Regular Article
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 37, No. 1, pp. 52-61, March, 2021
https://doi.org/10.7747/JFES.2021.37.1.52

JFES
Journal of Forest and Environmental Science

Identification, Enzymatic Activity, and Decay Ability of Basidiomycetous Fungi Isolated from the Decayed Bark of Mongolian Oak (*Quercus mongolica* Fisch. ex Ledeb.)

Manh Ha Nguyen[1,2], Dae Ho Kim[1], Ji Hyun Park[3], Young Ui Park[4], Moo Yeul Lee[4], Myeong Hee Choi[4], Dong Ho Lee[4] and Jong Kyu Lee[1,*]

[1]*Tree Pathology and Mycology Laboratory, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea*
[2]*Forest Protection Research Center, Vietnamese Academy of Forest Sciences, Hanoi 100 000, Vietnam*
[3]*Department of Forest Insects and Diseases, National Institute of Forest Science, Seoul 02455, Republic of Korea*
[4]*Green Space Corporation, Gwacheon 13821, Republic of Korea*

Abstract

Decay fungi can decompose plant debris to recycle carbon in the ecosystem. Still, they can also be fungal pathogens, which can damage living trees and/or wood material and cause a large amount of timber loss. We isolated and identified basidiomycetous fungi from the decayed bark of Mongolian oak wrapped with sticky roll traps. The degrading enzyme activities were then tested for all fungal isolates. The decay ability of selected isolates was assessed based on the weight loss of wood discs after inoculating with culture suspension of decay fungi under the different humidity levels. A total of 46 basidiomycetous fungal isolates belonged to 12 species, and 10 genera were obtained from Jong Myo (16 isolates), Chang Kyung palace (7 isolates), Cheong Gye (10 isolates), and Gun Po (13 isolates). *Gymnopus luxurians* was the most dominant fungus in the present study, and this species distributed in all survey sites with 9 isolates in Jong Myo, followed by 3 isolates in Chang Kyung palace, while Cheong Gye and Gun Po had only 1 isolate each. Among 46 isolates, 44 isolates secreted at least one enzyme, while 25 isolates produced both cellulase and phenol oxidase enzymes, and 2 isolates produced neither. The assessment of decay ability by artificial inoculation indicated that the weight loss of wood discs was significantly influenced by humidity conditions when inoculated with bark decay fungi. The percent weight losses by *G. luxurians* inoculation in RH of 90-100% and RH of 65-75% were 4.61% and 2.45%, respectively. The weight loss caused by *Abortiporus biennis* were 6.67% and 0.46% in RH of 90-100% and RH of 45-55%, respectively. The humidity reduction approach should be applied for further studies to control the growth and spread of bark decay fungi on the trunks wrapped with sticky roll traps.

Key Words: Basidiomycetous fungi, decayed bark, *Gymnopus luxurians*, sticky roll trap, weight loss

Received: January 17, 2021. Revised: January 28, 2021. Accepted: January 28, 2021.

Corresponding author: Jong Kyu Lee

Tree Pathology and Mycology Laboratory, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea

Tel: +82-33-250-8364, Fax: +82-33-259-5617, E-mail: jongklee@kangwon.ac.kr

Introduction

Decay fungi, including destroying fungi, staining fungi, and surface molds, have caused serious rot and/or discoloration for woods and other lignocelluloses (Schmidt 2006; Brischke and Unger 2017). In which, wood-destroying fungi were considered as bioremediation agents to recycle carbon in nature through lignin mineralization (Anastasi et al. 2013; Seweta et al. 2013; Pournou 2020). Depending on the decay mechanism of wood components, these fungi were distinguished into three groups namely brown rot fungi, white rot fungi, and soft rot fungi (Schmidt 2006; Anastasi et al. 2013; Seweta et al. 2013; Brischke and Unger 2017). Both brown and white rot fungi mainly belongs to basidiomycota, while soft rot fungi belongs to ascomycota and deuteromycetes (imperfect fungi) (Brischke and Unger 2017). Brown rot fungi have capacity to degrade hemicellulose and cellulose, but not lignin, whilst white rot fungi could degrade lignin as well as hemicellulose and cellulose (Lee and Oh 1998; Schmidt 2006; Seweta et al. 2013; Brischke and Unger 2017). Softwoods are easily attacked by most brown rot fungi, while white rot fungi more frequently attack hardwoods (Brischke and Unger 2017). These fungi damage living trees and cause a large amount of timber loss in forestry every year (Seweta et al. 2013). Brown and white rot fungi also cause changes in wood products such as sorption and electrical properties (Brischke et al. 2019). Previous studies indicated that silvicultural practices and forest ecosystems influenced the diversity and distribution of wood decay fungi (Marcot 2017). It was estimated that more than 100 species belongs to basidiomycota decayed woody plants and timber in North America (Seweta et al. 2013), while 1582 species, including 341 species in ascomycota and 1241 species in basidiomycota were recorded in Italy (Saitta et al. 2011). There were 28 species of wood decay fungi obtained from several parks in Hong Kong with the most abundant species namely *Phellinus noxius*, *Ganoderma gibbosum*, and *Auricularia polytricha* (Ding et al. 2020). In Korea, wood decay fungi have been studied since the last century (Lee and Oh 1998). Investigation of wood decay fungi in 47 different locations of Korea has identified 365 species, of which, *Trametes* and *Trametes versicolor* was the most dominant genus and species, respectively (Kim et al. 2018). Studying decay fungi on shade trees in urban areas was also conducted recently, and the photos and descriptions of 16 decay fungal species with high frequency in Korea were provided with the brief descriptions on the biology of wood decay (Lee 2020).

It has been indicated that reproduction and development of wood decay fungi are influenced by several environmental factors such as humidity, oxygen, temperature, pH, and so on (Schmidt 2006; Seweta et al. 2013; Zabel and Morrell 2020). Moisture conditions are favorable for growth and spread of wood decay fungi (Seweta et al. 2013). In addition, the humidity accelerates wood degradation, and then mechanical properties and strength of wood are closely related to humidity (Wang et al. 2020). Therefore, the humidity reduction approach could effectively prevent the development of wood decay fungi and could delay wood degradation processes (Wang et al. 2020; Zabel and Morrell 2020). Moreover, *Streptomyces* species could be used as a potential biocontrol agent against wood decay fungi such as *Trametes versicolor* and *Gloeophyllum trabeum* (Kim 2014; Jung et al. 2018).

The bark is defined as all tissues outside of the cambium. It consists of inner bark (living phloem), periderm, and outer bark (dead phloem) (Schweingruber et al. 2019). The key functions of bark are to protect tree trunks against not only mechanical influences but also forest fires; moreover, the inner bark plays an important role in the transport and storage of photosynthates (Bold et al. 2020). However, bark is normally damaged by beetles such as weevils, longhorn and ambrosia beetles (Vega and Hofstetter 2015; Tittiger and Blomquist 2016). Once these beetles attack bark tissues, they occupy bark tissues from initial decline to decay (Lindgren and Raffa 2013). In addition, longhorn and ambrosia beetles were recorded as insect vectors that transported pathogens into trees causing pine and oak wilt diseases (Kim et al. 2009; Futai 2013; Oguro et al. 2015). In Korea, oak wilt disease caused by *Raffaelea quercus-mongolicae* was widespread nationwide, and this pathogen was vectored by the ambrosia beetle, *Platypus koryoensis* (Kim et al. 2009; Lee et al. 2020). To select potential microbial isolates for biocontrol of oak wilt pathogen, endophytic fungi and actinomycetes were screened *in vitro* for inhibiting mycelial growth, sporulation, and spore germination of oak wilt fungus (Hong et al. 2018; Nguyen et al. 2020). Another

study indicated that using culture suspension of *Streptomyces blastmyceticus* can prevent oak wilt by tree injection methods (Lee et al. 2018). In addition, wrapping of the sticky roll trap around the trunk was also applied as one of the management strategies for oak wilt disease to exclude the possible transmission of oak wilt pathogen by the ambrosia beetle (Yi et al. 2017). However, when sticky roll traps were treated on oak trees, bark tissues within the wrapped trap were always wet during the rainy season, and thus provided favorable condition for the rapid growth and development of decay fungi. Hence, this study was conducted to (i) isolate and identify decay fungi from the decayed bark; (ii) test and compare the degrading enzyme activities of the decay fungi; (iii) compare decay ability of dominant species by artificial inoculation and incubation on the sterilized wood discs under the different humidity levels.

Materials and Methods

Sample collection and isolation of decay fungi

Decayed bark tissues and fruiting bodies were collected from the trunks of Mongolian oak (*Q. mongolica*), wrapped with sticky roll traps in 4 survey sites: Jong Myo and Chang Kyung palace in Seoul, and Cheong Gye and Gun Po in Gyeonggi province, Korea.

Isolation of bark decay fungi was based on the previous study (Thompson 2004) using a selective medium, MYBDA (malt and yeast extracts with benomyl and dichloran, and 3 antibiotics). The composition of the MYBDA medium (per liter) included malt extract (10 g), yeast extract (2 g), agar (20 g), and distilled water (966 mL). After autoclaving at 121°C for 15 min, and cooled to 55°C, fungicides and antibiotics were then added with sterile equipment as follow: 2 mL benomyl solution (2 mg/L), 2 mL dichloran solution (8 mg/L), 10 mL chlortetracycline-HCL (60 mg/L), 10 mL streptomycin sulfate (30mg/L), and 10 mL penicillin G (30 mg/L). All purified fungal isolates were examined for the formation of clamp connections under a compound light microscope.

Molecular identification of bark decay fungi

DNA extraction was applied for fungal isolates with clamp connection formation using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA), following instructions of the manufacturer. The final 25 μL pure DNA eluted from each isolate. Each amplification was conducted in a total volume of 50 μL mixed solution including 39.9 μL distilled water, 5.0 μL buffer (10X), 3.0 μL dNTP mix (2.5mM), 0.4 μL of each primer ITS1F and ITS4 (100 pmol), and 0.3 μL e-Tag DNA polymerase (5 U/μL) (SolGent Co., Ltd. Daejeon, Korea). The mixed solution was placed on ice and pipet 49 μL to each PCR tube containing 1 μL fungal DNA template from pure cultures. PCR reaction was incubated in a Takara PCR Thermal Cycler (Takara Bio Inc., Shiga, Japan) with an initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 0.5 min at 58°C and extension for 2 min at 72°C. All reaction will be ended a final extension of 7 min at 72°C. Negative control was treated without a fungal DNA template. The concentration and quality of the amplicons was evaluated by electrophoresis on 0.8% agarose gels (SeaKem LE Agarose, Lonza, ME, USA). PCR product was mixed with Dyne LoadingSTAR® (Dyne Bio Inc., Gyeonggi, Korea) followed by a rate in the volume of 5:1 before loading samples, and run the gels with Advance Mupid-One Submarine electrophoresis system® (Takara Bio Inc., Shiga, Japan) in 1X TAE buffer (Bioneer Corporation, Daejeon, Korea). After running, gels were placed on SL-20 High Performance DNA Image Visualizer® (SeouLin Bioscience Co., Ltd., Gyeonggy, Korea) to immediately examine bands by comparing with GeneRuler 1kb DNA ladder® (Thermo Fisher Scientific, Vilnius, Lithuania). Standard amplicons were purified and sequenced in both directions by Macrogen Inc. (Seoul, Korea). The obtained sequences were edited using Lasergene® version 7.0 (DNASTAR, Inc., Madison, WI, USA). The edited sequences were compared with sequences available in the NCBI GenBank database using BLAST searches to find the sequence with high similarity.

Cellulase activity test

Cellulase activity was examined based on the method of previous study (Kim 2014) using Basal medium containing Carboxymethyl Cellulose (CMC). The final composition of basal medium (per liter) included $C_4H_{12}N_2O_6$ (5 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $CaCl_2 \cdot 2H_2O$ (1 mg), Yeast extract (0.1 g), CMC (20 g), agar (16 g), and

distilled water (1 L). The Basal medium was autoclaved at 121°C for 20 min and transferred to 9-cm-diameter Petri plates. A plug (5-mm diameter) of active mycelia was taken from culture plates of bark decay fungi with a cork borer, and then inoculated on the center of plates. After incubating at 25°C in the dark for 5 days, all plates were stained with Congo red solution (0.5%) for 15 min, followed by washing with sterilized water, and finally flooded with NaCl solution (1M) for 15 min. The clearing zone formed around the mycelium outer edge of fungal isolates showed their cellulase activity. The assessment of cellulase activity was divided into a rating scale with 4 levels as follows: no activity (-) if no clearing zone; weak activity (+) if 0 < clearing zone ≤ 2 mm; moderate activity (++) if 2 mm < clearing zone ≤ 5 mm; strong activity (+++) if clearing zone ≥ 5 mm.

Phenol oxidase activity test

Detecting secretion of phenol oxidase by bark decay fungi was conducted on the Bavendamm medium (Lee and Oh 1998; Kim 2014). Bavendamm medium was prepared by mixing two kinds of media. Medium A consisted of 5 g tannic acid and 200 mL distilled water, while medium B included 15 g malt extract, 20 g agar, and 800 mL distilled water. The medium A and B were autoclaved separately at 121°C for 20 min and then mixed after cooling to 50°C. The Bavendamm medium was adjusted pH to 4.5 with 1M NaOH solution before transferring to 9-cm-diameter Petri plates. A plug (5-mm diameter) of active mycelia was taken from culture plates of bark decay fungi with a cork borer, and then inoculated on the center of plates. All plates were incubated at 25°C in the dark for 5 days. The formation of a color zone on the Bavendamm medium showed phenol oxidation of bark decay fungi. The level of phenol oxidase activity was assessed based on the intensity of the color zone as follows: no activity (-) if no color zone; weak activity (+) if light brown; moderate activity (++) if brown; strong activity (+++) if dark brown.

Wood disc preparation and culture suspension inoculation

Wood discs (5 cm thick, 10 cm diameter) were freshly cut from Mongolian oak trunks in Chuncheon campus of Kangwon National University. Wood discs with bark tissue were dried for 168 hours at 75°C and measured dry weight. These discs were then autoclaved 121°C for 60 min, and cooled in the clean bench. The wood discs were distributed evenly by weight for each treatment. Five wood discs were prepared for each treatment and placed in each clean plastic box.

Two fungal isolates were selected for the experiment. In which, isolate BDF01 (*Abortiporus biennis*) had the fastest mycelial growth, while isolate BDF09 (*Gymnopus luxurians*) was the most common bark decay fungus in our study. Bark decay fungi (BDF) were shake-cultured in potato dextrose broth at 25°C for 4 days. After then, culture media containing mycelia were homogenized using a blender to make culture suspension. The bark of each wood disc in each clean plastic box was inoculated with 100 mL culture suspension by sprayer. For the control, 100 mL sterilized water (SW) was inoculated by the same method. These plastic boxes were incubated at 25°C under 2 relative humidity (RH) conditions, 50% and 100% for 45 days. To set relative humidity, sterilized water was added to clean paper towel placed in the bottom of plastic box. Relative humidity was automatically monitored every day during the experiment period using humidity monitoring equipment (HOBO U12-012 data logger). After 45 days of incubation, wood discs were gently washed to remove mycelia and then dried for 168 hours at 75°C and measured dry weight. The loss of dry weight of each wood disc was calculated by the following equation (Jung et al. 2018):

$$\text{Loss of dry weight (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where: *W1* is dry weight of wood disc before inoculating with BDF/SW.
W2 is dry weight of wood disc after inoculating with BDF/SW for 45 days.

Statistical analyses

The significant difference in loss of dry weight among treatments was tested by using one-way ANOVA followed by Tukey's HSD test with a 5% probability level. All statistical analyses and graphs were conducted in IBM SPSS statistics version 24 for Windows.

Table 1. Identification of bark decay fungi isolated from decayed bark or fruiting body grown on the trunk of Mongolian oak

No.	Isolates no.	Location[a]	Source	The closest Genbank taxa	Identity (%)	ITS accession no.
1	1-1(1)	A	Fruiting body	*Psathyrella candolleana*	97	MK996305
2	1-1(2)					
3	1-2(1)	A	Fruiting body	*Fuscoporia senex*	89	MN523249
4	1-2(2)					
5	1-2(3)	A	Fruiting body	*Abortiporus biennis*	99	KP135300
6	1-3(1)	A	Fruiting body	*Ganoderma gibbosum*	100	MK280717
7	1-3(3)					
8	2(1)	A	Fruiting body	*Gymnopus luxurians*	98	KM496469
9	2(2)					
10	3(1)	A	Fruiting body	*Gymnopus luxurians*	99	MN523269
11	4(1)	A	Bark tissue	*Gymnopus luxurians*	98	MN523269
12	4(2)					
13	6-1(1)	A	Fruiting body	*Gymnopus luxurians*	93	KM496469
14	6-1(2)					
15	6-2(2)	A	Bark tissue	*Gymnopus luxurians*	92	KM496469
16	6-2(4)					
17	2B(4)	B	Bark tissue	*Psathyrella sp.*	97	MK226172
18	4B(1)	B	Bark tissue	*Gymnopus luxurians*	100	MN523269
19	5B(1)	B	Bark tissue	*Gymnopus luxurians*	93	KM496469
20	5B(2)					
21	6B(1)	B	Bark tissue	*Psathyrella candolleana*	100	MK247759
22	6B(3)					
23	7B(3)	B	Bark tissue	*Psathyrella candolleana*	99	MK247759
24	3(1)	C	Bark tissue	*Mycena guzmanii*	96	MG926695
25	3(3)					
26	5(4)	C	Bark tissue	*Gymnopus sp.*	100	KF251055
27	7(3)	C	Bark tissue	*Gymnopus luxurians*	100	KM496469
28	8(1)	C	Bark tissue	*Perenniporia koreana*	100	MK992847
29	8(4)					
30	8(5)					
31	9(1)	C	Bark tissue	*Perenniporia koreana*	100	KJ156313
32	9(2)					
33	9(3)					
34	2-1(4)	D	Bark tissue	*Gymnopus luxurians*	100	KM496469
35	2-2(3)	D	Fruiting body	*Xylodon ovisporus*	100	MK992859
36	2-2(4)					
37	2-3(1)	D	Bark tissue	*Radulomyces copelandii*	86	MK172829
38	2-3(3)					
39	2-3(4)					
40	2-5-2(4)	D	Bark tissue	*Xylodon ovisporus*	100	MK269312
41	2-5-2(5)					
42	2-7-1(1)	D	Fruiting body	*Xylodon ovisporus*	100	MK269311
43	2-7-1(3)					
44	2-7-1(4)					
45	2-7-2(1)	D	Fruiting body	*Emmia lacerata*	100	MN341828
46	2-7-2(2)					

[a]Location: A (Jong Myo); B (Chang Kyung palace); C (Cheong Gye); D (Gun Po).

Results and Discussion

Isolation and identification

A total of 46 basidiomycetous fungal isolates (19 isolates from fruiting bodies, 27 isolates from decayed bark tissues) were isolated from the decayed bark of Mongolian oak trunks. The detailed information of these fungal isolates was shown in Table 1. The obtained ITS sequences resulted in 12 fungal species with a similarity range from 86% to 100% compared to NCBI GenBank database (Table 1). Among these isolates, 44 fungal isolates has been identified to 10 species belonging 10 different genera, while other 2 isolates only identified at the genus level (Table 1; Fig. 1). There were 16 isolates belonged to 5 species obtained from Jong Myo, while these figures in Chang Kyung palace were 7 isolates and 3 species, respectively. Although the number of fungal isolates obtained from Cheong Gye and Gun Po was different (10 and 13 isolates, respectively), the number of species in both areas was similar as 4 species (Table 1, Fig. 1).

Gymnopus luxurians was the most common fungus in our study, and this species presented in all study sites with 9 isolates in Jong Myo, followed by 3 isolates in Chang Kyung palace, while Cheong Gye and Gun Po had only 1 isolate each (Fig. 1). *Abortiporus biennis* (1 isolate), *Fuscoporia senex* (2 isolates), and *Ganoderma gibbosum* (2 isolates) only occurred in Jong Myo, while *Psathyrella candolleana* was found in both Jong Myo and Chang Kyung palace, with the number of 2 and 3 isolates, respectively (Fig. 1). *Psathyrella* sp. (1 isolate) only appeared in Chang Kyung palace, while *Gymnopus* sp. (1 isolate), *Mycena guzmanii* (2 isolates), and *Perenniporia koreana* (6 isolates) only presented in Cheong Gye (Fig. 1). On the other hand, *Emmia lacerate* (2 isolates), *Radulomyces copelandii* (3 isolates), and *Xylodon ovisporus* (7 isolates) were only obtained from Gun Po (Fig. 1). In general, the species composition of bark decay fungi was relatively different among study sites. Tree age structure, mature forests, and regenerating forests also influenced the species composition of wood decay fungi (Hopkins 2007). Moreover, the diversity and distribution of wood decay fungi had differences among forest types, regions, and elevations (Kim et al. 2018).

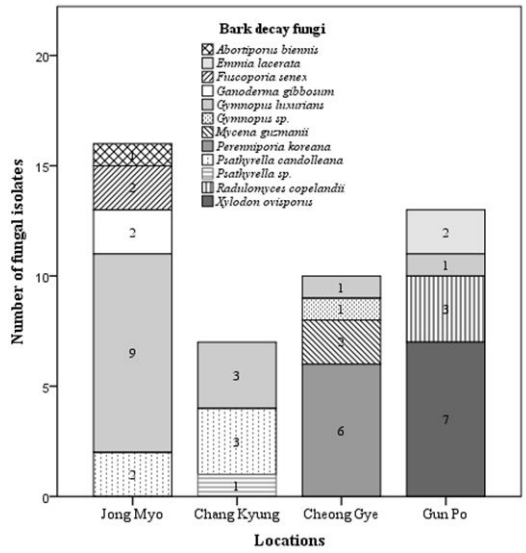

Fig. 1. Diversity and distribution of bark decay fungi isolated from Mongolian oak in different locations.

Enzyme production by bark decay fungi

Forty-four isolates out of 46 isolates secreted at least one enzyme, while 25 isolates produced both cellulase and lignin-degrading enzymes, and 2 isolates produced neither (Table 2). For cellulase activity, 21 isolates had no activity and 14 isolates had weak activity distributed in all 4 survey sites (Fig. 2, 3). Four isolates with moderate activity were found in both Jong Myo (2 isolates) and Chang Kyung palace (2 isolates), while there were 7 isolates with strong activity only obtained from Cheong Gye (Fig. 2, 3). Phenol oxidase was produced by all bark decay fungi isolated, excepted 2 isolates belonged to *Xylodon ovisporus* from Gun Po (Table 2, Fig. 4, 5). Among these fungi, 9 isolates had a weak activity, while 29 isolates showed a moderate activity and 6 isolates exhibited strong activity (Fig. 4, 5).

Enzyme activity tests indicated that the dominant fungus, *G. luxurians*, had a moderate phenol oxidase activity and a weak cellulase activity or no activity among tested isolates (Table 2). *G. luxurians* was known as a biodegradation agent of forest litter (Mallerman et al. 2019), therefore, this fungus could damage wood and bark tissues of living trees. Six isolates of *P. koreana* had the strongest cellulase and phenol oxidase activity, and this fungus was only isolated from bark tissues in Cheong Gye (Table 1 and 2). *P. koreana* was recorded in the previous study as a new wood-rotting basidiomycetous fungus distributed in Gyeonggi and

Table 2. Cellulase and phenol oxidase activities of bark decay fungi isolated from Mongolian oak

No.	Isolates		Bark decay fungi	Cellulase activity*	Phenol oxidase activity*
	Original code	New code			
1	1-2(3)	BDF01	*Abortiporus biennis*	+	++
2	2-7-2(1)	BDF02	*Emmia lacerata*	+	++
3	2-7-2(2)	BDF03	*Emmia lacerata*	+	++
4	1-2(1)	BDF04	*Fuscoporia senex*	+	++
5	1-2(2)	BDF05	*Fuscoporia senex*	+	++
6	1-3(1)	BDF06	*Ganoderma gibbosum*	-	++
7	1-3(3)	BDF07	*Ganoderma gibbosum*	-	++
8	2(1)	BDF08	*Gymnopus luxurians*	+	++
9	2(2)	BDF09	*Gymnopus luxurians*	+	++
10	3(1)	BDF10	*Gymnopus luxurians*	+	++
11	4(1)	BDF11	*Gymnopus luxurians*	-	++
12	4(2)	BDF12	*Gymnopus luxurians*	-	++
13	6-1(1)	BDF13	*Gymnopus luxurians*	-	++
14	6-1(2)	BDF14	*Gymnopus luxurians*	+	++
15	6-2(2)	BDF15	*Gymnopus luxurians*	-	++
16	6-2(4)	BDF16	*Gymnopus luxurians*	+	++
17	4B(1)	BDF17	*Gymnopus luxurians*	-	++
18	5B(1)	BDF18	*Gymnopus luxurians*	-	++
19	5B(2)	BDF19	*Gymnopus luxurians*	-	++
20	7(3)	BDF20	*Gymnopus luxurians*	+	++
21	2-1(4)	BDF21	*Gymnopus luxurians*	+	++
22	5(4)	BDF22	*Gymnopus sp.*	+++	++
23	3(1)	BDF23	*Mycena guzmanii*	-	++
24	3(3)	BDF24	*Mycena guzmanii*	-	++
25	8(1)	BDF25	*Perenniporia koreana*	+++	+++
26	8(4)	BDF26	*Perenniporia koreana*	+++	+++
27	8(5)	BDF27	*Perenniporia koreana*	+++	+++
28	9(1)	BDF28	*Perenniporia koreana*	+++	+++
29	9(2)	BDF29	*Perenniporia koreana*	+++	+++
30	9(3)	BDF30	*Perenniporia koreana*	+++	+++
31	1-1(1)	BDF31	*Psathyrella candolleana*	++	+
32	1-1(2)	BDF32	*Psathyrella candolleana*	++	+
33	6B(1)	BDF33	*Psathyrella candolleana*	++	+
34	6B(3)	BDF34	*Psathyrella candolleana*	+	+
35	7B(3)	BDF35	*Psathyrella candolleana*	+	+
36	2B(4)	BDF36	*Psathyrella sp.*	++	+
37	2-3(1)	BDF37	*Radulomyces copelandii*	-	+
38	2-3(3)	BDF38	*Radulomyces copelandii*	-	+
39	2-3(4)	BDF39	*Radulomyces copelandii*	-	+
40	2-2(3)	BDF40	*Xylodon ovisporus*	-	-
41	2-2(4)	BDF41	*Xylodon ovisporus*	-	-
42	2-5-2(4)	BDF42	*Xylodon ovisporus*	-	++
43	2-5-2(5)	BDF43	*Xylodon ovisporus*	-	++
44	2-7-1(1)	BDF44	*Xylodon ovisporus*	-	++
45	2-7-1(3)	BDF45	*Xylodon ovisporus*	-	++
46	2-7-1(4)	BDF46	*Xylodon ovisporus*	-	++

*Level of enzyme activity: no activity (-); weak activity (+); moderate activity (++); strong activity (+++).

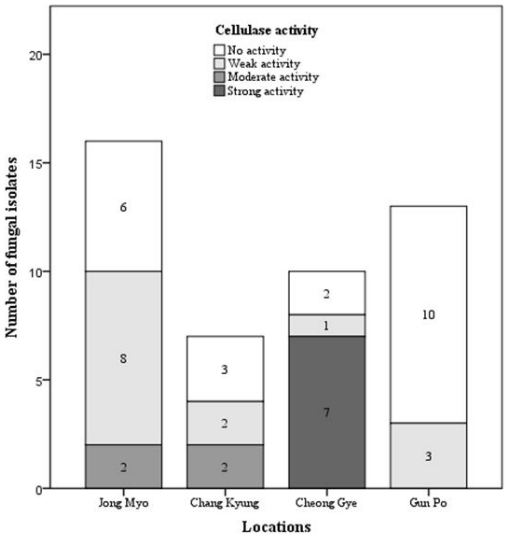

Fig. 2. Cellulase activity of bark decay fungi distributed in different locations.

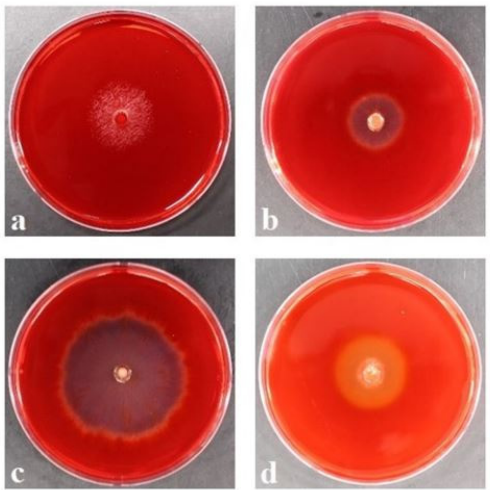

Fig. 3. Levels of cellulase activity of bark decay fungi on Cellulose Basal medium. (a) no activity (BDF37), (b) weak activity (BDF08), (c) moderate activity (BDF31), (d) strong activity (BDF25).

Gangwon provinces, Korea (Jang et al. 2015). Biological characteristics of *P. koreana* should be conducted in the further study.

Bark decay evaluation by selected fungal isolates

G. luxurians was the most common fungus and distributed in all survey sites. On the other hand, *A. biennis* had the fastest-growing mycelium, and this species has been recorded as a white rot fungus in oak trees (Glaeser

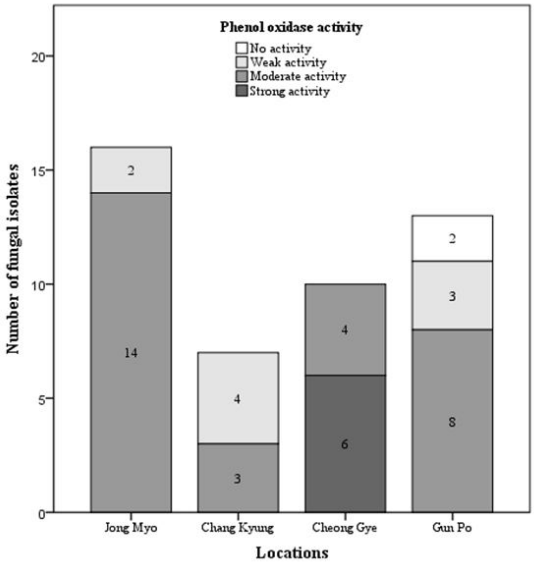

Fig. 4. Phenol oxidase activity of bark decay fungi distributed in different locations.

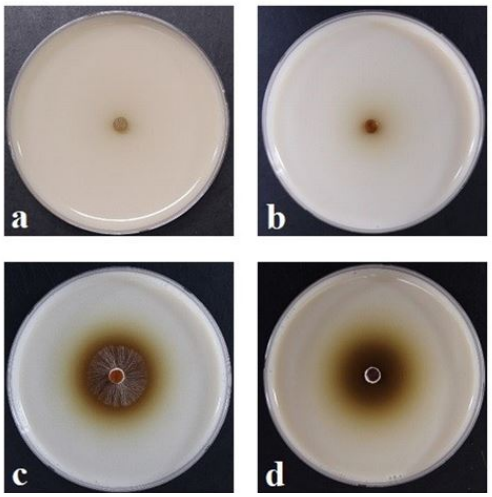

Fig. 5. Levels of phenol oxidase activity of bark decay fungi on Bavendamm medium. (a) no activity (BDF40), (b) weak activity (BDF33), (c) moderate activity (BDF11), (d) strong activity (BDF28).

and Smith 2010). Thus, two isolates of white rot fungi, *G. luxurians* and *A. biennis*, were selected for the evaluation of decay ability on bark tissue of Mongolian oak. The results of these experiments were shown in Fig. 6. For the experiment of *A. biennis*, the relative humidity was recorded by HOBO® U12-012 data logger fluctuated from 90% to 100% in 100%-designed treatments and from 45% to 55%

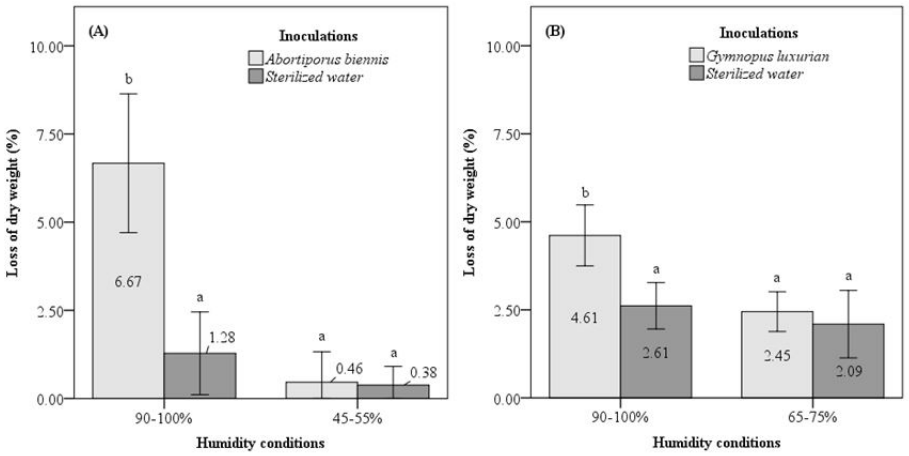

Fig. 6. The percentages of dry weight loss of wood discs after inoculating with culture suspension of bark decay fungi (A: *Abortiporus biennis,* B: *Gymnopus luxurians*) or sterilized water under different humidity levels. The incubation condition and period were at 25°C in the dark for 45 days. Different letters indicate a significant difference ($p < 0.05$) among treatments by Tukey's HSD test. The error bars mean the standard deviation of data.

in 50%-designed treatments (Fig. 6A). However, these data in the experiment of *G. luxurians* fluctuated from 90% to 100% and 65% to 75%, respectively (Fig. 6B).

In general, both *A. biennis* and *G. luxurians* caused a range of weight losses from wood discs with differences between humidity conditions (Fig. 6). *A. biennis* caused more than 5 times of weight loss than the control in RH of 90-100%, with the percentages of 6.67% and 1.28%, respectively (Fig. 6A). There was a significant difference ($p < 0.05$) between fungal inoculation and the control in RH of 90-100%, but it was not significantly different ($p > 0.05$) in RH of 45-55% (Fig. 6A). The weight loss caused by *A. biennis* was also significantly different ($p < 0.05$) between two humidity conditions. There were very low percentages of weight loss in RH of 45-55%, with 0.46% and 0.38% in fungal inoculation and the control, respectively (Fig. 6A). *G. luxurians* also caused significantly higher weight loss ($p < 0.05$) in RH of 90-100% compared to other treatments (Fig. 6B). The percent weight losses by *G. luxurians* inoculation in RH of 90-100% and RH of 65-75% were 4.61% and 2.45%, respectively, while the percentages of treated controls were 2.61% and 2.09%, respectively (Fig. 6B).

Our results showed that the weight loss of wood discs was significantly influenced by humidity conditions when inoculated with bark decay fungi. The weight losses caused by both *A. biennis* and *G. luxurians* in RH of 90-100% were higher than those in RH of 45-55% and RH of 65-75%, respectively. Some previous studies indicated that mycelial spread and fruiting body formation of wood decay fungi were affected by air humidity and wood moisture content (Schmidt 2006; Seweta et al. 2013). Wood decay fungi might not develop on the wood surface without moisture, and woods will not be decayed in the dry conditions (Seweta et al. 2013). In addition, the spore germination rate of wood decay fungi was normally higher by high humidity (Schmidt 2006). To the best of our knowledge, this is the first study that was conducted to identify bark decay fungi colonized in the decayed bark of oak trees, wrapped by sticky roll traps. The present study suggested that applying approaches to remove moisture or prevent wetting could minimize the damage severity of bark tissue caused by bark decay fungi and optimize the mechanical control efficiency of oak wilt disease using sticky roll traps.

Acknowledgements

This study was partially supported by Research projects from National Institute of Forest Science (No. 120200483) and National Research Foundation of Korea (NRF-2017 R1D1A3B03033191).

References

Anastasi A, Tigini V, Varese GC. 2013. The Bioremediation Potential of Different Ecophysiological Groups of Fungi. In: Fungi as Bioremediators (Goltapeh EM, Danesh YR, Varma A, eds). Springer, Heidelberg, pp 29-49.

Bold G, Langer M, Börnert L, Speck T. 2020. The Protective Role of Bark and Bark Fibers of the Giant Sequoia (*Sequoiadendron giganteum*) during High-Energy Impacts. Int J Mol Sci 21:

3355.
Brischke C, Stricker S, Meyer-Veltrup L, Emmerich L. 2019. Changes in Sorption and Electrical Properties of Wood Caused by Fungal Decay. Holzforschung 73: 445-455.
Brischke C, Unger W. 2017. Protection of the Bio-Based Material. In: Performance of Bio-based Building Materials (Jones D, Brischke C, eds). Woodhead Publishing, Cambridge, pp 187-247.
Ding S, Hu H, Gu JD. 2020. Diversity, Abundance, and Distribution of Wood-Decay Fungi in Major Parks of Hong Kong. Forests 11: 1030.
Futai K. 2013. Pine Wood Nematode, *Bursaphelenchus xylophilus*. Annu Rev Phytopathol 51: 61-83.
Glaeser JA, Smith KT. 2010. Decay Fungi of Oaks and Associated Hardwoods for Western Arborists. Western Arborist 2010: 32-46.
Hong AR, Yun JH, Yi SH, Lee JH, Seo ST, Lee JK. 2018. Screening of Antifungal Microorganisms with Strong Biological Activity against Oak Wilt Fungus, *Raffaelea quercus-mongolicae*. J For Environ Sci 34: 395-404.
Hopkins AJM. 2007. The taxonomy and ecology of wood decay fungi in *Eucalyptus obliqua* trees and logs in the wet sclerophyll forests of southern Tasmania. PhD thesis. University of Tasmania, Tasmania, Australia. (in English)
Jang Y, Jang S, Lim YW, Kim C, Kim JJ. 2015. *Perenniporia koreana*, a New Wood-Rotting Basidiomycete from South Korea. Mycotaxon 130: 173-179.
Jung SJ, Kim NK, Lee DH, Hong SI, Lee JK. 2018. Screening and Evaluation of *Streptomyces* Species as a Potential Biocontrol Agent against a Wood Decay Fungus, *Gloeophyllum trabeum*. Mycobiology 46: 138-146.
Kim KH, Choi YJ, Seo ST, Shin HD. 2009. *Raffaelea quercus-mongolicae* sp. nov. Associated with *Platypus koryoensis* on oak in Korea. Mycotaxon 110: 189-197.
Kim NK, Kim DH, Han SK, Cha DS, Lee JK. 2018. Diversity and Distribution of Wood Decay Fungi in Korea. J For Environ Sci 34: 126-135.
Kim NK. 2014. Genetic diversity, wood decaying patterns and biological control of *Trametes versicolor* isolates. PhD thesis. Kangwon National University, Chuncheon, Korea. (in Korean)
Lee DH, Son SY, Seo ST, Lee JK. 2020. Investigation of the Mating-type Distribution of *Raffaelea quercus-mongolicae* in South Korea. For Pathol 50: e12590.
Lee JH, Hong AR, Yun JH, Seo ST, Lee JK. 2018. Prevention of Oak Wilt by Tree Injection of Culture Suspension of an Antifungal Microorganism, *Streptomyces blastmyceticus* against Oak Wilt Fungus, *Raffaelea quercus-mongolicae*. J For Environ Sci 34: 376-381.
Lee JK, Oh ES. 1998. Screening of White Rot Fungi with Selective Delignification Capacity for Biopulping. Korean J Mycol 26: 144-152.
Lee JK. 2020. Brief Description of Wood Decay and the Common Decay Fungi on Shade Trees in Urban Areas. 32pp. Tree Diagnostic Center, Kangwon National University, Chuncheon, Korea.
Lindgren BS, Raffa KF. 2013. Evolution of Tree Killing in Bark Beetles (Coleoptera: Curculionidae): Trade-offs between the Maddening Crowds and a Sticky Situation. Can Entomol 145: 471-495.
Mallerman J, Itria R, Babay P, Saparrat M, Levin L. 2019. Biodegradation of Nonylphenol Polyethoxylates by Litter-basidiomycetous Fungi. J Environ Chem Eng 7: 103316.
Marcot BG. 2017. A review of the role of fungi in wood decay of forest ecosystems. Res. Note. PNW-RN-575, Portland, OR, USDA For. Ser. Pacific, pp31.
Nguyen MH, Yong JH, Sung HJ, Lee JK. 2020. Screening of Endophytic Fungal Isolates against *Raffaelea quercus-mongolicae* Causing Oak Wilt Disease in Korea. Mycobiology 48: 484-494.
Oguro M, Imahiro S, Saito S, Nakashizuka T. 2015. Mortality Due to Japanese Oak Wilt Disease and Surrounding Forest Compositions. Data Brief 5: 208-212.
Pournou A. 2020. Wood Deterioration by Aquatic Microorganisms. In: Biodeterioration of Wooden Cultural Heritage: Organisms and Decay Mechanisms in Aquatic and Terrestrial Ecosystems (Pournou A, ed). Springer, Cham, pp 177-260.
Saitta A, Bernicchia A, Gorjón SP, Altobelli E, Granito VM, Losi C, Lunghini D, Maggi O, Medardi G, Padovan F, Pecoraro L, Vizzini A, Persiani AM. 2011. Biodiversity of Wood-Decay Fungi in Italy. Plant Biosyst 145: 958-968.
Schmidt O. 2006. Wood and Tree Fungi: Biology, Damage, Protection, and Use. Springer, Berlin.
Schweingruber FH, Steiger P, Börner A. 2019. Bark Anatomy of Trees and Shrubs in the Temperate Northern Hemisphere. Springer International Publishing, Cham.
Srivastava S, Kumar R, Singh VP. 2013. Wood Decaying Fungi. LAP Lambert Academic Publishing, Saarbrücken.
Thompson TA. 2004. A study of basidiomycetes isolated from coarse woody debris and contiguous soil horizons in a mixed deciduous-conifer forest in New Hampshire, United States of America. PhD thesis. University of New Hampshire, Durham, USA. (in English)
Tittiger C, Blomquist GJ. 2016. Pine Bark Beetles. Academic Press, London.
Vega FE, Hofstetter RW. 2015. Bark Beetles: Biology and Ecology of Native and Invasive Species. Academic Press, London.
Wang J, Cao X, Liu H. 2020. A Review of the Long-term Effects of Humidity on the Mechanical Properties of Wood and Wood-based Products. Eur J Wood Prod doi: 10.1007/s00107-020-01623-9. [Epub ahead of print]
Yi SH, Lee JH, Seo ST, Lee JK. 2017. In vivo Pathogenicity Test of Oak Wilt Fungus (*Raffaelea quercus-mongolicae*) on Oriental Chestnut Oak (*Quercus acutissima*). J For Environ Sci 33: 342-347.
Zabel RA, Morrell JJ. 2020. Factors Affecting the Growth and Survival of Fungi in Wood (fungal ecology). In: Wood Microbiology: Decay and Its Prevention (Zabel RA, Morrell JJ, eds). 2nd ed. Academic Press, San Diego, pp 99-128.