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Original article

An aeromycological study of various wooden cultural heritages in Korea

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ABSTRACT

Korea has many wooden cultural heritages (WCHs), which should be preserved, along with various other cultural properties. WCHs, however, have undergone biodeterioration because of various fungal attacks in the past centuries; this type of biodeterioration is one of the significant problems faced during preservation of WCHs. To prevent this damage, it is important to investigate the fungal diversity of the WCHs. This aim of this study was to analyze the diversity of airborne fungi at 3 WCHs in Korea: Yeonghwadang (YHD; open building) and Juhamnu (JHN; closed building) in Changdeokgung Palace Complex located in Seoul and Unbong hyanggyo (UH; closed building) in Namwon. The airborne fungi were isolated twice in spring (March) and summer (August) using the gravity settling culture plate method and were identified using morphological and molecular techniques. There were differences in fungal diversity depending on the geographical location, climatic conditions, and the open or closed status of a building. During spring, in the open and closed buildings, a total of 671 fungal isolates (20 genera and 25 species) were collected in YHD and 125 isolates (19 genera and 25 species) were isolated in JHN. In summer, 175 isolates (11 genera and 12 species) and 66 isolates (12 genera and 13 species) were collected from YHD and JHN, respectively. The number of fungal isolates was greater in the open building than in the closed WCHs, but these buildings had similar fungal diversity. In UH, 180 isolates (13 genera and 15 species) were recovered in spring season and 58 isolates (14 genera and 17 species) in summer. There was no significant difference in the number of fungal isolates, but the fungal diversity was different depending on the environmental factors. Finally, fungal diversity was richer in spring than in summer because dusty and windy weather in spring was conducive to the release and transmission of fungal spores. In summer, there were a substantial number of basidiomycetes probably because their spores germinate better at higher temperatures and humidity.

1. Research aims

The present study was focused on preservation of wooden cultural heritages (WCHs) in Korea against fungal biodeterioration. The first aim was to analyze the diversity of airborne fungi at 3 WCHs in Korea, Juhamnu (JHN; closed building) and Yeonghwadang (YHD; open building) in Changdeokgung Palace Complex in Seoul and Unbong hyanggyo (UH; closed building) in Namwon. These buildings are under different environmental conditions and are managed in different ways such as open to the public or closed. The second aim was to determine the relationship of fungal diversity with environmental and geographic factors. Understanding of

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fungal diversity between various WCHs could provide useful clues for the selection of target fungi for preventive measures.

2. Introduction

Since ancient times, wood has generally been considered as a great source of building materials because of its firmness and endurance. Numerous architects have used wood in Korea. However, it is inevitable that WCHs would be exposed to outdoor environment, and subjected to high humidity and temperature, in the course of several centuries. This situation is expected to aggravate the deterioration problem. Recently, serious biological deterioration of WCHs around the world was continuously reported along with growing concerns about preservation [1–3]. Among the risks, fungal attacks are considered one of the main causes of damage [4]. There are four types of fungal damage. One is decay caused by basidiomycetes and three other types of damage are soft rot, sapstain, and surface mold caused by ascomycetes [5]. This fungal damage can decrease the aesthetic value and cause serious structural problems. Thus, the causes of deterioration, such as climatic effects, moisture, and pollutants, should be eliminated and their effects mitigated. Appropriate measures should be taken to protect WCHs.

Generally, fungal diversity in indoor and outdoor environments is closely related to air particulate matter because fungal spores are airborne. Therefore, constant air circulation is a particularly important factor for the fungal diversity and preservation of WCHs. To enhance the value of WCHs, there have been several efforts to protect WCHs from biodeterioration. The European Council has implemented policies to reduce air pollution and other deterioration factors [6]. In 2009, the World Health Organization explained indoor biological pollution caused by the problem of excess moisture and recommended air quality guidelines [7]. In Korea, the first law on the protection of the cultural heritage was passed in 1962. Furthermore, there are many restoration and repair projects at WCHs, but scientifically valid protection techniques have not been developed yet.

Nowadays, in Korea, some WCHs are open to the public to help the people to learn about and understand the wisdom of ancestors and to offer enjoyment and appreciation to public users, other WCHs are closed to the public in order to reduce the inherent risks associated with air pollution and inappropriate handling. The diversity and growth of fungal species depend on the outdoor environment including the presence of water that comes from atmospheric precipitation, condensation phenomena, and dampness of the soil as well as indoor relative humidity influenced by the exchange with the outdoor environment and by weather changes. Although an open building is well ventilated and exposed to outdoor air, when the doors of WCHs are closed, the airflows are blocked, resulting in high humidity levels and risk of mold growth. Therefore, identification and assessment of biological risks in open and closed WCHs are needed for systematic protection. Management of these risks is expected to be facilitated by characterization of the differences between the environmental effects and biodeterioration.

In order to preserve WCHs from the fungal biodeterioration, it is necessary to determine the kinds of fungal species that are found in WCHs and mechanism of WCHs damage caused by fungi in combination with environmental factors such as temperature and humidity. Although numerous attempts are made to enhance our understanding of inhabiting fungi involved in deterioration of WCHs [8,9], the data on WCHs in Korea have yet not been collected thoroughly. Therefore, the aim of this study was to investigate the fungal diversity at 3 WCHs: JHN and YHD in Changdeokgung Palace Complex and UH in Namwon. In addition, we compared the observed fungal diversity among different climatic conditions.

3. Materials and methods

3.1. Sampling sites

JHN and YHD in Changdeokgung Palace Complex, which is located in Seoul (37°58′N, 126°99′E) were selected to compare the diversity of indoor airborne fungi between buildings with closed and open doors. All doors of YHD are always open, and those of JHN are closed all year around (Fig. 1). JHN is about 30 m away from YHD, so airborne spore levels of the outdoor environment of these 2 buildings are not different.

Fig. 2 shows UH which is located in Namwon (35°25′N, 127°31′E), Jeollabuk-do, was selected to compare the diversity of indoor airborne fungi depending on geographical locations. The fungal diversity of UH, which is a closed building, was compared with that of JHN. The difference in geographical coordinates between JHN and UH, including the altitude above the sea level, may affect fungal diversity because the climatic conditions of the 2 locations are different.

3.2. Isolation of the fungi

Indoor airborne fungal spores were collected at the 3 WCHs twice in spring (May) and summer (August) using the gravity settling culture plate method. Fifteen petri dishes were exposed to the air for 30 minutes in each building. The petri dishes were placed on the floor. The culture medium was 2% malt extract agar (MEA), composed of 20 g of Difco malt extract and 15 g of Difco agar per 1 L of distilled water, with 100 ppm streptomycin to inhibit bacterial growth. After the exposure, the plates were sealed with parafilm and incubated at room temperature for 2 weeks, and the mycelial margins were routinely sub-cultured onto new plates to obtain pure cultures. For the preferential isolation of the basidiomycetes, 2% MEA with 4 ppm benomyl and 100 ppm streptomycin was used. After further purification, most of the airborne fungal species were identified to the genus or species level according to morphological and molecular biological characteristics.

3.3. DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was extracted from fungal mycelia using the Accuprep Genomic DNA Extraction Kit (Bioneer, Korea). The nuclear ribosomal DNA (rDNA) gene cluster was amplified by PCR using the Accupower PCR Premix Kit (Bioneer). The nuclear internal transcribed spacer (ITS) region including the 5.8S rDNA gene was amplified by means of the following primer set: ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′)/ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [10]. In addition, partial large subunit (LSU) was sequenced to identify basidiomycetes more accurately. According to Vigalys and Hester (1990) [11], partial LSU gene with primer set LROR (5′-ACCGCGTGAACTTAAGC-3′)/LR3 (5′-GGTCCGTGTTTCAAGAC-3′) was amplified. PCR conditions for the ITS and LSU regions were as follows; a 7 min at 95 °C, followed by 30 cycles of 40 s at 95 °C, 40 s at 51 °C, and 1 min 20 s at 72 °C, with the final extension for 7 min at 72 °C. The PCR products were purified using a PCR purification kit (Bioneer) and sequenced by the MACROGEN DNA Synthesis Sequencing Facility (Seoul, Korea). All sequences were compared with reference strains by BLAST search of the GenBank database [12]. The sequences were deposited in GenBank under accession numbers shown in Table 1.