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Cha, Joo Young

Field Science Center for Northern Biosphere, Hokkaido University

Lee, Sang Yong

Division of Forest Resource, College of Forest and Environmental Sciences, Kangwon National University

Im, Sangjun

Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University

Ohga Shoji

Laboratory of Forest Resources Management, Division of Forest Environment Sciences, Department of Agro-Environment Sciences, Faculty of Agriculture, Kyushu University

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## Fungal Species Associated with Litter–mycelial Mat Formation in an *Abies holophylla* Forest in Korea

Joo Young CHA<sup>1</sup>, Sang Yong LEE<sup>2</sup>, Sangjun IM<sup>3\*</sup>  
and Shoji OHGA

Laboratory of Forest Resources Management, Division of Forest Environmental Sciences,  
Department of Agro–environmental Sciences, Faculty of Agriculture,  
Kyushu University, Fukuoka, 811–2415, Japan  
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We examined the fungal species associated with litter–mycelial mat formation in a Korean *Abies holophylla* forest. The 60 recovered isolates comprised 20 fungal taxa, including 17 ascomycetes, 1 zygomycete, 1 basidiomycete, and 1 deuteromycete. The most abundant species was *Peziza* sp., followed by *Mortierella* sp., *Chaetomium globosum*, *Xylaria* sp.–1, *Xylaria* sp.–2, and *Coniochaeta velutina*. *Peziza* sp., *C. globosum*, and *Xylaria* spp. produced colored zones on guaiacol medium, indicating lignin degrading ability. Nine of the 10 ligninolytic fungi were ascomycetes associated with formation of the litter–mycelial mat in the *A. holophylla* forest litter layer. These results indicate that ascomycetes play an important role in the formation of litter–mycelial mats in forest litter layers, and the period of mat formation seems to be the early to middle stages of decomposition in forest ecosystems.

### INTRODUCTION

Litter decomposition, a key ecological process responsible for the transformation of organic matter and energy from vegetation to the soil in a variety of ecosystems (Xu *et al.*, 2011), plays an important role in nutrient cycles and controls primary production in forest ecosystems (Petersen and Luxton, 1982; Vitousek, 1982; Aerts, 1997; Clark *et al.*, 1998; Onyekwelu *et al.*, 2006; Pandey *et al.*, 2007). Among the main groups of decomposers (i.e., animals living in soil, bacteria, and fungi), fungi are critical and indispensable (Swift *et al.*, 1979; Cooke and Rayner, 1984) because they can decompose plant–derived lignin–rich polymers and humus (Ayato *et al.*, 2005). The profile and structure of decomposing fungal communities in forest litter and the frequency of occurrence of fungi have been reported as key factors in the decomposition process (Osono and Takeda, 2002). However, the temporal and spatial distribution patterns of fungi are determined by litter quality (Swift, 1976).

The litter layer significantly reduces soil water loss by evaporation and the diurnal amplitude of soil temperature by providing daytime shade and limiting nighttime heat loss (Park *et al.*, 1998). Moreover, surface litter that develops on the soil surface intercepts both throughfall and streamflow in forest catchments (Putuhena and Cordery, 1996). Miller (1977) reported that 1–3 kg m<sup>–2</sup> of liquid water can typically be stored in forest vegetation, and a similar amount can be retained on the forest floor. On the other hand, transient accumulation of litter may

decrease soil water by blocking water movement into the soil and allowing for increased evaporation.

In Korea, projects to restore the nation's forests began in the 1960s, resulting in dramatic increases in the planted forested area. Most of these plantations are now 40–50 years old, and forest management practices such as thinning and pruning have largely been abandoned due to the shift from forest fuels to fossil fuels associated with economic development beginning in the 1970s. As a result, the thickness of the litter layer has gradually increased in these forests. At the same time, water resources supplied from these forests have gradually decreased and streams are drying up except in the rainy season.

Fungi produce water–repelling aerial molds and elaborate fruit–bodies such as mushrooms and polypores (Wosten *et al.*, 1999). Filamentous fungi colonize moist substrates such as wood and have to breach the water–air interface to grow in air (Wosten *et al.*, 1999). Moreover, organic substances leached from plant litter and produced by microbial mycelium can induce water repellency (Hudson *et al.*, 1994; DeBano *et al.*, 1998). Most research on water repellency in forest soils, however, has focused on ectomycorrhizal fungi (Unestam, 1991; Smits *et al.*, 2003). Few studies have observed water repellency in saprophytic fungi (Wosten *et al.*, 1999). More knowledge of fungal composition and function is needed to more effectively understand water resources in forest ecosystems.

As litter layers increase in thickness in plantations and form litter–mycelial mats, soil water loss may increase because the mats may block water movement into the soil. Moreover, the hydrophobicity of the filamentous fungi forming litter–mycelial mats is an important factor in the movement of rainwater, such as the occurrence of surface water on the litter layer, in forest stands. The present study sought to clarify the fungal species associated with litter–mycelial mat formation and to supply

<sup>1</sup> Field Science Center for Northern Biosphere, Hokkaido University, Nayoro, 096–0071, Japan

<sup>2</sup> Division of Forest Resource, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon, 200–701, Korea

<sup>3</sup> Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, 151–921, Korea

\* Corresponding author (E–mail: junie@snu.ac.kr)

fundamental data concerning the function of these species in forest ecosystems. The term litter–mycelial mat used herein refers to layers of fresh to fermented litter fixed by fungal mycelia, including those of white–rot humus, as described by Hintikka (1970). Hintikka (1970) reported that the humus and litter around the fruit bodies of certain saprophytic fungi are almost always pale-colored and often covered with whitish mycelia, and that this whitish humus is quiet common in Finnish forest soils.

MATERIALS AND METHODS

Study site

This study was conducted in the Kwangrung Forest of the National Arboretum, Pochun–kun, Kyongki Province, Korea (37°45' N, 127°9' E). The site is an *Abies holophylla* Maxim. plantation located on a north-east–facing slope of 12°. The plantation was established in 1976. The average height and diameter at breast height (DBH, 1.3 m) were 18 m and 15 cm, respectively. The overstory of this forest consisted of *A. holophylla* at a density of 2500 trees ha<sup>–1</sup> with little understory present (Table 1). A well–developed organic layer consisting of litter, fermentation, and humus layers and ranging in thickness from 2 to 6 cm was present over mineral soil, with granite gneiss in places.

**Table 1.** Stand conditions of the study site of *Abies holophylla* forest

Density (trees/ha)	DBH* (cm)	H* (m)	Slope degrees	Elevation (m)
2500	15.6±6.3	18±1	13	158

\*Values are mean and standard deviation.

Litter sampling and fungal isolation

Twenty samples of litter–mycelial mats ranging in thickness from 1 to 3.5 m were collected using sterilized tweezers and placed in plastic bags. Sampling locations were randomly selected.

Fungi were isolated from *A. holophylla* needles using a modified washing method (Arnold *et al.*, 2007). Needle surfaces were sterilized by sequential immersion in 70% ethanol (2 min), 0.5% NaOCl (2 min), and sterilized distilled water (5 s). Needles were surface–dried under sterile conditions and on sterilized filter paper before placement on either 2% malt–extract agar (MEA) or 2% MEA + benomyl (1 µl ml<sup>–1</sup>), which suppresses hyphal growth except for that of basidiomycetes. Six needles from each sample were placed on both kinds of medium for fungal isolation. Plates (9 cm in diameter) were sealed, checked daily for hyphal growth, and incubated at 25 °C in darkness for up to 2 weeks. Any fungal hyphae appearing on the plates were isolated onto PDA plates and incubated. Four weeks after isolation, pure cultures on PDA plates were used in DNA extraction.

Identification of fungal species

Fungal DNA from the mycelia of isolates was extracted using a DNeasy Plant Mini kit (QIAGEN, USA)

according to the manufacturer's instructions. DNA amplification of the ITS regions, including the 5.8S rDNA, was performed on a MyCycler thermal cycler (BioRad, USA) using Takara Ex Taq (TAKARA, Japan) with a specific primer for higher fungi ITS 1f (Gardes and Bruns, 1993) and the universal primer ITS4 (White *et al.*, 1990). We used the following PCR amplification conditions: 94 °C (3 min), followed by 30 cycles of 94 °C (30 s), 50 °C (30 s), and 72 °C (2 min), and a final hold at 72 °C (10 min). We determined the quality and quantity of the PCR products by agarose gel electrophoresis using QA–Agarose™ (MP Biomedical, USA).

Each PCR product was purified using a QIA quick PCR purification kit (QIAGEN, USA) according to the manufacturer's instructions, and sequenced with primers ITS 1f and ITS4. Sequencing reactions were conducted by Solgent Co., Ltd. (Daejeon, Korea). Fungal sequences were compared with the GenBank database at the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov>) and UNITE (<http://unite.ut.ee>) using the nucleotide–nucleotide basic local alignment search tool algorithm (BLAST program). Sequences were aligned with closely matched reference sequences and submitted to neighbor–joining (NJ) analysis at the NCBI for taxonomic interpretation at the species, genus, and family levels. Sequences were considered to be identified at the species level when more than 97% identity with reference sequences derived from sporocarps of the same species was obtained and made a single clade with the specimen in NJ trees. When less than 97% identity with reference sequences derived from sporocarps was obtained, sequences were considered to be identified at the genus or family level based on phylogenetic tree interpretation.

Test of lignin–degrading ability

Ligninolytic activity was estimated by plate tests on wood powder medium supplemented with guaiacol (Nishida *et al.*, 1988; Cha, 1995; Miyamoto *et al.*, 2000). Fungal discs were cut with a 4–mm diameter corkborer from the actively growing colony margins of isolates maintained on PDA. These discs were incubated on agar plates containing 0.01% (v/v) guaiacol, 0.2% (w/v) birch (*Betula platyphylla* var. *japonica* Hara) wood powder (100–mesh–pass), and 1.8% (w/v) agar. Plates were incubated at 25 °C. Medium pH was adjusted to 5.0 before autoclaving at 121 °C for 15 min, and guaiacol was added after the medium had cooled to 50–60 °C. All tests were performed in 9–cm diameter disposable Petri dishes containing 15 ml of medium per dish, and four replicates were performed. After 2 weeks, the diameters of the colonies and surrounding colored zones were measured in two orthogonal directions.

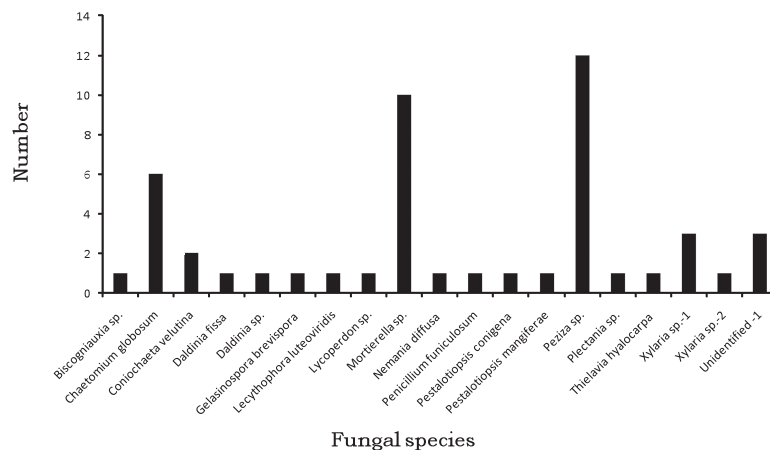
RESULTS

Identification of fungal species

A total of 60 isolates comprising 20 fungal taxa were recovered from the litter–mycelial mats of the *A. holophylla* forest (Table 2). Of these taxa, we identified 10

**Table 2.** Possible identities of fungal species from litter–mycelial mat in an *Abies holophylla* forest

Possible identity based on BLAST and phylogenetic analysis	BLAST match by the highest similarity		
	Definition	Accession no.	Overlapped sequence
<b>Basidiomycetes</b>			
<i>Lycoperdon</i> sp.	<i>Lycoperdon perlatum</i> isolate	EU622257	533/552 (96%)
<b>Ascomycetes</b>			
<i>Biscogniauxia</i> sp.	<i>Biscogniauxia mediterranea</i> isolate	AF280625	492/517 (95%)
<i>Chaetomium globosum</i>	<i>Chaetomium globosum</i> isolate	FJ772001	563/567 (99%)
<i>Coniochaeta velutina</i>	<i>Coniochaeta velutina</i> strain	GQ154545	536/536 (100%)
<i>Daldinia fissa</i>	<i>Daldinia fissa</i>	AF176981	563/566 (99%)
<i>Daldinia</i> sp.	<i>Daldinia petriniae</i>	AF176975	557/575 (96%)
<i>Gelasinospora brevispora</i>	<i>Gelasinospora brevispora</i>	AY681196	552/554 (99%)
<i>Nemania diffusa</i>	<i>Nemania diffusa</i>	FJ438909	507/510 (99%)
<i>Nemania</i> sp.	<i>Nemania serpens</i> isolate	EF155504	562/595 (94%)
<i>Penicillium funiculosum</i>	<i>Penicillium funiculosum</i>	GQ221866	554/558 (99%)
<i>Pestalotiopsis conigena</i>	<i>Pestalotiopsis conigena</i>	AY687301	527/528 (99%)
<i>Pestalotiopsis mangiferae</i>	<i>Pestalotiopsis mangiferae</i> isolate	GU722595	541/543 (99%)
<i>Peziza</i> sp.	Uncultured mycorrhizal fungus <i>Peziza</i>	AY656939	544/586 (92%)
<i>Plectania</i> sp.	<i>Plectania milleri</i> voucher	EU652355	493/537 (91%)
<i>Thielavia hyalocarpa</i>	<i>Thielavia hyalocarpa</i> genes	AB470856	563/565 (99%)
<i>Xylaria</i> sp.–1	<i>Xylaria</i> sp.	GQ461725	578/582 (99%)
<i>Xylaria</i> sp.–2	<i>Xylaria</i> sp.	GQ906941	567/570 (99%)
Unidentified	Fungal endophyte sp.	EU686201	511/521 (98%)
<b>Zygomycetes</b>			
<i>Mortierella</i> sp.	<i>Mortierella</i> sp.	FJ810149	608/608 (100%)
<b>Deuteromycetes</b>			
<i>Lecytophora luteoviridis</i>	<i>Lecytophora luteoviridis</i> strain	DQ404354	561/564 (99%)

**Fig. 1.** Number of fungal species isolated from litter–mycelial mat in an *Abies holophylla* forest.

and 9 samples at the species and genus levels, respectively, and 3 unidentified fungal species were matched to sequences of known sporocarps. The 20 species comprised 17 ascomycetes (belonging to 12 genera and 1 unidentified species), 1 zygomycete, 1 basidiomycete, and 1 deuteromycete. The most abundant species was *Peziza* sp. (belonging to 12 isolates), followed by *Mortierella* sp. (belonging to 10 isolates), *Chaetomium globosum* Kunze (belonging to 6 isolates), unidentified fungal species (belonging to 3 isolates), *Xylaria* sp.–1 (belonging to 3 isolates), *Xylaria* sp.–2 (belonging to 2 isolates), and *Coniochaeta velutina* (Fuckel) Cooke (belonging to 2 isolates). The other fungal species were present as single

isolates (Fig. 1). *Chaetomium globosum*, *Lycoperdon* sp., *Mortierella* sp., *Pestalotiopsis mangiferae* (Henn.) Steyaert, *Peziza* sp., and unidentified fungal species also showed mycelial growth on medium containing benomyl.

#### Lignin–degrading ability

Ten of the 19 identified species produced dark red zones on the medium containing guaiacol (Table 3). These species showed mycelial growth with coloration on the medium. Two species, *Gelasinospora brevispora* R. S. Khan & J. C. Krug and *Thielavia hyalocarpa* Arx, showed mycelial growth without coloration, while the other seven species showed no coloration and no myce-

**Table 3.** The ligninolytic ability of fungal species from litter–mycelial mat in an *Abies holophylla* forest

Fungal species	Colony diam <sup>a</sup>	Coloration zone diam <sup>b</sup>
<i>Biscogniauxia</i> sp.	≤40.5±0.6	40.5±0.6
<i>Chaetomium globosum</i>	≤39±1.2	39±1.2
<i>Coniochaeta velutina</i>	≤11.5±1	11.5±1
<i>Daldinia fissa</i>	0±0	0±0
<i>Daldinia</i> sp.	0±0	0±0
<i>Gelasinospora brevispora</i>	23±1.6	0±0
<i>Lecythophora luteoviridis</i>	≤22±4.8	22±4.8
<i>Lycoperdon</i> sp.	0±0	0±0
<i>Mortierella</i> sp.	0±0	0±0
<i>Nemania diffusa</i>	0±0	0±0
<i>Nemania</i> sp.	0±0	0±0
<i>Penicillium funiculosum</i>	0±0	0±0
<i>Pestalotiopsis conigena</i>	≤23.5±1.9	23.5±1.9
<i>Pestalotiopsis mangiferae</i>	≤37±1.2	37±1.2
<i>Peziza</i> sp.	≤29.5±1.9	29.5±1.9
<i>Plectania</i> sp.	≤17.5±0.6	17.5±0.6
<i>Thielavia hyalocarpa</i>	8±0	0±0
<i>Xylaria</i> sp.–1	≤13.5±5.1	13.5±5.1
<i>Xylaria</i> sp.–2	≤19.8±0.5	19.8±0.5
<i>Armillaria singula</i> *	≤30±0	30±0
<i>Bondarzewia montana</i> *	≤15.3±0.5	15.3±0.5

<sup>a</sup> The mean of four replications in colony diameter (mm) on agar medium containing wood powder and guaiacol after 2 wk (±SD)

<sup>b</sup> The mean of four replications in coloration zone diameter (mm) on agar medium containing wood powder and guaiacol after 2 wk (±SD)

\* White rotting fungus of wood for comparison

lial growth on the medium. All species that produced coloration were ascomycetes. The species that did not produce coloration comprised a basidiomycete (*Lycoperdon* sp.), a zygomycete (*Mortierella* sp.), and seven ascomycete species (*Daldinia fissa* Lloyd, *Daldinia* sp., *G. brevispora*, *Nemania diffusa* (Sowerby) Gray, *Nemania* sp., *Penicillium funiculosum* Thom, and *T. hyalocarpa*). For comparison, *Armillaria singular* Cha and Igarashi and *Bondarzewia montana* (Quél.) Sing., fungal species with lignin-degrading ability that are known to cause white-rot of wood (Cha, 1995; Igarashi, 2009), also exhibit mycelial growth and coloration on this medium.

## DISCUSSION

Many species of litter-decomposing fungi have been found in different forest types (Christensen, 1989; Yao and Yang, 1997). Fifty-seven taxa consisting of 18 ascomycetes and 39 deuteromycetes were identified using direct identification methods in rain forest (Parungao *et al.*, 2002). In subtropical forest, Song *et al.* (2004) reported 67 fungal taxa comprising 56 deuteromycetes, 3 zygomycetes, 5 ascomycetes, and 3 unidentified fungi. Osono *et al.* (2002) found 70 species of fungi in temperate forest, and Ando and Suzumura (1986) identified 77 fungal species in subalpine coniferous forest in Japan. The species from these studies included non-basidiomycetes such as ascomycetes, zygomycetes, and deuteromycetes. Although basidiomycetes are known to func-

tion in lignin decomposition (Miyamoto *et al.*, 2000) and are associated with production of white-rot humus in the forest litter layer (Hintikka, 1970), non-basidiomycetes are considered to play an important role in the formation of the litter–mycelial mat. Thus, species composition may gradually change to include more basidiomycetes in late decomposition stages.

In this study, 20 species were found in the litter–mycelial mat of an *A. holophylla* forest in Korea. Our results suggest that the ascomycete species were involved in litter–mycelial mat formation. The most abundant species, i.e., *Peziza* sp., followed by *Mortierella* sp. and *C. globosum*, are considered to play important roles in fermentation as well as decomposition of litter. Ascomycete, deuteromycete, and zygomycete species such as *Mortierella* sp. and *Chaetomium* sp. result in litter weight loss (Osono and Takeda, 2002; Song *et al.*, 2010). *Peziza* is a large genus of saprophytic cup fungi that grow on the ground, rotting wood, or dung. Therefore, the role of *Peziza* sp. in the litter decomposition process and litter–mycelial mat formation should be clarified in detail.

The dominant species in this study, with the exception of *Mortierella* sp., produced colored zones on guaiacol medium, indicating their lignin degrading ability, as described by Nishida *et al.* (1988). Nine of the 10 ligninolytic fungi were ascomycetes associated with the formation of the litter–mycelial mat in *A. holophylla* forest. Other species were nonligninolytic fungi, including cellulolytic and sugar fungi whose hyphal growth is generally faster than that of ligninolytic fungi (Osono, 2007).



Within the ligninolytic fungi, Osono (2007) reported that ascomycetes are more abundant than basidiomycetes in freshly fallen leaves, but ascomycetes gradually decrease in abundance during decomposition and are replaced by basidiomycetes in later stages. Thus, ascomycetes likely play an important role in litter-mycelial mat formation in the litter layer in early to middle stages of decomposition. These stages will be maintained until the ascomycetes are replaced by basidiomycetes, the timing of which depends on soil moisture conditions or the activity and distribution of soil animals.

Although filamentous fungi have largely been excluded from hydrophobicity measurements (Hazen, 1990), some filamentous fungi have been classified as hydrophilic or hydrophobic based on indirect observations, such as their resistance to flooding, growth behavior on agar plates, excretion of phenols, and production of aerial hyphae and spore (Stenstrom, 1991). Smits *et al.* (2003) quantitatively analyzed the hydrophobicity of mycelial mats of filamentous fungi based on contact angle measurements. They found that deuteromycetes were generally hydrophilic, while ascomycetes and basidiomycetes were mainly hydrophobic. In the present study, ascomycetes associated with litter-mycelial mat formation accounted for 17 of the 20 isolated species. Their hydrophobic characteristics will be clarified in future forest ecosystem studies.

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