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Fungal Catastrophe of a Specimen Room: Just One Week is Enough to Eradicate Traces of Thousands of Animals

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Abstract
Indoor fungi obtain carbon sources from natural sources and even recalcitrant biodegradable materials, such as plastics and synthetic dye. Their vigorous activity may have negative consequences, such as structural damage to building materials or the destruction of precious cultural materials. The animal specimen room of the Seoul National University stocked 36,000 animal resources that had been well-maintained for over 80 years. Due to abandonment without the management of temperature and humidity during the rainy summer season, many stuffed animal specimens had been heavily colonized by fungi. To investigate the fungal species responsible for the destruction of the historical specimens, we isolated fungi from the stuffed animal specimens and identified them at the species level based on morphology and molecular analysis of the β-tubulin (*BenA*) gene. A total of 365 strains were isolated and identified as 26 species in *Aspergillus* (10 spp.), *Penicillium* (14 spp.), and *Talaromyces* (2 spp.). *Penicillium brocae* and *Aspergillus sydowii* were isolated from most sections of the animal specimens and have damaged the feathers and beaks of valuable specimens. Our findings indicate that within a week of mismanagement, it takes only a few fungal species to wipe out the decades of history of animal diversity. The important lesson here is to prevent this catastrophe from occurring again through a continued interest, not to put all previous efforts to waste.

Keywords Animal specimen · *Aspergillus* · Biodegradation · Fungi · *Penicillium* · *Talaromyces*

Introduction

Fungi can be divided into saprotrophs, symbionts, and pathogens based on how they obtain nutrients (Deacon et al., 2006). Of them, saprotrophic fungi obtain carbon and energy sources from nonliving organic matter, such as plant debris and carcasses. They can also decompose artificially synthesized polymer materials, such as plastic (Muhonja et al., 2018; Srikanth et al., 2022) and synthetic dye (Wesenberg et al., 2003). As saprotrophic fungi produce a wide range of depolymerase enzymes to degrade different polymers, they play an important role in the nutrient cycle in nature and occupy various ecological niches on earth.

The indoor environment is a well-known habitat for fungi (Sánchez Espinosa et al., 2021). Many carbon sources are available, such as building materials, textiles, and food products (Amend et al., 2010; Flannigan et al., 2002). Fungi can actively decompose these carbon sources, especially when the humidity is high during the rainy season. Fungi cause structural damage to building materials (Kauserud et al., 2007; Schmidt, 2007) and can also destroy precious cultural materials. The contamination and destruction of cultural materials by fungi have been previously reported on paintings and ironworks displayed in museums (de Carvalho et al., 2018; Sterflinger, 2010; Wiszniewska et al., 2009) and historical records in libraries (Fitri et al., 2016; Swapna & Lalch, 2017). Some indoor fungi are detrimental as they cause sick house syndrome (Straus et al., 2003) or illnesses, such as allergies (Fukutomi & Taniguchi, 2015; Żukiewicz-Sobczak, 2013), asthma (Baxi et al., 2019; Hogaboam et al., 2000; Sharpe et al., 2015), and infectious diseases (Douglas, 2003).

Dominant fungal genera in indoor environments include *Aspergillus*, *Penicillium*, and *Talaromyces* (de Carvalho et al., 2018; Treves & Martens, 2010). Culture-based studies have forecasted the dominance of these three genera in a wide range of indoor environments, from medical

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facilities in Turkey to the libraries of Pakistan (Demirel et al., 2017; Hassan et al., 2021). Amplicon-based studies on environmental DNA have revealed the proportion of these genera in indoor environments to be more than one-third of the mycobiota (Amend et al., 2010; Visagie et al., 2014). A wide range of carbon sources, from building materials to dust (Flannigan et al., 2002), has been considered to be responsible for the succession of these genera in indoor environments. Many *Aspergillus* species are found in numerous indoor environments, such as buildings in the USA and hospitals in Iran, and cause asthma through volatile compound (VOC) production (Hedayati et al., 2010; Mousavi et al., 2016; Omebeyinje et al., 2021). *Penicillium* is also a widely known indoor mold. *Penicillium brevicompactum* and *P. citrinum* are common examples of molds isolated from many indoor environments (Rojas & Aira, 2012; Scott et al., 2008). In contrast to *Aspergillus* and *Penicillium*, *Talaromyces* is relatively less studied but is often reported in indoor studies (Chen et al., 2016; Peterson & Jurjević, 2017). *Aspergillus*, *Penicillium*, and *Talaromyces* account for about 93% of species within Eurotiales, a large order in Ascomycetes (Houbraken et al., 2020). Multiple morphological characteristics distinct to each genus are prominent, but accurate identification of species within each genus is difficult due to common micro-morphological characteristics shared among species. Thus, despite the immense damage they cause in indoor environments, identification of the exact species responsible through morphology is challenging. With this, molecular identification using the β-tubulin (*BenA*) gene has become a standardized solution to species identification (Visagie et al., 2014), in addition to examining morphological features.

In the animal specimen room of the Seoul National University, specimens of immersed marine animals and valuable stuffed specimens that are over 80 years old are stored. After the professor in charge retired in February 2021, the specimen room was left unmaintained until the summer of the same year. During the rainy summer season, the specimen room was exposed to high temperatures and humidity. In just one week, the floor, walls, and stuffed animal specimens were heavily colonized by fungi. Given its long history and the collection of valuable specimens, the animal specimen room provided an intriguing environment for a fungal study. To date, little is known about fungal damage in stuffed biological materials, such as animal specimens and skeleton bones (Pinzari et al., 2020). This study aimed to identify the fungi that colonized the historical specimens. The results show that several fungal species can destroy historically valuable specimens within days. This highlights the need for attentive and consistent management of historical specimens.

Materials and Methods

Sampling and Isolation

The animal specimen room (an area of 176.09 m^2) was relocated in 2007 from Building 20 to the basement of Building 24 at the Seoul National University. Humidity and temperature have been controlled using an air conditioner and dehumidifier to maintain a temperature of approximately 23 °C and 20% humidity. However, it was left unmanaged for approximately five months due to the manager's retirement last February 2021. There was heavy rain over the summer, causing the temperature and humidity of the animal specimen room to remain high for approximately a week due to the malfunction of dehumidifiers and air conditioners. The room temperature spiked to over 30 °C, and the measuring device malfunctioned, so it was impossible to ascertain the precise humidity value. However, since the environmental condition was similar to the outside temperature in Seoul at the time, it could be assumed that the average humidity was around 70% based on KMA Weather Data Service (https://data.kma.go.kr/climate/RankState/selectRankStatisticsDivisionList.do, accessed on 2022.11.18). As a result, the animal specimen room was rapidly covered with fungi (Fig. 1).

We isolated fungi from 21 substrates: five from the room environment and 16 from the animal specimens (Fig. 1 and Table S1). Substrates from the animal specimens were further divided into 16 specific sections, including beak, feather, skin, and nails, among others (Table S1). Sampling of the visible fungal colonized parts in the animal specimen room was performed using sterile swabs. It was then spread to dichloran rose bengal chloramphenicol media (DRBC; Difco). The airborne fungi were sampled by leaving DRBC media open for approximately an hour in the room. The DRBC media were then incubated at 25 °C, and single colonies were transferred to potato dextrose agar (PDA; Difco) media. Pure fungal isolates were stored in a 20% glycerol stock in the form of PDA media slants at − 80 °C at the Seoul National University Fungus Collection.

Molecular Identification

All fungal isolates were morphologically grouped based on colony characteristics on PDA (e.g., growth rate, colony shape, color, texture of colony, etc.). Representatives selected from each group were used for molecular identification. Genomic DNA was extracted from isolates using the AccuPrep Genomic DNA extraction kit (Bioneer Co.), following the manufacturer's instructions with a

Fig. 1 Photographs of the internal environment of the animal specimen room. **A** Room environment (wall, plastic caps, floor, and shelves) and **B** animal specimens (fish, falcon, and wildcat) colonized by fungi

slight modification of using CTAB buffer instead of a TL buffer. Polymerase chain reaction (PCR) was performed on a C1000 thermal cycler (Bio-Rad) using the AccuPower PCR premix (Bioneer Co.). The primer set Bt2a and Bt2b (Glass and Donaldson, 1995) was used to amplify the protein-coding gene *BenA*. Sequencing was performed at Macrogen using an ABI PRISM 3700 Genetic Analyzer (Life Technologies).

The generated sequences were manually proofread and edited using MEGA7 (Kumar et al., 2016) and were deposited in GenBank (Fig. 3). Molecular identification was performed using the in-house Maximum-Likelihood tree-based pipeline with the species database for each genus.

Results

The most frequently isolated fungi were *Aspergillus* and *Penicillium*, and very few *Cladosporium* and *Trichoderma* were detected. Thus, we focused on identifying the *Aspergillus* and *Penicillium* species in this study. A total of 365 isolates of *Aspergillus* and *Penicillium* were obtained from all substrates (Table 1), of which 185 strains were isolated from the specimen room environment (Fig. 2), and 180 strains were isolated from the animal specimens (Fig. 2). These fungal strains were identified as 26 species based on *BenA* sequence analysis: 10 *Aspergillus* spp., 14 *Penicillium* spp., and 2 *Talaromyces* species that were preliminarily identified as *Penicillium* based on their growth morphological characteristics on PDA. They showed more than 97% similarity and formed a monophyletic clade with the corresponding species in the phylogenetic tree (data not shown). Among the three genera, *Penicillium* constituted the greatest number of species (14 species with 257 strains), followed by *Aspergillus* with 10 spp. (95 str.), and *Talaromcyes* with 2 spp. (13 str.).

Ten species were isolated from more than four substrates, and the rest were sampled from a restricted substrate (Fig. 3). The most diverse fungi were detected in the air and plastic caps (Fig. 3 and Table S1). Although several species were found in the feather and skin of the animal samples, mainly one or two species dominated. One to seven fungal species were isolated from each of the sixteen types of animal specimens. The substrate fish included ten different specimens, in which a total of sixteen fungal species were detected.

Although most species were isolated from both the room environment and the animal specimens, some were only detected from either substrate type. Seven species were isolated only from the room environment: *Aspergillus ochraceus*, *Aspergillus* cf. *protuberus*, *Penicillium coffeae*, *P. polonicum*, *P. rubens*, *P. terrigenum*, and *Talaromyces wortmannii*. Furthermore, seven species were only isolated from the animal specimens: *A. amoenus*, *A. miraensis*, *P. crustosum*, *P. exsudans*, *P. steckii*, *P. sumatraense*, and *T. scorteus* (Fig. 3). *Penicillium brocae* (146 strains) was the species confirmed from the most diverse substrates, being isolated from 29 specific sections of substrates, such as plastic caps, and even in air sampling, a large number of strains were isolated as a single colony (Fig. 3). It was also isolated from various sections in the animal specimens, such as the beak, feather, nail, skin, and teeth (Table S1).

Table 1 The information of the species type and the number of strains isolated from each sampling site of substrates

Substrate type	Sampling site	Fungal species	Strain no.
Room environment	Air	*Aspergillus creber*	1
		A. jensenii	5
		A. protuberus	4
		A. tennesseensis	5
		Aspergillus cf. *protuberus*	1
		Penicillium brevicompactum	2
		P. brocae	33
		P. copticola	4
		P. hispanicum	1
		P. polonicum	1
		P. steckii	4
		P. sumatraense	2
		Penicillium cf. *citreonigrum*	1
		Talaromyces scorteus	1
	Floor	*A. amoenus*	2
		P. brevicompactum	1
		P. brocae	2
		P. citrinum	1
		P. rubens	1
	Plastic	*A. creber*	3
		A. ochraceus	1
		A. protuberus	2
		A. sydowii	10
		A. westerdijkiae	6
		P. brevicompactum	6
		P. brocae	14
		P. coffeae	2
		P. copticola	6
		P. hispanicum	1
		P. rubens	3
		P. steckii	22
		P. sumatraense	16
		P. terrigenum	4
		T. wortmannii	1
	Shelves	*P. brocae*	4
		P. steckii	1
		T. scorteus	1
		T. wortmannii	2
	Wall	*A. creber*	2
		A. protuberus	2
		A. sydowii	3
		P. rubens	1
Animal samples	Feather	*A. jensenii*	4
		A. protuberus	1
		A. sydowii	2
		A. tennesseensis	3
		P. brocae	14
		Penicillium cf. *citreonigrum*	1
		P. citrinum	1
		P. steckii	1
		T. scorteus	1

Table 1 (continued)

Substrate type	Sampling site	Fungal species	Strain no.
	Beak	*A. creber*	1
		A. jensenii	1
		A. sydowii	3
		P. brocae	8
		P. hispanicum	1
		T. scorteus	1
	Nail & Teeth	*A. creber*	1
		A. jensenii	2
		A. sydowii	1
		A. tennesseensis	1
		P. brocae	10
		P. steckii	1
	Skin (leg skin, fish surface, turtle skin, etc.)	*A. amoenus*	3
		A. creber	5
		A. jensenii	6
		A. miraensis	1
		A. protuberus	1
		A. sydowii	7
		A. tennesseensis	4
		P. brocae	61
		Penicillium cf. *citreonigrum*	3
		P. citrinum	1
		P. copticola	1
		P. crustosum	1
		P. exsudans	1
		P. hispanicum	4
		P. steckii	13
		P. sumatraense	4
		T. scorteus	5

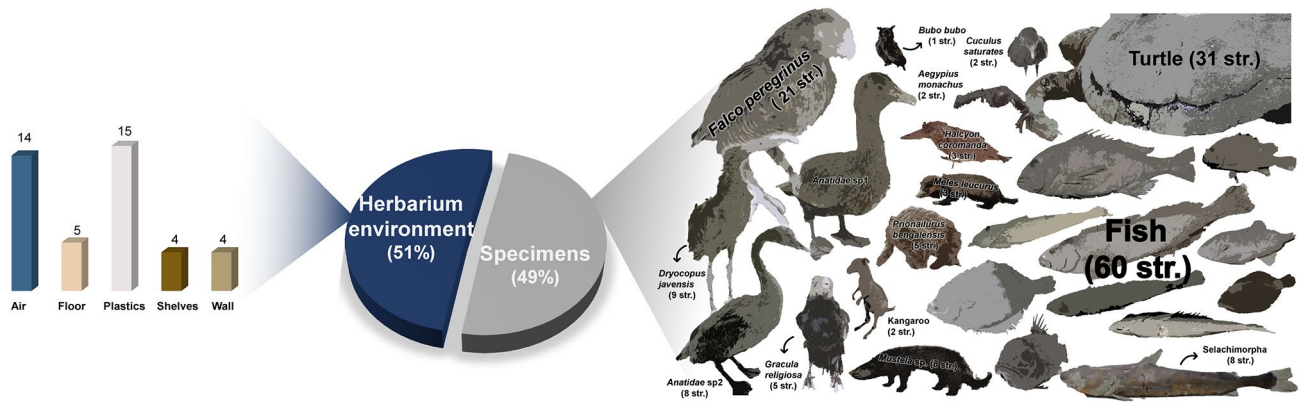

Fig. 2 Proportion of isolated fungal strains per substrate. The numbers of fungal strains isolated from each type of room environment are shown on the bar graph on the left. The size of the animal specimen on the right is proportional to the number of fungal strains isolated from it. The scientific or common names of the specimens and the number of fungal isolates from each specimen are also shown

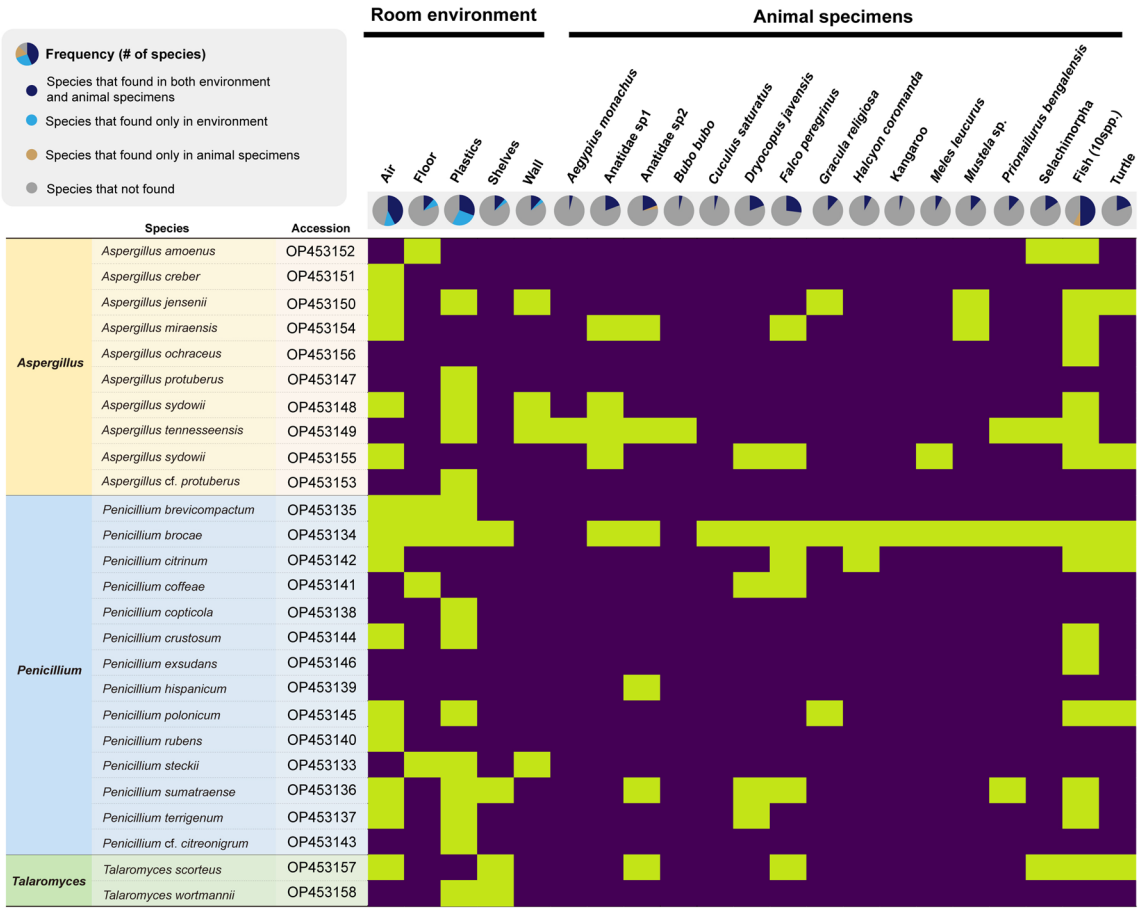

Fig. 3 Occurrence of species in each environment. The frequency of each species is represented by a pie chart, with the colors of the slices indicating whether the species is present in either or both substrate types (room conditions and animal specimens). The heat map shows the presence (yellow) and absence (purple) of the species in each sampling location (substrate) The column next to the species name shows the accession number (*benA*) for each representative

Aspergillus tennessensis and *Penicillium sumatraense* were also found from many substrates of the room environment and animal specimens, following *P. brocae*. Similarly, *A. creber*, *A. jensenii*, *P. hispanicum*, and *T. scorteus* were isolated from various substrates.

Most fungal species were isolated from both the room environment and animal specimens, but three species were exclusively found from the animal specimens (Anatidae sp.2 and fish specimens) (Fig. 3). On the contrary, some fungal species that were not confirmed from the animal specimens were isolated from the substrates categorized as part of the room environment. Most of these species (*Aspergillus creber*, *A. protuberus*, *Aspergillus* cf. *protuberus*, *Penicillium copticola*, *P. rubens*, and *Penicillium* cf. *citreonigrum*) were only found from one type of substrate, while *P. brevicactum*, *P. steckii* and *Talaromyces wortmannii* were found from two or three types of substrates, all including plastic (Fig. 3).

Discussion

The Seoul National University animal specimen room was a vast animal diversity archive that housed a massive amount of animal resources (approximately 36,000 species). These resources carried valuable information about the past biodiversity in various classifications, including invertebrates, fish, mammals, and birds, among others. However, a short period of abandonment has placed these valuable resources at risk of deterioration due to fungal invasion. Most fungal species identified in this study have been previously reported from the indoor environment (Amend et al., 2010; Visagie et al., 2014). In this study, we isolated 26 fungal species (365 strains) of *Aspergillus*, *Penicillium*, and *Talaromyces* that completely covered the animal specimen room and were detected from both the room environment and animal specimens. The common

fungal composition of the two types might be due to the dispersal of spores from and to the specimens and the air (Adams et al., 2013), as fungal spores can easily disperse through the air (Golan & Pringle, 2017; Lacey, 1996). In addition, the sealed room with high temperature and moisture could have sustained an optimal condition for fungal growth and sporulation.

Of the 26 species confirmed in this study, *P. brocae* was found to have overwhelmingly grown in the animal specimen room. It is a monoverticillate species first reported in coffee berry borers collected at coffee plantations in Mexico (Peterson et al., 2003). This species was later isolated from the fresh tissue of the marine mangrove plant *Avicennia marina* at Hainan Island, China (Meng et al., 2014), a Fijian sponge species (Bugni et al., 2003), and the surface of Thai rice grains in Thailand (Shiratori et al., 2017). Its dominance in the indoor environment has also been reported from a tannery (Treves & Martens, 2010), which is an enclosed interior environment similar to the environment in this study. *P. brocae* and *A. sydowii* have been reported to degrade some forms of plastic, such as polyvinyl chloride, or a plasticizer (Ali et al., 2014; Pradeep et al., 2013). *A. sydowii* and *P. steckii* are also known for their ability to degrade some types of keratin (Nikitina et al., 2017; Kotwal & Sumbali, 2016), which is a major component of feathers. Thus, these species appear to thrive on feathers, beaks, nails, and plastics, decomposing them for nutrients.

In this study, five species (*A. amoenus*, *A. miraensis*, *A. protuberus*, *P. brocae*, and *T. scorteus*) are newly reported from Republic of Korea despite the active ecological research conducted in the country from various environments, such as the air (Kim et al., 2010; Oh et al., 2014), freshwater (Heo et al., 2019; Lim et al., 2021), soil (Lee et al., 2003; Park et al., 2020), and marine environment (Lee et al., 2016; Park et al., 2014). However, the exact route of introduction of these species into the animal specimen room remains unclear.

In addition, the health of the researchers studying in the animal specimen room is at risk. Although most animal specimens followed the suitable sample preparation process before, fungi hidden in the hairs or scales were inevitable. The spores and VOCs that these hidden fungi produce can be a big concern. Among the fungi identified in this study, *A. sydowii* (Borgohain et al., 2019) and *P. citrinum* (Mok et al., 1997; Walsh et al., 2004) have been reported to cause diseases in humans. For *P. brocae*, which may produce mutagenic metabolites (Shiratori et al., 2017), immediate action is required to determine the extent of the spread of this species and the potential damage it can cause in other indoor environments. However, as most other species have not been evaluated for their biohazardous activities, continuous monitoring of environments is essential to prevent the unknown consequences of these species when they spread in the local environment or come into contact with people.

Fungi are called 'the planet's biggest killers' (Jones, 2013) that can put other organisms at risk of extinction, as seen from a potato blight (Cox & Large, 1960) and amphibian extinction (Rödder et al., 2009). Furthermore, the destruction caused by and the continuous spread of fungi are not easy to prevent. As shown by the present study, approximately 80 years of animal history had been lost in just a week because of less than 100 species of fungi. We can prevent this catastrophe from occurring again with continued interest. As biological specimens serve as evidence of evolution, researchers should value and preserve them. Succeeding researchers should also maintain continuous monitoring and management to value the efforts of past researchers.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflict of Interest The authors have no conflicts of interest.

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