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Note

Microfungi potentially disfiguring CCA-treated wood

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Abstract

The work reported here investigated the fungal community inhabiting from the CCA-treated radiata pine board stored at the yard of a commercial treatment plant in Incheon, Korea. From five treated boards, 22 fungal species were isolated and then characterized using both traditional morphology and molecular identification. The species identified included 16 ascomycetes and six basidiomycetes. Ascomycetous fungi predominated over basidiomycetous fungi in the surface area of the CCA-treated board and comprised nearly 90% of all isolates. Among all the isolates, five microfungi, *Phoma herbarum*, *Phoma glomerata*, *Cladosporium oxysporum*, *Penicillium stoloniferum*, and *Cladosporium sphaerospermum*, were the dominant species. These species could be candidate 'tolerant and potential staining' species that colonize, stain and/or degrade CCA-treated radiata pine board.

Keywords: Basidiomycetes; CCA-treated wood; Microfungi; Soft rot; Staining fungi

1. Introduction

Chromated copper arsenate (CCA) preservative is accepted as one of the most effective treatments for protecting wood against fungi, insects and marine borers. In high hazard situations, these chemicals perform similarly to creosote and provide a service life of 30 years or more. However, CCA-treated wood is generally known to contain higher levels of both decay and non-decay fungi than wood treated with oilborne chemicals (Morrell et al., 1988). Therefore, many comparative ecological studies have been carried out to determine the establishment of decay mycoflora on untreated and CCA-treated wood in ground contact.

Many researchers have suggested that in untreated and CCA-treated wood, microorganisms have a similar pattern of colonization and succession, comprising first bacteria, primary moulds, staining fungi and soft rot fungi, followed by basidiomycetes and secondary moulds. However, the rate of colonization is significantly slower in CCA-treated wood (Butcher, 1968; Clubbe, 1980; Clubbe and Levy, 1982; Eaton and Hale, 1993; Choi, 2004). Bacteria grow rapidly and are the first to establish in wood and for a very short time they are the dominant mycoflora. However, bacteria do not change the wood structure significantly. Except copper-tolerant fungi such as *Antrodia* species, *Wolfiporia cocos*, and *Fomitopsis palustris* (Clausen et al., 2000; unpublished data), most of the basidiomycetes are not tolerant to CCA preservatives and do not decay treated wood at ground contact. Problems in CCA-treated service wood arise from the activities of CCA-tolerant soft rot fungi that are able to degrade cellulose (Eaton and Hale, 1993; Kim et al., 2005a). Because primary moulds and staining fungi are also potential soft rot fungi, it is likely that most of them might be tolerant to CCA. Long-term storage of CCA-treated wood in close-stacked after treatment could create serious concerns for utilities. Stain development and emergence of moulds in CCA-treated wood surface lower product quality and may result in economical losses.

Intensive work has been carried out to investigate the mycoflora of treated wood and to improve the composition of CCA solutions by adding fungicides that prevent wood against moulds and staining fungi growth (Cofta et al., 2004). However, very little information on the fungi colonizing CCA-treated wood in Korea is available. Thus it is necessary to develop a better database on the fungal

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diversity of CCA-treated wood and to accurately identify CCA-tolerant fungi, especially those able to cause stain on the surface of treated wood.

The first survey of fungi colonizing CCA-treated wood was conducted from radiata pine (*Pinus radiata*) boards stored at the commercial treatment plant yard in Incheon. We identified the isolates, at the species level, using morphological characteristics, as well as phylogenetic analysis of the ribosomal DNA (rDNA) gene sequences.

2. Materials and methods

In the fall of 2003, five CCA-treated radiata pine board samples (5×10 cm and 180 cm in length) were randomly selected at the storage yard of a commercial treatment plant in Incheon, Korea. The samples treated with CCA Type C (retention: 3.5 kg/m^3) were closely stacked above ground for about 4 months before shipment. Their surfaces were discoloured by microorganisms in patches. From each board, 12 small pieces (approximately 1×1 cm and 1–2 mm in depth) were removed from treated board surface. Samples were placed on (1) 2% malt extract agar (MEA, 20 g Difco malt extract, 15 g Difco agar, and 1000 mL distilled water) with 100 ppm ampicillin for general fungal flora, and (2) 2% MEA with 4 ppm benomyl and 100 ppm ampicillin for basidiomycetes (Clubbe and Levy, 1982). The ampicillin inhibits bacterial growth. Inoculated plate media were incubated at room temperature.

After further purification, most of the fungi isolated from the specimens were identified to the genera or species level using morphological, physiological and DNA characteristics with reference cultures. For light microscopy, fungal structures were mounted in water and observed using a Zeiss Axioplan light microscope. Morphological characteristics were identified based on descriptions of Nobles (1965), Barnett and Hunter (1987), and Wang and Zabel (1990). Fungal isolates were stored in both sterile water at 4 °C and 20% (V/V) glycerol solution at −80 °C for further studies. They were deposited in the Korea University Culture Collection (KUC).

To confirm our morphological results, we also sequenced the rDNA of representative isolates. Fungal DNA extraction and PCRs were performed using the techniques described by Lim et al. (2005). To PCR-amplify the internal transcribed spacer (ITS) regions, we used the fungal universal primers (ITS5 and ITS4) for the ascomycetes, and the reverse primer (ITS4B) with ITS5 primer for the basidiomycetes (Gardes and Bruns, 1993). Fungal DNA sequences were then compared with datasets from GenBank (Table 2).

3. Results

A total of 69 isolates associated with CCA-treated radiata pine board were obtained from board stored at the yard of a Preservative Timber Company about 5 months after treatment (Table 1).

Six genera and fifteen species were identified as the Fungi Imperfecti colonizing the treated wood. The species were from *Phoma*, *Cladosporium*, *Penicillium*, *Aureobasidium*, *Phialophora*, and *Trichoderma*. Among these species, *Phoma herbarum* was the most frequently isolated, accounting for 27.5% of the isolates; it was followed by *Phoma glomerata*, *Cladosporium oxysporum*, *Penicillium stoloniferum*, and *Cladosporium sphaerospermum*, representing 17.4%, 8.7%, 8.7%, and 5.8%, respectively. In addition, the other fungi occurred at low isolation frequency. They were *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Phialophora* sp., *Penicillium atrovirens*, *Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicillium verrucosum*, *Phialophora melinii* like, *Trichoderma harzianum*, and *Trichoderma koningii*. One ophiostomatoid fungus, *Hyalodendron* sp., causing sapstain on wood, was isolated at low frequency (1.4%).

Table 1
Isolation frequency of fungi isolated from CCA-treated radiata pine boards

Fungal species	No. of isolates	% Frequency of isolates
Ascomycetes		
Phoma herbarum	19[a]	27.5
Phoma glomerata	12[a]	17.4
Cladosporium oxysporum	6[a]	8.7
Penicillium stoloniferum	6[a]	8.7
Cladosporium sphaerospermum	4[a]	5.8
Aureobasidium pulullans	2	2.9
Cladosporium cladosporioides	2	2.9
Phialophora sp.	2	2.9
Hyalodendron sp.	1	1.4
Penicillium atrovirens	1	1.4
Penicillium brevicompactum	1	1.4
Penicillium funiculosum	1	1.4
Penicillium verrucosum	1	1.4
Phialophora mellini-like	1	1.4
Trichoderma harzianum	1	1.4
Trichoderma koningii	1	1.4
Basidiomycetes		
Ceriporia lacerata	2	2.9
Irpex lacteus	2	2.9
Funalia trogii	1	1.4
Phanerochaete sp.	1	1.4
Unknown basidiomycete 1	2	2.9
Number of total isolates	69	

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

Eight basidiomycetes isolates were obtained on MEA with benomyl/ampicillin. They represented about 12% of all the isolates. Using morphological and molecular methods, we recognized at least five different fungal taxa (Table 1). They were *Ceriporia lacerata*, *Irpex lacteus*, *Phanerochaete* sp., *Funalia trogii*, and an unknown basidiomycete. The frequency of occurrence of each fungus was somewhat low.

According to the Camargo's index (Table 1), the data showed that *P. herbarum*, *P. glomerata*, *C. oxysporum, P. stoloniferum*, and *C. sphaerospermum* were the dominant species in CCA-treated radiata pine board (Table 2).

4. Discussion

4.1. Staining and mould fungi, diversity, damage, and tolerance

This was the first comprehensive survey of fungal species colonizing CCA-treated radiata pine board stored in above