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Fungi associated with bamboo and their decay capabilities

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Abstract

Biological deterioration is the most important issue facing the use of bamboo as a bioresource. To identify the fungi responsible for bamboo degradation, isolates were obtained from three decaying bamboo species and evaluated. A total of 16 genera and 18 species of fungi were isolated and identified. The major fungi causing serious damage are *Trametes versicolor* and *Arthrinium arundinis*, which caused the largest weight losses in tests, of approximately 21.6% and 17.9%, respectively. This investigation confirms that the natural durability of bamboo in outdoor utilization is low.

Keywords: bamboo; bamboo-degrading fungi; biodeterioration; decay capability; soft rot; white rot.

Introduction

In the past, bamboo has played an important role in the daily life of people living in tropical countries and it has been also used for the production of pulp and paper (Kim et al. 2008). Since the 1970s, the emergence of plastic products reduced the dependence upon bamboo and its cultivation decreased. Nevertheless, bamboo has good physical properties and can be produced at a relatively low cost. However, the development of high value-added products is needed to make its cultivation economic (Deshpande et al. 2000).

Theoretically, bamboo could be employed in outdoor services and as landscaping material. However, outdoor uses of bamboo are avoided owing to its inferior weather resistance. To change this situation, study of the biological deterioration of bamboo has to be intensified. Studies in the past decade in Taiwan have focused on maintaining the natural green color of bamboo (Chang and Lee 1996; Chang et al. 2002), but the improvement of its resistance against biological deterioration was neglected hitherto.

In Korea, three bamboo species are widely distributed and commercially important: (1) giant bamboo (*Phyllostachys bambusoides*), (2) Hachiku bamboo (*Phyllostachys nigra* var. *henonis*), and (3) Moso bamboo (*Phyllostachys pubescens*). Their resistance to biological deterioration has not yet been investigated. Before the development of preservative treatments for bamboo, it is necessary to know the fungi responsible for the outdoor damage as determined for commercially important conifer trees in Korea (Kim et al. 2005a,b, 2007, 2009). Among others, the different decay patterns produced by fungi have to be investigated, following the information reported by Kim et al. (2008). This study aims to identify bamboo-decay fungi, and determine their decay capability to bamboo.

Materials and methods

Isolation and identification of bamboo-decay fungi

For fungal isolation, several small bamboo chips were obtained from materials in outdoor service. Giant bamboo (*Phyllostachys bambusoides*), Hachiku bamboo (*Phyllostachys nigra* var. *henonis*), and Moso bamboo (*Phyllostachys pubescens*) were in examined. Samples were collected from Jinju, Seoul, and nearby areas. Bamboo chips were taken from visibly decayed parts. For isolation, samples were placed on 2% malt extract agar (MEA, 20 g Difco (Detroit, MI, USA) malt extract, 15 g Difco agar, and 1 l distilled water) containing 100 ppm streptomycin, which inhibits bacterial growth. For cultivation of basidiomycetes, samples were applied to 2% MEA containing 4 ppm Benomyl (Sigma-Aldrich, St. Louis, MO, USA) and 100 ppm Ampicillin (Sigma-Aldrich) (Clubbe and Levy 1977). Plates were incubated at room temperature for several weeks. To obtain pure cultures, fungi were routinely sampled from the mycelial margins and subcultured onto new plates. After further purification, reference cultures were used to identify most of the fungal isolates to a genera or species level via morphological, physiological, and DNA characteristics. Isolates were deposited in the Korea University Culture Collection (KUC).

To confirm the morphological results, the rDNA of representative isolates was sequenced. Fungal DNA extraction and PCR were performed using the techniques described by Lim et al. (2005). To amplify the internal transcribed spacer (ITS) regions, the fungal universal primers (ITS5 and ITS4) were applied for ascomycetes, whereas the ITS5 and ITS4B reverse primer were used for basidiomycetes (White et al. 1990; Gardes and Bruns 1993). PCR products were purified by a Qiaquick PCR Purification Kit (Qiagen Inc., Mississauga, ON, Canada). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer Inc., Foster City, CA, USA) at the DNA Synthesis and Sequencing Facility, MACROGEN (Seoul, Korea). All of the nucleotide sequences determined in this work have been deposited in GenBank, and their accession numbers are presented in Table 1.

Table 1 Fungi isolated from bamboo used outdoors.

Isolate no.	GenBank acc. no.	No. of isolates	Closest matched fungal species in databases (acc. no.)	S^b (%)
Ascomycetes				
KUC4035	HM008926	36[a]	*Alternaria alternata* (EU807867)	100
KUC4003	HM008923	25[a]	*Arthrinium arundinis* (FJ914693)	99.4
KUC4095	HM008931	3	*Cladosporium cladosporioides* (EU030342)	100
KUC4042	HM008927	4	*Cosmospora consors* (EF121861)	97.2[c]
KUC4094	HM008930	3	*Curvularia lunata* (DQ337381)	100
KUC4001	HM008922	3	*Fusarium proliferatum* (EU839366)	100
KUC4096	HM063434	2	*Penicillium minioluteum* (EU030364)	99.8
KUC4008	HM008924	2	*Phoma glomerata* (FJ481024)	100
KUC4010	HM008925	1	*Phoma macrostoma* (DQ474111)	99.8
KUC4053	HM008928	1	*Sporothrix* sp. (AY618685)	92.7
KUC1274	HM008920	6	*Trichoderma citrinoviride* (EU280098)	100
KUC3025	HM008921	7	*Trichoderma koningii* (AF456923)	100
Basidiomycetes				
KUC8715	HM008936	9	*Ceriporia lacerata* (DQ912694)	99.7
KUC8713	HM008934	1	*Hypochnicium lyndoniae* (DQ309069)	92.1[c]
KUC8709	HM008937	2	*Irpex lacteus* (EU273517)	99.9
KUC8710	HM008933	7	*Phanerochaete sordida* (EU047805)	100
KUC8703	HM008932	9	*Schizophyllum commune* (EF155505)	99.4
KUC8714	HM008935	6	*Trametes versicolor* (AY309017)	99.7
Total number of isolates		127		

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

[b]Similarity scores from pair-wise alignments of sample sequences with the closest BLAST match or reference strains.

[c]Fungi cannot be identified with certainty, thus they should be designated as *Cosmospora* sp. and *Hypochnicium* sp., respectively.

Decay capabilities of bamboo-degrading fungi isolated in this study

The decay capabilities of the isolated ascomycetes and basidiomycetes were tested by means of the soft rot and the decay test, respectively (Table 1). Decay tests for white-rot fungi were conducted for 12 weeks according to the soil-block test procedures described in the American Wood-Preservers' Association Standard E10-01 (AWPA 2004). The soft-rot capability of ascomycetes isolates was tested by means of a modified vermiculite burial test (Nilsson 1973; Kim et al. 2005b) and the weight loss was determined 20 weeks after incubation. Small bamboo blocks (20 mm wide and 20 mm long, with an original thickness of approximately 5 mm) were tested in both cases. The weight loss caused by different fungi was evaluated by analysis of variance (ANOVA, SAS software from SAS Institute 2002).

Results and discussion

Isolation and identification of fungi that decay bamboo

A total of 127 fungal isolates representing 16 genera and 18 species were isolated from the bamboo samples obtained at outdoor locations. The fungi most frequently isolated were ascomycetes (73.2%; 12 species), followed by basidiomycetes (26.8%; six species). *Alternaria alternata* represented the majority of ascomycetes isolates and a quarter of all isolates, whereas *Ceriporia lacerata* and *Schizophyllum commune* were the basidiomycetes isolated in common. In comparison with previous studies (Chang and Lee 1996; Chang et al. 2002), basidiomycetes were isolated frequently in this study. Accordingly, preservative treatment should focus on basidiomycetes for extending the service life of bamboo in outdoor applications.

Ascomycetes

Among the 93 ascomycetes isolates, 12 species representing 10 genera have been identified. The most frequent isolates were *A. alternata* (28.3%), followed by *Arthrinium arundinis* (19.7%), *Trichoderma koningii* (5.5%), *Trichoderma citrinoviride* (4.7%) and *Cosmospora* sp. (3.1%). Some of the isolates in this study, such as *Acrimonies* sp., *Arthrinium* sp., and *Fusarium* spp., are known species causing bamboo disease that have been reported in the USA and in various locations in South Asia (Rahman 1978; Rahman and Khisha 1983). The isolates *Cladosporium* sp., *Penicillium* sp., and *Phoma* spp. have also been identified in bamboo from Thailand, whereas *Arthrinium* sp. and *Fusarium* spp. have been isolated in Bangladesh (Thongkantha 1999). Isolates of *Acremonium* sp., *Phialophora* sp., *Trichoderma* sp., and *Verticillium* sp. have been reported previously by Zhang et al. (1996). Although these isolates are considered to be involved in bamboo deterioration, previous studies have not elabo-

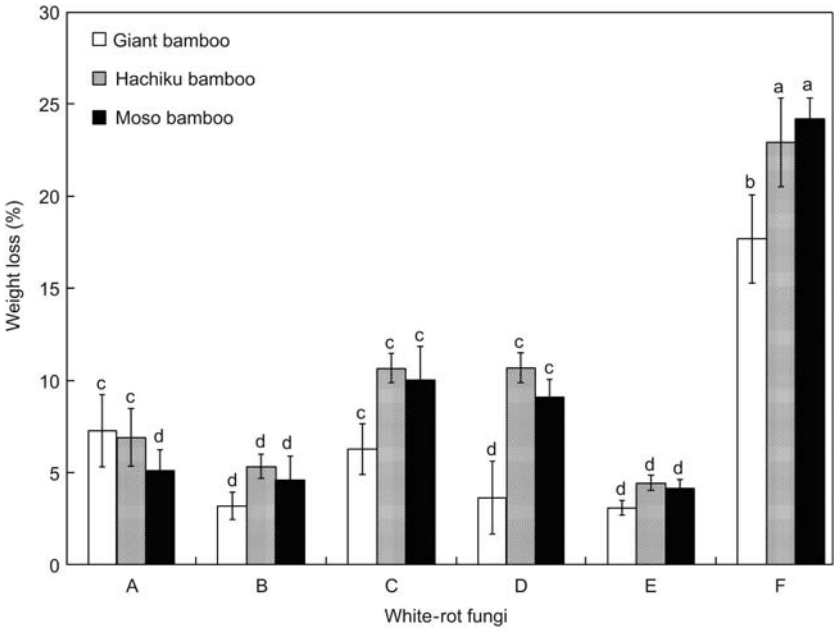

Figure 1 Average weight loss caused by white-rot fungi in three bamboo species. Error bars represent means ± standard deviation. Different letters indicate a significant difference ($P < 0.05$). A, *Ceriporia lacerata*; B, *Hypochnicium* sp.; C, *Irpex lacteus*; D, *Phanerochaete sordida*; E, *Schizophyllum commune*; F, *Trametes versicolor*.

rated clearly which fungi actually causes the white rot and soft rot in bamboo in Korea. The dominant species in the study, *A. alternata*, has been identified previously as a commonly found species (Kim et al. 2010). Although the dominant species *A. arundinis* has often been collected from species of *Arundinaria* in other countries (Hyde et al. 2001), the species identified in this study has not been recorded previously on bamboo in Korea.

Basidiomycetes

Among the 14 basidiomycetes isolates obtained, a total of six genera have been identified that included six species. *C. lacerata* and *S. commune* showed the highest frequencies of isolation (7.1%), followed by *Phanerochaete sordida* (5.5%) and *Trametes versicolor* (4.7%). These species were also isolated in a previous study (Kim et al. 2005a, 2009), in which it was assumed that common wood-rotting fungi in Korea might be also harmful to bamboo. Remarkably, all basidiomycetes isolated in this study belong to a group of white-rot fungi which are capable of utilizing all structural components in woody cell walls. *S. commune* is a common basidiomycete which is found throughout the world and it was identified easily from a fruiting body on the decaying bamboo. Although basidiomycetes have rarely been isolated from bamboo in other countries, they represented 26.8% of all isolates in this study. It can be concluded that preservative treatments might be effective at extending the life of bamboo products.

Decay capabilities of bamboo-degrading fungi

Giant bamboo showed the highest resistance to fungal deterioration for all the fungi tested, except *C. lacerata*. The Moso and Hachiku bamboos did not reveal significant differences ($P < 0.05$) in resistance to fungal decay, regardless of fungal species. Among the white-rot fungi isolated, the greatest weight loss was caused by *T. versicolor* (21.6%), regardless of bamboo species, followed by *Irpex lacteus* (9.0%) and *P. sordida* (7.8%) (Figure 1).

Giant bamboo has the highest resistance to soft rot. With the exception of *Phoma glomerata*, the Moso and Hachiku bamboos exhibited no significant differences ($\alpha = 0.05$) in their resistance to any of the soft rots. Among the soft-rot fungi isolated, *A. arundinis* (17.9%) caused the highest rate of weight loss, followed by *A. alternata* (6.7%). There were no significant differences ($P < 0.05$) in weight loss caused by the other soft rot species (Figure 2). *A. arundinis* was found to be very common and caused both staining and rotting. Most of the black spots seen on the surface of stained bamboo were morphologically identified as *A. arundinis*. Thus, it can be inferred that this species should be considered an important target for control treatments of bamboo.

Giant bamboo does not differ greatly from other bamboo species growing in Korea, concerning density, thickness, and lignin content (So 1997). Therefore, these properties might not be the main indicators for the elevated resistance to decay of this bamboo. Future work should address the factors behind the better resistance of giant bamboo.

Conclusions

In total, 18 fungal species belonging to 16 genera were isolated from bamboo used outdoors. Among them, 76.2% of isolates were ascomycetes and 23.8% basidiomycetes. In decay tests, the greatest weight losses were observed for

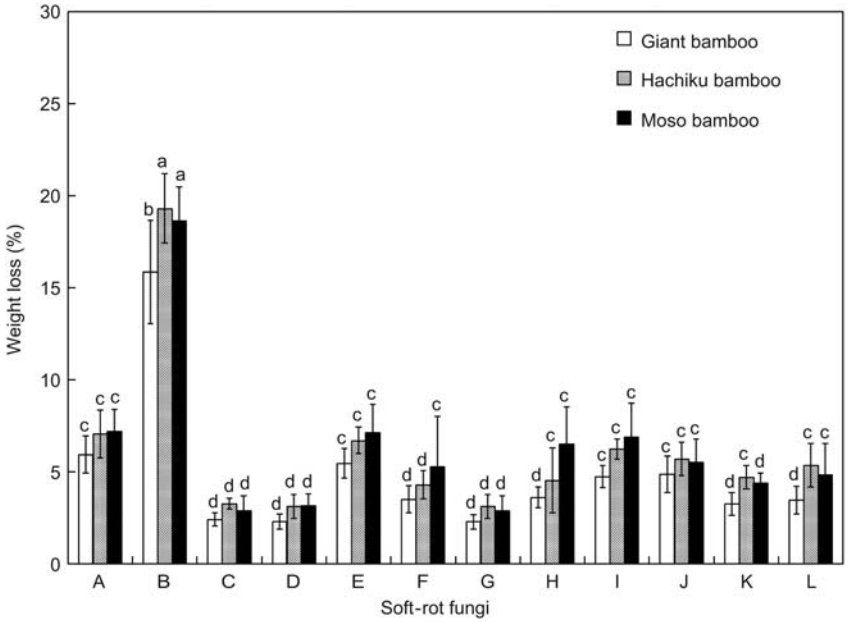

Figure 2 Average weight loss caused by soft-rot fungi in three bamboo species. Error bars represent means ± standard deviation. Different letters indicate a significantly difference ($P < 0.05$). A, *Alternaria alternata*; B, *Arthrinium arundinis*; C, *Cladosporium cladosporioides*; D, *Cosmospora* sp.; E, *Curvularia lunata*; F, *Fusarium proliferatum*; G, *Penicillium minioluteum*; H, *Phoma glomerata*; I, *Phoma macrostoma*; J, *Sporothrix* sp.; K, *Trichoderma citrinoviride*; L, *Trichoderma koningii*.

bamboo samples associated with *T. versicolor*, whereas in the soft-rot test, the greatest weight loss occurred with *A. arundinis*, regardless of the bamboo species tested. Although *T. versicolor* and *A. arundinis* were the second most dominant species of basidiomycetes and ascomycetes isolated, respectively, they might be the major agents of decay.

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