Basic Analyses of Aquatic Biodiversity in the Fenghe-river

Si-dan Lu
Institute of water science
Beijing Normal University
Beijing， China
e-mail: 1554619997@qq.com

Ai-zhong Ding.
Institute of water science
Beijing Normal University
Beijing， China
e-mail: ading@bnu.edu.cn

Yu-jiao Sun*
Institute of water science
Beijing Normal University
Beijing， China
e-mail: sun201405@163.com

Lei Wang
Institute of water science
Beijing Normal University
Beijing， China
e-mail: wlqueen@126.com

Xuan Zhao
Institute of water science
Beijing Normal University
Beijing， China
e-mail: wuyou0107@m.deu.cnail.bnu

Abstract—Fenghe river is a nature river originating from north of QinlingMountains,it is fed by glaciers and rain. On its way flowing, changes of its quality are varied, because of the Point and Non-point Source Pollution around. The aquatic biodiversity is a good indicator of the ecosystem that reflect water system's inner characteristics. The community structure of different aquatic microorganisms in Fenghe river were analyzed employing Illumina Genome Analyzer. Changes of the microbial communities are well connected with the water quality, which is also reflected on Taxonomic status of phyla. In the research, 7792 OTUS were get ,which were classified into 24 phyla, The most dominant phyla detected in stream habitats were Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. Also, 897 genus and 15 dominate genus were analyzed specially. The clustering results showed that Acinetobacter, Yersinia, and Pseudomonas form dominant genus community throughout the Fenghe river and all of them belong to phylum of Proteobacteria. The results of diversity analysis show that the diversity of polluted water is corrected with pollution level.

Keyword-Fenghe-river; microbial communities; Illumina Genome sequencing; biodiversity; pollution level

I. INTRODUCTION

Fenghe river is fed by rain and glaciers, The upstream water quality meets requirements of Surface water I-II class standard. Point and Non-point Source Pollution's entrance make the water quality more complicated [1]. In the last twenty years, pollution of the upstream became worse, but quality of the downstream is substantial improved, while problem with nitrogen pollution is still a tough nut.

Four typical research sections have been set through the Fenghe river to collect water samples and to analyze water quality, The place where the first sampling point S-1 was set at is Yuantou, it is the source of Fenghe river. The next sampling point S-2 was set at the place Guangpingsi, no tributaries from point S-1 to S-2 feed into the main flow. Fengyukou is the place where the third sampling point S-3 was set at, into here a tributary from west falls; the fourth sampling point S-4 was set at Qingduzheng, here two other large tributaries feed in, brought more loading of pollutant.

Metagenomic sequencing technology is a advanced tool used for sequencing, which can detect the whole genome in a short time, Metagenomic sequencing technologys is in its process of being widely popularized these years. Using this method, time of sequencing is shorten because we do not need to culture microorganisms first, samples analyzed are got directly from environment and this technology is broadening our understanding of microbial metabolic potential. Via modern biological technology, research of microbial community structure are done, statisticalanalysis helped by statistical software are also finished.

As an important part of aquatic ecosystem, microorganisms are decomposing, transforming and saving materials and energy, producing in the food chain, having a special meaning in the flux of energy through ecosystems and microorganisms ' diversity tell us how the water quality is ,what pollution exist in the river and the degree of contamination.

II. MATERIALS AND METHODS

A. Setting of experimental sections

Collection of the samples should be set at every tributaries of Fenghe as much as possible, the river flows from south to north until it reaches another river. Fig .1 shows the sample sections of the points exactly.

Figure 1. The sampling section positions

B. Collecting water samples

For DNA extraction, a water sample was collected in a 5L sterilized glass bottle and then filtered through 0.22 μ m pore-sized cellulose acetate filters under steady pressure of 0.03MPa. The filters were immediately frozen and then stored at -20°C until DNA extraction.

C. DNA extraction and MiSeq sequencing of 16S rRNA gene amplicons

DNA extraction was extracted using Ezup genomic DNA extraction kit for soil,DNA concentration and quality were checked using a NanoDrop Spectrophotometer. Extracted DNA was diluted to 10 ng/μL and stored at -40oC for downstream use.

Universal primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') with 12 nt unique barcode was used to amplify the V4 hypervariable region of 16S rRNA gene for pyrosequencing using Miseq sequencer[2]. The PCR mixture contained 1x PCR buffer, 1.5 mM MgCl2, each deoxynucleoside triphosphate at 0.4 (M, each primer at 1.0 (M and 0.5 U of Ex Taq (TaKaRa, Dalian) and 10 ng soil genomic DNA. The PCR amplification program included initial denaturation at 94oC for 3 min, followed by 30 cycles of 94°C for 40 s, 56°C for 60 s, and 72°C for 60 s, and a final extension at 72°C for 10 min. Conduct two PCR reactions for each sample, and combine them together after PCR amplification. PCR products were subjected to electrophoresis using 1.0% agarose gel. The band with a correct size was excised and purified using SanPrep DNA Gel Extraction Kit and quantified with Nanodrop. All samples were pooled together with equal molar amount from each sample. The sequencing samples were prepared using TruSeq DNA kit according to manufacture's instruction. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30% of final DNA amount) as described in the Illumina library preparation protocols, and then applied to an Illumina Miseq system for sequencing with the Reagent Kit v2 2×250 bp as described in the manufacture manual.

D. Pyrosequence data analysis

The sequence data were processed using QIIME Pipeline – Version 1.7.0. All sequence reads were trimmed and assigned to each sample based on their barcodes. The sequences with high quality (length > 150 bp, without ambiguous base 'N' , and average base quality score > 30) were used for downstream analysis. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold. The aligned ITS gene sequences were used for chimera check using the Uchime algorithm [3].

III. RESULTS AND DISCUSSION

A. Environmental condition

TABLE I. THE SAMPLING SECTION INFORMATION TABLE

sample	number
Yuantou	S-1
Guanpingsi	S-2
Fengyukou	S-3
Qingduzheng	S-18

TABLE II. CHARACTERISTICS OF THE FENGHE RIVER WATER SAMPLES

sample	DO mg/L	NH4-N	NO3-N mg/L	COD mg/L	BOD mg/L	TP mg/L
S-1	8.25	0.017	0.774	2.8	0	0
S-2	8.35	0.025	1.039	3.8	0	0
S-3	8.37	0	1.625	3.8	0	0
S-18	7.94	0	3.115	6.1	2	0.02

Eutrophication and waterbloom have become important water pollution problems yet to be solved in many countries around the world including China. Nitrogen and phosphorus are critical nutrients in aquatic ecosystems. Correspondingly, the contribution of nitrogen and phosphorus in the eutrohpication and waterbloom has been paid great attention in the study of mechanism and treatment of eutrophication and waterbloom, this is why we studied the parameters..

From upstream to downstream, from sampling point S-1 to S-2, no tributaries feed in Fenghe river, non-point source pollution and point source pollution that are along the river way make a bigger chemical oxygen demand loading[4].Concentration of ammonia nitrogen and potassium nitrate increase, biological oxygen demand loading, total phosphorusand dissolved oxygen stay almost the same; from sampling point S-2 to S-3, influenced by one tributary, ammonia nitrogen decrease, potassium nitrate increase, and other parameters stay the same; from sampling point S-3 to S-18, many tributaries come in and riverine input add to the pollution, DO decrease ,COD

loading, BOD loading, total phosphorus and potassium nitrate increase much, others stay almost the same.

TABLE III. ESTIMATED OTU RICHNESS, DIVERSITY INDICES, AND ESTIMATED SAMPLE COVERAGE OF THE BACTERIAL 16S RRNA LIBRARIES OF THE FENGHE RIVER WATER SAMPLES

Sample	Reads	OTUs	Chao1	H'	E
S-1	25544	1504	2998.62	4.341	0.8
S-2	25450	1821	3009.41	6.199	0.94
S-3	25299	2044	3659.65	6.681	0.96
S-18	25507	1803	3870.89	5.121	0.86

B. Pyrosequencing Results

The parameter Chao1 shows richness of microorganisms[5], from sample S-1 to sample S-18, richness of the water keeps growing, though in a unconspicuous way, it can be concluded that because of the tributaries fed in the river and the estuary sediment, some more microorganisms were brought into the river and increased richness of the samples.

The parameter E is evenness of aquaticmicroorganisms,, it indicates a good shape of these samples because they are all above number 0.8, showing that water in these sampling points is mixed and steady well to some extent. But in another way, numbers of E increased from sample S-1 to S-3, meaning evenness is improved by the increasing COD loading moderately, but from sample S-3 to S-18, number of E decreased, meaning that when the COD loading reached a maximum number, the balance of the system was broken a bit and the too much dominated species suppressed other microorganisms. It can be concluded that increase of COD loading helps to improve evenness within certain limits and evenness decrease out of this limits

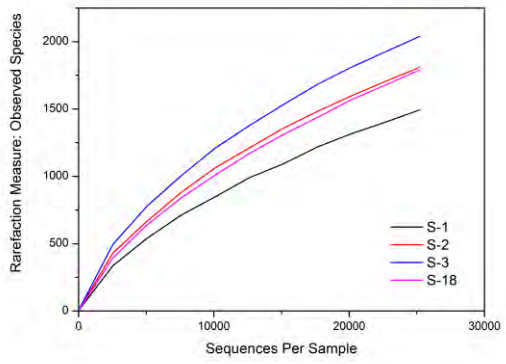

Figure 2. Dilution degrees curve shows the values above.

The rarefaction curves tapered off but did not reach a plateau, indicating that the entire bacterial community cannot be revealed even with large amount of data. As can be seen in the rarefaction curves exhibited similar patterns across all samples

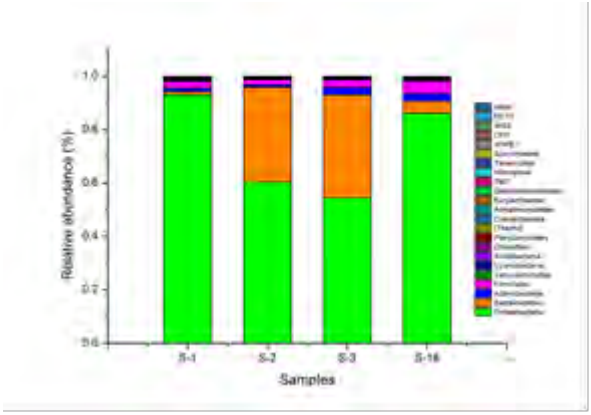

Figure 3. All the phyla of the samples

These samples share the same phyla like *Proteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes, Proteobacteria* is the most dominate phylum of them, the biggest phylum of all the phyla and belong to ammoniaoxidizingbacteria[6]. This result is normal with water like Fenghe river, The proportion of *Proteobacteria* is 93% in sample S-1,60% in sample S-2, 54% in sample S-3 and 86% in sample S-18.

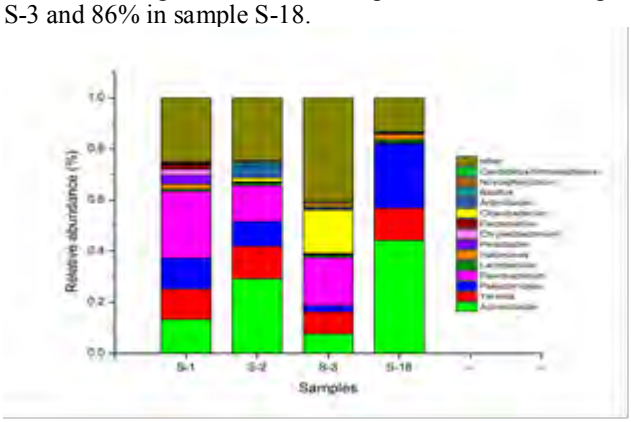

Figure 4. Dominate genus of the samples

The dominate genus are Acinetobacter, Yersinia, and Pseudomonas, and there have other hundreds kinds of genus exist in the water.

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteo bacteria. Acinetobacter species are not motile and oxidase-negative, and occur in pairs under magnification. They are important soil organisms, where they contribute to the mineralization of, for example, aromatic compounds. Acinetobacter species are a key source of infection in debilitated patients in the hospital, in particular the species Acinetobacter baumannii [7].

Pseudomonas is Gram-negative bacterium, Their ease of culture in vitro and availability of an increasing number of Pseudomonas strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include P. aeruginosa in its role as an opportunistic human pathogen, the plant pathogen P. syringae, the soil bacterium P. putida, and the plant growth-promoting P. fluorescens [8].

Yersinia is a genus of bacteria in the family Enterobacteriaceae, Yersinia species are Gram-negative, rod-shaped bacteria, a few micrometers long and fractions of a micrometer in diameter, and are facultative anaerobes.

Some members of Yersiniaare pathogenic in humans; in particular, Y.pestis is the causative agent of the plague. Rodents are the natural reservoirs of Yersinia; less frequently, other mammals serve as the host. Infection may occur either through blood (in the case of Y. pestis) or in an alimentary fashion, occasionally via consumption of food products (especially vegetables, milk-derived products, and meat) contaminated with infected urine or feces [9].

Cloacibacterium is a dominant genus appeared in S-3 while little in other samples, This genus in the family Flavobacteriaceae was new to science when it was described in 2006.it is a signal from Untreated wastewater.

It is supposed that outfalls of wastewater were nearby upstream of S-3 In terms of genus category. compare sample S-1 and S-2, chemical oxygen demand loading increase from the former to the later and it changed the most dominate genus from 26% Flavobacterium to 29% Acinetobacter, while amount of Acinetobacter decrease to14% in sample S-2; The most dominate genus is Acinetobacter in sample S-4 that is at percent of 44%. Compared with others, S-18 has the biggest COD loading. Conclusion is: amount of Acinetobacter is related with COD loading in the water.

Comparing the four samples, S-18 is the only sample that has phosphorus pollution, and by analyzing all the dominate genus of four samples, Flavobacterium, a nitrogen fixing bacteria [10], is not dominate only in sample S-18 and the content is 3.6%. Conclusion is : Flavobacterium is related with content of phosphorus pollution, it is effected by the over grew of Acinetobacter and Acinetobacter.It is also effected by Water quality difference that is caused by point and none-point pollution, making the environmental sensitive organisms change. But this result cannot reflect all kinds of microorganismspecies in river, some uncultured organisms need to be researched

IV. CONCLUSIONS

As the river flows to downstream, some point-source of pollution feed into the Fenghe river along its way , three larger tributaries also change the kinds of pollutant and the pollutant load, the water quality becomes more and more complex. The microorganism communities in the river develop with these changes. On this basis, we can conclude that:

1,The most dominant phyla detected in stream habitats were Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes, microbialbiomass of Actinobacteria increase in water with more COD loading.

2, Acinetobacter, Yersinia, and Pseudomonas are three most dominate genus that can be find all in the river, all these most dominate genus belong to phylum of Proteobacteria.

3, Evenness increase along with increase of COD loading in water to a certain degree, and when COD loading is too much, evenness will decrease.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China.

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