Research Article

The Vertical Distribution of Sediment Archaeal Community in the “Black Bloom” Disturbing Zhushan Bay of Lake Taihu

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Using the Illumina sequencing technology, we investigated the vertical distribution of archaeal community in the sediment of Zhushan Bay of Lake Taihu, where the black bloom frequently occurred in summer. Overall, the Miscellaneous Crenarchaeotal Group (MCG), Deep Sea Hydrothermal Vent Group 6 (DHVEG-6), and Methanobacterium dominated the archaeal community. However, we observed significant difference in composition of archaeal community among different depths of the sediment. DHVEG-6 dominated in the surface layer (0–3cm) sediment. Methanobacterium was the dominating archaeal taxa in the L2 (3–6cm) and L3 (6–10cm) sediment. MCG was most abundant in the L4 (10–15cm) and L5 (15–20cm) sediment. Besides, DHVEG-6 was significantly affected by the concentration of total phosphorus (TP). And loss on ignition (LOI) was an important environmental factor for Methanobacterium. As the typical archaeal taxa in the surface layer sediment, DHVEG-6 and Methanobacterium might be more adapted to abundant substrate supply from cyanobacterial blooms and take active part in the biomass transformation. We propose that DHVEG-6 and Methanobacterium could be the key archaeal taxa correlated with the “black bloom” formation in Zhushan Bay.

1. Introduction

Archaea have traditionally been recognizable as extremophiles. However, culture-independent approaches such as 16S rRNA gene sequence analysis have shown Archaea can colonize vast reaches of the earth [1, 2]. Previous studies have extensively studied the methanogenic community in the freshwater lakes. And a review article indicates Methanomicrobiales and Methanosarcinales usually dominate the methanogenic community in freshwater sediment [3]. The uncultured archaeal groups of Miscellaneous Crenarchaeotic Group (MCG) and Deep Sea Hydrothermal Vent Group 6 (DHVEG-6) were also detected in freshwater lakes [4, 5]. The ubiquitous MCG is reported to contribute significantly to carbon and nitrogen cycling within the environments [6, 7]. DHVEG-6 was detected dominating in wastewater treating bioreactors [8], which indicated it might be heterotrophic and contribute to nutrient cycling. As a result of their diverse function and their ubiquity, Archaea may play a critical role in driving global biogeochemical cycles and maintaining the health of the freshwater environment.

“Black bloom” is a phenomenon in lakes, rivers, or seashores, which is characterized as hypoxic and malodorous [9, 10]. It often occurs during the summer after severe algae blooms and has become a serious ecological problem in water environments [11]. Lake Taihu is a large shallow eutrophic freshwater lake [12]. Serious cyanobacterial blooms frequently occurred in some lake zones of Lake Taihu, as a result of eutrophication [13]. As a result of cyanobacterial blooms, the “black bloom” happened frequently from 2007 to 2011 in Meiliang Bay, Gonghu Bay, and Zhushan Bay of Lake Taihu [14]. To date, researches on microbial diversity in the black bloom occurring lake zones have mainly focused on Bacteria. Clostridium, Desulfovibrio, and Comamonadaceae were found to be the main biological factor contributing to lacustrine black bloom [15, 16]. However, little is known about
the diversity and vertical distribution of archaeal community in the lake sediment.

In this study, we investigated archaeal community composition in the sediment of the black bloom occurring area of Lake Taihu by using the next-generation sequencing method of Illumina. We particularly want to know whether the archaeal community composition is different among different layers of the sediment, given the difference in quality and quantity of organic materials among different sediment layers resulting from degradation of cyanobacterial blooms. We also want to see if there are some key sediment archaeal taxa contributing to the black bloom in Zhushan Bay.

2. Materials and Methods

2.1. Site Description and Sample Collection. Lake Taihu is a large shallow eutrophic lake with an area of 2338 km² and an average depth of 1.9 m, located in the Yangtze River Delta (30°55.667'–31°32.967'N, 119°52.533'–120°36.167'E). Zhushan Bay is one of the most eutrophic bays in north of Lake Taihu, where the black bloom frequently occurred in summer.

Three sediment cores (8.6 cm inner diameter, 25 cm length) were collected from Zhushan Bay (31°23.705’N, 120°02.176’E) on July 9, 2010. The sediment cores were immediately transported to lab on ice. Once arriving at the laboratory, the sediment cores were sliced as 0–3 cm, 3–6 cm, 6–10 cm, 10–15 cm, and 15–20 cm. The three replicates for the five layers were mixed as thoroughly as possible. 0–3 cm, 3–6 cm, 6–10 cm, 10–15 cm, and 15–20 cm were labeled as L1, L2, L3, L4, and L5, respectively. Then, the five samples for DNA extraction were stored at −20°C and those for analysis of soil chemical properties at 4°C.

2.2. Physicochemical Analysis. The physicochemical properties of its overlying water were investigated using the YSI 550A instrument. Chlorophyll a (Chla) of sediment was determined using the HP8452 UV-Vis spectrophotometry. Total phosphorus (TP) was analyzed by molybdenum antimony resistance-colorimetric method. Total nitrogen (TN) was analyzed by Kjeldahl method. Total organic carbon (TOC) was analyzed by potassium dichromate oxidation-ferrous sulphate titrimetry method. Moisture content (MC) of sediment was measured after drying to constant weight at 105°C. Loss on ignition (LOI) was analyzed by heating at 550°C for 2 h. The TP, TN, TOC, MC, and LOI of sediment were analyzed according to Bao (2000) [17].

2.3. DNA Extraction, PCR Amplification, and Sequencing. Before DNA extraction, freeze-drying of the sediment samples at −53°C was performed in a freeze dryer (LABCONCO, 2.5 L). DNA was extracted from the five sediment layers in three replicates, using a FastDNA spin kit for soil (MP Biomedicals LLC, Ohio, USA). And according to the instruction, 0.5 g dried sediment was used for the DNA extraction. The quality and size of the DNA were checked by electrophoresis on 1% agarose gels.

The extracted DNA was amplified with the archaeal domain-specific primer set 519f (5'-CAGCMGCAGGCTAA-3') [18] with barcode, and 915r (5'-GTGCTCCCCGCAATTCCTCC-3') [19]. Protocol and conditions for polymerase chain reaction (PCR) were according to Coolen et al. (2004) [20]. The amplicons were purified by Gel Extraction Kit (Takara Bio, Dalian, China). The size of amplicons was checked by electrophoresis on 2% agarose gels. The purity and quantity of amplicons were assessed using the Nanodrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Finally, the amplicons for different samples were pooled in equimolar ratios for sequencing analysis. Library preparation and DNA sequence analysis using an Illumina MiSeq paired-end 300 bp protocol (Illumina, Inc., San Diego, CA, USA) were performed at Shanghai BIOZERON Biotechnology Co., Ltd. (Shanghai, China). The raw sequence data was submitted to the ENA database (PRJEB10387).

2.4. Sequence Data Processing. Trimmomatic software was used to process the raw sequence data for quality control [21]. The PE reads were overlapped to assemble the final tag sequences with minimum overlap length as 10 bp. We removed all of the sequences that contained more than one ambiguous basis “N,” those that contained any errors in the forward or reverse primers, and those with more than 0.2 mismatch ratio within the overlap region. Tail base of reads with quality values below 20 was filtered, and the variable tags (overlapped length minus primers and barcodes) that were shorter than 50 bp were also removed. The obtained clean sequences were then analyzed at the Qiime platform [22]. The clean sequences were screened for chimeras using Usearch [23]. After the raw sequence data processing, sequences with length between 301 bp and 500 bp were used for the following analysis (see Figure S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/8232135). Then, operational taxonomic unit (OTU) grouping was performed using Usearch software at 97% similarity. In order to filter the bacterial sequences during the assigning taxonomy process, an archaeal database was made by extracting archaeal sequences from the Silva database (release119 http://www.arb-silva.de/). The database was provided by the Shanghai BIOZERON Biotechnology Co., Ltd. (Shanghai, China). Taxonomic data was then assigned to each representative sequence against the archaeal database at 97% similarity, using the RDP Classifier (http://sourceforge.net/projects/rdp-classifier/). Total of 17,358 sequences which cannot find affiliations were filtered out during this process.

2.5. Statistical Analysis. To perform the downstream analyses, the OTU matrix must be normalized to account for uneven sample sums. The normality of OTU data was performed using the command of “normalize.shared”, and the number of sequences for each sample was set as 19910, referring to the smallest group. The α-diversity of the archaeal community was indicated by the inverse Simpson diversity index. Besides, the sample coverage and the number of observed OTUs (Sobs) were also provided in Table 3. The normality of OTU data and analysis of the α-diversity of the archaeal community were performed using Mothur software [24]. The coverage percentage was calculated according to
Table 1: Some physicochemical characteristics of the five sediment layers.

| Sample | Chla (µg/kg) | TP (g/kg) | TN (g/kg) | TOC (g/kg) | LOI (%) | MC (%) |
|--------|--------------|-----------|-----------|------------|---------|--------|
| L1     | 616.99       | 1.13      | 1.23      | 12.35      | 2.15    | 51.08  |
| L2     | 704.84       | 0.93      | 1.35      | 13.54      | 6.21    | 48.74  |
| L3     | 309.51       | 0.59      | 0.83      | 8.32       | 2.47    | 47.49  |
| L4     | 110.86       | 0.53      | 0.86      | 8.64       | 2.18    | 46.92  |
| L5     | 159.64       | 0.66      | 0.82      | 8.25       | 1.26    | 44.08  |

Table 2: The top 13 most abundant families in all of the samples from the five sediment layers.

| Class | Family | Percentage (%) |
|-------|--------|----------------|
| MCG   | MCG_norank | 22.78          |
| Halobacteria | DHVEG-6 | 20.10         |
| Unclassified Archaea | Unclassified Archaea | 19.94 |
| Methanobacteria | Methanobacteriaceae | 14.22 |
| Unclassified Euryarchaeota | Unclassified Euryarchaeota | 7.50 |
| Halobacteria | MEG | 2.36 |
| Group C3 | Group C3_norank | 1.80 |
| Thermoplasmata | ASC21 | 1.54 |
| Halobacteria | DSEG | 1.33 |
| Thermoplasmata | MBGD and DHVEG-1 | 1.12 |
| Methanomicrobia | ANME-1a | 0.58 |
| Methanomicrobia | Methanosarcinaceae | 0.54 |
| SCG | SCG_norank | 0.53 |

Note. "Percentage" was the average value of all samples; Miscellaneous Crenarchaeotic Group (MCG); Deep Sea Hydrothermal Vent Gp-6 (DHVEG-6); Miscellaneous Euryarchaeotic Group (MEG); Deep Sea Euryarchaeotic Group (DSEG); Marine Benthic Group D (MBGD); anaerobic methanotroph (ANME).

3. Results

3.1. Physicochemical Properties. The physicochemical properties of overlying water were shown in Table S1. Environmental parameters changed largely among the five sampling layers of Zhushan Bay sediment (Table 1). The average concentration values of all environmental parameters were observed much higher in the surface layers (L1-L2, 0–6 cm) than the deeper layers (L3–L5, 6–20 cm). The highest concentration values of Chla, TN, TOC, and LOI were observed in L2 (3–6 cm) sediment, while the concentration values of TP and MC were highest in L1 (0–3 cm) sediment.

3.2. Archaeal Community in Zhushan Bay Sediment. Results of taxonomic analysis indicated the top three archaeal classes were Halobacteria (24.42%), MCG (22.78%), and Methanobacteria (14.22%), except unclassified Archaea (Figure 1(a)). The archaeal community was dominated by Halobacteria in the L1 (0–3 cm) sediment. In the L2 (3–6 cm) sediment, methanogen (Methanobacteriaceae and Methanomicrobiota) was main component of archaeal community. In the L3 (6–10 cm) sediment, the relative abundance of MCG obviously increased. And in the L4 (10–15 cm) and L5 (15–20 cm) sediment, MCG dominated in the archaeal community.

As the dominating methanogen, Methanobacteria consisted of seven OTUs (Figure 1(b)). These seven OTUs were affiliated to the genera of Methanobacterium and Methanobrevibacter. The composition of Methanobacteria did not change significantly among the five sediment layers. And Methanobacterium_OTU5 was the most abundant OTU in all of the five sediment layers.

Additionally, MCG_norank, DHVEG-6, and Methanobacteriaceae were the top three dominating families in the sediment, except unclassified Archaea (Table 2). DHVEG-6 was affiliated to Halobacteria, while Methanobacteriaceae was affiliated to Methanobacteria.
### Table 3: α-Diversity analysis for archaeal community of the five sediment layers (standard deviations of replicates are in brackets).

| Sample | Coverage (%) | Sobs  | Inverse Simpson |
|--------|--------------|-------|-----------------|
| L1     | 98.00 (0.15) | 1641 (134) | 83.19 (7.96) |
| L2     | 99.25 (0.09) | 809 (177)  | 15.70 (6.49)  |
| L3     | 99.17 (0.47) | 891 (310)  | 30.11 (3.90)  |
| L4     | 99.60        | 798      | 63.35          |
| L5     | 98.62 (0.42) | 1212 (363) | 54.78 (15.92) |

### 3.3. Statistical Analysis.

Coverage estimate indicated that the archaeal 16S rRNA gene libraries for each sample were large enough to capture the total estimated OTUs (Table 3). And the average values of inverse Simpson index suggested that the L2 (3–6 cm) sediment harbored the lowest diversity of archaeal community, while the highest diversity of archaeal community was found in the L1 (0–3 cm) sediment.

Global ANOSIM comparison indicated the overall archaeal community composition among the five sediment layers was significantly different ($R$ statistic = 0.932, $P = 0.001$). Observed difference was qualitatively displayed in the RDA plot, where relative similarities among sediment layers were presented by clusters of layer-specific data points (Figure 2(a)). Besides, result of RDA at the OTU level showed the first axis explained 22.65% of total microbial variance and the second axis 10.01%. The Monte Carlo permutation test at the OTU level showed the environmental factors were significantly related to archaeal community distribution (Pseudo-$F = 1.93$; significance level = 0.001). Envfit test at the OTU level suggested Chla and TP were significant at the 0.001 level ($P = 0.001$), while LOI was significant at the 0.05 level ($P = 0.002$). The RDA plot at the OTU level indicated TP was a more important factor for the archaeal community in the L1 sediment, while LOI was a more important factor for the archaeal community in the L2 and L3 sediment. Archaeal community in the L1 and L2 sediment was both affected by the Chla. Moreover, result of RDA at the family level showed the first axis explained 25.42% of total microbial variance and the second axis 15.36% (Figure 2(b)). The Monte Carlo permutation test at the family level showed the environmental factors were significantly related to archaeal community distribution (Pseudo-$F = 2.98$; significance level = 0.001). Envfit test at the family level suggested Chla and TP were significant at the 0.001 level ($P = 0.001$), while LOI was significant at the 0.05 level ($P = 0.004$). The RDA plot at the family level indicated TP was an important factor for DHVEG-6 and MEG, while LOI was an important
Figure 2: (a) The relationship of archaeal community composition among the five sediment layers with the major environmental factors at the OTU level. Redundancy analysis (RDA) plot was drawn by RDA1 and RDA2. (b) The most abundant 13 families were correlated with the major environmental factors (MCG: Miscellaneous Crenarchaeotic Group; DHVEG-6: Deep Sea Hydrothermal Vent Gp-6; MEG: Miscellaneous Euryarchaeotic Group; DSEG: Deep Sea Euryarchaeotic Group; MBGD: Marine Benthic Group D; ANME: anaerobic methanotroph).

Cluster analysis of the top 13 most abundant families identified specific families; those were differentially distributed among the five sediment layers (Figure 3). DHVEG-6 was most abundant in the L1 (0–3 cm) sediment. Methanobacteriaceae and Methanosarcinaceae were most abundant in the L2 (3–6 cm) sediment. Methanobacteriaceae was more abundant in the L3 (6–10 cm) sediment, compared with the ones in L1, L4, and L5 sediment. Group C3_norank and Deep Sea Euryarchaeotic Group (DSEG) were most abundant in the L4 (10–15 cm) sediment. MCG_norank and anaerobic methanotroph (ANME)-1a were most abundant in the L4 (10–15 cm) and L5 (15–20 cm) sediment.

4. Discussion

4.1. Significant Vertical Heterogeneity of Archaeal Community in Zhushan Bay Sediment. Significant difference of overall archaeal community composition among the five sediment layers was found ($P = 0.001$), which may result from the decreasing nutrients from the surface layers to the deep layers (Figure 2). The vertical heterogeneity of archaeal community was also observed in another lake zone of Lake Taihu [29].

DHVEG-6 differentially distributed among the five sediment layers and was most abundant in L1 sediment (Figure 3). DHVEG-6 was known as haloarchaea previously, as it had been detected in hydrothermal sediment [30], deep sea methane seep sediment [31], hypersaline [32], and shallow saline [33] lakes. However, DHVEG-6 was also detected in the water of freshwater lake [5] and in municipal wastewater treating methanogenic bioreactors [34]. Moreover, it is found that DHVEG-6 was the predominant uncultured archaeal community in wastewater treatment sludge, being most abundant in the nitrogen-/phosphate-removing wastewater treatment sludge [8]. This might indicate some groups of DHVEG-6 were more adaptive to high substrate supply, and...
phosphate might be a key factor to it. And the result of this study also indicated members of DHVEG-6 were significantly affected by TP (Figure 2(b)). More organic matters and higher level of TP in the surface sediment may favor the DHVEG-6.

*Methanobacteriaceae* differentially distributed among the five sediment layers and was most abundant in L2 and L3 sediment (Figure 3). Besides, *Methanosarcinaceae* was also the most abundant family in the L2 sediment (Figure 3). However, previous studies revealed that the Methanomicrobia usually dominated the methanogenic communities in freshwater sediment, while the *Methanobacteria* occurred scarcely [3], using clone libraries and/or fluorescent in situ hybridization methods. *Methanobacteria* were usually found to be the predominant methanogen in the wastewater treatment sludge or municipal solid waste landfill [35]. It could be noted that strains and type strains of *Methanobacterium* and *Methanosarcinaceae* were commonly cultivated from freshwater lakes by using media with high substrate concentrations [36, 37]. And in this study, 99.99% sequences of *Methanobacteriaceae* were affiliated to *Methanobacterium* (Figure 1(b)). These indicated *Methanobacterium* and *Methanosarcinaceae* were more adaptive to high carbon concentrations. And the result of this study also indicated members of *Methanobacterium* and *Methanosarcinaceae* were significantly affected by TP (Figure 2(b)). Moreover, members of *Methanobacterium* were adapted to the habitats with high substrate supply, such as wastewater treatment sludge [8, 35]. Besides, DHVEG-6 was significantly affected by the concentration of phosphate, and labile organic matters were an important environmental factor for *Methanobacterium* (Figure 2(b)). Moreover, members of *Methanobacterium* were strictly anaerobic *Archaea* [36]. However, more work is required to enable a better understanding of the roles of DHVEG-6 in Zhushan Bay sediment. And the dominating uncultured species of *Methanobacterium_OTU5* (Figure 1(b)) was expected to be further understood by isolation and culture methods. DHVEG-6 and *Methanobacterium* might actively take part in the degradation of cyanobacterial biomass, contributing to the black bloom in Zhushan Bay.

5. Conclusion

This is the first study to investigate the vertical distribution of archaeal community in a “black bloom” disturbing area using high throughput sequencing technology. Our work revealed that the vertical distribution of archaeal community in the 0–20 cm sediment was significantly heterogeneous. DHVEG-6 and *Methanobacterium* dominated in the surface sediment, which might be the key archaea taxa correlated with the “black bloom” occurrence in Zhushan Bay, Lake Taihu. This work shed light on the contribution of *Archaea* to the black bloom formation in this high-risk area of cyanobacterial blooms. More work is needed to get a better understanding of the roles of DHVEG-6 and uncultured *Methanobacterium* in the fast nutrient cycling in the surface sediment of this area.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Xianfang Fan and Peng Xing made the same contribution to this work.

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