Open Access Library Journal
2019, Volume 6, e5315
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Research on the Microbial Community Structure of Wooden Relics and the Preservation of Water Environment: Illumina MiSeq Sequencing

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How to cite this paper: Tang, L., Xing, J.J., Lei, Q., Qiu, Z.M., Zhang J. *and* Ma, L.A. (2019) Research on the Microbial Community Structure of Wooden Relics and the Preservation of Water Environment: Illumina MiSeq Sequencing. *Open Access Library Journal*, **6**: e5315.
https://doi.org/10.4236/oalib.1105315

Received: March 6, 2019
Accepted: March 22, 2019
Published: March 25, 2019

Abstract

[Objective] Microorganisms are an important disease in archaeological wood lacquer. The effect of single microorganism on the erosion of wooden relics is small, but the microbial community is more harmful to the wooden relics. Therefore, it is very important to acquaint the microbial community structure of wooden relics and their preservation environment. **[Methods]** Illumina MiSeq sequencing method was used in this study to explore wood relics and full water environmental microbial community structure. **[Results]** The results showed that the level of wood samples was distributed in 17 phyla containing 6 dominant bacteria, and water samples in 21 containing 7 dominant phyla. In the family level, the wood samples were distributed in 64 with 8 dominant families, while the water samples were distributed in 93 with 10 dominant families. At the level of the genus, the wood samples were distributed in 72 with 8 dominant genera, while the water samples were distributed in 105 with 10 dominant genera. **[Conclusion]** Most of the microbes in the wood samples are the same as those in the water environment, but there are differences in the community distribution.

Subject Areas

Environment Science

Keywords

Illumina MiSeq Sequencing, Wood Lacquer, Microbial Community, Dominant Phylum, Dominant Family, Dominant Genera

1. Introduction

A lot of research shows that bacteria can use carbohydrates from wood to survive in a long-term anoxic subterranean environment [1] [2] [3]. Most of the wooden relics in the middle and south of China are in the full water environment [4], so the method of immersion preservation is adopted after unearthed. Wooden relics are thought corroded by microorganisms after they are unearthed [5] [6], so it is necessary to know the diversity of microbial communities in wood lacquers.

At present, researchers judge the microbes in wooden relics mainly by separation, purification and identification, which deviate greatly from the original habitat (accounting for only 0.1% to 10% of the total number of environmental microbes) [7]. In order to solve this problem, DNA fingerprinting technology, phospholipid fatty acid analysis, gene chip and other microbial community detection methods came into being, but all have the shortcomings of low flux and small information volume [8]. High throughput sequencing has many advantages, such as wide coverage, high accuracy, low cost, long sequencing and bidirectional sequencing [9].

Using Illumina MiSeq sequencing characteristics to understand the microbial community structure of wood lacquer preserved with water is of great significance for analyzing the corrosion causes and disease information of wood lacquer.

2. Materials and Methods

2.1. Sampling and Sample Handling

Samples were collected with sterilizing tweezers from the waterlogged wooden lacquer (No.F446) in Jingzhou Museum 0.3 - 0.5 g sample was cut, ground, and added with sterile water at 1:10 ratio. Suspensions were extracted after centrifugation for several times. Then the processed samples were stored at 4˚C. The water samples were named W1, W2, W3, W4, and the wood samples were named W5, W6, W7, W8.

2.2. DNA Extraction, Polymerase Chain Reaction and Illumina MiSeq Sequencing

DNA was extracted from samples using the improved method for CTAB [10]. The extracted DNA was amplified using a set of bar-coded primers 515F and 806R. The PCR products from each sample were pooled together with equal molar concentrations, and then sequenced within Illumina Miseq platform.

2.3. Sequencing Analyses

The sample DNA was sent to the biological Mdt InfoTech Ltd. of NOA, Beijing, to carry out high throughput sequencing of Illumina MiSeq 2500. The sequencing results were compared with the gene bank of NCBI, and the bacteria were divided into different OTUs based on the homology of 97%. In order to evaluate

the diversity of microbial community composition, cluster analysis was conducted on the detected OTU based on the Bray-Curtis distance. Using QIIME software to calculate the Alpha diversity index of the sample, including the Chao1 value and the Shannon index [11].

3. Results and Analyse

3.1. Quality Analysis of Sequencing Results

A total of 178,312 raw sequences were obtained; after filtering, 177,847 high-quality 16SrRNA gene sequences with an average length of 251 - 254 bp were recovered.

3.2. Analysis of Alpha Diversity in Microbial Communities

The diversity of microbial community is related to the number of microorganisms and the uniformity of distribution. Figure 1 shows that the Shannon index and pielou evenness of water samples are larger than those of wood samples. However, the Simpson index of wood samples is larger than that of water samples. Therefore, the species of microorganisms in water samples were larger than those in wood samples, but the distribution of microorganisms in wood samples is even.

3.3. Sample Community Composition Analysis

3.3.1. Analysis of Microbial Community Composition at Phylum Level

Wood samples were distributed on 17 phyla, and water samples were distributed on 21 phyla. In addition, wood samples had 6 dominant phyla (relative abundance > 1%), while 7 dominant phyla (relative abundance > 1%) were found in

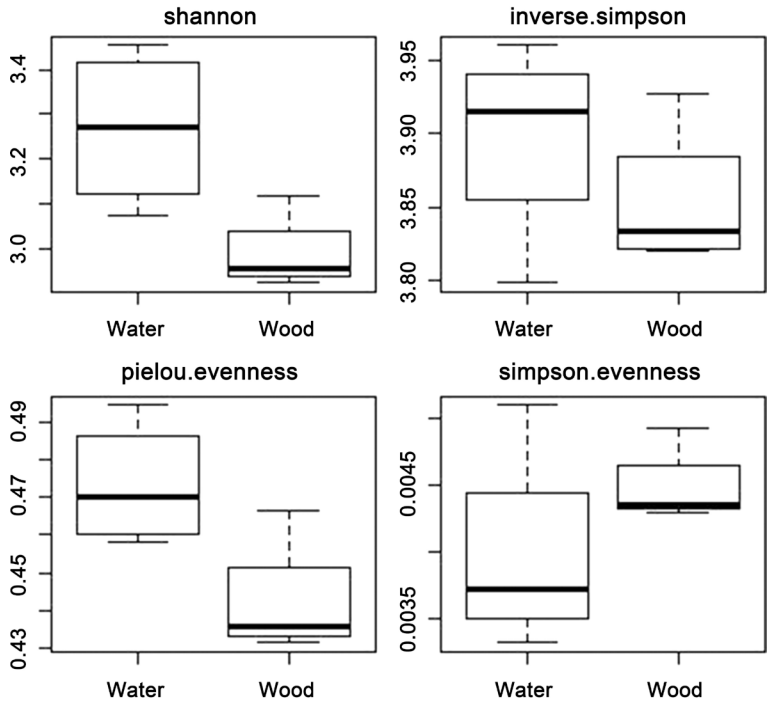

Figure 1. Alpha-diversity of samples.

water samples. As can be seen from Figure 2, there is a certain difference in the distribution of microbes in the wood samples and water samples in phylum. The relative abundance of *Acidobacteria* in wood samples was extremely significantly than that in water samples ($P < 0.05$), and the relative abundance of *Planctomycetes* and *Nitrospira* in water samples was extremely significantly than that in wood samples ($P < 0.01$).

3.3.2. Analysis of Microbial Community Composition at Family Level

The bacteria in the water samples are distributed in 93 families, and the wood samples are distributed in 64 families. In addition, wood samples had 8 dominant families (relative abundance > 1%), while 10 dominant families (relative abundance > 1%) were found in water samples. Water samples and wood samples in 11 families with significant differences among the relative abundance > 1% for a total of six families. Respectively: *Caulobacteraceae*, *Hyphomicrobiaceae*, *Planctomycetaceae*, *Sinobacteraceae*, *Nitrospiraceae*, *Erythrobacteraceae*. Figure 3 shows that there are differences in the community structure distribution of the eight samples. But the internal differences of wood samples and water samples are small.

3.3.3. Analysis of Microbial Community Composition at Genus lEvel

The total number of bacteria at genus in wood samples is 72, and in water samples is 104. The dominant genera in (relative abundance > 1%) the wood sample are 8, and the proportion is 42.15%. The dominant genera in water sample is 10, and the proportion is 30.60%. The rare genera (relative abundance < 0.1%) in the wood sample are 48 and the proportion is 1.45%. And the water sample is 66 and the proportion is 1.90%. It is known from Table 1 that there is no significant difference between the wood samples and the water samples in the distribution of the dominant genus. However, there are significant differences in the distribution of all the bacteria at genus and the rare bacteria in the wood and water samples. In addition, there is a significant difference in the proportion of the distribution of the dominant and the rare genus. It suggests that the distribution of wood samples and water samples is great different in the distribution of bacteria.

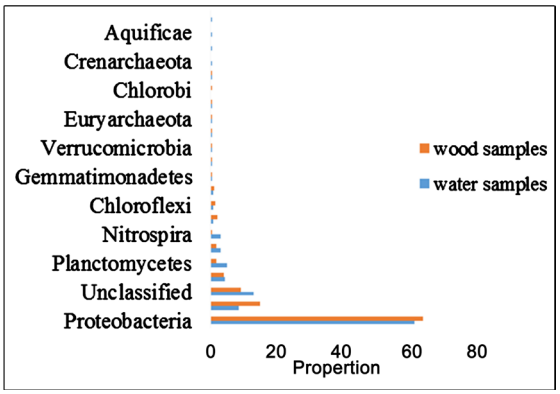

Figure 2. Comparison of wood samples and water environment on the distribution in phylum.

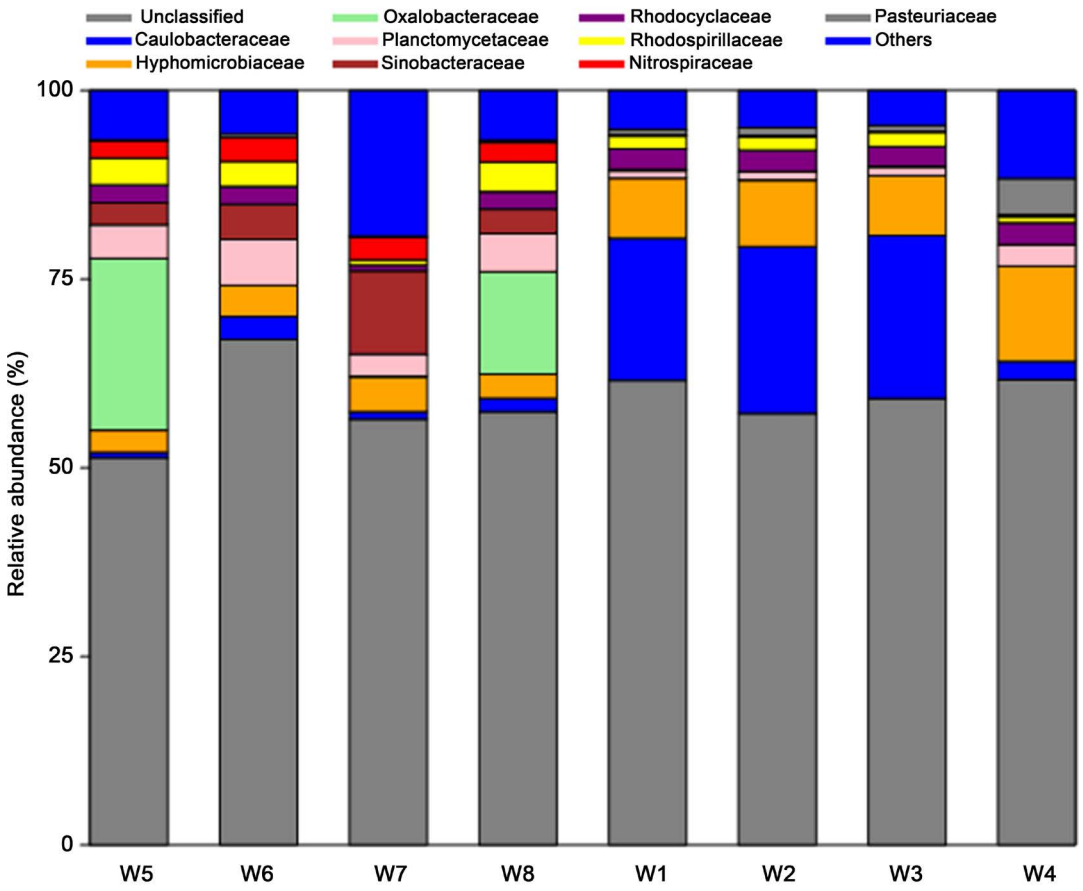

Figure 3. Analysis of the composition of community structure of wood samples and water samples.

Table 1. The distribution of the bacteria in wood samples and water samples.

	Total number	Number of dominant genus	Proportion of dominant genus (%)	Number of rare genus	Proportion of rare genus (%)
W1	73	7	42.29	49	1.44
W2	76	6	43.95	52	1.34
W3	70	6	43.6	49	1.59
W4	69	12	38.76	43	1.42
W5	92	9	37.39	51	1.9
W6	104	9	22.64	63	1.92
W7	98	11	30.72	68	1.67
W8	124	11	31.65	80	2.12
P-Value ($P < 0.05$)	0.004	0.196	0.012	0.034	0.005

3.4. Analysis of Beta Diversity in Microbial Communities

DCA analysis method was adopted to this research, because it can not only analyze the data matrix composed of coverage and quadrat, but also can analyze data matrix by frequency and quadrat, biomass and composition analysis of the

sample [12]. As shown in **Figure 4**, the wood samples and water samples are clearly divided into two categories. In addition, the four sampling points of the wood sample are more concentrated than the water samples. It suggests that there is a certain difference between the microbial community structure in the wood samples and the water samples.

4. Discussion

The unearthed lacquer is kept in a full water environment and will be affected by the microorganism in the water environment. Based on Illumina MiSeq sequencing, the bacteria in the wood samples were detected in 17 bacteria phyla and 94 families and 106 bacteria genera while the bacteria in the wood samples were detected in 21 bacteria phyla and 65 families and 72 bacteria genera. Through calculation and analysis, there are 6 dominant phyla and 10 dominant families and 10 dominant genera in water samples, and the wood samples contain 7 dominant phyla, 8 dominant families and 8 dominant genera. The results showed that the smaller the classification level, the diversity of microbial community structure diversity between water samples and wood samples are greater. And there are significant differences in the microbial community structure of the two samples at the level of the genus.

Many scholars have done the study of microorganism on wooden lacquer. ZJ Zhao [13] and others conducted microbial studies on the site of the Chengdu coffin, and isolated *Pseudomonas*, *Flavobacterium*, *Proteur*, and *Cellulomonas*. [14] detected the bacteria in the burial environment of the Han Tombs in Siyang,

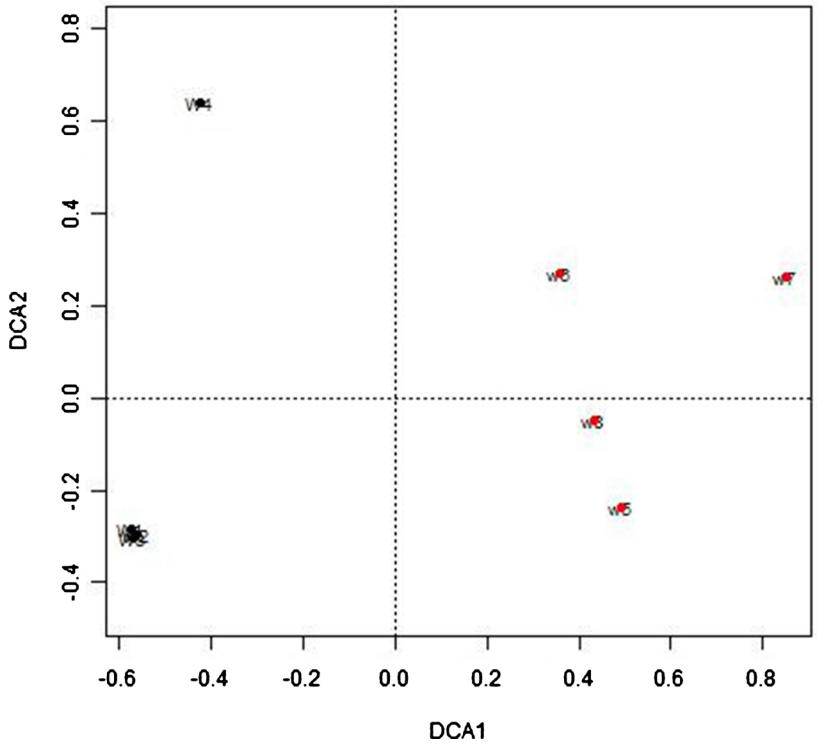

Figure 4. DCA analysis of wood samples and water samples.

and found the presence of *Pseudomonas*. Furthermore, [15] studied bacterial diversity of waterlogged in 108 samples by DGGE; the results showed that *Cytophaga-Flavobacterium-Bacteroides* (CFB) complex and the *Pseudomonas* group were commonly recovered, with relatives of the *Cellvibrio* and *Brevundimonas* groups also present, and most of these bacteria were detected in this study. The distribution of microbes in the lacquer of the full water preservation wood has regional differences and similarity.

Moreover, [16] isolated and identified the bacteria on the wooden lacquer F446 and the bacteria in the water conservation environment. The bacteria isolated from the wood samples belonged to 5 genera, the dominant bacteria were *Bacillus*, the bacteria isolated from the water samples belonged to 9 genera, and the dominant bacteria were *Brevis*. [17] selected 32 strains of bacteria from F455 wood lacquer belonging to 4 genera, the dominant genus was *Bacillus*, and 9 species of bacteria were in water samples, of which 9 were *Brevis.*

At present, the study of microbial community of full water preserved wooden lacquer in world is still in a relatively blank stage. However, by microorganism pure culture method, after isolation and identification, it can be found that the microbial diversity of water lacquer wares is much larger than that of wood samples. Therefore, in order to delay the corrosion process of wooden lacquer, it is suggested to preserve the wooden lacquer with aseptic water, and regularly replace the water to keep the number of microbes in the water environment in a low state.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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