggesting the similar water quality in the two lakes. The distributions from sampling stations (Z1 to Z4) were dispersed on the right of the second axis, suggesting a greater fluctuation of water quality in Lake Zixia.

DGGE analysis

Figure 2 shows the DGGE banding pattern of the bacterioplankton community structure from three different lakes. Each sample contained several separated DGGE bands, however, the numbers and migration position of the separated bands were different and several specific DGGE bands could be observed. According to the output result of Gelcompar II, the average numbers of DGGE bands separated from Lake Pipa, Lake Zixia and Lake Taihu were 15, 21.3 and 20.6, respectively. Sampling stations of T2, T3, T5 and Z2 maintained the most number of DGGE bands (23 bands), compared with station P2, which possessed the least number of DGGE bands (14 bands). The Student's *t*-test demonstrated that the number of DGGE bands obtained from the Lake Pipa samples were significantly lower than those of the samples from the other two lakes ($P < 0.05$).

Additionally, the unweighted pair group method with arithmetic mean (UPGMA) was carried out to investigate the similarities of bacterioplankton community structure among different samples (Figure 3). All the eleven samples clustered into four main groups. Group I included the two samples collected from Lake Pipa, the four samples collected from Lake Zixia formed group II and the Lake Taihu samples made up group III (T2 and T3) and group IV (T1, T4 and T5).

Results of canonical correspondence analysis (CCA) are shown in Figure 4. The first axis maintained the highest eigenvalue of 0.454. Therefore, the fluctuation of environmental factors along the first axis would induce the largest effects on the bacterioplankton community

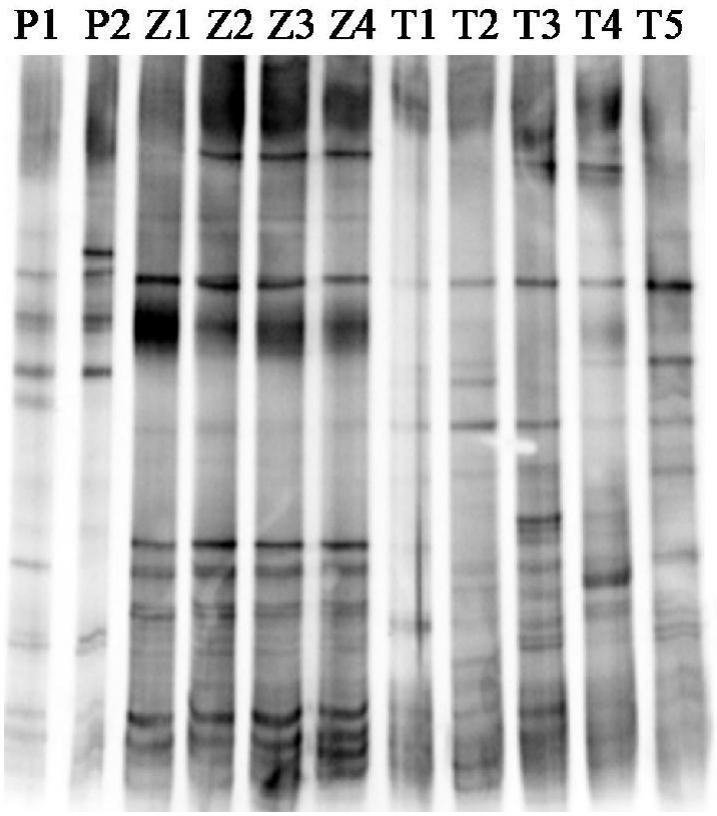


Figure 2. DGGE pattern of the bacterioplankton community structure from three different lakes.

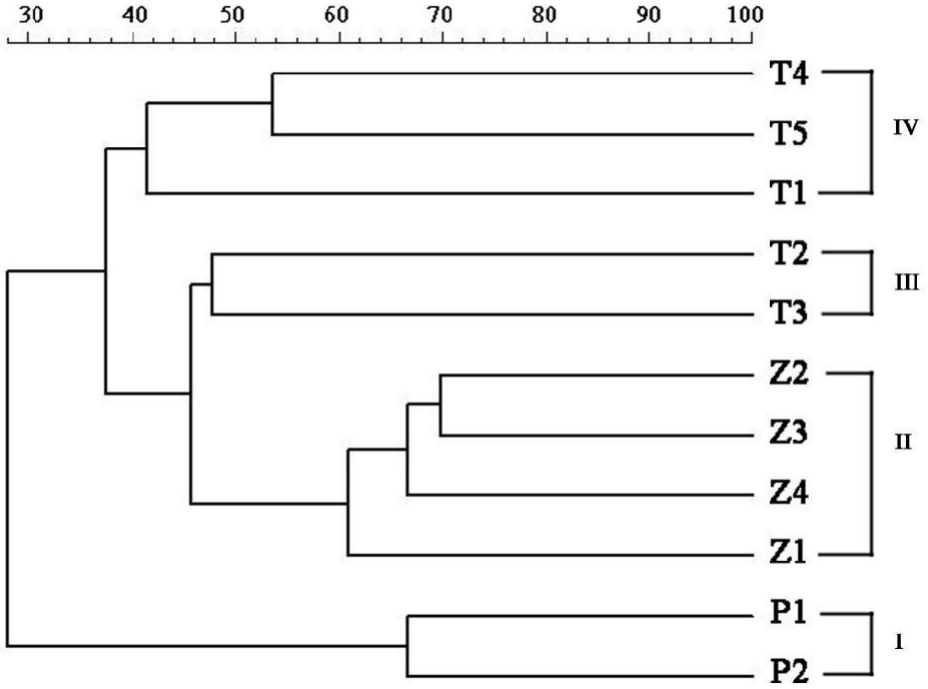


Figure 3. Cluster analysis of the DGGE samples collected from three different lakes based on the unweighted pair group method with arithmetic mean (UPGMA).

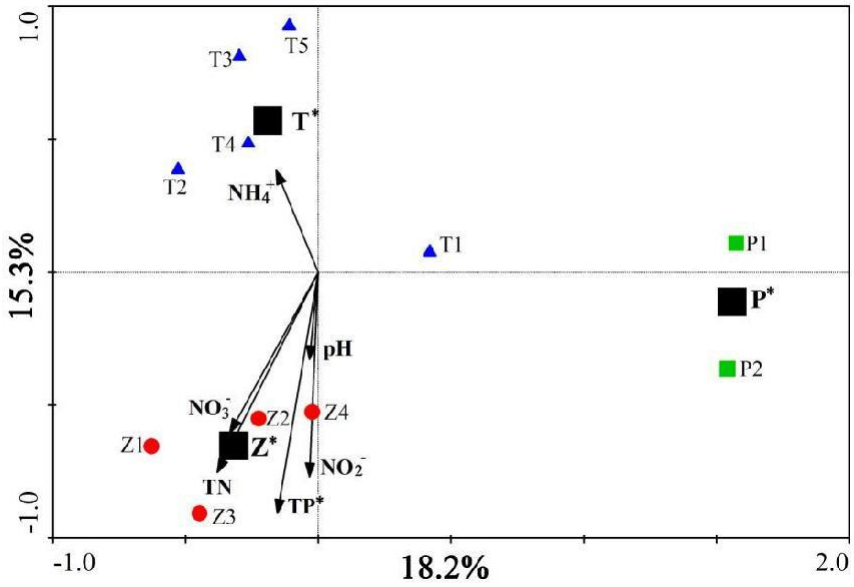


Figure 4. Relationship between water qualities and bacterioplankton community structure from three different lakes revealed by canonical correspondence analysis (CCA).

structure. The first and second axes combined explained 33.5% of the environmental variables. Arrows represented environmental variables and samples collected from different lakes were indicated with different symbols. In Figure 4, the samples from three different lakes formed individual cluster although T1 to T5 was relative scattered. Environmental variables that significantly affected the bacterioplankton community structure, such as TP and sampling lakes, are marked with asterisks.

Clone library analysis

There are totally 41, 41 and 39 non-chimeric 16S rRNA sequences obtained from the Lake Taihu, Lake Zixia and Lake Pipa clone library, respectively (Table 2). Phylogenetic analysis results indicated that the three libraries differed markedly even at the phylum and subphylum levels. Nearly similar percentages of clones affiliated with alpha-, beta- and gammaproteobacteria were observed in the Lake Pipa library. However, betaproteobacteria was the dominating bacterial group compared with alpha - and gammaproteobacteria in the Lake Zixia library. More than 35% of the Lake Pipa library was from the cyanobacteria phylum, indicating that it was the most important group in this lake. Lower percentages of clones affiliated with OD1, Gemmatimonadetes and Planctomycetes were present in the Lake Taihu library, whereas these groups were not observed in the libraries of Lake Zixia and Lake Pipa.

Statistical comparison results of homologous and heterologous coverage curves based on the LIBSHUFF program are shown in Table 3. Comparisons between the Lake Taihu clone library of and the libraries from the other lakes revealed significant differences (P = 0.001). Comparison between Lake Pipa and Lake Zixia libraries yielded a P value of 0.033 (the Lake Pipa library is homologous), suggesting some overlap between the two libraries. In contrast, a P value of 0.003 (the Lake Pipa library is heterologous) was also observed, suggesting that the Lake Zixia library contained more taxa that were not found in the Lake Pipa library.

There are totally 16, 15 and 13 clones affiliated with Proteobacteria obtained from the Lake Taihu, Lake Zixia and Lake Pipa library, respectively (Table 2). These Proteobacteria clones were highly diverse (Figure 5A) and many of the obtained sequences were affiliated with the previously reported freshwater clusters (Mueller-Spitz et al., 2009). For example, alphaproteobacteria detected in this study were affiliated with environmental sequences previously obtained from Lake Taihu (GenBank Accession No.: EU373202) and Xiangjiang River estuary (GenBank Accession No.: HQ132387).

Betaproteobacteria and gammaproteobacteria were also affiliated with previous sequences isolated from Lake Zixia (GenBank Accession No.: GU323668), Lake Michigan (GenBank Accession No.: EU642146) and an Austrian oligo-mesotrophic lake (GenBank Accession No.: AM849429). Other than Proteobacteria, dominating bacterial groups such as Bacterioidetes, Cyanobacteria and Actinobacteria were also detected in this study (Figure 5B). Bacterioidetes sequences related with

Table 2. Phylogenetic analysis of clone library of bacterioplankton in Lake Taihu, Lake Zixia and Lake Pipa.

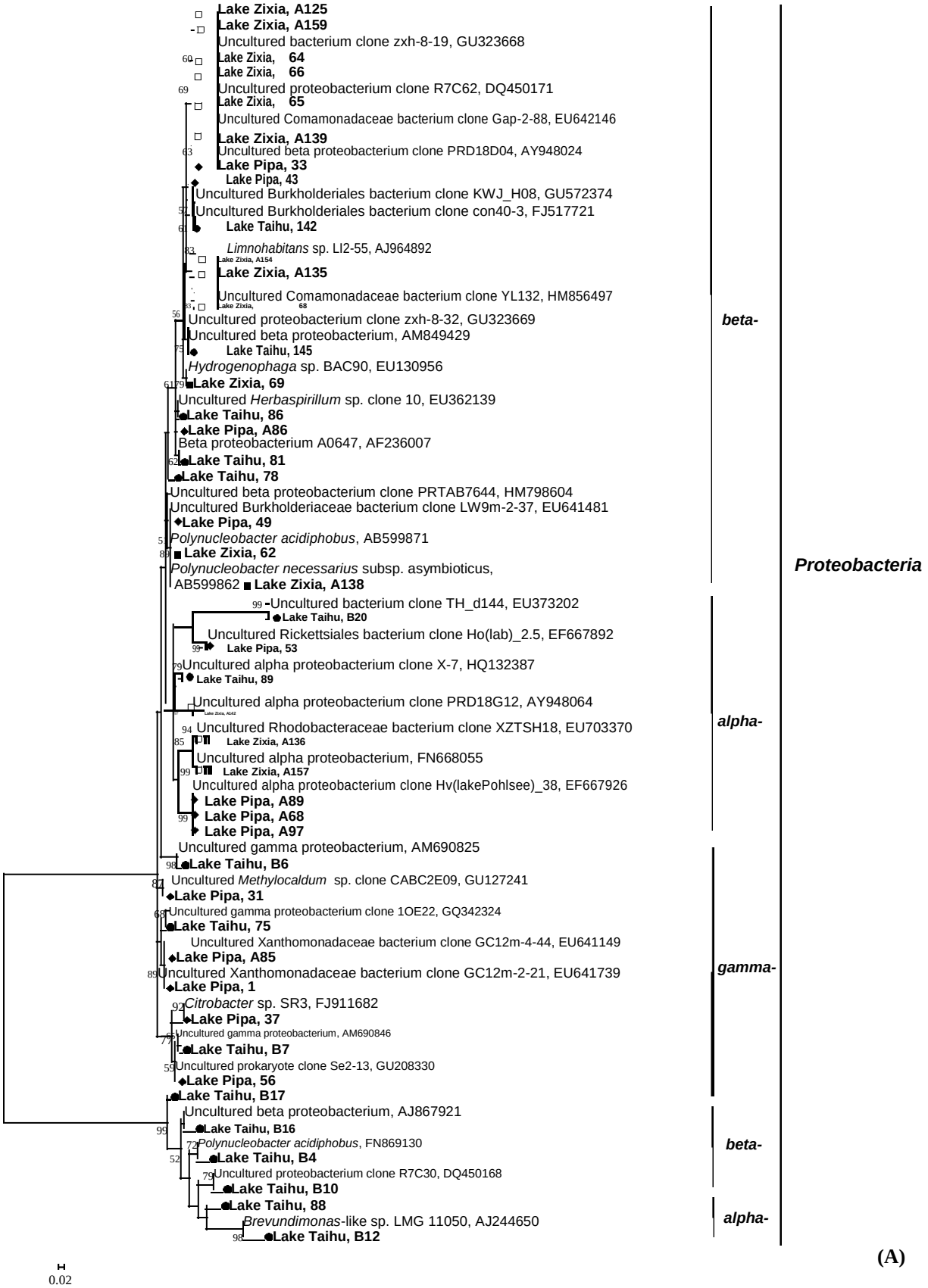
Bacteria group	Lake Taihu		Lake Zixia		Lake Pipa	
	No. of clones	Frequencies (%)	No. of clones	Frequencies (%)	No. of clones	Frequencies (%)
Alphaproteobacteria	2	4.9	3	7.3	4	10.3
Betaproteobacteria	8	19.5	12	29.2	4	10.3
Gammaproteobacteria	6	14.6	N.D.	N.D.	5	12.8
Bacteroidetes	7	17.1	9	22.0	8	20.5
Actinobacteria	6	14.6	12	29.3	3	7.7
Verrucomicrobia	4	9.8	N.D.	N.D.	1	2.6
Cyanobacteria	5	12.2	5	12.2	14	35.9
OD1	1	2.4	N.D.	N.D.	N.D.	N.D.
Gemmatimonadetes	1	2.4	N.D.	N.D.	N.D.	N.D.
Planctomycetes	1	2.4	N.D.	N.D.	N.D.	N.D.
Total	41	100	41	100	39	100

N.D: not detected.

Table 3. LIBSHUFF comparative analyses of clone libraries. *P* value < 0.05 was considered as significantly different. Numbers in brackets represent the number of clones in each library. X: Homologous library; Y: Heterologous library.

Library	*P*-value
Lake Taihu (41) and lake Zixia (41)	
XY	0.001
YX	0.001
Lake Taihu (41) and Lake pipa (39)	
XY	0.001
YX	0.001
Lake Zixia (41) and Lake pipa (39)	
XY	0.003
YX	0.033

species including uncultured or cultured bacteria isolated from freshwater ecosystem, such as Lake Taihu (GenBank Accession No.: EU373118, HQ905090 and DQ235055), Lake Michigan (GenBank Accession No.: EU640182) and Lake Dongping (GenBank Accession No.: FJ612348).


Lake Zixia, A125
Lake Zixia, A159
Uncultured bacterium clone zxh-8-19, GU323668
Lake Zixia, 64
Lake Zixia, 66
Uncultured proteobacterium clone R7C62, DQ450171
Lake Zixia, 65
Uncultured Comamonadaceae bacterium clone Gap-2-88, EU642146
Lake Zixia, A139
Uncultured beta proteobacterium clone PRD18D04, AY948024
Lake Pipa, 33
Lake Pipa, 43
Uncultured Burkholderiales bacterium clone KWJ_H08, GU572374
Uncultured Burkholderiales bacterium clone con40-3, FJ517721
Lake Taihu, 142
Limnohabitans sp. LI2-55, AJ964892
Lake Zixia, A154
Lake Zixia, A135
Uncultured Comamonadaceae bacterium clone YL132, HM856497
Lake Zixia, 68
Uncultured proteobacterium clone zxh-8-32, GU323669
Uncultured beta proteobacterium, AM849429
Lake Taihu, 145
Hydrogenophaga sp. BAC90, EU130956
Lake Zixia, 69
Uncultured Herbaspirillum sp. clone 10, EU362139
Lake Taihu, 86
Lake Pipa, A86
Beta proteobacterium A0647, AF236007
Lake Taihu, 81
Lake Taihu, 78
Uncultured beta proteobacterium clone PRTAB7644, HM798604
Uncultured Burkholderiaceae bacterium clone LW9m-2-37, EU641481
Lake Pipa, 49
Polynucleobacter acidiphobus, AB599871
Lake Zixia, 62
Polynucleobacter necessarius subsp. asymbioticus, AB599862
Lake Zixia, A138
Uncultured bacterium clone TH_d144, EU373202
Lake Taihu, B20
Uncultured Rickettsiales bacterium clone Ho(lab)_2.5, EF667892
Lake Pipa, 53
Uncultured alpha proteobacterium clone X-7, HQ132387
Lake Taihu, 89
Uncultured alpha proteobacterium clone PRD18G12, AY948064
Lake Zixia, A142
Uncultured Rhodobacteraceae bacterium clone XZTSH18, EU703370
Lake Zixia, A136
Uncultured alpha proteobacterium, FN668055
Lake Zixia, A157
Uncultured alpha proteobacterium clone Hv(lakePohlsee)_38, EF667926
Lake Pipa, A89
Lake Pipa, A68
Lake Pipa, A97
Uncultured gamma proteobacterium, AM690825
Lake Taihu, B6
Uncultured Methylocaldum sp. clone CABC2E09, GU127241
Lake Pipa, 31
Uncultured gamma proteobacterium clone 1OE22, GQ342324
Lake Taihu, 75
Uncultured Xanthomonadaceae bacterium clone GC12m-4-44, EU641149
Lake Pipa, A85
Uncultured Xanthomonadaceae bacterium clone GC12m-2-21, EU641739
Lake Pipa, 1
Citrobacter sp. SR3, FJ911682
Lake Pipa, 37
Uncultured gamma proteobacterium, AM690846
Lake Taihu, B7
Uncultured prokaryote clone Se2-13, GU208330
Lake Pipa, 56
Lake Taihu, B17
Uncultured beta proteobacterium, AJ867921
Lake Taihu, B16
Polynucleobacter acidiphobus, FN869130
Lake Taihu, B4
Uncultured proteobacterium clone R7C30, DQ450168
Lake Taihu, B10
Lake Taihu, 88
Brevundimonas-like sp. LMG 11050, AJ244650
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gamma-
beta-
alpha-
Proteobacteria
0.02

(A)

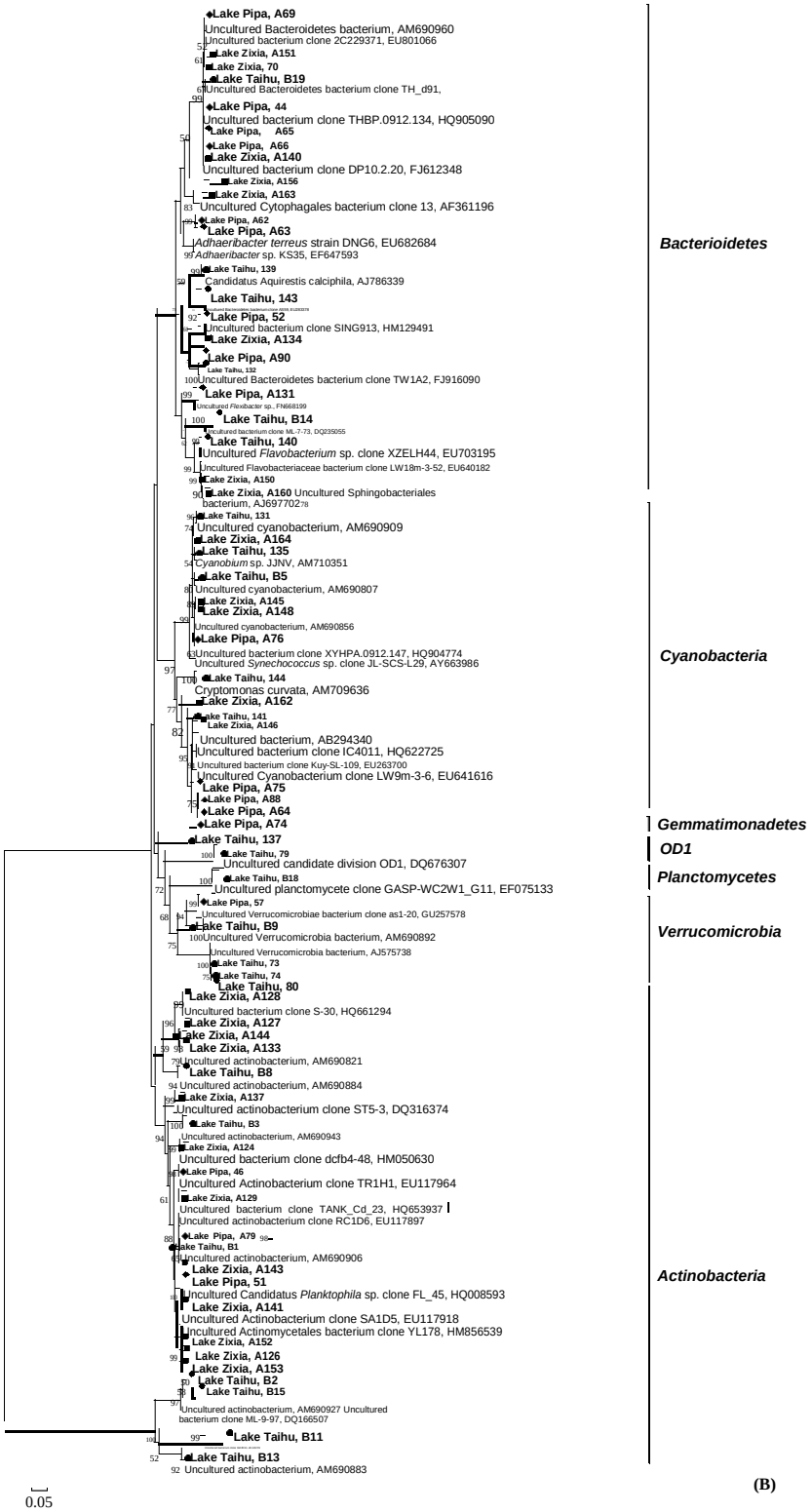

Figure 5. Neighbor-joining phylogenetic trees for phylum *Proteobacteria* (A) and all other phyla (B) detected in Lake Taihu, Lake Zixia and Lake Pipa. Numbers at nodes represent the percentages of bootstrap resamplings based on 1000 replicates; only the values higher than 50 are presented. Sequences from this work were in bold faces, with (●) labels for bacterial clones detected in Lake Taihu, (■) and (◆) labels for bacterial clones detected in Lake Zixia and Lake Pipa, respectively.

DISCUSSION

Compared with the culture-dependent methods, the 16S rRNA based molecular biological methods such as DGGE could shed light on the bacterial community structure in environmental samples at more accurate and comprehensive levels. These methods could also provide information about the bacterial groups which are with important ecological functions but could not be cultivated (Muyzer et al., 1993; Zeng et al., 2008). Additionally, multivariate analysis has been widely used to investigate the relationship between environmental factors and bacterial community structure. Results of previous studies demonstrated that nitrogen, phosphorus and pH were the key factors that driving the dynamics of bacterioplankton community structures (Rooney-Varga et al., 2005; Haukka et al., 2006).

All the three lakes involved in this study are in eutrophic status. Concentrations of nitrogen and phosphorus associated parameters detected in Lake Zixia were significantly higher than those of the other two lakes. The PCA results indicated that the physicochemical parameters of lake water at three lakes formed individual clusters (Figure 1) and sampling stations from Lake Zixia were all distributed in the right part of the Figure, which resulted from the higher nitrogen and phosphorus nutrient concentrations in this lake (Table 1).

Our statistical analysis revealed sampling lakes followed by TP were the most influential parameters responsible for the observed differences in bacterioplankton community structure (Figure 4). Influences of different lakes may be attributed to the different water retention time of the three lakes. Lindström et al. (2005) found that lake water retention time was an important explanatory variable for the bacterioplankton community composition. The formation of stable bacterioplankton community composition in lake takes time. Previous results demonstrated that bacterioplankton community composition was related to water flow and the import of bacterial cells from the drainage area (Lindström and Bergström, 2004). In a lake with short retention time, the community can be expected to be shaped by the import of river and or terrestrial bacteria, whereas bacterioplankton community in a system with a long retention time is shaped by the internal processes in lake (Lindström et al., 2006).

The effect of TP on the diversity and composition of bacterioplankton communities has been previously reported. Lindström and Bergström (2005) pointed out that TP concentration statistically explained the differences between the microbial community compositions in two different drainage areas. Li et al. (2005) also reported that the number of phosphate dissolving and decomposing bacteria was directly correlated to the total phosphorus concentration in Guanting reservoir. Except for TP, other nitrogen associated environmental factors did not affect the bacterioplankton community significantly. Nutrient levels are significantly correlated with the biomass of phytoplankton, which would indirectly influence the community of bacterioplankton (Lindström, 2000).

Cluster and LIBSHUFF analysis results revealed the presence of three distinct kinds of bacterioplankton communities at three lakes. Wu et al. (2007) also reported the bacterioplankton community structure varied strongly between the macrophytes-dominated and phytoplankton-dominated ecological areas in Lake Taihu. In the constructed three clone libraries, Lake Taihu maintained the most microbial diversity of bacterioplankton. This could be attributed to the large area and lower depth of Lake Taihu. The wind driven re-suspension of sediment particles into the water column would result in a pronounced spatial and chemical heterogeneity in the water column (Simon et al., 2002), which is expected to form many additional ecological niches for bacterioplankton.

Eight groups of bacterioplankton were detected in the threeclonelibraries,includingProteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, Verrucomicrobia, OD1, Gemmatimonadetes and Planctomycetes. Proteobacteria and Bacteroidetes groups were the most commonly found bacterial groups in eutrophic freshwater ecosystems (Trusova and Gladyshev, 2002). In our study, 44 and 24 clones obtained from three lakes belonged to Proteobacteria and Bacteroidetes, respectively. For Proteobacteria, 24 of our obtained sequences belonged to the betaproteobacteria subgroup, nine to the alpha- subgroup and eleven to the gamma- subgroup. Betaproteobacteria was also shown to be the most commonly abundant group in many freshwater systems (Wu et al., 2007), whereas alpha- and gammaproteobacteria appear to be the dominant groups in higher salinity environments (Henriques et al., 2006). Dai et al. (2005) found that gammaproteobacteria group was the most abundant bacterial group isolated from Lake Taihu with culture dependent methods. Jaspers et al. (2001) reported that the majority of strains isolated from a eutrophic lake in Germany belonged to Bacteroidetes, and rapid shifts in the diversity of this group were observed during the phytoplankton bloom process. Two strains of Bacteroidetes capable of lysing cyanobacteria were isolated from the eutrophic Brome Lake, suggesting that the Bacteroidetes group might play an important role during the cyanobacterial bloom (Rashidan and Bird, 2001).

A new cyanobacterial cluster not previously detected in freshwater habitats was revealed (Figure 5B). This novel freshwater taxon may result from an understudy of bacterial diversity in freshwater habitats. On the other hand, the new discovered cyanobacterial clusters also indicated the bacterioplankton diversity in freshwater habitats is still far away from complete coverage (Hahn,

2006).

Another bacterial group that is commonly present in freshwater ecosystems all over the world is Actinobacteria (Kolmonen et al., 2004). In addition to the present study, Actinobacteria was also widely existed in four small lakes of Sweden and the author assumed that these particular Actinobacteria were of major importance for the composition of the bacterioplankton communities (Lindström, 2001). Zwisler et al. (2003) reported that Actinobacteria appeared present throughout the year at 3 m depth of Lake constance.

In summary, the data obtained in this study, based on cluster analysis of DGGE pattern, clone libraries and LIBSHUFF analysis obtained in this study demonstrated that the composition of the bacterioplankton community composition in three lakes was significantly different. TP played an important role in driving the variations of bacterioplankton community composition. The three lakes involved in this study were all in eutrophic, therefore, future field studies with the aim of explaining variations in community composition should include as many different lake types as possible.

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