Original Article

Comparison of Bacterioplankton Communities in Three Heavily Polluted Streams in China*

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Abstract

Objective To compare the bacterioplankton communities in streams exposed to pollution of different types.

Methods The bacterioplankton communities in three selected heavily polluted streams were investigated by using terminal-restriction fragment length polymorphism (T-RFLP) analysis in combination with 16S rRNA gene clone library analysis.

Results Both T-RFLP and 16S rRNA gene clone library revealed a great difference in bacterioplankton community composition in the different streams.

Conclusion This work might provide some new insights into bioremediation of heavily polluted streams.

Key words: Bacterioplankton community; Terminal-restriction fragment length polymorphism (T-RFLP); Streams; Clone library

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INTRODUCTION

Among aquatic ecosystem, the bacterial community is relatively sensitive to environmental perturbations[1], and also plays a key role in the reduction of organic matters and the remineralization of nutrients[2]. The knowledge about the composition of the bacterial community is important if bioremediation is to be applied as an effective technology. Most of previous studies on freshwater bacterioplankton communities have focused on lakes[3-5] and large rivers[6-8]. Aquatic communities in small streams are usually more sensitive to environmental perturbations than in lakes and large rivers. Moreover, since small streams exchange physical, chemical and biological masses with large rivers, reduction of organic matters and nutrients by aquatic communities in small streams is also important for maintaining the healthy ecosystems in downstream lakes or large rivers. However, little attention has been paid to the bacterioplankton communities in small streams.

DNA profiling techniques using 16S ribosomal (16S rRNA) genes as phylogenetic markers have proven to be more rapid and economical methods to assess microbial diversity than nucleic acid sequencing[9]. Among available DNA profiling techniques, terminal-restriction fragment length polymorphism (T-RFLP) analysis is a powerful tool used to monitor changes in the structure and composition of microbial communities[10-11]. T-RFLP has been successfully applied in numerous studies on microbial communities in many aquatic ecosystems[12-15]. When it is coupled with 16S rRNA clone library analysis, additional specific information on the composition of microbial communities can be obtained[16].

In China, the booming industrial development

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along with rapid population growth has been deteriorating the water quality of lakes, rivers and ponds in many regions. Small streams are heavily polluted by industrial discharge, urban sewage, rural runoffs or their combination. These small streams commonly contain high amounts of organic matters and nutrients of different types. However, to the authors' knowledge, so far no information has been available on the composition of the bacterioplankton communities in these highly polluted streams. Previous researchers have indicated that nutrients could affect the structure of the bacterioplankton communities in lakes and large rivers[3,5,17]. Therefore, it could be assumed that the structure of the bacterioplankton communities in small streams might also be dependent on pollution of different types they are exposed to. The objective of the present study is to compare the bacterioplankton communities in several polluted streams, exposed to pollution of different types, by using T-RFLP in combination with 16S rRNA gene clone library analysis. This may be useful for the choice of suitable bioremediation technologies for different streams.

MATERIALS AND METHODS

Sampling Sites and Water Quality

In this study, the middle reaches of three small streams (Figure 1), the Wujin River (31°.42.480 N, 120°.03.194E), the Cailing River (31°.42.280N, 119°.59.331E), and the Caoli Canal (31°.38.353N, 120°.06.749E), were sampled in August, 2009. The three shallow streams (depth, 2-3 m) are all located in Changzhou and Wuxi regions, Jiangsu Province. The Cailing River and the Caoli Canal are the upstreams of the Wujin River. The Cailing River is heavily polluted by untreated or ineffectively treated discharge from the textile and electroplating industry. The heavy pollution in the Caoli Canal is mainly linked to rural runoffs. However, industrial discharge, urban sewage and rural runoffs mutually deteriorate the water quality of the Wujin River.

Samples were collected 0.2-0.3 m below the water surface by using acid-rinsed and autoclaved polycarbonate bottles. They were sampled within 2-3 hours on the same day. Water samples were immediately transported to the laboratory and stored in the dark at 4 °C until use. Samples for dissolved organic carbon concentration were filtered through 0.45 μm pore-size Whatman GF/F filters and analyzed with a Shimadzu TOC 5 000. Other physicochemical parameters, such as dissolved oxygen, pH, and total nitrogen and total phosphorus, were determined according to the standard methods described by China Environmental Protection Agency (2002)[18]. The aquatic physicochemical parameters of the three streams are shown as in Figure 2.

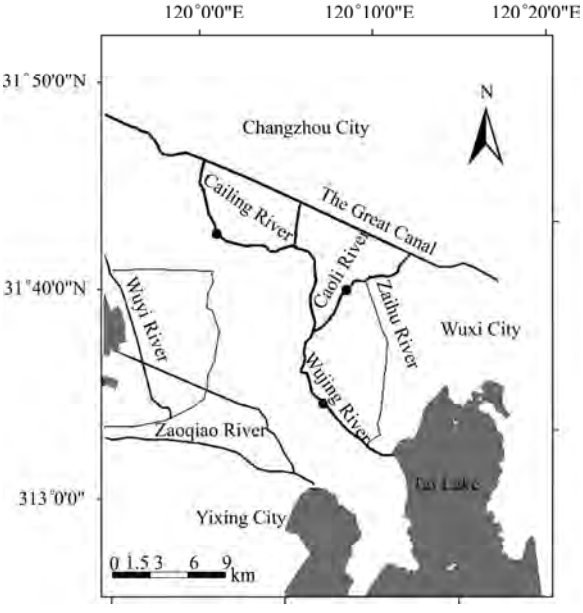

Figure 1. The geological locations of sampling sites.

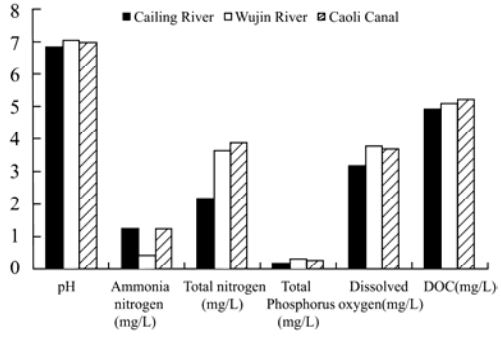

Figure 2. The aquatic physicochemical parameters of the three streams.

T-RFLP Community Profiles

To analyze bacterial diversity, samples (300 mL) in triplicate were filtered through 0.22 μm pore-size membranes (diameter 50 mm; Millipore). The membrane filter was cut into quarters with a sterile scalpel, and each quarter was stored at -20 °C for further molecular analysis. DNA from each filter type was extracted separately with the DNA extraction kit (Biocolor BioScience & Technolgy Company, China) by following the manufacturer's instructions. 16S rRNA genes were amplified by using universal eubacterial

primers 27F-FAM (5'- GAGTTTGATCMTGGCTCAG-3', 5' end-labeled with carboxyfluorescine) and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Tiangen BioTech, China)[19]. 300 ng of PCR products were purified with QIA quick PCR purification kit (Qiagen Inc, German) by following the manufacturer's instructions and digested with *Hae*III[20]. The TRF patterns were detected using an ABI 3730 DNA Analyzer (Applied Biosystems). Triplicate profiles from separate DNA extractions and separate PCR reactions for each sample were compared to identify the reproducible fragments. T-RFs under 100 fluorescent units or smaller than 39 bp were excluded from the further analysis. T-RFs that differed by less than 1 bp were clustered. Phylotype richness (*S*), Shannon diversity index (*H*), and evenness (*E*) were calculated, as previously described[20].

Cloning and Sequence Libraries

The DNA template used for construction of the clone library was the same as that used for T-RFLP analysis. The PCR conditions were the same as the above-mentioned, except that the forward primer was unlabeled. The PCR products were purified and cloned into pMD19-T vector (Takara Corp, Japan) following the manufacturer's instructions. 40-50 clones for each sample were sequenced for this study. Out of 93 successfully sequenced clones, 30 were from the Wujin River, 27 from the Caoli Canal, and 36 from the Cailing River. The partial 16S rRNA gene sequences obtained in this study were deposited with GenBank under the accession numbers of HQ271255-HQ271347. The partial 16S rRNA gene sequences were compared with sequences of public databases by using NCBI Blast. Sequences showing highest matches in the Blast search were obtained from GenBank database. The Ribosomal Database Project analysis tool "classifier" was utilized to assign taxonomic identity[21].

RESULTS

T-RFLP Analysis

The number of different ribotypes for each sample was between 9 and 36 (Table 1). In total, we detected only 3 distinct terminal restriction fragments (T-RFs) (40 bp, 66 bp, and 70 bp) shared among all samples. Moreover, there were 11 distinct T-RFs shared by samples from the Wujin River and the Caoli Canal. The abundant ribotypes (with a relative T-RFs abundance no less than 5%) were 49 bp (5.1%) and 70 bp (78.7%) in samples from the Cailing River, 40 bp (7.3%) ,70 bp (16.7%) , and 215 bp (5.6%) in samples from the Wujin River, and 65 bp (7.2%), 70 bp (5.5%), 251 bp (12.5%), and 622 bp (29.7%) in samples from the Caoli Canal. Therefore, 70 bp was the only abundant ribotype shared among all the three water samples. For richness, Shannon-Weiner diversity index and evenness index, samples from the Wujin River had the highest values (Table 1). The lowest values for samples from the Cailing River reflected the predominance of the 70 bp. Altogether, the calculated ecological indices revealed big differences in microbial community composition among the different water samples.

Table 1. Ecological Indices of Microbial Communities in the Three Streams

Ecological Indice	Cailing River	Wujin River	Caoli Canal
Phylotype richness (S)	9(0)[a]	36(1)[a]	28(1)[a]
Shannon-Weiner diversity index(H')	1.35(0.06)[a]	3.37(0.14)[a]	2.72(0.09)[a]
Evenness index (E)	0.61(0.03)[a]	0.94(0.08)[a]	0.82(0.05)[a]

Note. [a]Standard deviation from the mean (n = 3).

Phylogeny

The phylum distribution of bacterioplankton communities in the three streams is shown in Figure 3. The clones recovered from the Wujin River were distributed across phyla as follows: Cyanobacteria 26.7%, Betaproteobacteria 23.3%, Alphaproteoba cteria 16.7%, Bacteroidetes 16.7%, Gammaproteo bacteria 6.7%, unknown phylum 6.7%, and Firmicutes 3.3%. The major phylum types of clones recovered from the Cailing River were Betaproteobacteria 44%, Bacteroidetes 33%, Gammaproteobacteria 11.1%. However, the major phylum types of clones from the Caoli Canal were Betaproteobacteria 42.8%, Alphaproteobacteria 39.3%, and Gammaproteobacteria 10.7%. Therefore, Betaproteobacteria was the only major phylum group shared among all the three streams. One of the most unusual findings was the absence of Cyanobacteria from the other two streams although it was the largest phylum group in the Wujin River.

Among the total of thirty five Betaprote obacteriaial clones from the three streams, twenty seven could be classified with confidence (80%) to the genus level: fifteen as *Malikia*, eight as *Hydrogenophaga*, two as *Acidovorax*, one as *Burkholderi*, and one as *Macromonas*. Most of Betaproteobacteriaial clones from the Cailing River and the Caoli Canal belonged to *Malikia* and *Hydrogenophaga* respectively. However, among the three Betaproteobacteriaial clones with classified

genus, two also belonged to *Hydrogenophaga*. Thirteen Alphaproteobacterial clones could be classified to the genus level: seven as *Rhodobacter*, two as *Novosphingobium*, and the other four as

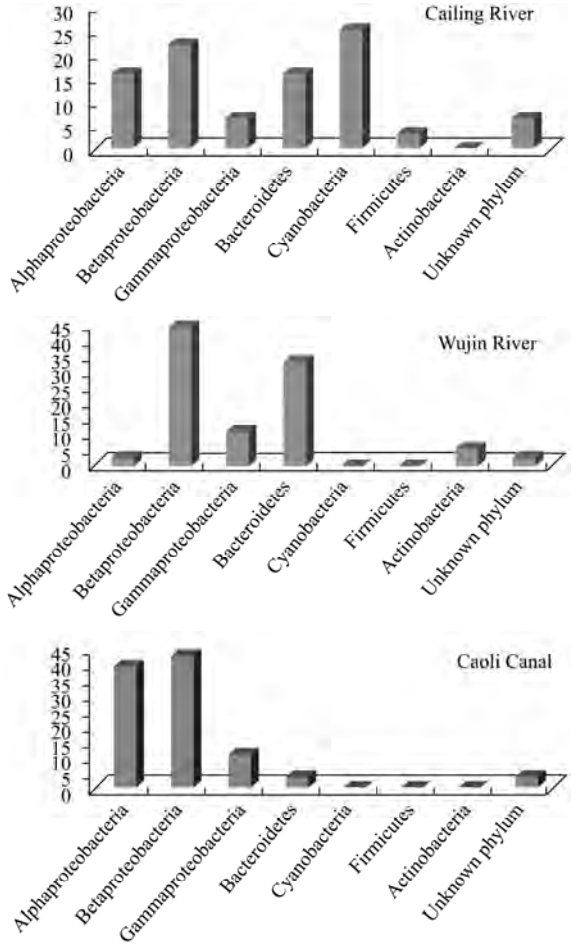

Figure 3. Comparison of the quantitative contribution of the clones affiliated with different phyla and sub-phyla to the total number of clones from the three streams. Clones not classified to any known phylum are included as unknown phylum.

Methylocystis, *Phenylobacterium*, *Chelatococcus,* and *Sphingomonas* respectively. However, among the six Gammaproteobacterial clones with classified genus, two wasas *Rheinheimera*, two as *Thermomonas*, and the other two as *Acinetobacter*. For the total of thirteen Betaproteobacteriaial clones with classified genus, eleven belonged to *Flavobacterium*. Eight Cyanobacterial clones had a big genus diversity, including four belonging to *Bacillariophyta* and the other four to *GpIIa*, *GpXI*, *GpXIII*, and unclassified genus respectively.

Of the bacterial clones recovered from the Cailing River, 47.2% were related with a high percent of similarity (more than 97% similarity to clone sequence within the GenBank database), 36.1% with a middle percent of similarity (95%-97%), and 16.7% with a low percent of similarity (less than 95%). However, of the clones recovered from either the Wujin River or the Caoli Canal, less than 40% was related with a high percent of similarity.

DISCUSSION

The availability of nutrients can affect the structure of the bacterioplankton communities[3,17]. Wei et al.[5] revealed that the temperature, BOD_5, ammonia nitrogen, total nitrogen, total phosphorous, and dissolved oxygen significantly influenced the bacterioplankton community composition in Chaohu Lake, a highly eutrophic lake in China. However, since the differences in determined physicochemical parameters among the three streams were minor, it was still difficult to relate these limited physicochemical properties to the diversity of microbial populations. The big differences in the ecological indices might be partly attributed to the pollutants of different types the three streams were exposed to. The Cailing River was heavily polluted by discharges from untreated or ineffectively treated wastewaters from the textile and electroplating industry. To the authors' knowledge, there has been no report concerning microbial communities in streams heavily polluted by industrial discharges. The toxic organic and inorganic pollutants from the industrial discharges might narrow the diversity of the bacterioplankton community in the Cailing River, reflecting its lowest values of the ecological indices.

Freshwater bacteria are commonly affiliated with the phyla Cyanobacteria, Bacteroidetes, Alpha-, Beta-, and Gamma-Proteobacteria, Actinobacteria and Verrucomicrobia[22], usually referred to as "globally distributed" freshwater phylogenetic clusters"[8]. In this study, most of the bacteria in the aquatic systems belonged to these phylum groups. The Cyanobacterial clones from the Wujin River showed big genus diversity, with a majority belonging to *Bacillariophyta*. *Bacillariophyta* has been found in the highly eutrophic Lake[23], natural plain water bodies[24], and acidified streams[25].

Fifteen of the sixteen Betaproteobacteriaial clones from the Cailing River fell into the genus of *Malikia*. However, little information is available for *Malikia*. Moreover, six Betaproteobacteriaial clones from the Caoli Canal and two from the Wujin River belonged to the genus of *Hydrogenophaga*.

Hydrogenophaga are present in various aquatic habitats, such as river sediment[26], biofilms of polluted river[27], soda pond[28], and eutrophic lake[29].

Alphaproteobacteriaial clones belonging to the genus of *Rhodobacter* could be recovered from the two streams. *Rhodobacter* are present in various habitats, such as polluted and unpolluted river waters and the discharged effluents[30], soda lake[31], brackish lake water[32], and industrially polluted pond[33]. Usually, *Rhodobacter* grows under anaerobic conditions[32]. In this study, due to the heavy pollution in these streams, the dissolved oxygen of each sampling site was low and could be depleted quickly along the depth (data not shown). The anaerobic zone below the sampling site may favor the growth of *Rhodobacter*. The navigation of small ships in the streams might push *Rhodobacter* in the deeper anaerobic zone to the shallower zone.

Most of these clones affiliated to phylum of Bacteroidetes belonged to the genus of *Flavobacterium*. *Flavobacterium* have been isolated from rivers[34], lakes[35], ponds[36], and wastewater treatment plants[37]. *Flavobacterium* sp. has also been immobilized for bioremediation of contaminated surface water with a very effective reduction of chemical oxygen demand[38]. Presumably, the existence of *Flavobacterium* in this study might have links to organic reduction in the streams.

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