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NOTE

Assessment of decay risk of airborne wood-decay fungi II: relation between isolated fungi and decay risk

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Abstract The relationship between the taxa of airborne fungi and the decay risk was investigated. Airborne fungi in 1,000 l of air were trapped on Japanese cedar disks, and incubated in a damp container kept at 26°C. After 16-week incubation, filamentous fungi grown on the disks were isolated and DNA extracted from each isolate was amplified with the primers ITS4/ITS5. The DNA sequences of the amplified products were determined and compared to the sequence data of GenBank to determine the species or genus according to a BLAST search. This search revealed that the isolate consisted of 5 major taxa, namely *Bjerkandera* sp., *Phanerochaete* sp. (A), *Phanerochaete* sp. (B), *Polyporales* sp. *Polyporus arcularius*, and 6 minor ones. Statistical analysis revealed that the major taxa were trapped on the disks in similar weather conditions except for *Bjerkandera* sp., which was trapped at a cooler temperature. The analysis also proved the disks to which *Phanerochaete* spp. or *Polyporales* sp. were attached showed higher mass loss. It is concluded that, under these experimental conditions, related species of *Phanerochaete sordida* play an important role in increasing the decay risk caused by airborne wood-decay fungi.

Keywords Decay risk · Airborne fungi · Air sampler · Identification · White rot

Introduction

Utilizing harvested wood and extending the service life of wooden products are reportedly important means of mitigating global warming [1–3]. With this in mind, many investigative studies have been carried out regarding efforts to prolong the service life of wooden products [4–8].

Conversely, research into the factors that accelerate carbon dioxide gas emissions from wooden products has also been performed, and has revealed that one of the key biological factors for accelerating emissions is wood-decay fungi classified in Basidiomycota [9, 10]. Basidiospores released from Basidiomycota are transferred to wood by wind, water, and insects [11–13]. Fungal hyphae grow from the basidiospores, and degrade the wood cell-wall components with enzymes and/or low molecular weight compounds [14, 15]. Therefore, it is important to investigate the characteristics of the spores and hyphae in order to estimate the decay risk of wood especially in above-ground applications.

In our previous paper [16], we discussed a novel method consisting of the direct air sampling of airborne wood-decay fungi to wood disks using an air sampler, followed by incubation in a conditioned room. The method proved useful in assessing the decay risk of wood for above-ground applications and revealed that under experimental conditions, airborne fungi in 1,000 l of air caused a mass loss of 255 mg on average for 16-week incubation at 26°C and the decay risk rose on cloudy days.

On the other hand, it remained still unclear that which fungi responsible for increasing decay risk of wood for

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above-ground applications. Therefore, this study aimed at revealing fungal species responsible for increasing decay risk in our experimental conditions. Taxa of the isolated fungi and the relation between the decay risk and the identified taxa are discussed.

Materials and methods

All materials and experimental conditions including mass loss determination were the same as those described in the previous paper [16]. Air sampling was carried out at a height of about 100 cm using a BIOSAMP MBS-1000 air sampler (Midori Anzen, Tokyo, Japan) at the same site on the grounds of the Forestry and Forest Products Research Institute in Tsukuba, from June to November 2008. Two hundred one damp Japanese cedar (*Cryptomeria japonica*) disks, about 3-mm thick and 7.8 cm in diameter, were used as a fungal medium to trap airborne wood-decay fungi. Japanese cedar disks exposed to 1,000 l of air by the air sampler were incubated for 16 weeks. Fungal strains grown on the disks whose initial mass and mass loss during the incubation were 5,530 and 255 mg, respectively, were isolated [16].

Isolated filamentous fungi were incubated on cellophane membranes placed on potato dextrose agar plates at 25°C for 1–2 weeks. The cultural appearances were then checked by visually in order to select fungal strains for further studies in accordance with the following rules:

1. Compare the culture morphology of all the strains isolated from each Japanese cedar disk.
2. Select all strains showing different cultural appearances.
3. Select one strain from all of the strains showing the same cultural appearance.

The mycelia of the selected strains were harvested from each plate by scratching the surface of the membranes with a sterilized spatula and placing them in 1.5-ml tubes. The mycelia were frozen at −80°C for more than 30 min and lyophilized. The freeze-dried mycelia were then ground to a fine powder using a sterile pipette tip.

DNA was extracted from the powder using a DNeasy extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

The primers ITS4/ITS5 [17] were used to amplify a portion of the ITS region. Each 20-μl reaction mixture contained 10 ng of template DNA (or no DNA template for a negative control), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each primer, 2.5 mM of each dNTP, and 0.5 U Takara Ex Taq (Takara, Tokyo, Japan). The PCR condition was as follows: 94°C for 1 min, 30 cycles at 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min, respectively. The PCR products were purified with MicroSpin Columns and Sephacryl S-300 (GE Healthcare, Piscataway, NJ, USA) and sequenced bidirectionally with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to ensure accuracy. Sequences were assembled and manually edited using Bioedit [18] and Chromas Pro (Technelysium Pty Ltd, Australia). The sequences excluding 18S and 28S ribosomal RNA region were clustered into the operational taxonomy units (OTUs) that shared 99% or greater sequence similarity using clustalX [19]. All sequences of representative OTUs were subjected to similarity searches against those deposited in GenBank using Blastn (nucleotide Basic Local Alignment Search Tool). One representative sequence of each OTUs was deposited in GenBank (Accession No. AB638337-AB638347, Table 1). The phylogenetic affinity of the sequence was also considered.

All statistical analyses in the relationship between fungi and other factors, such as weather conditions and sampling date, were performed using JMP 8.0 software (SAS Institute, Cary, North Carolina, USA) [20] with the significance level set at 0.01.

Results

Identification of airborne wood-decay fungi

Two hundred ninety-three strains of airborne wood-decay fungi were successfully isolated from 110 Japanese cedar disks. Those 293 strains were firstly screened by cultural appearance, and 119 strains were finally selected. The selected 119 strains were subjected to BLAST searches of the GenBank using DNA sequences amplified with ITS4/ITS5 [17].

The result of the BLAST searches indicated that all selected strains were divided into 11 identical taxa, namely *Bjerkandera* sp., *Ceriporia* sp., *Irpex* sp., *Phanerochaete* sp. (A), *Phanerochaete* sp. (B), *Phanerochaete* sp. (C), *Polyporales* sp., *Polyporus arcularius*, *Sistotreme* sp., *Trametes hirusuta* and *Trametes versicolor* (Table 1).

The identified taxa firstly had their decay types determined, namely white-rot or brown-rot fungi, by a literature survey. The survey on the identified taxa suggests all of them except for *Polyporales* sp. are white-rot fungi (Table 1). In addition, *Polyporales* sp. is probably a white-rot fungus because the DNA sequence of the fungus shows the nearest match to that of the white-rot fungus, *Flavodon flavus*.

Relationship between taxa and decay risk

Mass losses of the Japanese cedar disks were compared between the disks on which the identified taxa grew and

Table 1 Frequency and taxa designation of fungal taxa recovered from Japanese cedar disks

Incidence (%)[a]	Taxa	Accession no.	Nearest match Taxa[b]	Nearest match Acc. no.[b]	Similarity (%)	Decay type
12.4	*Bjerkandera* sp.	AB638337	*Bjerkandera adusta*	HQ327995	100	W[d]
1.0	*Ceriporia* sp.	AB638338	*Ceriporia lacerata*	HM595573	98	W[d]
0.5	*Irpex* sp.	AB638339	*Irpex lacteus*	AB079265	98	W[d]
5.0	*Phanerochaete* sp. (A)	AB638340	*Phanerochaete sordida*	HQ608013	99	W[d]
18.4	*Phanerochaete* sp. (B)	AB638341	*Phanerochaete sordida*	HM595562	100	W[d]
3.0	*Phanerochaete* sp. (C)	AB638342	*Phanerochaete sordida*	HM595569	99	W[d]
10.9	*Polyporales* sp.	AB638343	*Flavodon flavus*	FJ010207	98	(W)[e]
6.0	*Polyporus arcularius*	AB638344	*Polyporus arcularius*	GU207249	97	W[f]
0.5	*Sistotrema* sp.	AB638345	*Sistotrema* sp.[c]	GU062211	99	W[d]
0.5	*Trametes hirusuta*	AB638346	*Trametes hirusuta*	HQ435865	100	W[f]
1.0	*Trametes versicolor*	AB638347	*Trametes versicolor*	HM595570	99	W[f]

[a] Incidence was obtained by dividing number of disks from which each fungus was isolated by the total number of disks

[b] Taxa and GenBank accession numbers that show the highest similarity

[c] The species is not reported in the GenBank database

[d] White rot determined by genus

[e] Probably white rot suggested by closest match fungal taxon

[f] White rot determined by species

those did not grow. The mean mass loss and standard deviations of the disks on which the identified taxa grew was 313 ± 156 mg while that of the disks from which no taxa were isolated was 180 ± 170 mg. Statistical analysis using *T* test revealed there is a significant difference between these two groups.

Studies were hereafter carried out on the following 5 major taxa, namely *Bjerkandera* sp., *Phanerochaete* sp. (A), *Phanerochaete* sp. (B), *Polyporales* sp. and *Polyporus arcularius*, that were isolated from more than 10 disks.

The relation between the mean mass losses and the 5 taxa is shown in Fig. 1. The Tukey–Kramer test reveals that the identified taxa can be divided into three groups from mass loss cause by the fungi: the first group consists of *Phanerochate* sp. (A), *Polyporales* sp. and *Phanerochaete* sp. (B); the second consists of *Phanerochaete* sp. (B) and minor fungi that were observed in few disks; and the third consists of *Bjerkandera* sp., *Polyporus arcularius* and the minor fungi.

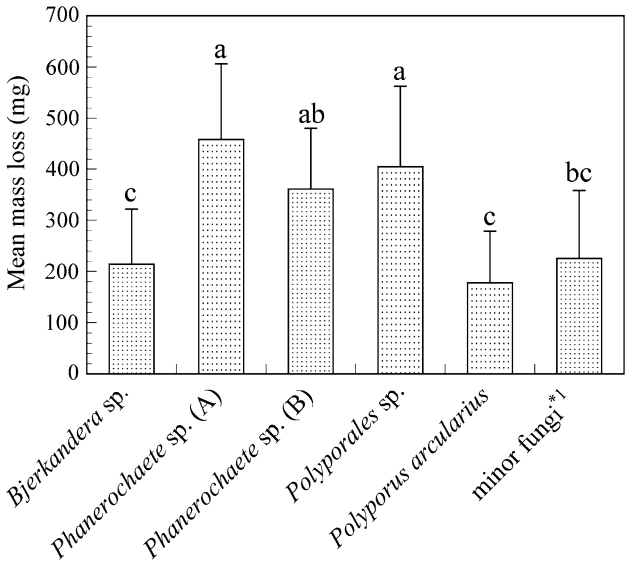

Fig. 1 Identified taxa and mass loss. The mean mass loss of Japanese cedar disks during the 16-week incubation was calculated for each of the identified taxa. The mean mass losses (+standard deviations) marked by the same letter do not differ significantly according to the Tukey–Kramer test ($P < 0.01$). *1: minor fungi consist of 6 identified fungi that were isolated from a few disks (see Table 1)

Relationship between taxa and sampling date

The cumulative frequency of each taxon plotted against the sampling dates, with the graph indicating some seasonal difference when each taxon was trapped (Fig. 2). For example, *Polyporus arcularius* and *Bjerkandera* sp. were mainly found in early summer and late October to November, respectively. The graph also shows that all identified taxa were found during the period June to November.

Relationship between taxa and weather factors

Figure 3 shows an example that indicates the relationship between weather factors and the occurrence of the identified fungus. The Tukey–Kramer test reveals that *Bjerkandera* sp. was trapped at a cooler sampling period than the four other identified taxa. The test also clarifies that the

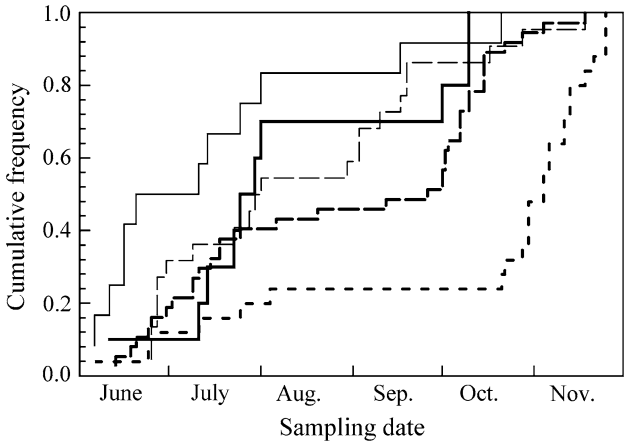

Fig. 2 Cumulative frequency of fungal occurrence against sampling date. *Thick dotted line Bjerkandera* sp., *thick line Phanerochaete* sp. (A), *thick dash line Phanerochaete* sp. (B), *thin dash line Polyporales* sp., *thin line Polyporus arcularius*

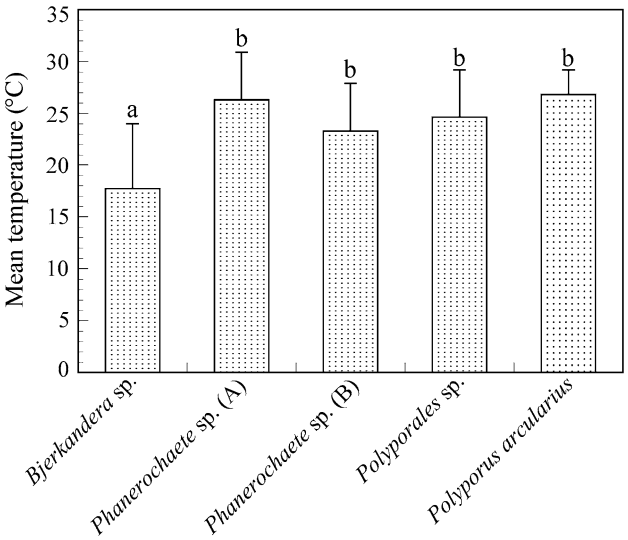

Fig. 3 Identified taxa and air temperatures at sampling time. The mean air temperatures of the sampling time were calculated for each of the identified taxa. The mean temperatures (+standard deviations) marked by the same letter do not differ significantly according to the Tukey–Kramer test ($P < 0.01$)

mean, maximum and minimum temperatures of the days on which *Bjerkandera* sp. was trapped were lower than those of the days on which other four taxa were trapped (Table 2). Conversely, other weather factors listed in Table 2 are revealed not to affect the occurrence of each identified taxon.

Discussion

Although our previous paper revealed the relationship between weather factors and decay risk, fungal species that increased decay risk remained unclear. Therefore, the present study aimed to clarify fungal species responsible for increasing decay risk and relation between fungal species and weather factors.

Table 2 Relation between weather factors and fungal incidence

Investigated weather factors		Influence[a]
Temperature (°C)	Value at sampling period	+
	Mean value on sampling day	+
	Maximum value on sampling day	+
	Minimum value on sampling day	+
	Daily range	
Relative humidity (%)	Value at sampling period	
	Mean value on sampling day	
	Maximum value on sampling day	
	Minimum value on sampling day	
	Daily range	
Atmospheric pressure (hPa)	Value at sampling period	
	Mean value on sampling day	
	Maximum value on sampling day	
	Minimum value on sampling day	
	Daily range	
Solar radiation (MJ/m^2)	Value at sampling period	
Sunshine duration (h)	Value on sampling day	

[a] Plus symbols indicate that the weather factors significantly affect the fungal occurrence of some fungi

We selected 119 strains from 293 strains by their cultural appearance from 110 Japanese cedar disks. In other words, only one fungus was dominant in above 90% of disks though an average of 2.7 strains was subcultured from each disk.

BLAST searches of the GenBank using DNA sequences amplified with ITS4/ITS5 indicate that all selected strains are divided into 11 identical taxa shown in Table 1. Except for *Polyporus arcularius* and *Trametes* spp., 8 taxa could not be determined because there are many species with similar DNA sequences comparable to that showing the nearest match listed in Table 1. They are similar to those found in decayed wood in above-ground applications [21, 22].

Using the identified taxa, we firstly checked their decay types. In our previous paper, we investigated the decay type based on the color reaction between each fungal strain and guaiacol, and concluded about one-third of the isolated strains was probably classified as white-rot fungi [16]. However, the survey on the identified taxa suggests all of them except for *Polyporales* sp. are white-rot fungi

(Table 1). In addition, *Polyporales* sp. is probably a white-rot fungus because the DNA sequence of the fungus shows the nearest match to that of the white-rot fungus, *Flavodon flavus*.

Although it is unclear why the inconsistency takes place between the conclusion in the previous paper and this study, the incubation period in the previous study might affect the conclusion. We incubated for 5 days to check the color reaction while Miyamoto et al. incubated for 2 weeks in the cited paper [23]. Increasing the incubation period may result in an increase in phenol oxidase production and a change of color.

The efficacy of sampling and isolation of airborne wood-decay fungi was firstly checked. As mentioned previously, we identified 11 taxa from 110 disks, despite using 201 disks for the sampling. Presumably, part of the important fungi may be lost during the subculturing processes on a PDA medium containing biocides. To check this hypothesis, mass losses of the Japanese cedar disks were compared between the disks from which the identified taxa were selected and those not selected. The mean mass loss of the disks from which the taxa were identified was about twice as larger as that of the disks from which no taxa were isolated. Statistical analysis using *T* test revealed a significant difference between these two groups. In conclusion, important taxa that influence decay risk can be successfully identified in this experimental procedure even though some unimportant fungi might be missed during the subculturing processes.

The relation between the 5 major taxa and the mean mass losses was statistically analyzed to specify the taxa responsible for increasing decay risk. The Tukey–Kramer test reveals the identified taxa can be divided into three groups from mass loss cause by the fungi, and that *Phanerochate* sp. (A), *Polyporales* sp. and *Phanerochaete* sp. (B) have the highest decay activity among the isolated taxa.

To confirm when the 5 major taxa were trapped, occurrence of the major taxa and sampling date were also investigated. The graph plotting cumulative frequency of the major taxa against the sampling date clearly indicates that they appeared throughout the sampling period. This result is good in accordance with our former result that there was no significant correlation among sampling months. The reason that the decay risk was almost identical for each month from June to November [16] is due to the presence of several airborne wood-decay fungi throughout the sampling period.

The effect of weather factors on the occurrence of the 5 major taxa was also investigated. The Tukey–Kramer test reveals that only temperature affected the occurrence of the major taxa. *Bjerkandera* sp. was trapped at cooler conditions than the four other major taxa (Table 2) while other weather factors listed in Table 2 do not affect the occurrence of each identified taxon.

To summarize the above discussions, *Phanerochaete* sp. (B) are concluded to be the fungi responsible for increasing decay risk in our experimental conditions because of its abundance (Table 1) and decay activity (Fig. 3). The DNA sequences of the ITS region of *Phanerochaete* sp. (B) are very similar to that of *Phanerochaete sordida*, which has been well known as a species distributed worldwide [24], mineralizing lignin [25], producing manganese peroxidase [26] and decolorizing dyes [27]. Related species of *P. sordida* play an important role in increasing the decay risk caused by airborne wood-decay fungi under these experimental conditions.

Our novel method reported in the previous paper [16] and the following DNA sequence analysis discussed here is a useful method to specify the fungi contributing to wood decay in above-ground applications and the relationships between such contributing fungi and other conditions such as weather factors.

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