FUNGI COLONIZING DOUGLAS-FIR IN COOLING TOWERS: IDENTIFICATION AND THEIR DECAY CAPABILITIES

Gyu-Hyeok Kim†

Professor
Division of Environmental Science and Ecological Engineering
Korea University
Seoul, Korea, 36701

Dae-Sun Son

Research Scientist
Research & Development Center
Eagon Industrial Co., Ltd,
Korea

and

Jae-Jin Kim

Research Associate
Department of Wood Science
University of British Columbia
Vancouver, BC
Canada V6T 1Z4

(Received June 2004)

ABSTRACT

This study was performed to identify microfungi isolated from chromated copper arsenate (CCA) treated-Douglas-fir members in cooling towers, and to test for their capacities to cause weight loss, anatomical damage, and strength losses in Douglas-fir and Keruing heartwood. Among 26 fungal species isolated, *Acremonium* sp., *Fusarium* spp., *Trichoderma* spp., *Phialophora* spp., and *Alternaria alternata* were most frequently isolated, constituting approximately 75% of all isolates. Half of the fungi, representing about 60% of all isolates, caused soft-rot damage. Microscopic examination revealed that most of the fungi eroded the cell wall (Type 2 damage), and soft-rot types did not differ with wood species. Strength reductions by fungal attack were not significant compared to controls although one fungal species (*Monocillium* sp. KUC 3016) produced significant strength loss on Douglas-fir, and three species (*Gonabotrys simplex*, *Phialophora mutabilis* KUC 3022, and *Phialophora mutabilis* KUC 3039) caused significant strength loss on Keruing. The results indicate that some soft-rot fungi can affect wood properties significantly, and their potential to affect the service life of wood members in cooling towers must be considered.

Keywords: Cooling tower, Douglas-fir, Keruing, soft-rot, anatomical damage, weight loss, maximum crushing strength.

INTRODUCTION

High moisture levels and elevated temperatures in cooling towers limit the growth of many Basidiomycetous fungi. However, the wood in this environment is often attacked by Ascomycetes and Deuteromycetes that cause soft-rot. CCA-treated Douglas-fir (*Pseudotsuga menziesii*) and Keruing (*Dipterocarpus* spp.) are generally used for the construction of cooling towers in Korea, but it appears that their service life is often shortened due to severe soft-rot attack. This attack might be attributed to low preservative retention, which is lower than toxic threshold values for soft-rot fungi, because the heart-

† Member of SWST.

wood of Douglas-fir and Keruing is at least moderately resistant to preservative treatment. Soft-rot fungi are generally more tolerant to preservative than Basidiomycetes (Daniel and Nilsson 1988).

While the role of soft-rot fungi in decay of cooling towers is well known (Savory 1954), there is little work on the identities of the fungal species associated with this decay in Korea. As a first step, therefore, we conducted a survey of microfungi colonized wood members in cooling towers and their decay capabilities.

MATERIALS AND METHODS

Isolating fungi and their identification

Ten CCA-treated Douglas-fir beams in the cooling tower of a semiconductor plant were selected for sampling microfungi. The beams had been in service for at least 6 years and had been subjected to a continuous contact with warm water (35° to 45°C). Using an increment borer, two cores, 5 mm in diameter by 30 mm long, were extracted from each beam on both wide faces at mid-length. The outer surface decayed portion of each core was discarded, the remaining portion flamed to eliminate surface contamination, and placed onto 2% malt extract agar (MEA) amended with 100 ppm tetracycline to inhibit bacteria growth. The plates were incubated at room temperature for four weeks. Any fungi growing from the wood core were sub-cultured onto fresh MEA plates to obtain pure cultures. The fungi were identified by means of the available literature (Arx 1981; Barnett and Hunter 1998; Carmichael et al. 1980; Cole and Kendrick 1973; Ellis 1971; Ellis 1976; Hawksworth 1996; Ramirez 1982; Raper and Fennell 1973; Rifai 1969; Sutton 1980; Wang and Zabel 1990), based on their colony morphology and micromorphology.

Decay testing

The soft-rot capability of the selected isolates was tested by a modified vermiculite burial test (Nilsson 1973) and measured by weight loss, anatomical damage, and maximum crushing strength loss. Douglas-fir and Keruing heartwood blocks measuring 10 by 20 by 20 mm (T × R × L) were oven-dried for 24 h at 103°C, weighed (to 0.001 g), immersed in distilled water, and held under vacuum until saturation. Glass jars (500 ml) were filled with 20 g of vermiculite, and two blocks of each wood species were placed on the vermiculite with transverse surfaces facing upward. An additional 20 g of vermiculite were added, and 100 ml of a nutrient solution (6 g NH_4NO_3, 4 g K_2HPO_4, 5 g KH_2PO_4, 4 g $MgSO_4 \cdot 7H_2O$, 2.5 g glucose, and 0.1 mg thiamine-HCL in l,000 ml of distilled water) were added. Each jar was then capped and autoclaved for 30 min at 121°C, cooled overnight, and re-autoclaved for 30 min at 121°C.

After cooling, each jar was inoculated with 20 ml mycelial suspension of the test fungus in 2.5% malt extract solution. The inoculum source was a 5-mm-diameter disc cut from the edge of an actively growing culture of the test fungus and dispersed by vortexing in the malt extract solution under sterile conditions. Each of the selected isolates was tested on 10 blocks. The chambers were incubated at 28°C for 12 weeks; then the test blocks were removed and cleaned of adhering mycelium. Two blocks were aspirated immediately in formaline-aceto-alcohol (FAA) for later sectioning, and the eight remaining blocks were air-dried for 48 h, oven-dried for 24 h at 103°C, and weighed to determine weight loss during fungal exposure.

After weight loss was determined, the test blocks were reconditioned to 12 ± 1% moisture content of 12 ± 1%, and maximum crushing strength (compression strength parallel to grain) was measured using a Universal Testing Machine at a machine head speed of 2 mm/min. Maximum crushing strength was chosen since this property is of critical importance in cooling towers.

The FAA-treated blocks were thoroughly rinsed in distilled water, and surface sections (transverse, radial, and tangential) were cut (15-20 μm thick) using a sliding microtome. The sections were examined microscopically for evidence of soft-rot attack with the light microscope and scanning electron microscope.

RESULTS AND DISCUSSION

A total of 69 fungi representing 15 genera and 26 taxa were isolated from 20 cores using MEA (Table 1). *Acremonium* sp. was the most frequently isolated genus, occurring in 23.2%, followed by *Fusarium* spp., *Trichoderma* spp., *Alternaria alternata*, and *Phialophora* spp. representing 20.3%, 11.5%, 10.1%, and 10.1% of all isolates, respectively. *Acremonium* spp. are one of typical soil-inhabiting fungi that are known to cause soft-rot. This genus was reported to be isolated from a *Tabebuia* sp. cooling tower in Brazil with relatively high frequencies (Borazolin et al. 1998); however, it was not isolated from redwood cooling towers (Morrell and Smith. 1988). The genus *Fusarium* has been reported in other cooling towers (Eaton 1972; Udaiyan and Manian 1991), and would be considered as primary colonizers in wood that has been recently installed in cooling towers (Udaiyan and Manian.1991). *Trichoderma* spp. has also been isolated with high frequencies in the previously mentioned studies (Eaton 1972; Udaiyan and Manian 1991). *Alternaria alternata* is a well-known sapstain fungus, but has also been isolated from a redwood cooling tower as a soft-rot fungus (Morrell and Smith 1988). The genus *Phialophora*, which contains many species capable of soft-rot attack, has a worldwide distribution occurring in preservative-treated wood in ground contact (Nilsson and Henningsson 1978; Wong et al. 1992; Zabel et al. 1991) and in cooling towers (Borazolin et al. 1998; Eaton 1972; Morrell and Smith 1988). Although the remaining fungi were isolated with low frequencies, *Aurebasidium pullulans*, *Epicoccum purpurascens, Gonatobotrys simplex*, and *Pestalotia guepini* can produce soft-rot damage (Duncan and Eslyn 1966; Morrell and Smith 1988; Morrell and Zabel 1985; Nilsson 1973). *Chaetomium globosum*, however, was not isolated in this study although it is well known as one of the most common soft-rot fungi in cooling tower environments.

TABLE 1. *Identity and isolation frequency of microfungi from CCA-treated Douglas-fir beams in cooling tower.*

Fungal taxa	No. of isolates	% frequency of isolates[1]
Acremonium sp.	16	23.2
Fusarium sp. KUC 3003[2]	8	11.6
Alternaria alternata	7	10.1
Fusarium sp. KUC 3007	6	8.7
Phialophora mutabilis sp. KUC 3022	3	4.3
Cladosporium cladsporioides	3	4.3
Aspergillus niger	2	2.9
Pestalotia guepini	2	2.9
Phialophora sp. KUC 3024	2	2.9
Trichoderma harzianum	2	2.9
Trichoderma viride	2	2.9
Trichoderma sp. KUC 3030	2	2.9
Aurebasidium pullulans	1	1.4
Epicoccum purpurascens	1	1.4
Gonatobotrys simplex	1	1.4
Monocilium indicum	1	1.4
Monocilium sp. KUC 3016	1	1.4
Paecilomyces variotti	1	1.4
Penicillium sp. KUC 3018	1	1.4
Penicillium sp. KUC 3019	1	1.4
Penicillium sp. KUC 3020	1	1.4
Phialophora mutabilis sp. KUC 3039	1	1.4
Phialophora sp. KUC 3041	1	1.4
Trichoderma sp. 3026	1	1.4
Trichoderma sp. KUC 3029	1	1.4
Ulocladium botrytis	1	1.4

[1] Percent frequency is based on 69 isolates.
[2] Korea University Collection Number.

One half of taxa isolated comprising about 60% of all isolates, caused soft-rot damage. Although weight and strength losses differed between Douglas-fir and Keruing, the type of soft-rot damage associated with each fungal species remained constant (Table 2). Microscopic examination revealed that most of the fungi eroded the cell wall (Type 2 damage). Exceptions to these were *Gonabotrys simplex*, which formed diamond-shaped cavities in the cell wall (Type 1 damage); *Phialophora* sp. KUC 3041 and *Phialophora mutabilis* KUC 3039, which caused both Type 1 and 2 soft-rot damage in our tests. Type 2 damage is by far the more common type of soft-rot attack (Nilsson 1973), so it is not surprising that most of our isolates produced this type of attack. Morrell and Smith (12) reported that the genus *Phialophora* caused Type 1, Type 2, or both Type 1 and 2 soft-rot damage according to species; however, Type 1 damage could not be observed in this study. One species, *Phialophora* sp. KUC 3024, caused no detectable damage to the wood cell wall.

TABLE 2. *Weight loss, maximum crushing strength (MCS), and type of soft-rot attack of Douglas-fir and Keruing heartwood blocks after exposure to soft-rot fungi isolated from CCA-treated Douglas-fir cooling tower.*[1]

	Douglas-fir			Keruing		
Fungal taxa	Weight loss (%)	MCS (kg/cm^2)	Soft-rot type[3]	Weight loss (%)	MCS (kg/cm^2)	Soft-rot type
Control	0.16 (0.22) f[2]	375.0 (40.1) b	—	0.23 (0.22) g	639.4 (40.1) d	—
Acremonium sp. KUC 3036	2.29 (0.20) bcd	349.2 (36.7) ab	2	0.61 (0.34) fg	621.0 (16.0) cd	2
Alternaria alternate	3.03 (0.59) a	329.6 (38.5) ab	2	0.90 (0.42) efg	615.6 (37.7) cd	2
Aurebasidium pullulans	2.18 (0.42) bcd	349.7 (37.7) ab	2	0.48 (0.38) g	622.0 (28.4) cd	2
Chaetomium globosum[4]	1.91 (0.28) de	338.3 (52.5) ab	1, 2	11.81 (0.59) a	407.0 (38.5) a	1, 2
Epicoccum purpurascens	1.66 (0.55) e	366.2 (36.8) ab	2	0.51 (0.28) g	628.9 (52.5) cd	2
Fusarium sp. KUC 3007	2.53 (0.44) b	337.1 (37.2) ab	2	0.74 (0.17) fg	613.9 (44.1) cd	2
Gonabotrys simplex	2.37 (0.31) bc	331.4 (43.3) ab	1	10.67 (0.17) b	471.5 (48.1) b	1
Monocillium sp. KUC 3016	2.44 (0.16) b	322.3 (49.6) a	2	1.29 (0.20) def	610.3 (36.7) cd	2
Penicillium sp. KUC 3019	2.55 (0.17) b	350.0 (48.1) ab	2	0.85 (0.41) fg	611.7 (33.4) cd	2
Pestalotia guepini	1.96 (0.34) cde	369.0 (16.0) ab	2	1.61 (0.31) de	596.8 (43.3) cd	2
Phialophora mutabilis KUC 3022	1.97 (0.17) cde	329.4 (44.1) ab	2	4.10 (0.44) c	488.5 (37.2) b	2
Phialophora mutabilis KUC 3039	2.15 (0.41) bcd	346.3 (33.4) ab	1, 2	3.49 (0.16) c	582.5 (49.6) c	1, 2
Phialophora sp. 3041	2.41 (0.43) b	337.1 (51.4) ab	1, 2	1.77 (0.43) d	626.0 (51.4) cd	1, 2
Trichoderma harzianum	1.68 (0.38) e	349.6 (28.4) ab	2	0.24 (0.55) g	631.9 (36.8) cd	2

[1] Values represent the mean of eight replicates, while values in parentheses represent one standard deviation.
[2] Means in the same column followed by the same letters do not differ significantly by Duncan's multiple range method ($\alpha = 0.05$).
[3] Type 1 soft-rot damage appears as diamond-shaped cavities, and Type 2 damage as cell-wall erosion.
[4] Known soft-rot fungus, used as a reference standard.

Aspergillus niger and *Paecilomyces variotii* did not produce soft-rot attack after 12 weeks incubation on either wood species, although these species are reported to cause Type 2 soft-rot damage on redwood (Morrell and Smith 1988). This may be explained by the attack pattern of these fungi. The attack of tracheids or fibers may have been delayed until readily available simple carbon compounds within the ray cells were utilized. It appears that structural carbohydrates of Douglas-fir and Keruing were not degraded within the duration of incubation. *Fusarium* sp. KUC 3003, *Phialophora* sp. KUC 3024, *Penicillium* sp. KUC 3018, *Penicillium* sp. KUC 3020, *Monocillium indicum*, *Trichoderma viride* KUC 3026, *Trichoderma viride* KUC 3028, *Trichoderma* sp. KUC 3029, *Trichoderma* sp. KUC 3030, *Ulocladium botrytis*, *Cladosporium cladosporioides*, failed to cause soft-rot damage on either wood species.

Each of the 13 fungi in Douglas-fir and only six fungi in Keruing caused significant weight losses compared to controls (Table 2). *Alternaria alternata* (Type 2 damage) and *Gonabotrys simplex* (Type 1 damage) caused the highest weight losses in Douglas-fir and Keruing, respectively. All of the fungi, with the exception of *Trichoderma harzianum* and *Epicoccum purpurascens*, caused substantially higher weight losses than a reference fungus, *Chaetomium globosum* in Douglas-fir, but all of the fungi caused lower weight losses than *Chaetomium globosum* in Keruing. The majority of fungal species, with the exception of *Phialophora mutabilis* KUC 3022, *Phialophora mutabilis* KUC 3039, *Gonabotrys simplex* and *Chaetomium globosum* caused higher weight losses on Douglas-fir than on Keruing. This was unexpected because softwood timbers are more resistant to soft-rot attack than hardwoods (Purslow and Williams 1979).

While weight loss and anatomical damage can be used to detect soft-rot fungi, a more useful measure of potential damage to wood members in cooling towers would be reduction in maximum crushing strength. With the single exception of *Monocillium* sp. KUC 3016, none of the fungi produced significant strength losses in Douglas-fir, while only *Gonabotrys simplex*, *Phialophora mutabilis* KUC 3022, and *Phia-*

lophora mutabilis KUC 3039 produced significant reduction in Keruing (Table 2). However, isolates, which produced low weight and strength losses, could potentially influence wood properties over a longer incubation period. With the exception of *Gonabotrys simplex*, *Phialophora mutabilis* KUC 3022, and *Phialophora mutabilis* KUC 3039, strength reductions due to fungal attack were similar between Douglas-fir and Keruing. The relatively small strength loss in Douglas-fir heartwood compared to Keruing was not surprising, since hardwoods are more susceptible to soft-rot attack because of their higher percentages of ray cells and lower lignin content. Weight and strength losses were reasonably well correlated in Keruing ($R^2 = 0.89$), but were poorly correlated in Douglas-fir ($R^2 = 0.30$).

CONCLUSIONS

A variety of microfungi were isolated from CCA-treated Douglas-fir beams exposed for up to 6 years in a cooling tower. One half of these fungi were capable at some level of soft-rot attack on both Douglas-fir and Keruing heartwood samples in laboratory decay tests, although the effects were often small.

ACKNOWLEDGMENT

This study was conducted with a special grant provided by Korea University.

REFERENCES

ARX, J. A. VON. 1981. The genera of fungi sporulating in pure culture. 3rd ed., J. Cramer, Vaduz, Germany. 424 pp.

BARNETT, H. L., AND B. B. HUNTER. 1998. Illustrated genera of imperfect fungi. Macmillan Publishing Co., New York, NY. 218 pp.

BORAZOLIN, S., M. TOMAZELLO, AND I. H. SCHOENLEIN-CRUSIUS. 1998. Soft-rot in *Tabebuia* sp. wood used in water cooling tower: Identification and degradation capacity of the fungi. Int. J. Res. Group Wood Preserv., Document No. IRG/WP/98-10253. Stockholm, Sweden.

CARMICHAEL, J. W., W. B. KENDRICK, I. L. CONNERS, AND L. SIGLER. 1980. Genera of hyphomycetes. The University of Alberta Press, Edmonton, Canada. 386 pp.

COLE, G. T., AND B. KENDRICK. 1973. Taxonomic studies of *Phialophora.* Mycologia 65:661–688.

DANIEL, G. F., AND T. NILSSON. 1988. Studies on preservative tolerant *Phialophora* species. Int. Biodeter. Bull. 24: 327–335.

DUNCAN, C. G., AND W. E. ESLYN. 1966. Wood-decaying Ascomycetes and *Fungi imperfecti.* Mycologia 58:642–645.

EATON, R. A. 1972. Fungi growing on wood in water cooling towers. Int. Biodeter. Bull. 8:39–48.

ELLIS, M. B. 1971. Dematiaceous Hyphomycetes. Commonw. Mycol. Inst., Kew, Surrey, UK. 608 pp.

———, 1976. More Dematiaceous Hyphomycetes. Commonw. Mycol. Inst., Kew, Surrey, UK. 507 pp.

HAWKSWORTH, D. L., P. M. KIRK, B. C. SUTTON, AND D. N. PEGLER. 1996. Dictionary of the fungi. Int. Mycol. Inst. Surrey, UK. 616 pp.

MORRELL, J. J., AND S. M. SMITH. 1988. Fungi colonizing redwood in cooling towers: Identities and effects on wood properties. Wood Fiber Sci. 20(2):243–249.

———, AND R. A. ZABEL. 1985. Wood strength and weight losses caused by soft-rot fungi isolated from treated southern pine utility poles. Wood Fiber Sci. 17(1):132–143.

NILSSON, T. 1973. Studies on wood degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica No. 104.

———, AND B. HENNINGSSON. 1978. *Phialophora* species occurring in preservative treated wood in ground contact. Mat. und Org. 13:297–313.

PURSLOW, D. F., AND N. A. WILLIAMS. 1979. A laboratory examination of the comparative resistance to decay of certain timbers treated with a copper/chrome/arsenic preservative. Mat. und Org. 14:117–130.

RAMIREZ, C. 1982. Manual and Atlas of the Penicillia. Elesvier Biomed. Press., New York, NY. 874 pp.

RAPER, K. B., AND D. I. FENNELL. 1973. The Genus *Aspergillus*. Robert E. Krieger Publishing Company, Huntington, NY. 686 pp.

RIFAI, M. J. 1969. A Revision of the Genus *Trichoderma*. Commomw. Mycol. Inst. Mycological Pap. No.116. 56 pp.

SAVORY, J. G. 1954. Breakdown of timber by ascomycetes and *fungi imperfecti.* Ann. Appl. Biol. 44:336–347.

SUTTON, B. C. 1980. The Coelomycetes. Commonw. Mycol. Inst., Kew, Surrey, UK. 696 pp.

UDAIYAN, K., AND S. MANIAN.1991. Fungi colonizing wood in cooling tower water system at the Madras fertilizer company, Madras, India. Int. Biodetr. Biodegrad. 27(4):351–371.

WANG, C. J. K., AND R. A. ZABEL. 1990. Identification manual for fungi from utility poles in the Eastern United States. Allen Press Inc., Lawrence, KS. 356 pp.

WONG, A. H. H., R. B. PEARCE, AND S. C. WATKINSON. 1992. Fungi associated with groundline soft-rot decay in copper-chrome-arsenic treated heartwood utility poles of Malaysian hardwoods. The Int. Res. Group Wood Preserv. Document No. IRG/WP/1567-92. Stockholm, Sweden.

ZABEL, R. A., C. J. K. WANG, AND S. E. ANAGNOST. 1991. Soft-rot capabilities of the major microfungi isolated from Douglas-fir poles in the Northeast. Wood Fiber Sci. 23(2):220–237.