Holzforschung, Vol. 63, pp. 633–638, 2009 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/HF.2009.099

Primary and secondary decay fungi on exposed pine tree logs in the forest

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Abstract

The successional diversity of basidiomycetous fungi was studied on Korean pine (*Pinus koraiensis*) and pitch pine (*Pinus rigida*) logs that were left exposed on the forest floor based on fungal surveys conducted after 18, 42, and 54 months of exposure. A total of 131 basidiomycetous isolates were recovered from the logs and grouped by their cultural morphology. Fungal identification was achieved by a BLAST search of partial nuclear large subunit ribosomal DNA sequences in GenBank. The results of the 18 month survey revealed that the isolation frequency and fungal diversity were higher for pitch pine than Korean pine. The dominant species found on Korean and pitch pine logs during the first survey were *Hypochnicium karstenii* and *Phlebiopsis gigantea*, respectively. In the 42 and 54 month surveys, the primary species were replaced by *Hypochnicium eichleri*, *Phanerochaete velutina*, *Phlebia radiata*, *Rhizochaete* sp., and *Trametes versicolor*. These results showed that decay fungi have host preference for woody materials that vary according to the species of tree being colonized.

Keywords: basidiomycete fungi; decay fungi; host preference; pine log; *Pinus koraiensis*; *Pinus rigida*; successive species.

Introduction

Fungi that are capable of degrading lignin, cellulose, and hemicelluloses in wood are primarily basidiomycetes, with the exception of a few xylariaceous ascomycete species. These wood-rotting basidiomycetes have specific preferences for wood in certain stages of decomposition (Renvall 1995). Rayner and Webber (1984) and others divided wood degrading fungi into primary and secondary decayers. Many of the primary decay fungi colonize an unoccupied substrate and metabolize most of the available nutrient resources and then die back. This process enables succession by fungi that can obtain nutrients from the residual materials. The majority of primary decayers of logs are saprotrophs, such as *Stereum sanguinolentum*, *Trichaptum abietinum*, and *Phlebiopsis gigantea* (Renvall 1995). However, primary decayers also include a few pathogenic species, such as *Heterobasidion annosum*, *Onnia leporina*, and *Inonotus obliquus* (Stenlid 1993). Secondary decayers are organisms that colonize a substrate that has already been occupied by other organisms because they outcompete the original organisms (Renvall 1995; Holmer et al. 1997). Although wood decayers are replaced in a species-specific fashion, most secondary decay fungi seem to appear and disappear without any obvious correlations to other decayers (Rayner and Boddy 1988).

The general pattern of the colonization of wood inhabiting fungi during the deterioration of logs, logging slash, natural debris, fire-killed trees, and wind-thrown trees has been reported from Europe and North America (Findlay 1966; Shigo 1967; Butcher 1968; Coates and Rayner 1985; Chapela et al. 1988; Renvall 1994). Similar studies have been conducted to evaluate the logs and dead stumps in forest and woods in Korea (Kim et al. 2005). However, few studies evaluated the relationship between primary and secondary decayers. To establish integrated control strategies for wood degradation, it is necessary to understand all stages of the decay process. Accordingly, characterizing the entire decay process rather than focusing on only a few organisms degrading the wood may provide a better understanding of the rate of wood decomposition.

In this study, primary and secondary basidiomycetous decay fungi were established on logs of two major Korean coniferous species, Korean pine (*Pinus koraiensis* Siebold et Zucc.) and pitch pine (*Pinus rigida* Mill.), that were left exposed on the forest floor for 3.5 years. Because the decay fungi do not always form fruiting bodies, they were cultured and then identified by DNA sequences.

Materials and methods

Fungal isolation and identification by traditional methods

In March 2003, several healthy Korean pine and pitch pine trees were cut at the forestry research facility of Korea University in Yangpyeong. Five disease-free logs (15–20 cm in diameter and 2 m in length) between 40 and 45 years of age were selected from each tree species and then left exposed on the ground without peeling the bark at two different sites (0.5 km apart). Three fungal surveys of the exposed logs were then conducted at 18, 42, and 54 months. During the initial survey at 18 month, 12 cores were removed 30 cm from both ends and near the middle of each log using an increment borer. For the second and third surveys, the fungal isolation was performed by removing wood chips from the decayed areas of the logs with a knife because the logs were severely decayed. The cores and chips

were placed individually in a plastic bag and cultured on 2% malt extract agar (20 g Difco malt extract, 15 g Difco agar, and 1000 ml distilled water) that contained 4 ppm benomyl and 100 ppm ampicillin (Clubbe and Levy 1977). The plates were incubated at room temperature for several weeks. Each different type of the mycelia was routinely subcultured onto new plates to obtain pure culture. The isolates were then grouped based on their morphologies and identified based on their cultural (Nobles 1965; Stalpers 1978) and molecular characteristics.

Fungal identification using molecular methods

Fungal genomic DNA was prepared from mycelium using the method described by Lim et al. (2005). PCR amplification of the 5′ partial region of the nuclear large subunit rDNA (nLSU rDNA) – based on the LROR and LR3 primers (http://www.biology.duke.edu/fungi/mycolab/primers.htm) – was conducted by means of the method described by Kim et al. (2004). The PCR products were purified with a PCR Clean-up Kit (Mo Bio, USA), then sequenced with an ABI 3700 automated sequencer (Perkin-Elmer, Foster City, CA, USA) at the MACROGEN DNA Synthesis and Sequencing Facility (Seoul, Korea). In most cases, two or more isolates from each group were sequenced. All of the nucleotide sequences determined in this study have been deposited in GenBank under the accession numbers shown in Tables 1 and 2.

Identification of the decay fungi based on the sequences of the nLSU rDNA region was achieved via a BLAST search of GenBank (Wheeler et al. 2007). Closely matching sequences available in GenBank were then downloaded and aligned with our nLSU rDNA sequences by Clustal X (Thompson et al. 1997) and then manually adjusted by PHYDIT version 3.2 (http://plaza.snu.ac.kr/jchun/phydit/).

Results and discussion

A total of 131 basidiomycetous isolates were obtained from Korean pine logs (49 isolates) and pitch pine logs (82 isolates). Among them, only three isolates, *Heterobasidion annosum*, *Schizophyllum commune*, and *Sistotrema brinkmannii*, were identified at the species level based on morphology. *Heterobasidion annosum* was easily identified based on the presence of oedocephaloid conidiophores. *Schizophyllum commune* was identified by its mat cottony, locally wooly colony morphology, and hyphae with minute projections on the walls. *Sistotrema brinkmannii* cultures produced a smooth fruiting body in cultures grown on media and uniform basidia with six sterigmata (Nobles 1965; Stalpers 1978). The remaining isolates were placed in 26 groups based on macro- and microscopic characters, such as mycelial shape, mycelial color, growth rate, hyphal type, and the presence of clamp connections.

Two or more representative isolates of each group were sequenced. The nLSU rDNA regions ranged from 600 to 650 bp. The nLSU rDNA sequences allowed most of the 26 groups of morphologically unidentifiable fungal isolates to be linked to established genera or species (Tables 1 and 2). The ITS sequence is regarded as an excellent tool for identifying unknown fungi to broad species groups or genera (Horton and Bruns 2001) and to identify even very closely related species (Schmidt and Moreth 2002); however, the nLSU rDNA region sequence can also be used successfully for fungal identification (Kernaghan et al. 2003; Tedersoo et al. 2003; Hunt et al. 2004). We have demonstrated that fungal isolates from wood products (collected on a playground) are conspecific with greater than 97.9% similarity based on nLSU rDNA sequences (Kim et al. 2005). In this study, we assigned species name to our isolates when pairwise similarity scores from BLAST searches were greater than 98.0%, except *Mucronella calva* and *Phanerochaete* species. For example, even though the similarity of a nLSU rDNA region sequence to *Phanerochaete calotricha* is greater than 98%, the identification of the fungus could not be determined based on the sequence data alone. This is because *Phanerochaete* species are morphologically similar (Burdsall 1985) and phylogenetically close-related (Paulus et al. 2000; Lim 2001; De Koker et al. 2003). Therefore, for these species, phylogenetic knowledge as well as morphological consideration is required to confirm their identification. Morphological characterization of the isolates and their sequence analysis allowed us to distinguish the 131 isolates into 19 genera and 29 species, including an unknown basidiomycete (Tables 1 and 2).

A total of 49 isolates, including 14 genera and 18 species, were obtained from Korean pine logs (Table 1). Most of these species were corticioid fungi, although four isolates were poroid fungi. Five species were obtained during the first survey, and the most frequent species was *Hypochnicium karstenii*. Eight and ten species were isolated during the second and the third surveys, respectively. In the second survey, the *Phanerochaete* species were the most frequently isolated, whereas common white rot decay fungi *Cerrena consors*, *Irpex lacteus*, and *Trametes versicolor* were isolated in the third survey. The results of this study show that the five species of the first survey were replaced with other species in the second and third surveys. In addition, a greater diversity of fungi was recovered during the second and third surveys.

A total of 82 isolates were recovered from the pine pitch logs, including 23 species distributed among 15 genera (Table 2). Corticioid decay fungi – *Phlebiopsis gigantea*, *Phlebiella* sp. 1, and *Hypochnicium cremicolor* – were the most prevalent species recovered during the first survey. However, these species were not recovered during the second and third surveys, which were dominated by *Hypochnicium eichleri* and *Phlebia radiata*, respectively. Although the species diversity decreased over time on the pitch pine logs, it was still higher than the diversity of fungi recovered from the Korean pine logs.

According to Camargo's index (Table 1), the dominant species differed between the species of pine tree logs evaluated in this study. Initially, the freshly cut trees were colonized by primary decayers, such as *H. karstenii*, *Rhizochaete* sp., and *S. brinkmannii* on the Korean pine logs and *H. cremicolor*, *Phlebiella* sp. 1, *P. gigantea*, and an unknown basidiomycete (KUC8302) on the pitch pine logs. However, while the primary saprotrophs recovered from Korean pine were restricted to decay fungi which are associated with conifers, those that were recovered from pitch pine were more diverse and included *Schizophyllum commune* and *Trametes versicolor*, which are known to decay both hardwoods and softwoods (Eaton

Table 1 Basidiomycetes isolated from Korean pine logs exposed to the forest floor.

Isolate no.	GenBank accession	No. of isolates			Closest matched fungal species in Blast (LSU) (GenBank acc. no.)	Identity (%)[b] (LSU)	Fungal identity	Rot type[c]	Family[d]
		18 months	42 months	54 months					
KUC8407	FJ471540	–[a]	–	1	*Ceriporia lacerata* (AY858357)	558/560 (99.6)	*Ceriporia lacerata*	W	PP
KUC8416	FJ471541	–	–	1	*Cerrena consors* (AY515338)	576/578 (99.7)	*Cerrena consors*	W	PP
KUC8362	FJ471542	–	1	2	*Hypochnicium eichleri* (AY858365)	583/583 (100)	*Hypochnicium eichleri*	W	C
KUC8343	FJ471543	10*	–	–	*Hypochnicium karstenii* (DQ677510)	579/584 (99.1)	*Hypochnicium karstenii*	W	C
KUC8368	FJ471544	–	2	1	*Hypholoma subviride* (AF261631)	616/616 (100)	*Hypholoma subviride*	W	ST
KUC8411	FJ471545	–	–	1	*Irpex lacteus* (AY858353)	559/560 (99.8)	*Irpex lacteus*	W	PP
KUC8367	FJ471546	–	1	–	*Mucronella calva* (AJ406588)	576/612 (94.1)	*Mucronella calva*	B	CL
KUC8405	FJ471547	–	–	2	*Phanerochaete sordida* (KCTC26213)	583/583 (100)	*Phanerochaete sordida*	W	C
KUC8370	FJ471558	–	1	–	*Phanerochaete sordida* (AY858368)	568/583 (97.4)	*Phanerochaete* sp. 1	W	C
KUC8361	FJ471549	–	2	–	*Phanerochaete sordida* (AY858368)	560/583 (96.1)	*Phanerochaete* sp. 2	W	C
KUC8369	FJ471550	–	4*	2	*Phanerochaete velutina* (DQ679917)	578/582 (99.3)	*Phanerochaete velutina*	W	C
KUC8406	FJ471551	–	–	1	*Phlebia radiata* (AY858369)	583/583 (100)	*Phlebia radiata*	W	C
KUC8357	FJ471552	2	4*	–	*Rhizochaete radicata* (AY219392)	599/614 (97.6)	*Rhizochaete* sp.	W	C
KUC8341	FJ471553	3	1	–	*Sistotrema brinkmannii* (AY858375)	561/561 (100)	*Sistotrema brinkmannii*	W	C
KUC8360	FJ471554	1	–	–	*Stereum hirsutum* (AY858376)	579/583 (99.3)	*Stereum hirsutum*	W	C
KUC8413	FJ471555	–	–	4*	*Trametes versicolor* (AY858378)	582/582 (100)	*Trametes versicolor*	W	PP
KUC8349	FJ471556	1	–	–	*Tulasnella calospora* (DQ388044)	531/535 (99.3)	*Tulasnella calospora*	W	T
KUC8401	FJ471557	–	–	1	*Helicogloea* sp. (AY512848)	530/601 (88.2)	Unknown basidiomycete	–	–
Total isolates		17	16	16					

[a]Not isolated.

[b]Identity (%) was represented by matched nucleotide/compared nucleotide.

[c]W, white rot type; B, brown rot type (Gilbertson and Ryvarden 1986, 1987; Ginns and Lefebvre 1993).

[d]C, Corticiaceae; CL, Clavariaceae; PP, Polyporaceae; ST, Strophariaceae; T, Tulasnellaceae (Gilbertson and Ryvarden 1986, 1987; Ginns and Lefebvre 1993).

*Dominant species. A species is considered dominant if $P_i > 1/S$, where P_i (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species present in the community (Camargo 1993).

Table 2 Basidiomycetes isolated from pitch pine logs exposed to the forest floor.

Isolate no.	GenBank accession	No. of isolates			Closest matched fungal species in Blast (LSU) (GenBank acc. no.)	Identity (%)[b] (LSU)	Fungal identity	Rot type[c]	Family[d]
		18 months	42 months	54 months					
KUC8421	FJ471558	–	–[a]	1	*Cerrena consors* (AY515338)	576/578 (99.7)	*Cerrena consors*	W	PP
KUC8320	FJ471559	1	–	–	*Heterobasidion annosum* (DQ384592)	572/580 (98.6)	*Heterobasidion annosum*	W	PP
KUC8321	FJ471560	3	–	–	*Hypochnicium cremicolor* (DQ677506)	579/589 (98.3)	*Hypochnicium cremicolor*	W	C
KUC8330	FJ471561	1	14*	–	*Hypochnicium eichleri* (AY858365)	583/583 (100)	*Hypochnicium eichleri*	W	C
KUC8319	FJ471562	1	–	–	*Hypochnicium karstenii* (DQ677510)	579/584 (99.1)	*Hypochnicium karstenii*	W	C
KUC8433	FJ471563	–	–	1	*Hypholoma subviride* (AF261631)	616/616 (100)	*Hypholoma subviride*	W	ST
KUC8381	FJ471564	–	1	–	*Mucronella calva* (AJ406588)	576/612 (94.1)	*Mucronella calva*	B	CL
KUC8419	FJ471565	–	–	1	*Phanerochaete affinis* (EU118652)	593/604 (98.2)	*Phanerochaete calotricha*	W	C
KUC8423	FJ471566	–	–	1	*Phanerochaete sordida* (KCTC26213)	583/583 (100)	*Phanerochaete sordida*	W	C
KUC8301	FJ471567	1	2	4	*Phanerochaete velutina* (DQ679917)	578/582 (99.3)	*Phanerochaete velutina*	W	C
KUC8323	FJ471568	1	–	–	*Phanerochaete sordida* (AJ406532)	568/583 (97.4)	*Phanerochaete* sp. 3	W	C
KUC8337	FJ471569	1	–	–	*Phlebia chrysocrea* (AY293199)	569/577 (98.6)	*Phlebia chrysocrea*	W	C
KUC8437	FJ471570	–	–	11*	*Phlebia radiata* (AY858369)	583/583 (100)	*Phlebia radiata*	W	C
KUC8325	FJ471571	5	–	–	*Phlebiella* sp. (AY858371)	578/578 (100)	*Phlebiella* sp. 1	W	C
KUC8389	FJ471572	–	1	–	*Phlebiella* sp. (AY858371)	572/578 (99.0)	*Phlebiella* sp. 2	W	C
KUC8303	FJ471573	13*	–	–	*Phlebiopsis gigantea* (AF141634)	574/585 (98.1)	*Phlebiopsis gigantea*	W	C
KUC8382	FJ471574	–	2	1	*Rhizochaete radicata* (AY219932)	609/613 (99.3)	*Rhizochaete radicata*	W	C
KUC8391	FJ471575	–	1	–	*Rhizochaete radicata* (AY219932)	599/614 (97.6)	*Rhizochaete* sp.	W	C
KUC8305	FJ471576	1	–	–	*Schizophyllum commune* (AF261587)	580/582 (99.7)	*Schizophyllum commune*	W	S
KUC8336	FJ471577	1	–	–	*Skeletocutis amorpha* (AY293214)	553/553 (100)	*Skeletocutis amorpha*	W	PP
KUC8318	FJ471578	1	–	2	*Trametes versicolor* (AY858378)	580/582 (99.7)	*Trametes versicolor*	W	PP
KUC8328	FJ471579	1	1	–	*Tulasnella calospora* (DQ388044)	531/535 (99.3)	*Tulasnella calospora*	W	T
KUC8302	FJ471580	3	2	2	*Helicogloea* sp. (AY512848)	530/601 (88.2)	Unknown basidiomycete	–	–
Total isolates		34	24	24					

[a]Not isolated.

[b]Identity (%) was represented by matched nucleotide/compared nucleotide.

[c]W, white rot type; B, brown rot type (Gilbertson and Ryvarden 1986, 1987; Ginns and Lefebvre 1993).

[d]C, Corticiaceae; CL, Clavariaceae; PP, Polyporaceae; ST, Strophariaceae; T, Tulasnellaceae (Gilbertson and Ryvarden 1986, 1987; Ginns and Lefebvre 1993).

*Dominant species. A species is considered dominant if $P_i > 1/S$, where P_i (frequency of species *i*/total frequency for all species) is the proportion of the total sample represented by species *i*, and *S* (species richness) is the number of competing species present in the community (Camargo 1993).

and Hale 1993), however, with a preference to hardwoods. These findings indicate that pitch pine may be more susceptible to decay fungi than Korean pine.

Phlebiopsis gigantea and *Phlebiella* species are well-known primary saprotrophs on conifers (Rishbeth 1963; Niemelä and Ryvarden 1983; Vampola 1991; Renvall and Niemelä 1992; Holmer et al. 1997), whereas *Hypochnicium karstenii* has not been reported as a primary saprobic species. This study reveals that the two dominant species, *H. karstenii* and *P. gigantea*, are important primary decayers of both species of pine trees in Korea. These species were replaced by secondary decay fungi including *H. eichleri*, *P. radiata*, *P. velutina*, *Rhizochaete* sp., and *T. versicolor*, which were the dominant species at the time of the second and third surveys. These results indicate that many of the primary decay fungi are able to grow on extensive areas of wood for long periods of time, but are subsequently replaced by secondary decay fungi (Renvall 1995). Although selective replacement of species of wood decay fungi has been reported (Erikkson 1958; Niemelä 1980), we were unable to determine if this happened in our study.

The data generated here also provided some results that were specific to this study (Tables 1 and 2). Specifically, the primary and secondary decayers were primarily corticioid and white rot fungi. It is well known that wood in contact with the ground is easily invaded by cord-forming species (Kirby et al. 1990). Therefore, it is not surprising that many corticioid fungi (approximately 64.3% of total species) were recovered from pine logs that were left exposed and in contact with the ground. It has been reported that many polypores play an important role in the deterioration of various wood products (Lemke 1964; Scheffer et al. 1984). Corticioid fungi have been suggested to play a role in the decomposition of wood products (Lemke 1964; Kim et al. 2005). Similarly, our group also found that corticioid fungi play an important role in the decomposition of conifer logs on the forest floor. In addition, with the exception of *Mucronella calva*, all basidiomycetes – that were identified in this study on the genus or species level – were white rot fungi, which are known to colonize freshly cut conifers that are left exposed on the forest floor. Butcher (1968) also suggested that white rot fungi generally precede brown rot fungi during the colonization of untreated softwoods that are in contact with the ground.

Another specific finding in this study is that the isolation frequency and fungal diversity is higher for pitch pine logs than for Korean pine logs. Accordingly, pitch pine may be susceptible to a wide range of decay fungi. Indeed, 23 and 18 species of basidiomycetous fungi were obtained from pitch pine and Korean pine logs, respectively. Of these species, 12 (approximately 41.4%) were found on both species of pines; two during the first survey, three during the second survey, and seven during the third survey. Overall, these findings demonstrate that both species of pines evaluated here host different mycoflora, which indicates that specific fungal species may be better adapted to the chemistry and physiology of a particular host. However, the variation among species may have been due to differences in the diversity of basidiomycetous fungi in the forest floor.

Acknowledgements

This work was supported by a special grant from Korea University, Korea and a grant titled “Origin of biological diversity of Korea: molecular phylogenetic analyses of major Korean taxa” funded by The National Institute of Biological Resources, Korean Government.

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Received November 23, 2008. Accepted May 5, 2009.
Previously published online June 29, 2009.