Fungal Community Analyses of a Pirogue from the Tang Dynasty in the National Maritime Museum of China

Fengyu Zhang¹, Lin Li¹, Mingliang Sun¹, Cuiting Hu¹, Zhiguo Zhang², Zijun Liu¹, Hongfei Shao³, Guanglan Xi² and Jiao Pan¹,*

¹ Ministry of Education Key Laboratory of Molecular Microbiology and Technology, Department of Microbiology, Nankai University, Tianjin 300000, China; 13212002263@163.com (F.Z.); 18222760929@163.com (L.L.); 16602660537@163.com (M.S.); hct20180725@126.com (C.H.); xxmuliu@163.com (Z.L.)
² National Center of Underwater Cultural Heritage, Beijing 100000, China; zzgwys@126.com (Z.Z.); xiguanglan@126.com (G.X.)
³ National Maritime Museum of China, Tianjin 300000, China; xinxi2005@126.com
* Correspondence: panjiao@nankai.edu.cn; Tel.: +86-022-2350-5961

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Abstract: The goal of this research was to analyze the fungal community responsible for the biodeterioration of a pirogue in the National Maritime Museum of China and to make recommendations for the protection of this artifact. Molecular identification of fungal strains isolated from the surface of the pirogue and the air of the storage room that were most closely related to Cladosporium, Penicillium, Talaromyces and Trichoderma spp. DNA extracted from the samples was sequenced on the Illumina MiSeq platform. The results showed that the predominant fungal genera present were Penicillium sp., Cladosporium sp. and Exophiala sp. Thereafter, cellulose degradation experiments were carried out on the predominant fungi screened by pure culturing. Finally, we tested the sensitivity of the predominant fungal isolates to four biocides. This work suggests that we should pay more attention to Penicillium sp. and Cladosporium sp. in the protection of wooden artifacts, and environmental control is recommended as the main means of protecting the pirogue.

Keywords: pirogue; biodeterioration; high-throughput sequencing; culture-dependent approach; cellulase activity

1. Introduction

The canoe, as the earliest water transportation vehicle in ancient China, has irreplaceable research value. By studying the canoes of different ages unearthed in different places, we can infer information about ancient people’s life scenes, manufacturing processes and use of tools.

The National Maritime Museum’s collection includes a canoe produced in the Tang Dynasty of China (618–907 A.D.) with a length of 13.8 m and a ventral diameter of approximately 95 cm. The canoe was unearthed in Guangdong province. This is China’s largest surviving canoe made by hollowing out a single piece of a wood trunk, and its overall preservation is basically complete. The wood has been identified as Rhodamnia dumetorum.

Biodeterioration can be defined as “any undesirable change in a material brought about by the vital activities of organisms” [1]. Bacteria, archaea, fungi, and lichens as well as insect pests are constantly causing problems in the conservation of cultural heritage because of their biodeteriorative potential [2]. As a natural material, wood can often be found to be biodeterioration by microorganisms. Even if wooden artifacts are preserved in museums, they will still be attacked by microorganisms.
For instance, fungal mycelia have been observed on the surface of a wooden staircase (1108 B.C.) in Hallstatt, which was designated as a UNESCO World Cultural Heritage site in 1997 [3]. The Nanhai No. 1 shipwreck, preserved in the Yangjiang Museum, was severely attacked by *Fusarium* sp. due to a change in the environment [4]. Fungi has also been discovered on wooden historical objects in a regional museum [5]. Zijun Liu et al. observed that a wooden desk dated to the Qing dynasty presented viable fungal contamination in the Tianjin Museum [6]. The ‘M2’ Mausoleum of the Dingtao King, the best-preserved large-scale huangchang ticou tomb, is located Shandong Heze City of China. In 2015, fungal community colonized the wooden tomb and spread to every chamber. Zijun Liu et al. identified 114 total genera that belonged to five fungal phyla via a combination of high-throughput sequencing and culture-dependent techniques [7]. In addition, Liuyu Yin et al. also revealed predominant fungal community on the wooden lacquer plates from the Nanhai No. 1 shipwreck [8].

Compared to bacteria, most fungi can grow in environments with a lower temperature and humidity, so they pose a greater threat to artifacts in a museum. In 2009, the bamboo slips from the Three Kingdoms period in the Jiandu Museum of China suffered from extreme deterioration. A series of experiments revealed that fungal community contributed to the degradation of the object [9]. Slow-growing ascomycetes such as those in the genera *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, and *Chaetomium* are able to inhabit and degrade all types of organic artifacts, including wood and paper [2]. Due to the high requirements for humidity in the environment among bacteria, their numbers will increase significantly only when libraries are flooded or material is damp [10]. It is of great significance to evaluate the degree of fungal deterioration of wooden relics for the development of cultural relic excavation plans and subsequent protection measures. At the same time, it is of great practical significance to evaluate whether the current protective measures for underground objects are evidence-based and reasonable.

The identification of microorganisms responsible for the biodeterioration of objects is the first step for protecting the cultural heritages. Traditional methods based on cultivation and microscopy techniques are expensive and time consuming. In recent years, the use of high-throughput sequencing analysis in biodeterioration of cultural heritage has gained much traction [11]. This method has been used in the detection and characterization of microorganisms in environments that are notoriously hard to culture from [12]. Therefore, many studies have investigated microbial communities responsible for the biodeterioration of objects using a combination of traditional methods and high-throughput sequencing analysis.

In this study, we identified the fungal community responsible for the biodegradation of a pirogue preserved in the National Maritime Museum of China through amplicon sequencing analysis and culture-based methods. To this end, we isolated major fungal strains and evaluated their ability to degrade cellulose. Furthermore, we performed enzyme activity tests on the main fungi that degrade cellulose. Finally, we tested the sensitivity of the main fungal isolates to four biocides—P91, Euxyl® K100, BIT 20N and Preventol® D7.

### 2. Materials and Methods

#### 2.1. Characteristics of the Pirogue

The evaluated pirogue from the Tang Dynasty was hollowed out from a single piece of wood, and its overall preservation is basically complete. It is 13.8 m long and has a diameter of approximately 95 cm (Figure 1A).

After the canoe entered the hall, the mud on the inside and outside of the vessel was cleaned away, and the vessel was then sprayed with common insecticides and desalinated. In a controlled environment with a temperature of 18–24 °C and humidity of 70%, the current condition of the canoe includes cracking, partial shedding, and the presence of pollutants on the surface of the hull. The damage includes deformation, incompleteness, fractures, decay, salt degradation, shedding, and shrinkage.
The pirogue has presented visible fungal contamination (Figure 1B). To more accurately reflect the condition of the canoe body, samples were collected from three locations on the boat (Figure 1C).

**Figure 1.** The overall preservation of the canoe and sample collection. The pirogue overall frame (A) and degradation situation (B). Sampling sites for microbiological analyses on the surface of the pirogue (C).

### 2.2. Sampling

Surface samples from the canoe showing visible mycelia were inoculated onto PDA medium with sterile cotton swabs. The sampling positions are marked with red circles as shown in Figure 1B. Then, these petri dishes were transported to the laboratory and placed in incubators for fungal isolation and identification. Samples for high-throughput sequencing were collected from the same points and dipped in sterile water prepared in advance. Microorganism aerosols were collected at four sites around the canoe to analyze the fungal community in the canoe’s preservation environment. Air samples were taken at a flow rate of 100 L min\(^{-1}\) with a ZR-2050 air sampler (Junray, China). Three petri dishes with PDA medium were used for cultivating fungi and incubated at 28 °C for 4–7 d.

### 2.3. Isolation and Identification of Fungi

After the petri dishes containing PDA were cultured for 4–7 d at 28 °C; colonies showing different appearances were transferred to new plates to obtain pure isolates, and then the mycelia of fungal isolates were used for DNA extraction via the CTAB method [13]. Molecular identification of fungal strains was performed by amplification of the ITS, 28S rRNA and RNA polymerase II largest subunit (RPB1) genes [14,15]. The primer sequences summarized in Table S1 were used for fungi
identification [16]. The PCR reaction programs are summarized in Table S1. The PCR product quality and sequence analyses were performed by GENEWIZ (Beijing, China).

2.4. High-throughput Sequencing Analysis

Microorganism DNA was extracted by using a Mo Bio Power Water® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, USA), and DNA purity and concentration (A260/A280 ratio) were assessed using the BioDrop µLite PC Spectrophotometer (Cambridge, UK). Then, the DNA extractions were used as a template for PCR amplification.

For the study of fungal community, internal transcribed spacer 1 (ITS1) was amplified with the universal fungal primers ITS5-1737F/ITS2-2043R [17,18] (Table S2). The amplification reaction mixture and PCR program were prepared as described Liu et al. [4]. Samples with amplicon bands in the range of 500–700 bp were selected for further analysis. Finally, the PCR products were used for library construction, and sequencing was performed on the Illumina HiSeq2500 PE250 platform. Specific methods of operation can be found in the literature described by Liu et al. [6].

2.5. Phylogenetic Tree Analysis

To authenticate genetic associations among identified fungal strains inferred by the ITS gene, the determined sequences were analyzed with the Molecular Evolutionary Genetics Analysis software package (MEGA, version 5.05) [19] and aligned using the Clustal W program included in MEGA v.7.0. We selected 20 species per genus, and for the molecular data, we obtained sequences of species exemplars from GenBank. Based on the adjacency method [20,21], MEGA 5.05 software was used to build the phylogenetic tree for the system. Confidence in the tree topology was estimated using the bootstrap method (1000 bootstrap replicates). The distance scale was estimated to be 0.2 and 0.5, respectively. Figtree v.1.4.3 software was used to visualize and edit the tree.

The raw sequencing data can be downloaded at the NCBI Sequence Read Archive (SRA) under study accession number SRP145540.

2.6. Test the Activity of Lignin-Degrading Enzyme and Cellulase

2.6.1. Lignocellulose-Degrading Enzyme and Cellulase Screening

PDA plates containing 0.04% (v/v) guaiacol were used for lignocellulose-degrading enzyme screening [22]. CMC plates with agar medium were prepared to test whether the isolated fungi produce cellulase. The CMC agar consisted of 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% MgSO₄, 0.05% KCl, 0.2% carboxymethylcellulose sodium (CMC-Na), 0.02% peptone, 1.7% agar and 1 L of tap water [23]. Specific methods of operation can be found in the literature described by Liu et al. [4]. Each test comprised three replicates.

2.6.2. Cellulase Production and Enzyme Assays

One milliliter solution containing $1 \times 10^8$ spores was placed in 500-mL flasks filled with 140 mL of cellulase-producing medium and incubated at 28 °C for 7 days, and enzymatic analysis was performed every 24 h. The medium contained: peptone 10 g L⁻¹, beef extract 5 g L⁻¹, KH₂PO₄ 2 g L⁻¹, MgSO₄·7H₂O 0.5 g L⁻¹, (NH₄)₂SO₄ 2 g L⁻¹, and carboxymethylcellulose sodium (CMC-Na) 10 g L⁻¹ as the carbon source. All cultivations were performed in triplicate. A 1-mL aliquot of the supernatant was obtained by centrifugation (12,000× g) for 5 min and then added to 1 mL of CMC-Na (1% w/v) dissolved in citrate buffer (pH 4.8, 0.05 mol L⁻¹). Cellulase activity was estimated by the DNS method, and the procedure was introduced by He et al. [24].

2.7. The Susceptibility of Isolated Fungi to Biocides

The selected biocides were P91, BIT 20N, Euxyl® K100 (Schülke, Norderstedt, Germany), and Preventol® D7 (Lanxess, Köln, Germany). All of these chemicals are isothiazolinone derivatives,
which are considered broad-range biocides. Pure fungal isolates were cultivated on PDA plates and incubated at 28 °C for 5 days. Then a certain amount of 0.1% Tween-80 solution was added to each plate, and the spores were scraped off. The spore suspension was centrifuged at 4500 rpm for 10 min and redissolved in sterile water. The spore suspension of the test fungus was inoculated onto PDA plates, and the sensitivity of the pure fungal isolates to biocides was tested by the disc diffusion method. The four biocides were applied at a 0.5% concentration to inhibit fungal growth on the culture plates. After incubation at 28 °C for 2 d, the diameters of the inhibition zones were measured in centimeters. Each antimicrobial test included three replicates, and sterile water was used as a control.

3. Results

3.1. Identification of Predominant Fungi by High-Throughput Sequencing

The fungal community composition was assessed by amplicon sequencing of three samples taken from different locations using the Illumina HiSeq2500 PE250 platform. Figure 1 shows the distribution of the predominant fungi in the three samples. The dominant phylum in the three samples was Ascomycota. Ascomycota was present in all three samples and accounted for 96.73%, 82.4% and 65.45% of the communities (Figure 2A). Basidiomycota also existed in three samples and accounted for 2.2%, 15.14%, and 30.85% of the communities. Zygomycota accounted for 0.82%, 1.28% and 1.55% of the communities in the three samples. Neocallimastigomycota (0.03%) was only detected in sample DMZ-11.

![Figure 2. Fungal community analysis by high-throughput sequencing. Distribution patterns of fungal phyla and relative abundances of the fungal genera among samples (A); relative abundances of the fungal genera among samples (B).](image-url)
The three samples presented large differences in the diversity and distribution of fungal genera (Figure 2B). *Penicillium* (36.63%) and *Chaetomium* (23.99%) accounted for a large proportion of DMZ-9, while DMZ-10 consisted largely of the genus *Exophiala* (29.15%). The DMZ-11 sample presented a relatively uniform distribution of the genera *Cladosporium* (26.4%), *Cryptococcus* (22.16%) and *Aspergillus* (20.84%). In addition, *Talaromyces*, *Alternaria*, *Humicola*, *Pestalotiopsis*, and *Trichosporon* were detected in the three samples, with prevalence values ranging from 0.04% to 10.9%.

### 3.2. Identification of Main Fungi by Culture-Dependent Approach

A total of 14 species of fungi were isolated from the hull and air using culture-dependent approach and molecular methods; six species were isolated from the hull, and eight species were isolated from the air (Table 1). Three of the six fungal species from the hull belonged to *Penicillium* sp., which accounted for 74% of all cultured fungal isolates from the hull samples. Two other species belonging to *Talaromyces* sp. accounted for 0.36%. One species belonging to *Cladosporium* accounted for 0.45% of all isolated genera. The strains isolated from air samples were most closely related to *Cladosporium* sp. (46.69%), *Penicillium* sp. (47.17%) and *Trichoderma* sp. (0.47%). To further elucidate the characteristics of these isolated fungi, the morphology of their hyphae and conidia was observed under a microscope (Figure S1).

| Fungi          | Closest Related Strain            | Accession Number | Similarity | Source |
|----------------|-----------------------------------|------------------|------------|--------|
| NKDMZ-1        | *Penicillium pimiteouiense*       | MK551157.1       | 97%        | hull   |
| NKDMZ-2        | *Penicillium sumatrense*          | KT310939.1       | 89%        | hull   |
| NKDMZ-3        | *Penicillium sumatrense*          | KT310939.1       | 89%        | hull   |
| NKDMZ-4        | *Cladosporium cladosporioides*    | HQ148094.1       | 98%        | hull   |
| NKDMZ-5        | *Talaromyces pinophilus*          | KY979508.1       | 99%        | hull   |
| NKDMZ-6        | *Talaromyces variabilis*          | KL216713.1       | 99%        | hull   |
| NKDMZ-9        | *Cladosporium cladosporioides*    | HM776418.1       | 98%        | air    |
| NKDMZ-10       | *Cladosporium cladosporioides*    | KX815294.1       | 97%        | air    |
| NKDMZ-11       | *Cladosporium cladosporioides*    | KY114882.1       | 98%        | air    |
| NKDMZ-12       | *Cladosporium cladosporioides*    | KX815294.1       | 96%        | air    |
| NKDMZ-13       | *Cladosporium cladosporioides*    | KX815294.1       | 96%        | air    |
| NKDMZ-14       | *Cladosporium uredinicola*        | JN088229.1       | 84%        | air    |
| NKDMZ-15       | *Penicillium sumatrense*          | KT310939.1       | 89%        | air    |
| NKDMZ-16       | *Trichoderma afroharzianum*       | KX357849.1       | 97%        | air    |

### 3.3. Phylogenetic Tree Analysis

From the above results, it was determined that *Penicillium* sp. and *Cladosporium* sp. accounted for the largest proportions of the fungal communities, so phylogenetic tree analysis of *Penicillium* and *Cladosporium* fungi was carried out. We selected some strains that are often found by researchers on museum artifacts, as well as two strains of *Penicillium* sp. preserved in our laboratory. Strain NK-NH3 (MH392741.1) was isolated from the surface of the lacquer from the Nanhai No. 1 shipwreck, and strain TJM-F5 (MH171487.1) was isolated from a leather suitcase from the Tianjin Museum.

The results showed that the ITS sequences of the four species of *Penicillium* sp. were not highly similar, and the sequence homology reached approximately 89%. Phylogenetic tree results showed that the four fungi belonging to *Penicillium* sp. presented relatively independent evolutionary branches, which was consistent with the results of sequence alignment. The ITS sequences of strain NKDMZ-3 and *Penicillium chrysogenum* (TJM-F5) showed a closer genetic relationship, while the ITS sequences of strain NKDMZ-15 and NK-NH3 were highly homologous (Figure 3A). In addition to strain NKDMZ-9, the ITS sequence similarity of other *Cladosporium* sp. was high, showing differences of only a few base pairs (Figure 3B).
Figure 3. Neighbor-joining phylogenetic tree of *Penicillium* sp. (A) and *Cladosporium* sp. (B). Each of the ITS gene sequences used was 520–540 bp. The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates. The scale bar indicates the expected number of substitutions/sites.
3.4. Degradation of Lignin and Cellulose by Fungal Isolates

The 14 fungi isolated from the pirogue and two strains of *Penicillium* sp. preserved in our laboratory were inoculated onto two different media to detect cellulase and lignocellulose-degrading enzyme activity. *Penicillium pimiteouiense* NKDMZ-1 exhibited lignocellulose degradation ability after all fungal isolates were cultured on PDA plates containing 0.04% (v/v) guaiacol at 28 °C for 11 d (Figure S2). After 3 d, 10 isolates (*Penicillium* sp., *Cladosporium* sp., *Trichoderma* sp.,) produced a clear zone around their colonies, indicating they show good cellulase activity (Figure S3).

The 10 fungi that showed the cellulose degradation capability were inoculated into a cellulase-producing medium, and their enzyme activity was determined every 24 h. As shown in Figure 4, cellulase activity varied with the fermentation time, and the times at which the cellulase activities of different fungi reached their peaks’ were different. *Penicillium pimiteouiense* NKDMZ-1 and *Penicillium sumatrense* NKDMZ-15 showed higher enzymatic activity compared to the other eight fungal isolates, reaching 0.0599 IU mg\(^{-1}\) and 0.0443 IU mg\(^{-1}\), respectively. It was noted that these two species of *Penicillium* sp. accounted for a large proportion of the results obtained through cultivation. The enzymatic activity was considerably lower in the other eight fungi.

![Figure 4](image-url)  
*(A)* Detection of the cellulase activity of 10 fungal isolates by DNS method. The 10 fungi which showed the cellulose degradation capability were assessed for cellulose activity every 24 h. Each fungus is represented by a different color.

![Figure 4](image-url)  
*(B)*

3.5. Sensitivity of Fungal Isolates to Four Biocides

In this study, a total of 10 fungi exhibited cellulose degradation capabilities, so these fungi may potentially present risk to the pirogue. Chemical methods such as the application of biocides are considered to be effective methods for controlling deterioration caused by microorganisms. Therefore,
we tested the sensitivity of cellulose-degrading fungi to four biocides. The information of biocides was provided in supplementary materials (Figure S5).

The experiment was performed using a paper diffusion method. The diameter of the inhibition zones was measured in centimeters (Figure S4). The four biocides were applied at a 0.5% concentration to inhibit fungal growth on the culture plates. This concentration was significantly lower than the concentration proposed by the manufacturer for common use, which is usually a concentration of 2%. It was observed that *Penicillium pimiteouiense* NKDMZ-1 and *Cladosporium cladosporioides* NKDMZ-13 were very sensitive to all four fungicide products, and almost no obvious inhibition zone was observed in *Trichoderma afroharzianum* NKDMZ-16. Among the four fungicide products, P91 and D7 exhibited better inhibitory effects and almost inhibited all fungi (Figure 5).

![Figure 5. Sensitivity of fungal isolates to four biocides. The suppression halo around the paper shows the effectiveness of the biocide against the fungal strain isolates. All fungal isolates were cultured at 28 °C for 2 d. (A) NKDMZ-1. (B) NKDMZ-13. (C) NKDMZ-2. (D) NKDMZ-3. (E) NKDMZ-10. (F) NKDMZ-14. (G) NKDMZ-15. (H) NKDMZ-16. (I) NK-NHE3(MH392741.1). (J) TJM-F5 (MH171487.1).](image)

4. Discussion

The study involved a 1300-year-old canoe that researchers are trying to protect in a glass structure in a museum. However, fungal mycelia have begun to colonize the surface of the canoe due to excavation work, transportation, storage, surveys, and exhibition activities. For this reason, we analyzed the fungal flora that propagated on the wood to examine possible biodegradative fungi that might become active under new storage conditions [3].

High-throughput sequencing revealed 105 fungal genera; Basidiomycota and Ascomycota accounted for almost all the reads for each sample, and the sum of the two proportions reached 95%. Many articles reported that Ascomycota, Zygomycota, Chytridiomycota, and Rozellomycota can be found on the wooden relics deteriorated by microorganisms [25–27].

High-throughput sequencing results showed that the most abundant fungi were *Penicillium, Exophiala, Cladosporium, Chaetomium, Cryptococcus*, and *Aspergillus*. Although the three samples exhibited different dominant fungal genera, the above fungal genera were present in all samples. Fungal genera *Penicillium, Cladosporium* and *Trichoderma* have been isolated from the hull and air samples using a culture-dependent approach. The high-throughput sequencing results showed that
the species composition was somewhat different from the results obtained from pure culture, probably because some fungi cannot grow on PDA or MEA medium.

Many researchers have detected the genera *Penicillium* and *Cladosporium* in the museum environment. Indoor fungi not only exist in the form of spores in the air but also colonize various materials as mycelia. Museums are colonized by various microbes, and the detailed lists of these species vary according to the type and organization of the museum, the type of exhibit, the indoor microclimate, and the local environment [2,28–30]. The most common colonizing fungal taxa are species of *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus*, and *Chaetomium*. Abe discovered fungal contamination on a painting stored in an art museum of Tokyo. Afterwards, *Cladosporium* and *Aspergillus* were detected in the room where the contaminated painting was stored [31]. Klavker et al. investigated the fungal growth on historical textiles preserved in Slovene museums and religious institutions, using culture-dependent techniques and molecular genus-specific barcodes. The dominant contaminant fungi belonged to the genus *Penicillium*, followed by *Aspergillus* and *Cladosporium* [32]. The genera *Penicillium*, *Aspergillus* and *Chaetomium* have been detected on organic objects such as wooden square stools and suitcases in a museum [6]. Piotrowska et al. observed that the most important degrading component taxa were fungi of the genera *Penicillium* and *Cladosporium* [33].

The culture-dependent identification results for the fungal aerosol samples showed that the predominant fungi in the air were *Penicillium* sp. (47.17%) and *Cladosporium* sp. (46.69%). In temperate climates, researchers have found that the predominant fungal spores in the air are *Cladosporium*, *Penicillium* and *Aspergillus*. The concentration of these fungal spores varies with the season [34]. The most common fungi in the air are *Penicillium*, *Cladosporium* and *Aspergillus* [35]. Skóra et al. determined microbial contamination in four museums, two libraries and two archives located in Poland. Fungal genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Chaetomium*, and *Trichoderma* have been isolated from air aerosols sampled from museum storerooms [30]. The prevailing fungal genera *Aspergillus*, *Penicillium* and *Cladosporium* were isolated from air samples by Borrego et al. [36]. The predominant fungi in the warehouses of museums and other buildings are *Aspergillus*, *Penicillium* and *Cladosporium* [37–39]. Thirty-four fungal taxa were isolated by Zielińska-Jankiewicz et al. [40], mainly from the *Aspergillus* and *Penicillium* genera.

In our study, *Penicillium* sp. and *Cladosporium* sp. were present in all samples and constituted the predominant fungal community. The data in Figure 4 clearly indicate that some members of these two fungal taxa were capable of synthesizing cellulase. Since they are not specific cellulase-producing strains, the cellulase activity of these fungi was not positive. However, these cellulose-degrading fungi may become active if the requirements for growth and environmental conditions become appropriate, so *Penicillium* sp. and *Cladosporium* sp. must be regarded as a threat to the pirogue.

In addition, *Trichoderma* was detected in air samples, accounting for 0.47% of the total number of fungi in the air. Piotrowska et al. detected *Trichoderma* from fungal aerosols taken from the library [33]. *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* are airborne fungal spores and are considered to be important causes of respiratory allergies [41]. Within the genus *Cladosporium*, *C. herbarum* and *C. cladosporioides* are the most commonly encountered species, in both outdoor and indoor environments [42]. Therefore, the presence of these fungi may affect the health of museum staff.

Given that microbial contamination of canoes is not serious, environmental control is recommended as the main means of inhibiting microbes because environmental conditions greatly affect the growth of microorganisms and the decomposition of wooden cultural artifacts. First, the temperature and humidity in the glass room where the canoe is stored should be constantly monitored. The temperature in the glass room where the canoe is stored is 18–24 °C, and the humidity is 67%. The excessive humidity is conducive to the colonization of the fungus on the wood. However, due to the serious cracking of the canoe, all environmental adjustments need to be considered from many perspectives. Second, the application of efficient and low-toxicity biocides may be considered to restrain fungal growth and mitigate biodeterioration. However, the chemical–physical nature of the artwork’s material and its aesthetic appearance should be taken into account [12].
5. Conclusions

High-throughput sequencing and culture-dependent approaches showed that *Penicillium* sp. and *Chaetomium* sp. accounted for a large proportion of the fungal community composition. Phylogenetic tree results showed that the four fungi belonging to *Penicillium* sp. presented relatively independent evolutionary branches, but the ITS sequence similarity of *Cladosporium* sp. was high. *Penicillium sumatrense* NKDMZ-1 and *Penicillium sumatrense* NKDMZ-15 showed high cellulose degradation abilities, so they may pose a potential threat to wooden materials. Although the four biocides tested in this study showed efficacy, environmental control is recommended as the main means of protecting this pirogue.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/19/4129/s1, Figure S1: Single colony morphology and micrographs of some fungal isolates, Figure S2: Degradation of lignin by fungal isolates, Figure S3: The cellulose-degrading fungi were screened by measuring the zone of clearance, Figure S4: The diameter of the inhibition zones was measured in centimeter, Figure S5: The information of biocides and active compounds. Table S1: Primer sequences used in this study, Table S2: PCR reaction programs.

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