JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2013. Vol. 7(4), p. 2783- 2789

Archaeal Community Structure and Quantity in the Oxygen Deficient Sediments from Three Water Supply Reservoirs

Zhang Hai-Han[1] and Huang Ting-Lin[1,*]

[1]School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, No.13, Yanta Road, Xi'an, 710055, Shaanxi Province, People's Republic of China.

(Received: 28 September 2013; accepted: 09 December 2013)

Archaea is an important microorganism distributed in the aquatic environmental conditions. To better understand the diversity of archaea in the water supply reservoir, the objective of this work was to evaluate and compare the sediment archaeal quantity and community diversity in three oligotrophic water supply reservoirs (JPR, ZCR and SBYR) using real time quantitative PCR (RT-qPCR) and high-throughput Illumina Miseq Sequencing (IMS) techniques. The results showed that archaeal 16S RNA gene copy per gram sediment were 6.09×10^4, 6.19×10^5 and 1.94×10^5 for JPR, ZCR and SBYR, respectively ($P<0.01$). Moreover, in total, 40941, 36552 and 31234 effective sequence reads of the sediment archaeal 16S rRNA gene were obtained using Illumina Miseq sequencing method from sediments of the ZC, SBY and JP reservoirs respectively. The highest Chao diversity index was observed in ZC reservoir, which was 2.80 times higher than that of the lowest JP reservoir. Methanosaeta, Methanospirillum, Methanosarcina, Methanobacterium, Methanosphaerula, Methanocella, Methanomethylovorans, Methanocorpusculum, Methanoculleus, Methanolinea, Methanosphaera and Thermofilum were observed in the sediments from three reservoirs. Methanosaeta was the dominated species in ZC and SBY reservoirs, and Methanosarcina was the dominate species in JP reservoir. Thermofilum was only found in JP reservoir. ZC reservoir had significantly greater operational taxonomic unit (OTU) richness. Heat map and principle component analysis (PCA) suggested that there was a distinct different sediment archaeal quantity and community compositions among three water supply reservoirs, which may be play an important role in driving nutrition transportation and shaping the water quality of water supply reservoirs.

Key words: Archaeal community, Water supply reservoir, Quantitative PCR, Illumina Miseq sequencing.

Archaea is the most abundant and diverse group of sediment microbes[1-2]. Compared with more literatures on soil archaea, we understand little about archaea living in aquatic oxygen-deficient sedimentation conditions[3-4]. Sediment microbial community represents the wondrous diversity habitats in the reservoir and is central to water ecosystem ecological functioning and restoration[5-6]. Consequently, there is an increasing need to understand the composition of archaea in the sediment of oligotrophic water supply reservoirs.

In our previous studies, BIOLOG, PCR-DGGE and clone sequence methods were combined used to exam sediment bacterial and fungal community functional diversity from SBY, TY and ZC water supply reservoirs[5-6]. However, these techniques have several limitations. For example, BIOLOG can be performed to determine aerobic cultivable bacterial and fungal species[7-9]. There are fewer bands can be showed in the DGGE gel.

* To whom all correspondence should be addressed.
E-mail: huangtinglin@xauat.edu.cn

More information of sediment microbial community was lost. With the development of molecular biological method, real time quantitative PCR (RT-qPCR) and next generation sequencing technology (NGST) are developed and they are powerful in determining the quantity and diversity of environmental microbial community composition. NGST allows us to deeply explore the structure and compositions of microbial communities based on the 16S or 18S ribosomal RNA sequences.

To date, there are three NGST platforms including Roche 454 FLX pyrosequencing, Illumina Miseq and Ion Torrent (PGM)[10]. These techniques have been commonly used to reveal various environmental microbial communities[11-13]. Meanwhile, numerous reports have been demonstrated that archaea can live in several aquatic ecosystems such as lakes, rivers, seas[14-18]. Unfortunately, no report has been focused on the oxygen-deficient surface sediment of the water supply reservoir with poor nutrition characteristics.

It is therefore the primary goal of present research was to determine the quantity and composition of sediment archaeal community from three different water supply reservoirs. To this end, quantitative PCR (qPCR) was used to investigate the sediment archaeal abundance, and Illumina Miseq sequencing (IMS) was also employed to compare sediment archaeal communities from JP, ZC and SBY reservoirs.

MATERIALS AND METHODS

Site Description and Sampling

The research was conducted in three different water supply reservoirs, named JPR, ZCR and SBYR. JPR and SBYR were located in Xi'an City, Shaanxi, Province, while ZCR was located in Zao Zhuang City, Shandong Province, China. The highest depths are 15-18 m, 45-50 m and 90-105 m for ZCR, SBYR and JPR, respectively[19-20]. Oxygen-deficient area is formed. Dissolved oxygen concentration is near to zero. As described in our previous studies, the surface sediments (0-30cm) were collected using a Petersen stainless steel grab sampler, put them in a small cooler with 8°C, and then transported to School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology (SEME-XAUAT) with 24 h. The sediment samples were stored at -20°C until microbial total DNA extraction process.

Sediment Microbial DNA Extraction

To extract sediment microbial total DNA, soil DNA Kit (Omega, USA) was selected[5]. As described of the manufacturer's recommendations, total sediment microbial DNA was extracted and examined by electrophoresis in 0.8% agarose gels. The extracted DNA samples were stored at -20°C.

Quantitative PCR Determination

To exam the relative abundance of archaea, we used real time quantitative PCR (qPCR) (SYBR Premix Ex Taq II, Takara). In this work, the archaeal primer set was ARC-787F (5'-GATTAGATACCCSBGTAGTCC-3') and ARC1059R (5'-GCCATGCACCWCCTCT-3'). Total volume was 25µl containing DNA template 0.5µl, primers 0.5µl, dNTP 0.5µl, 10×Taq Buffer 2.5µl , 25mM MgCl2 2µl, Taq 0.2µl, H2O18.3µl. The PCR process was 95°C for 3min, 35 cycles of 94°C for 30s, 56°C for 30s, 72°C for 30S, and then 72°C for 5min, the PCR products was 272 bp. Copy numbers of archaeal 16S gene were examined using external standards. A standard curve and cycle threshold values was constructed using 10 times serial dilutions. Melting curve (MC) analysis was determined from 55°C to 95°C. Average amplification efficiencies was 84.76% and amplification was linear ($R^2 = 0.999$).

Illumina Miseq Sequencing Determination

To determine the diversity of archaeal community, we used the Illumina Miseq sequencing technique[21]. V5-V6 16S rRNA gene region was employed to identify the archaeal species. The primer sets are F: 5'-AGGATTAGATACCCTGGTA-3', R: CRRCACGAGCTGACGAC-3'. Fusion of primers are: F: 5'-Index+AGGATTAGAT ACCCTGGTA-3', R: 5'-CRRCACGAGCTGACGAC-3'. PCR reactions contained 10 × reactions Buffer 2.50 µl, dNTP 2.00 µl, DNA 1.00 µl, primer F 1.00 µl, primer R 1.00 µl, Taq DNA polymerase 0.125 µl. PCR cycle parameters were 5 min at 94 °C; 25 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C; and final extension for 7 min at 72 °C. The multiplexed DNA libraries (10nM) were sequenced.

Statistical Analysis

In order to explore the diversity of sediment archaeal community structure, Chao index, ACE index, Shannon' diversity and Simpson diversity were used. Based on the operational

taxonomic units (OTUs) data (cut-off 0.03), diversity indexes were treated by mother (http://www.mothur.org/) software. One-way ANOVA with Tukey-Kramer HSD test was performed to evaluate the significant differences of archaeal abundance (real time qPCR) using DPS software (Version 6.4). Heat map was constructed by R software (Version 3.0.2). Principal component analyses (PCA) was used to compare the Illumina Miseq sequencing data.

RESULTS AND DISCUSSION

Abundance of Sediment Archaea

In this work, we found that there was abundance archaeal species in the sediments of water supply reservoirs. As shown in Figure 1, the results revealed that archaeal 16S RNA gene copy per gram sediment were 6.09×10^4, 6.19×10^5 and 1.94×10^5 for JPR, ZCR and SBYR, respectively ($P<0.01$) (Fig 1).

Community Diversity of Sediment Archaea

According to the Illumina Miseq sequencing data, the Chao indexes were 774, 245 and 276 for ZCR, SBYR and JPR. The highest abundance-based coverage estimators (ACE) index was observed in ZCR for 1544, and the lowest of that was observed in JPR. The Shannon's diversity of ZCR was 1.76 times higher than that of ZCR for 1.634. However, the Simpson diversity indexes were 0.134, 0.139 and 0.487 for ZCR, SBYR and ZCR, respectively (Table 1). As shown in Fig 2, *Methanosaeta*, *Methanospirillum*, *Methanosarcina*, *Methanobacterium*, *Methanosphaerula*, *Methanocella*, *Methanomethylovorans*, *Methanocorpusculum*, *Methanoculleus,* *Methanolinea,* *Methanosphaera* and *Thermofilum* were the dominated archaeal species harbored in the sediments from three reservoirs (Fig 2, Table 1). Methanobacterium was not significant different among three samples (Table 2). Methanosaeta was

Table 1. The richness index and phylogenetic diversity of sediment archaeal community in ZC, SBY and JP water supply reservoirs based on Illumina Miseq sequencing

Reservoirs	Chao index	ACE index	Shannon's diversity	Simpson diversity	Coverage
ZCR	774	1544	2.869	0.134	0.957
SBYR	245	440	2.683	0.139	0.938
JPR	276	325	1.634	0.487	0.943

ACE index represent abundance-based coverage estimators. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively.

Table 2. Summary of parsimony test (P-test) results for the comparison of archaeal communities among sediment samples from ZCR, SBYR and JPR

Taxon	ZCR vs JPR (P-value)	JPR vs SBYR (P-value)	SBYR vs ZCR (P-value)
Methanobacterium	NS	NS	NS
Methanocella	<0.01	0.01	NS
Methanocorpusculum	0.01	NS	0.01
Methanoculleus	0.01	NS	0.01
Methanolinea	0.01	NS	0.01
Methanomethylovorans	0.01	NS	0.01
Methanosaeta	0.05	0.05	0.05
Methanosarcina	NS	NS	0.05
Methanosphaera	0.01	NS	0.01
Methanosphaerula	0.01	0.01	0.05
Methanospirillum	0.05	NS	NS
Thermofilum	0.01	0.01	NS

NS represents not significant

significant different among samples. As shown in Fig 2, there were Methanosaeta (53.6%), Methanosarcina (25.6%), Methanospirillum (12.0%), Methanobacterium (7.2%), Methanosphaerula (0.8%), Methanocella (0.8%) distributed in SBY reservoir. Meanwhile, Methanosarcina (44.0%), Methanosaeta (24.0%), Methanospirillum (18.0%), Methanobacterium (12.0%), Thermofilum (2.0%) were observed in JP reservoir. For ZC reservoir, Methanosaeta (76.31%), Methanospirillum (10.48%), Methanosarcina (6.71%), Methanobacterium (3.46%), Methanosphaerula (1.68%), Methanocella (0.32%), Methanomethylovorans (0.32%), Methanocorpusculum (0.21%), Methanoculleus (0.21%), Methanolinea (0.21%), Methanosphaera (0.11%). Thermofilum was not found. Methanosaeta was the dominated species in ZC and SBY reservoirs. However, Methanosarcina was the dominate species in JP reservoir. The sequencing data indicate that the ZC can host species of archaea in numbers higher than the JP and SBY. ZC had significantly greater OTU richness. The Venn diagram shows operational taxonomic units (OTUs) found exclusively in ZC (172), in JP (39), in SBY[35] (Figure 3). Illumina Miseq sequencing targeting the 16S rRNA gene of archaea showed shifts in the composition of archaeal community. Heat map

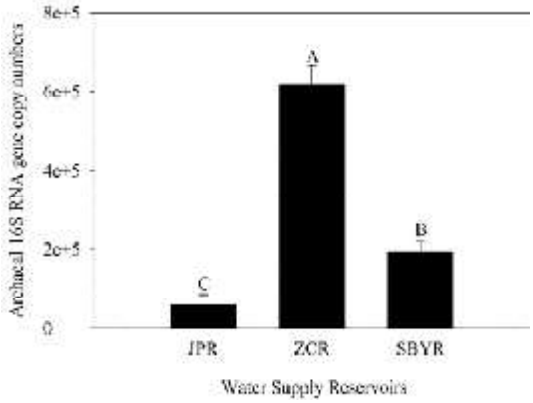

Fig. 1. Abundance of archaea species living in the sediment samples expressed as archaeal 16S RNA gene copy numbers per gram sediment from ZC, SBY and JP reservoirs. Different capital letter above the bars indicate significant difference by Tukey-Kramer HSD ($P<0.01$). Error bars represent standard deviations of triplicate. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively.

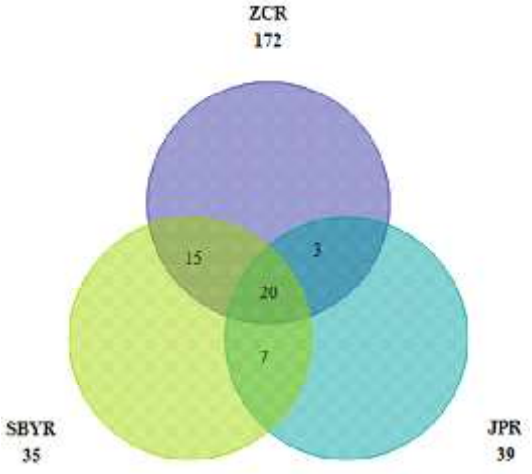

Fig. 3. Venn diagram shows the unique and shared OTUs of archaeal community from ZC, SBY and JP reservoirs. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively.

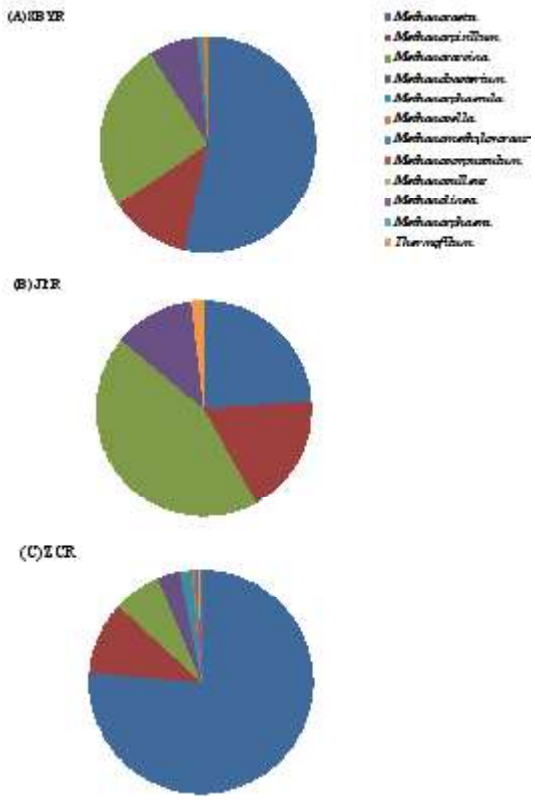

Fig. 2. Relative abundances of the dominant archaeal genus in the sediments of ZC, JP and SBY reservoirs, relative abundances are based on the proportional frequencies of archaeal 16S RNA sequences that can be classified at the genus level. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively.

colors indicate the relative percentage of archaea ranging within each sample. High and low OTUs were showed in Figure 5 (A). Heat map and principle component analyses (PCA) score plot revealed that there was a significant different sediment archaeal quantity and community compositions among three drinking water reservoirs (Figure 4, 5).

In the past few decades, several numbers of molecular approaches were developed to measure the sediment microbial community diversity from various aquatic environmental conditions. An increasing number of researches have been examined the archaeal species[14-18]. Consequently, there is an increasing need to understand archaea functioning. In aquatic ecosystems, sediment archaea is complex and varies in number and community composition. However, fewer works has been conducted to determine the quantity and diversity of archaeal community from the oligotrophic water supply

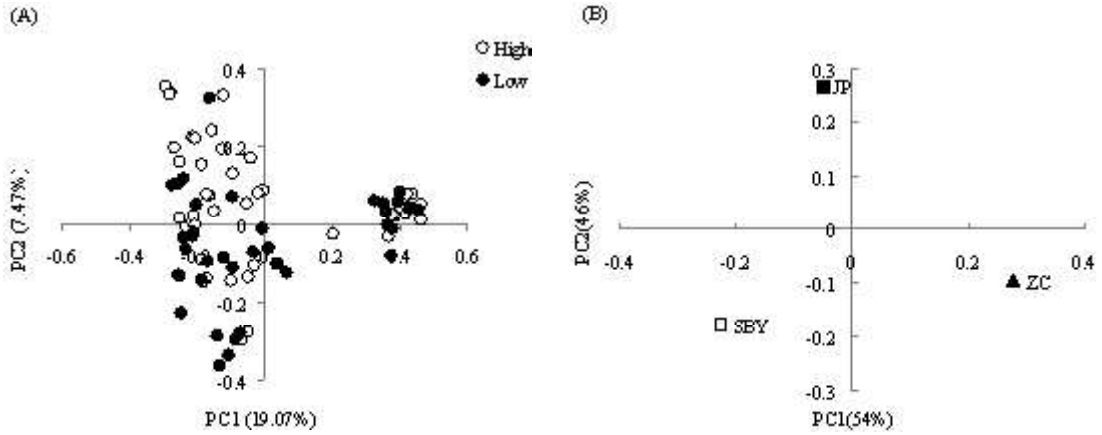

Fig. 5. Principle component analyses (PCA) scatter plots of sediment archaeal community diversity from ZC, SBY and JP reservoirs. (A) PC1 and PC2 explained the total variance for 19.07% and 7.47%. (B) PC1 explained the total variance for 54%, PC2 explained the total variance for 46%. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively.

reservoirs. In this study, we used molecular methods to explore the archaeal community, the composition of the archaeal community was monitored by Illumina Miseq sequencing of 16S rRNA genes and several valuable data was obtained. Koizumi *et al.*,[18] used PCR-DGGE to investigate sediment archaeal community structure in mesophilic freshwater lake, and suggested that the diversity of archaeal community did not drastically change through vertical changes, and sediment archaeal community in low-temperature has been underestimated. Similarly, Borrel *et al.*,[22] studied the depth changes of anoxic freshwater sediments archaeal communities of a freshwater meromictic Lake Pavin, France. The results showed that dramatically changes were observed in the archaeal community diversity along the sediment core. Green *et al.*,[23] examined sediment archaeal community diversity a sub-tropical polymictic reservoir, Lake Wivenhoe using cloning the 16S rRNA gene method, and revealed that the sediment

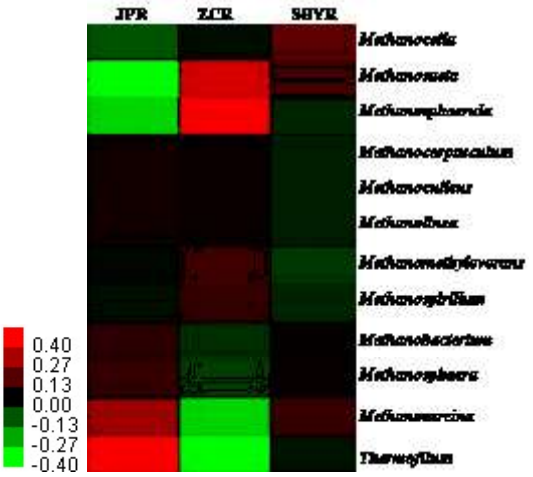

Fig. 4. Heat map diagram shows sediment archaeal community diversity from ZC, SBY and JP reservoirs. ZCR, SBYR, JPR represent ZC, SBY and JP water supply reservoir, respectively. Red and green colors (from -0.40 to 0.40) represent relative abundance of high and low OTUs, respectively.

archaeal community structure was shaped by the delivery of dissolved oxygen. In this study, JP and SBY reservoirs are near to each other, and they are oligotrophic drinking water reservoir, the water quality has been detecting regularly[19-20]. The sediment archaeal community might be influenced by water quality.

This work also provides further evidence that Illumina Miseq sequencing technology has shown great potential to reveal fresh water sediment archaeal community. Sediment microbial species such as archaea, bacteria and fungi also play an important role in regulation the water quality and pollutions released from the sediment to the surface water. It is necessary that sediment ammonia-oxidizing archaea, bacterial and fungal community and quantity should be determined simultaneously in the field experiments.

ACKNOWLEDGMENTS

The group sincerely expresses their gratitude to three reviewers for insightful suggestions on an earlier version of the work. The National Science and Technology Pillar Program (Grant No. 2012BAC04B02), the Doctoral Fund of Youth Scholar of Ministry of Education of China (Grant No. 20126120120002) and Shaanxi Postdoctoral Foundation Funded Projects funded this research.

REFERENCES

1. Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R. Examining the global distribution of dominant archaeal populations in soil. *ISME J*., 2011; **5**: 908-917.
2. Laurence, H., Mauro, T., Jakob, Z., Raffaele, P., Walter, W., John, P. Composition of bacterial and archaeal communities in freshwater sediments with different contamination levels (Lake Geneva, Switzerland). *Water Res.,* 2011; **45**: 1213-1228.
3. Leininger, S., Urich, D.A., Schloter, M., Schwark, L., Qi, J., Nicol, G.W. Archaea predominate among ammonia oxidizing prokaryotes in soils. *Nature*, 2006; **442**: 806-809.
4. Cao, H.L., Hong, Y.G., Li, M., Gu, J.D. Phylogenetic diversity and ecological pattern of ammonia-oxidizing archaea in the surface sediments of the Western Pacific. *Microb. Ecol*., 2011; **62**: 813-823.
5. Zhang, H.H., Huang, T.L., Liu, T.T. Sediment enzyme activities and microbial community diversity in an oligotrophic drinking water reservoir, eastern China. PLoS ONE, 2013; DOI: 10.1371/journal.pone.0078571.
6. Zhang, H.H., Huang, T.L., Chen, S.N., Guo, L., Liu, T.T., Yang, X. Microbial community functional diversity and enzymatic activity in the sediments of drinking water reservoirs, Northwest China. Desalin. Water Treat., 2013; DOI:10.1080/19443994.2013.827383.
7. Zhang, H.H., Huang, T.L., Yang, X., Ma, W.X. Soil dehydrogenase activity and bacterial community diversity in the water level fluctuation zone of a drinking water reservoir. *J. Pure Appl. Microbio*., 2013; **7**: 2451-2458.
8. Choi, K.H., Dobbs, F.C. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *J. Microbiol. Methods*., 1999; **36**: 203-213.
9. Zhang, H.H., Huang, T.L., Chen, S.N., Guo, L., Yang, X., Liu, T.T. Spatial pattern of bacterial community functional diversity in a drinking water reservoir, Shaanxi province, northwest China. *J. Pure Appl. Microbio.,* 2013; **7**: 1647-1654.
10. Loman, N. J., Misra, R.V., Dallman, T.J., Constantinidou, C., Gharbia, S.E., Wain, J., Pallen, M.J. Performance comparison of benchtop high-throughput sequencing platforms. *Nat. Biotechnol*., 2012; **30**: 434-439.
11. Luo, C., Tsementzi, D., Kyrpides, N., Read, T., Konstantinidis, K.T. Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample. PLoS ONE, 2012; **7**: e30087.
12. Christopher, Q., Anders, L., Thomas, P.C., Russell, J.D., Neil, H., Ian, M.H., Fiona, R., William, T.S. Accurate determination of microbial diversity from 454 pyrosequencing data. *Nature Methods*., 2009; **6**: 639-641.
13. Wirth, R., Kovács, E., Maróti, G., Bagi, Z., Rákhely, G., Kovács, K.L. Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnol. Biofuels.,* 2012; **5**: 41.
14. Li, Z.X., Jin, W.B., Liang, Z.Y., Yue, Y.Y., Lv, J.H. Abundance and diversity of ammonia-oxidizing archaea in response to various habitats in Pearl River Delta of China, a subtropical maritime zone. *J. Environ. Sci*., 2013; **25**: 1195-1205.
15. Lloyd K.G., Schreiber, L., Petersen, D.G., Kjeldsen, K.U., Lever, M.A., Steen, A.D., Stepanauskas, R., Richter, M., Kleindienst, S.,

Lenk, S., Schramm, A., Jørgensen, B.B. Predominant archaea in marine sediments degrade detrital proteins. *Nature*, 2013; **496**: 215-218.

16. Wang, P., Li, T., Hu, A.Y., Wei, Y.L., Guo, W.T., Jiao, N.Z., Zhang, C.L. Community structure of archaea from deep-sea sediments of the South China Sea. *Microb. Ecol*., 2010; **60**: 796-806.
17. Tang, C., Madigan, M.T., Lanoil, B. Bacterial and archaeal diversity in sediments of West Lake Bonney, McMurdo Dry Valleys, Antarctica. *Appl. Environ. Microbiol*., 2013; **79**: 1034-1038.
18. Koizumi, Y., Takii, S., Fukui, M. Depth-related change in archaeal community structure in a freshwater lake sediment as determined with denaturing gradient gel electrophoresis of amplified 16S rRNA genes and reversely transcribed rRNA fragments. *FEMS Microbiol. Ecol.*, 2004; **48**: 285-292.
19. Qin, C.H., Huang, T.L., Li, X. Studies on the seasonal variation and budget of nitrogen, phosphorus of the shibianyu reservoir. *J. Xi'an Univ. Arch. Tech.,* 2013; **45**: 111-116.
20. Ma, Y., Guo, Q.L., Huang, T.L., Tan, P. Response characteristics of water quality to the seasonal thermal stratification in Jin-pen reservoir along the Heihe river, Xi'an city in China. *J. Hydraul. Eng.*, 2013; **44**: 406-415.
21. Andrea, K.B., Michael, D.J.L, Jennifer, C.S., Gabriel, M.H., Josh, D.N. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Appl. Environ. Microbiol.,* 2011; **77**: 3846-3852.
22. Borrel, G., Lehours, A.C., Crouzet, O., Jézéquel, D., Rockne, K., Kulczak, A., Duffaud, E., Joblin, K., Fonty, G. Stratification of Archaea in the deep sediments of a freshwater meromictic lake: vertical shift from methanogenic to uncultured archaeal lineages. PLoS ONE., 2012; **8**: e43346.
23. Green, T.J., Barnes, A.C., Bartkow, M., Gale, D., Grinham, A. Sediment bacteria and archaea community analysis and nutrient fluxes in a sub-tropical polymictic reservoir. *Aquat. Microb. Ecol*., 2012; **65**: 287-302.