ISSN : 0974 - 7435 Volume 10 Issue 5

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 10(5), 2014 [1050-1056]

Microbial community diversity of typical rivers of Changzhou, Jiangsu province

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ABSTRACT

Microbial community structure analysis has developed on environmental science, environmental engineering, ecology and microbiology. We need to clarify the relationship between community structure and its growth environment. Based on the important role that microbial community structure played in the river, we use PCR and denaturing gradient gel electrophoresis (DGGE) with primer sets targeting the V3 region of 16S rDNA genes and bacterial abundance to reflect it. The results showed that: 1) Bacterial diversities in Chaizhibang river were significantly higher than others. Bacterial communities in the tests can be classified into: *Escherichia*, *Acinetobacter*, *Citrobacter*, *Trichococcus* and *Rhodobacter*. However, the typical rivers had dominantly higher proportions of *Escherichia* and *Acinetobacter*. 2) The bacterial abundance has been a significant increase with the pollution, the fluctuation is from 3.81×10^5 cfu/mL to 1.244×10^6 cfu/mL and average is 7.698×10^5 cfu/mL. The correlation analysis between the bacterial abundance and environmental factors reflect that COD_{Mn} and bacterial abundance had a significantly positive correlation. Next we will study the relationship between microbiology and water quality, also focus on the river water microbial communities with seasonal changes, the result provides a reference and guidance for future work.

KEYWORDS

Microbial community structure;
Bacterial abundance;
Diversity;
Water quality analysis;
PCR-DGGE;
16S rRNA gene.

INTRODUCTION

Changzhou is in the south of the Yangtze river, close to Taihu lake, in the center of the Yangtze river delta. Based on its particular geographic and economic position, with the rapid development of urbanization, the serious retention of river, the lag of water environment facilities, river ecosystem is disturbed and damaged by human activities. Water ecology and water environment has deteriorated seriously, to analysis the relationship between ecological habitat and water quality provides a reference and guidance for future work.

In aquatic ecosystems, microbes are the most sensitive and vulnerable to environmental impacted organisms. They are an important part of the biomass in the aquatic ecosystem, so as to influence the course of material cir-

culation and nutrient transmission[1]. The prerequisites of analysis the microbial activities process are to investigate the bacterial community structure in water. Researchers have been concerned about the coupling relationship between environmental factors and the change of the microbial community in aquatic ecosystems. We study bacterial abundance in different kinds of rivers by using traditional culture method. In this article, molecular biological techniques PCR-DGGE of 16S rRNA were applied to describe the dynamic changes of the microbial diversity and biomass. Finding out the impact of water quality on aquatic microbial groups were also expected.

MATERIALS AND METHODS

Site selection

Changzhou is the center of the Yangtze river delta, the central urban area is 24.3 km^2. There are 15 rivers in that region including the kind of the main river, connected river and beheaded river, most of them are IV or V. We set 10 sampling sites in the different types of river. 1# is the downstream from the Yangtze river located in Zaojiang river, it's a reference point. The main river Beitang river sets four sites, from upstream to downstream of the river named 2#, 3#, 4#, 5#. In the connected river Sanjing river sets 6#, 7#, 8#. Chaizhibang river is a heavy pollution river has two sites at both ends named 9#, 10#. Sampling sites are shown in Figure 1.

Water samples collection

We collect the water samples in June, 2013, water collected in 50 centimeters depth for count the total number of bacteria, samples were in the incubator with ice back to lab stored at 4°C until analysis. Water samples for extract total bacterial genomic DNA was stored at -20°C until analysis. To measure the water samples for other physical and chemical characteristics

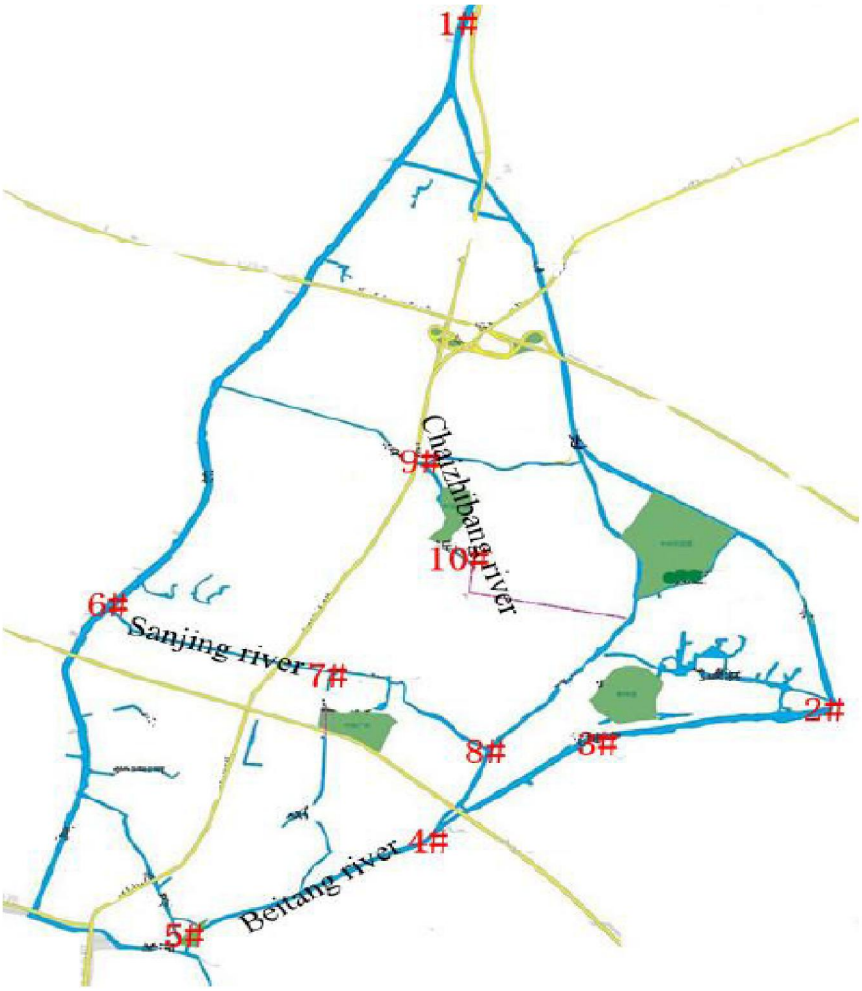

Figure 1 : Location of sampling sites in region of Xinbei, Changzhou

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need in the lab immediately.

Total bacterial genomic DNA extraction

The genomic DNA was extracted from the water samples were using the FastDNA® SPIN Kit (MP, Beijing, China), application spectrophotometer (Thermo Nanodrop, China) analysis the extracted DNA concentration. All DNA was stored at "20°C before further analysis[2].

PCR-DGGE analysis

The V3 regions of 16S rRNA genes were amplified with universal bacterial primers (341F: 5'-GTATTACCGCGGCTGCTGG-3', 534R: 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3') using the hot-start touchdown protocol described by Muyzer et al[3]., and the reaction mixtures and the amplification program described previously by Li et al[4].with minor modifications. The obtained PCR products were loaded onto 6% (W/V) acrylamide/bisacrylamide (37.5:1, W/W) gels containing a 40%–60% linear gradient of formamide and urea (where the 100% denaturant contained 40% (V/V) formamide and 7M urea)[5]. The electrophoresis was run for 7 hr at 150V and at a constant temperature of 60°C, using a DCode Universal Mutation Detection System (Bio-Rad Laboratories, USA). The similarities of PCR-DGGE DNA profiles were analyzed with Quantity One® analysis software.

Cloning, sequencing, and phylogenetic tree construction

DGGE gel strips of some distinguished bands among all treatments were excised and reamplified following previous PCR conditions. These PCR products were cloned by correcting insert selected fragments and sequenced by ABI 3730xl DNA Analyzer. These partial 16S rRNA gene sequences obtained from the National Center for Biotechnology Information (NCBI) database. Phylogenetic analyses were conducted under MEGA version 4.0 and the neighbor-joining trees were implemented by p-distance with 1000 replicates to produce bootstrap values[6].

Total bacteria count

Total bacteria count in water with the method of GB5750-85, Bacterial colonies were imaged by Shineso G6 Automated Colony Counter.

Basic physical and chemical properties testing

Temperature measured directly with water temperature meter; NH^{4+}-N testing by ultraviolet spectrophotometer; pH testing by Hach pH-meter equipped[7]. other physical and chemical properties all test with the method of national standard.

RESULT AND DISCUSSION

Basic physical and chemical properties of the sampling site

The water depth at each site of the main river is significantly deeper than the sites of others. Nutrients nitrogen in the water exist significant difference in different rivers, heavy pollution river 10# contains TN as high as 6.72, and DO is up to the maximum (TABLE 1). According to Environment Quality Standards for Surface Water (GB3838-2002), water from the upstream of zaojiang river is clean relatively, other rivers are V or worse V, the main exceed pollutants are $NH4^{+}$-N and TN. The severest is Chaizhibang river, followed by Sanjing river and Beitang river.

DGGE Profile of 16S rRNA gene fragments

Microbial diversity characteristics of the four river samples using DGGE bands pattern data are shown in TABLE 2. Diversity indices were useful as a first approach to estimate the diversity of microbial communities. In a micro ecology system, the more kinds of microorganisms, the higher the Evenness index (J) in species, and the Shannon index (H) is higher[8]. Diversity index consisted of two components: (1) the total numbers of species present or species richness and (2) the distribution of the number of individuals among those different species, called species evenness.

During four rivers, the sequence of Genotypic Richness (R) shows that Chaizhibang had higher numbers of bands, which were significant higher than Zaojiang, Beitang and Sanjing. No obvious differences of R were found between Zaojiang and Beitang, because of Beitang is the downstream of Zaojiang. The H value of the four rivers revealed the similar regulations. J of the four rivers had no significant difference. In general, R and H

index showed that the microbial diversities of heavy pollution river are higher than other sites.

TABLE 1 : Basic physical and chemical properties of the sampling sites

Site	Depth/ M	Temperature/ °C	Transparency/ cm	pH	COD_{Mn}/ mg/L	DO/ mg/L	TP/ mg/L	TN/ mg/L	$NH4^+$-N/ mg/L
1#	2.4	29.6	40	6.76	2.9	3.41	0.216	3.36	0.357
2#	3	31.1	60	7.06	8	8.76	0.251	3.63	2.45
3#	3.4	30.5	70	7	5.5	5.3	0.242	3.39	1.75
4#	3	29.5	40	7.09	3.4	4	0.183	3.14	0.925
5#	2.6	30.2	35	7.12	5.4	1.25	0.253	3.61	2.47
6#	1.37	30.9	40	7.04	2.5	1.27	0.288	4.18	2.46
7#	1.1	30.3	50	7.05	2.6	1.4	0.275	4.08	2.44
8#	1.34	30	33	7.23	2.8	1.96	0.228	3.72	1.86
9#	1.9	28.4	67	7	5.6	1.68	0.253	4.03	2.04
10#	1.29	32	66	7.33	4.2	11.04	0.107	6.72	0.139

TABLE 2 : Microbial diversity characteristics of the four rivers using DGGE bands pattern data

River	Shannon index (H)	Evenness index (J)	Genotypic richness (R)
Zaojiang	1.368	0.987	1.365
Beitang	1.369	0.988	1.366
Sanjing	1.079	0.982	1.028
Chaizhibang	1.609	1	1.737

PCR-DGGE and phylogenetic analysis of 16S rRNA gene

From the DGGE bands to be shown, electrophoresis bands basically represent the biodiversity, the brighter of the bands, the greater amounts and species of microorganisms. The relationship between amounts and species of microorganisms from different rivers is confirmed that the microbial diversity of information is concluded.

DGGE profiles of PCR-amplified 16S rRNA gene segments of DNA extracted that from the ten samples of typical rivers is shown in Figure 2. Visual inspection of the bacterial DGGE profiles revealed large numbers of bands per lane, whereas they failed to reveal great variation among different sampling sites.

It is observed from 1#, DGGE profile shows the characteristics of it has less bands and dominant obviously. With the change of the water environment and the heavily polluted, it shows the characteristics of the dominant bands decreased and total number increased. As the heavily polluted river 9#, 10# samples, bacteria showed a significant increase from them. This may be due to that 9# has a high incidence of algae, it limits the growth of some microorganisms, 10# in the residential areas, it changes water with sluice regularly, increased the community diversity of bacteria with providing more types fields for microorganisms. Bacteria groups close to 2#, 3#, 4#, 5#, 6#, due to they are all in the main river and the downstream of 1#. 7#, 8# become another group of microbial community structure, the characteristics of structure type of microorganisms provide an obvious indication for the change of the aquatic ecosystems.

We constructed a phylogenetic tree based on the 16S rDNA sequence data by Neighbor-Joining Method (Figure 3), compared them with the sequences available in the GenBank database, accounts for large proportion of microorganisms are *Escherichia* and *Acinetobacter*, the rest are *Citrobacter*, *Trichococcus* and *Rhodobacter*. *Escherichia* is the normal flora, used as the hygiene standard for drinking water, food or drugs. *Acinetobacter* mainly exists in water and soil, it is easy to survive in damp environment and the optimal temperature of 30°C. *Citrobacter* and *Trichococcus* are also the normal flora, they are the indigenous microorganisms that adapt to the warm and moist in water. DGGE band S11 in 10# is *Rhodobacter*, some can degrade environmental pollutants, to form some useful compounds by synthetic or transformation[9]. Based on 10# is in high levels of pollution and water quality into worse V, its existence is helpful for the purification of water quality.

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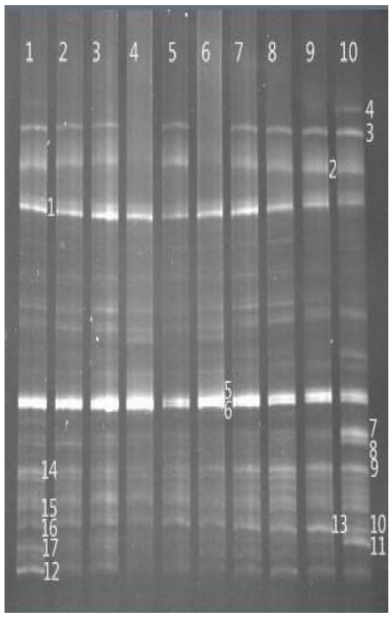

Figure 2 : Community profiles based on DGGE of the V3 region of the 16S rRNA gene

In order to compare the similarity between samples, calculated the similarity rectangular figure by Jaccard similarity index (Cj), according to the principle of Jaccard: When the Cj in 0.00-0.25 is dissimilarity highly, in 0.25-0.50 is dissimilarity, in 0.50-0.75 is similarity and in 0.75-1.00 is similarity highly. The results as shown in TABLE 3, the similarity index between different samples of bacteria is low, most are in dissimilarity and similarity, showed that bacterial species have relatively low similarity in typical rivers.

Different distribution of bacteria population in typical rivers

Results that the bacterial abundance showed the count of bacteria at different site has obvious distribution characteristics (Figure 4). In Chaizhibang with the algae arise frequently river, bacteria is significantly higher than the other rivers, the number is over 1.244×10^6

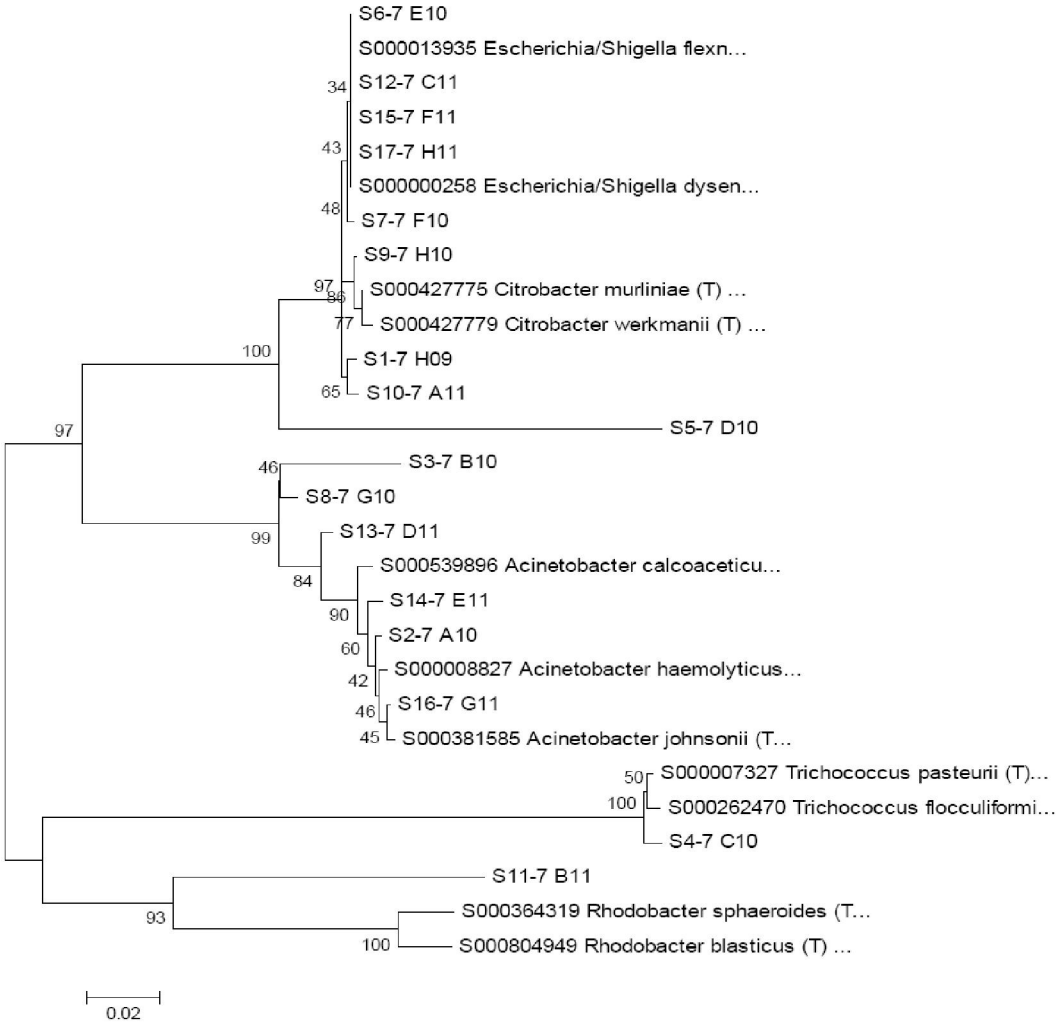

Figure 3 : Phylogenetic tree based on the 16S rDNA sequence data

cfu/mL and 1.004 ×10^6 cfu/mL, it is probably related to near residential areas, greatly influenced by life, production and other terrestrial pollution. By contrast, the bacterial abundance of Sanjing is significantly less, the minimum is 6$^{\#}$ by 3.81 ×10^5 cfu/mL. The average of bacteria fluctuation is 7.698 ×10^5 cfu/mL. As a whole, the bacterial abundance has biggish divergence in typical rivers.

TABLE 3 : The similarity index (Cj) of bacteria in different sampling sites

Sites	1$^{\#}$	2$^{\#}$	3$^{\#}$	4$^{\#}$	5$^{\#}$	6$^{\#}$	7$^{\#}$	8$^{\#}$	9$^{\#}$	10$^{\#}$
1$^{\#}$	1.0000	0.4286	0.3750	0.4286	0.4286	0.4286	0.4286	0.4286	0.4286	0.4444
2$^{\#}$		1.0000	0.4286	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000	0.3750
3$^{\#}$			1.0000	0.4286	0.4286	0.4286	0.4286	0.4286	0.4286	0.4444
4$^{\#}$				1.0000	0.5000	0.5000	0.5000	0.5000	0.5000	0.3750
5$^{\#}$					1.0000	0.5000	0.5000	0.5000	0.5000	0.3750
6$^{\#}$						1.0000	0.5000	0.5000	0.5000	0.3750
7$^{\#}$							1.0000	0.5000	0.5000	0.3750
8$^{\#}$								1.0000	0.5000	0.3750
9$^{\#}$									1.0000	0.3750
10$^{\#}$										1.0000

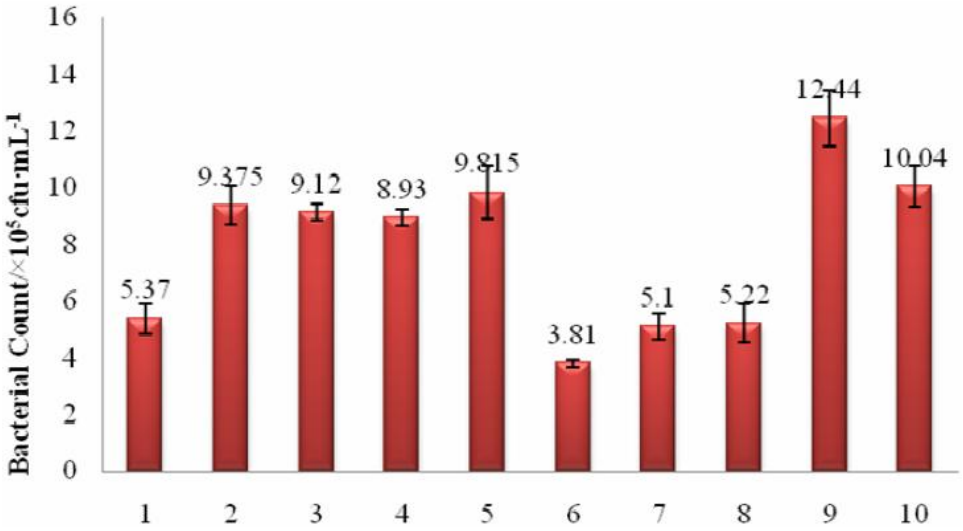

Figure 4 : Bacterial abundance at sampling sites

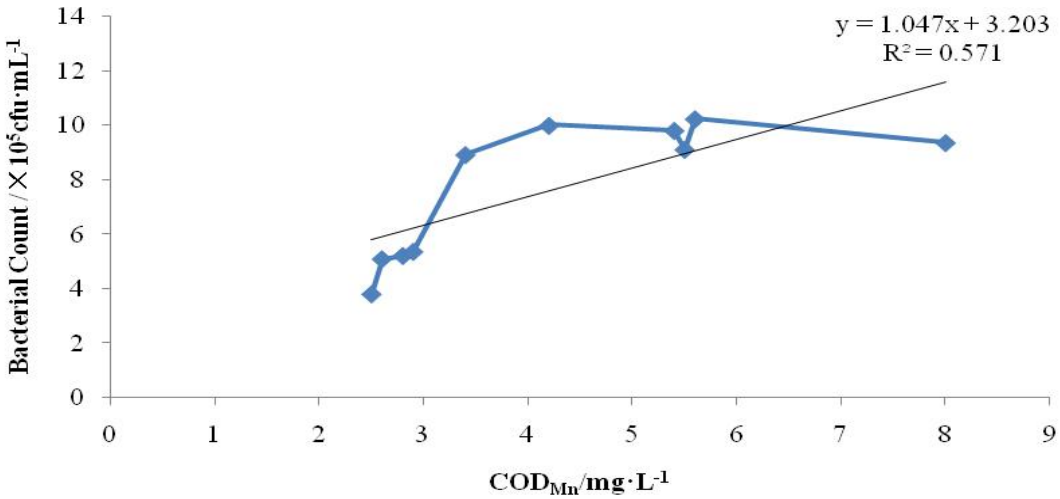

Figure 5 : The relationship between bacteria and COD$_{Mn}$ at sampling sites

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The correlation analysis between the bacterial abundance and environmental factors reflect that COD_{Mn} is an important factor affects the spatial and temporal distribution of the bacterial abundance. Bacterial reproduction rate increases with the COD_{Mn} increased (Figure 5), it is consistent with Xia et al. The bacterial abundance reflects the amount organic content in research area to a certain extent. Inorganic nitrogen is heavily utilized as nitrogen source, therefore it also exists a correlation with the bacterial abundance[10].

CONCLUSIONS

(1) Combining the method of PCR-DGGE with the bacterial abundance to research the diversity of bacterial community structure in aquatic ecosystem. Overcame some deficiencies of using DGGE, improved the accuracy of the experiment and ensured the reliability of the results.

(2) Bacterial diversities in Chaizhibang river were significantly higher than others. Bacterial communities in the tests can be classified into: *Escherichia*, *Acinetobacter*, *Citrobacter*, *Trichococcus* and *Rhodobacter*. However, the typical rivers had dominantly higher proportions of *Escherichia* and *Acinetobacter*. Microbial community diversity tends to be simple that makes the stable aquatic ecosystems become more vulnerable.

(3) To study the bacterial community structure in different rivers, the bacterial abundance has been a significant increase with the pollution, the fluctuation is from 3.81×10^5 cfu/mL to 1.244×10^6 cfu/mL and average is 7.698×10^5 cfu/mL. The correlation analysis between the bacterial abundance and environmental factors reflect that COD_{Mn} and bacterial abundance had a significantly positive correlation.

ACKNOWLEDGMENTS

This work was supported by Project of Graduate Student Innovation Research of Heilongjiang Province (YJSCX2012-190HLJ), National Major Science and Technology Program-water Body Pollution Control and Remediation (2012ZX07301-001-05) and Natural Science Foundation of China (51108145).

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