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

Zhang Hai-Han\textsuperscript{1} and Huang Ting-Lin\textsuperscript{1,*}

\textsuperscript{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.

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Archaea is the most abundant and diverse group of sediment microbes\textsuperscript{1-2}. Compared with more literatures on soil archaea, we understand little about archaea living in aquatic oxygen-deficient sedimentation conditions\textsuperscript{3-4}. Sediment microbial community represents the wondrous diversity habitats in the reservoir and is central to water ecosystem ecological functioning and restoration\textsuperscript{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\textsuperscript{5-6}. However, these techniques have several limitations. For example, BIOLOG can be performed to determine aerobic cultivable bacterial and fungal species\textsuperscript{7-9}. There are fewer bands can be showed in the DGGE gel.

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

* 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). These techniques have been commonly used to reveal various environmental microbial communities. Meanwhile, numerous reports have been demonstrated that archaea can live in several aquatic ecosystems such as lakes, rivers, seas. 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. 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℃, 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℃ until microbial total DNA extraction process.

**Sediment Microbial DNA Extraction**

To extract sediment microbial total DNA, soil DNA Kit (Omega, USA) was selected. 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℃.

**Quantitative PCR Determination**

To examine 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'-GCCATGACCCWCTCT-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℃ to 95℃. Average amplification efficiencies was 84.76% and amplification was linear (R² = 0.999).

**Illumina Miseq Sequencing Determination**

To determine the diversity of archaeal community, we used the Illumina Miseq sequencing technique V5-V6 16S rRNA gene region was employed to identify the archaeal species. The primer sets are F: 5'-AGGATTAGATACCCCTGGTA-3', R: CRRCACAGGTGACGC -3'. Fusion of primers are: F: 5'-Index+AGGATTAGATACCCCTGGTA-3', R: 5'CRRCACAGGTGACGC-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℃; 25 cycles of 30 s at 94℃, 30 s at 55℃ and 30 s at 72℃; and final extension for 7 min at 72℃. 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 $6.09 \times 10^4$, $6.19 \times 10^5$ and $1.94 \times 10^5$ 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

<table>
<thead>
<tr>
<th>Reservoirs</th>
<th>Chao index</th>
<th>ACE index</th>
<th>Shannon's diversity</th>
<th>Simpson diversity</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZCR</td>
<td>774</td>
<td>1544</td>
<td>2.869</td>
<td>0.134</td>
<td>0.957</td>
</tr>
<tr>
<td>SBYR</td>
<td>245</td>
<td>440</td>
<td>2.683</td>
<td>0.139</td>
<td>0.938</td>
</tr>
<tr>
<td>JPR</td>
<td>276</td>
<td>325</td>
<td>1.634</td>
<td>0.487</td>
<td>0.943</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Taxon</th>
<th>ZCR vs JPR (P-value)</th>
<th>JPR vs SBYR (P-value)</th>
<th>SBYR vs ZCR (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanobacterium</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Methanocella</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Methanocorpusculum</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Methanoculleus</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Methanolinea</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Methanomethylovorans</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Methanoseta</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Methanosarcina</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td>Methanosphaera</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Methanosphaerula</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Methanospirillum</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Thermofilum</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

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
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. 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 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. 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. 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. 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. 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.

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. 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.

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