Holzforschung, Vol. 66, pp. 883–887, 2012 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/HF.2011.187

The antagonistic properties of *Trichoderma* spp. inhabiting woods for potential biological control of wood-damaging fungi

Jaejung Lee[1], Nayoon Huh[1], Joo Hyun Hong[1], Beom Seok Kim[2], Gyu-Hyeok Kim[1] and Jae-Jin Kim[1,*]

[1] Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seongbuk-gu, Seoul, Korea

[2] Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seongbuk-gu, Seoul, Korea

* Corresponding author.
Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seongbuk-gu, Seoul, Korea.
E-mail: jae-jinkim@korea.ac.kr

Abstract

The antagonistic potential of *Trichoderma* spp. for biological control of wood-damaging fungi was investigated in the present paper. *In vitro* assays to investigate antifungal characteristics of *Trichoderma* spp. were conducted with various wood-damaging fungi. Exo-chitinase activity of the isolates was also measured. Three typical wood decayers and three sap-stainers served as target fungi. The antagonistic abilities of each *Trichoderma* species differed markedly according to the target fungus. The growth inhibition rates shown by the non-volatile metabolites against the wood decayers reached 100% for *Trichoderma harzianum* KUC1459. The antibiotics produced by *Trichoderma dorotheae* KUC5027, a recently reported species of *Trichoderma*, revealed strong antagonistic effects against sap-stainers. *Trichoderma gamsii* KUC1747 effectively inhibited the growth of all wood-damaging fungi in dual culture tests. The exo-chitinases of *Trichoderma longibrachiatum* KUC1540, *Trichoderma aureoviride* KUC1335, and *T. harzianum* KUC1459 showed significantly high activity.

Keywords: antagonistic ability; biological control; exo-chitinase; *Trichoderma*; wood-damaging fungi.

Introduction

The genus *Trichoderma* (teleomorph Hypocrea, Ascomycota) is well known for the production of biological control agents (BCAs) against several plant pathogens, as demonstrated by many studies (Weindling 1934; Highley and Ricard 1988; Murmanis et al. 1988; Doi and Yamada 1992; Rossman 1996; Bruce 1998; Kubicek and Harman 1998; Phillips-Laing et al. 2003; Benitez et al. 2004). Extensive efforts have been made to use these organisms in plant disease control. The beneficial effects of these organisms are based on combined mechanisms such as the secretion of antifungal secondary metabolites, mycoparasitism, and spatial or nutritional competition with target fungi (Kubicek and Harman 1998).

In the field of wood preservation, there are only a few studies on the application of *Trichoderma* in relation to those of crop preservation (Verma et al. 2007). Wood preservation by chemicals is relatively cheap and effectively prolongs the service life of wood (Rowell 1975). By contrast, the toxicity of heavy metals and other chemicals in wood preservatives are also a matter of serious health and environmental concerns (Cooper 1994; Lebow 1996; Hingston et al. 2001; Schultz et al. 2008). The intense research activities on developing and testing less problematic protective systems demonstrate the urgent need for innovation in this field (Namyslo and Kaufmann 2009; Verma et al. 2009; Lee and Cooper 2010; Pilgård et al. 2010; Robinson and Laks 2010; Tascioglu and Tsunoda 2010; Chirkova et al. 2011; Freitag et al. 2011; Pankras and Cooper 2011; Schultz and Nicholas 2011).

Biological control of wood-damaging pests could be a promising alternative, and in this context *Trichoderma* spp. with their high potential of BCA production could play an important role. In our previous study (Huh et al. 2011), 10 *Trichoderma* spp. from various wood substrates were identified by means of multigene phylogeny. The antagonistic properties were evolved in competition with other wood destroyers – such as wood-rotting and sap-staining fungi, or other molds – the expectation is justified that the *Trichoderma* isolated from wood does have the ability to effectively inhibit wood-damaging fungi.

The objectives of this study were to further investigate the biocontrol ability of indigenous wood-colonizing *Trichoderma* spp. against several wood destroyers by focusing on the inhibiting potential of BCAs of *Trichoderma* as potential agents for biological wood preservation. The BCAs were tested in detail as volatile and non-volatile metabolites. The exo-chitinase activity of *Trichoderma* crude enzymes was also evaluated.

Materials and methods

Fungal species

As potential antagonists, a total of 10 *Trichoderma* spp. isolated from woods in Korea were tested: *Trichoderma atroviride* KUC1335, *Trichoderma aureoviride* KUC1271, *Trichoderma citrinoviride* KUC5160, *Trichoderma dorotheae* KUC5027, *Trichoderma gamsii* KUC1747, *Trichoderma harzianum* KUC1459, *Trichoderma koningiopsis* KUC5063, *Trichoderma longibrachiatum* KUC1540,

Trichoderma pleuroticola KUC1279 and *Trichoderma viride* KUC5062. For detailed characteristics of these fungi, see Huh et al. (2011). The target fungi were: *Fomitopsis palustris* KUC8977, *Gloeophyllum trabeum* KUC8067, and *Trametes versicolor* KUC8936, and the sap-stainers *Ophiostoma floccosum* KUC2411, *Ophiostoma koreanum* KUC2071, and *Ophiostoma piceaperdum* KUC2929 (Kim et al. 2005a,b).

Test of non-volatile metabolites

The test was performed according to Dennis and Webster (1971a). A 5-mm disk of malt-extract agar (MEA) with 3-day-old *Trichoderma* was transferred to the center of a new MEA medium covered with a sterilized cellophane membrane. After 3 days of incubation, the cellophane with the growing mycelium of *Trichoderma* was removed. Each disk of target fungi grown for 3 days in MEA was immediately inoculated to the center of the remaining agar plate and incubated for 3 days at 26°C in darkness. The colony diameters of the test fungi were measured after this incubation period.

Test of volatile metabolites

The *Trichoderma* isolates were inoculated in MEA and incubated for 24 h. After incubation, the lid of the plate was replaced by the bottom of a 3-day-old MEA culture of the test fungi. The two halves of the plate were taped together. After 5 days of incubation, the diameter of the test fungi was measured. The tests were conducted in triplicate. All of the incubation procedures were performed under light-limited conditions to control the sporulation of *Trichoderma*. The method followed that of Dennis and Webster (1971b) with minor modifications.

Dual culture test

Trichoderma isolates were grown on 2% MEA for 7 days. Freshly grown mycelial plugs were then cut from the edge of the culture and inoculated on the edge of new MEA media. The test fungi taken from the edge of a 3- to 5-day-old MEA culture were inoculated on the opposite side of the same plate. The characteristics of the inhibition of the growth of the colony were observed after 10 days of incubation at 26°C. This determination was made based on all possible pairings of antagonists and test fungi for screening the antagonistic isolates. The degree of antagonism between *Trichoderma* and the target fungi in the dual culture test was scored on a scale of 1–5 (Bell et al. 1982).

Exo-chitinase assay

The *Trichoderma* spp. were grown in Richard's medium containing 10 g KNO_3, 5 g KH_2PO4, 2.5 g $MgSO_4 \times 7\ H_2O$, 2 mg $FeCl_3$, 1% insoluble polyvinylpyrrolidone, 150 ml V8 juice (Campbell Soup Co., Camden, NJ, USA), 10 g chitin from shrimp shells (Sigma Chemical Co., St. Louis, MO, USA), and 850 ml water at pH 6.0. Conidia suspensions of *Trichoderma* harvested after 7 days of growth on MEA media were inoculated in 100 ml Richard's medium in 250 ml Erlenmeyer flasks on a rotary shaker at 150 rpm at 25°C for 5 days. After incubation, the medium and the fungal mass were centrifuged at 8000 *g* for 10 min and were filtered through a glass fiber filter (Whatman GF/C, GE Healthcare, Buckinghamshire, UK). The filtrates were sterilized by filtration through a 0.45 µm filter. The final filtrates were used as the crude enzyme for the exo-chitinase assay. In this context, the release of *p*-nitrophenol from *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma Chemical Co.) was measured according to Tronsmo and Harman (1993). Briefly, 30 µl of the test samples were added to wells in a flat bottom well microtiter test plate. Then, 50 µl of substrate solution containing 300 µg ml^{-1} of *p*-nitrophenyl-*N*-acetyl-*β*-D-glucosaminide dissolved in 50 mM potassium phosphate (pH 6.7) was added. The plates were incubated at 50°C in a water bath for 10 min. Reactions were terminated by addition of 50 µl of Na_2CO_3 (0.5 M) and the absorbance at 405 nm was measured with a microplate reader (Sunrise, Tecan Austria GMBH, Austria). One unit of exo-chitinase activity was defined as the amount of enzyme that released 1 µmol of *p*-nitrophenol per ml enzyme per min under the conditions of the assay.

Statistical analysis

The significance of differences was tested by Tukey's multiple comparison test. The statistical significance of all results was determined by calculating the P-value ($P<0.05$). SAS version 8.4 was the software used for data analysis. The test was performed in triplicate experiments. The inhibition rates of non-volatile and volatile metabolites were calculated with the following formula: inhibition (%) = [(D1-D2)/D1]×100, where D1=the diameter of the target colony in the absence of the antagonist and D2=the diameter of the target colony in the presence of the antagonist.

Results and discussion

Non-volatile metabolites

The antifungal activity of the non-volatile metabolites of the *Trichoderma* spp. tested generally showed significant growth inhibition of three wood-decaying fungi (Table 1). An exception was the test with *T. aureoviride* against *G. trabeum*. *T. harzianum* has already proved to be an effective BCA against several plant-pathogenic fungi (Stasz et al. 1988). In addition, in the present study, *T. harzianum* completely inhibited the growth of all three wood-decaying fungi (100% inhibition). *T. dorotheae* and *T. viride* was also effective against *G. trabeum* (85.9% and 74.1% inhibition, respectively).

T. harzianum and *T. dorotheae* have a relatively high antagonistic ability against sap-stainers. The latter isolate represents a new *Trichoderma* species recently described by Samuels et al. (2006). Interestingly, *T. dorotheae* was effective in the inhibition test and this study is the first report in this regard. Furthermore, *T. dorotheae* produces a lower rate of wood discoloration than other *Trichoderma* spp. (Huh et al. 2011) and this characteristic increases the attractiveness of this organism as a potential BCA for wood protection. The inhibition rates of *T. dorotheae* against *O. floccosum* and *O. piceaperdum* were superior to those of *T. harzianum*. It can be concluded that *T. harzianum* is a strong antibiotic producer. Moreover, the isolated non-volatile metabolites of *T. dorotheae* have a high potential as BCA for wood protection.

Volatile metabolites

The rates of inhibition of wood-damaging fungi by volatile metabolites differed significantly according to the target fungus and the *Trichoderma* strain (Table 1). The highest inhibition of wood decayers was produced by *T. gamsii* against

Table 1 Exo-chitinase activity and the percentage of growth inhibition produced by *Trichoderma* spp. against wood-decaying and sap-staining fungi through non-volatile and volatile metabolites.

Antagonist	Exo-chitinase activity (U ml^{-1})	Percent growth inhibition of wood-damaging fungi (%)											
		Wood-decaying fungi						Sap-staining fungi					
		F. palustris		*G. trabeum*		*T. versicolor*		*O. koreanum*		*O. floccosum*		*O. piceaperdem*	
		NV[a]	V[b]	NV	V	NV	V	NV	V	NV	V	NV	V
T. atroviride	0.105^{E}	19.92^{D}	*-1.35*^{AB}	44.52^{E}	*5.87*^{A}	32.01^{D}	*0.12*^{CD}	40.56^{D}	1.31^{C}	11.05^{E}	*7.10*^{A}	70.43^{C}	*-3.20*^{BCD}
T. aureoviride	0.456^{AB}	9.81^{EF}	*-1.01*^{AB}	0.66^{GH}	*5.96*^{A}	6.66^{G}	*6.88*^{BC}	*-5.27*^{F}	1.76^{C}	11.79^{E}	*1.88*^{A}	50.83^{D}	*2.57*^{ABC}
T. citrinoviride	0.339^{D}	55.21^{B}	*-9.48*^{ABC}	54.65^{D}	*-4.50*^{B}	15.99^{F}	*2.70*^{CD}	73.38^{C}	-2.64^{F}	26.18^{C}	*-4.63*^{A}	20.54^{F}	-3.84^{D}
T. dorotheae	0.111^{E}	37.29^{C}	*-11.19*^{ABC}	85.86^{B}	*-1.94*^{AB}	52.72^{C}	14.89^{AB}	96.28^{A}	-1.68^{EF}	42.86^{B}	*3.03*^{A}	98.94^{A}	*-3.54*^{CD}
T. gamsii	0.368^{BCD}	10.77^{DE}	*-4.13*^{ABC}	5.54^{GH}	*-7.48*^{B}	7.98^{G}	19.08^{A}	*-6.89*^{F}	4.41^{A}	20.89^{CD}	*10.56*^{A}	35.07^{E}	*2.92*^{AB}
T. harzianum	0.445^{ABC}	100.00^{A}	*-11.40*^{BC}	100.00^{A}	*-3.52*^{B}	100.00^{A}	*-2.93*^{DE}	85.14^{B}	1.55^{C}	63.57^{A}	*-0.16*^{A}	83.51^{B}	*3.73*^{A}
T. koningiopsis	0.378^{BCD}	32.96^{C}	*-0.55*^{AB}	42.02^{E}	*0.76*^{AB}	36.18^{D}	*3.18*^{CD}	20.03^{E}	-1.20^{E}	26.43^{C}	*-3.41*^{A}	29.40^{E}	*2.98*^{AB}
T. longibrachiatum	0.484^{A}	30.76^{C}	*-2.77*^{ABC}	18.72^{F}	*-7.23*^{B}	24.83^{E}	*0.27*^{CD}	71.75^{C}	-6.12^{G}	20.78^{CD}	*-4.40*^{A}	22.33^{F}	4.95^{A}
T. pleuroticola	0.353^{CD}	47.90^{B}	*-5.93*^{ABC}	6.28^{G}	*-2.35*^{BC}	31.74^{D}	*5.74*^{BCD}	43.83^{D}	4.44^{A}	20.29^{D}	*1.63*^{A}	66.76^{C}	5.66^{A}
T. viride	0.323^{D}	47.92^{B}	-13.91^{C}	74.06^{C}	*0.44*^{AB}	84.63^{B}	-9.70^{E}	46.17^{D}	3.32^{B}	43.65^{B}	*10.23*^{A}	55.26^{D}	*3.02*^{AB}

[a]NV, percent growth inhibition by non-volatile metabolites of antagonists. [b]V, percent growth inhibition by volatile metabolites of antagonists. Numbers followed by the same letter in each column do not differ statistically (P<0.05) according to Tukey's multiple comparison test. Italics indicate that the values do not differ significantly from the control value.

Trametes versicolor. Of the wood decayers tested, *Trametes versicolor* was most sensitive to volatile metabolites from *Trichoderma*.

The growth inhibition in many of the pairs of *Trichoderma* and target fungus was below 0%. Accordingly, the growth of the target fungi was stimulated instead of inhibition. It is likely that the weak antifungal activities of the volatile metabolites from *Trichoderma* stimulated the defense mechanisms of the target fungi, which were able to overcome the antagonism by growing more rapidly. Growth stimulation of wood decayers also occurs by chemical wood preservatives in low concentrations (Schmidt 1977).

The inhibition rates of volatile metabolites are not closely related to those of non-volatile metabolites as the inhibition rate of the latter is more pronounced than that of the former. One of the volatile metabolites of *Trichoderma*, 6-*n*-pentyl-2*H*-pyran-2-one, was described as having an odor resembling that of coconut, and it is viewed as a BCA (Claydon et al. 1987). A coconut odor was detected for only three of the 10 *Trichoderma* spp. tested: *T. gamsii*, *T. atroviride,* and *T. pleuroticola*. However, *T. atroviride* and *T. pleuroticola* were not effective in inhibition tests. Only the volatile metabolites of *T. gamsii* show 19.1% growth inhibition against *Trametes versicolor* (Table 1). Similarly, Dennis and Webster (1971b) observed that an isolate of the *T. viride* group and one isolate of *Trichoderma koningii* that produced a coconut odor do not cause significant inhibition of the growth of *Rhizoctonia solani*. Therefore, the presence of a coconut odor cannot be interpreted as an indicator for the antibiotic ability of an isolate.

Dual culture tests

The tendency to decrease the spatial area occupied by the wood-damaging fungi was observed to be specific for the target fungi in dual culture tests of each *Trichoderma.* *F. palustris* was more weakly affected by the antagonists than the other target fungi. All of the *Trichoderma* tested cannot be ranked as a class 1 protection effect on Bell's scale in the dual culture with *F. palustris*. Four *Trichoderma* spp. (*T. aureoviride*, *T. gamsii*, *T. pleuroticola*, and *T. viride*) covered the entire surface of the plate, i.e., their overgrowth against the wood destroyers was complete.

Living *Trichoderma* on wood materials as a competitor against harmful fungi can cause discoloration of the wood surface, which is caused by the greenish spores of *Trichoderma*. Among the competitive antagonists in dual culture tests, *T. gamsii* showed favorable characteristics because it rarely produces spores on solid media or wood materials (Huh et al. 2011). Thus, *T. gamsii* can be considered as a BCA producer for wood with high potential.

Exo-chitinase assay

T. longibrachiatum exhibited the highest exo-chitinase activity (0.48 U ml^{-1}) among the *Trichoderma* spp., followed by *T. aureoviride* (0.46 U ml^{-1}) and *T. harzianum* (0.44 U ml^{-1}) (Table 1). The relationship between

Table 2 Evaluation of *Trichoderma* isolates against wood-damaging fungi in dual culture test according to Bell's scale[a] of antagonisms.

Antagonist	Wood decaying fungi			Sap-staining fungi		
	F. palustris	*G. trabeum*	*T. versicolor*	*O. koreanum*	*O. floccosum*	*O. piceaperdem*
T. atroviride	2	1	2	1	1	1
T. aureoviride	2	1	1	1	1	1
T. citrinoviride	3	2	2	2	3	2
T. dorotheae	2	1	1	1	2	2
T. gamsii	2	1	1	1	1	1
T. harzianum	2	2	1	2	1	1
T. koningiopsis	2	1	2	1	1	2
T. longibrachiatum	3	1	1	1	1	2
T. pleuroticola	2	1	1	1	1	1
T. viride	2	1	1	1	1	1

[a]Degree of antagonism as proposed by Bell et al. (1982): 1, antagonist completely overgrew the pathogen and covers the entire surface of the medium; 2, antagonist overgrew at least two-thirds of the surface of the medium; 3, antagonist and pathogen each colonized one-half of the surface of the medium (more than one-third and less than two-thirds), but neither organism appeared to be dominant; 4, pathogen colonized at least two-thirds of the surface of the medium and appeared to withstand encroachment; and 5, pathogen completely overgrew the antagonist and occupies the entire surface of the medium.

exo-chitinase activity and the percent inhibition produced by volatile or non-volatile metabolites against target fungi was not observed in this study. A similar finding was reported by Ordentlich et al. (1991) about the antagonistic activity of *Trichoderma* and chitin-degrading enzymes. *T. citrinoviride* was one of the least effective antagonists in the dual culture test (Table 2), but its chitinase activity was relatively high. The deviations between these results can be explained by differences concerning the mechanisms of spatial or nutritional competition and the effects of chitin-degrading enzymes.

The antibiotic metabolites of *T. aureoviride* and *T. longibrachiatum* did not show substantial activity. Their competitive performance in the dual culture test and their chitinase activities were not observable.

Conclusions

All *Trichoderma* spp. tested in this study showed antagonistic abilities against wood-decaying and sap-staining fungi. The extent of the antagonistic effects differed among the species tested. *T. harzianum* and *T. dorotheae* had the most effective antagonistic strains in the test of non-volatile metabolites. In dual culture, *T. gamsii* was effective against the target fungi and virtually showed no sporulation. Among the *Trichoderma* spp., *T. longibrachiatum*, *T. aureoviride*, and *T. harzianum* KU1459 had a relatively higher exo-chitinase activity than the others ($P<0.05$).

T. dorotheae did not produce noteworthy results in the chitinase assay or in the dual culture test against sap-stainers. However, it has some potential as a BCA producer, especially for use against sap-stainers. *T. gamsii* is a competitive BCA producer and could be effective as a living microorganism for wood preservation. The fungal species screened in this study are recommended as antagonistic organisms for the biological control of wood-damaging fungi. More detailed investigations of these fungal species are needed.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0008602) and the K2 research fund supported by Kyung Nong Corp.

References

Bell, D.K., Wells, H.D., Markham, C.R. (1982) In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72:379–382.

Benitez, T., Rincon, A.M., Limon, M.C., Codon, A.C. (2004) Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7:249–260.

Bruce, A. (1998) Biological control of wood decay. In: Forest Products Biotechnology. Eds. Bruce, A., Palfreyman, J.W. Taylor & Francis, London, pp. 251–266.

Chirkova, J., Andersone, I., Irbe, I., Spince, B., Andersons, B. (2011) Lignins as agents for bio-protection of wood. Holzforschung 65:497–502.

Claydon, N., Allan, M., Hanson, J.R., Avent, A.G. (1987) Antifungal alkyl pyrones of *Trichoderma harzianum*. Trans. Br. Mycol. Soc. 88:503–513.

Cooper, P.A. Disposal of treated wood removed from service: the issues. In: Proceedings of Environmental Considerations in Manufacture, Use, and Disposal of Preservative Treated Wood. Carolinas-Chesapeak Section, Forest Products Society, Madison, WI, USA, 1994.

Dennis, C., Webster, J. (1971a) Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57:25–39.

Dennis, C., Webster, J. (1971b) Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57:41–48.

Doi, S., Yamada, A. (1992) Preventing wood decay with *Trichoderma* spp. J. Hokkaido For. Prod. Res. Inst. 6:1–5.

Freitag, C., Morrell, J.J., Love, C.S. (2011) Long-term performance of fused borate rods for limiting internal decay in Douglas-fir utility poles. Holzforschung 65:429–434.

Highley, T.L., Ricard, J. (1988) Antagonism of *Trichoderma* spp. and *Gliocladium virens* against wood decay fungi. Material Organismen 23:157–169.

Hingston, J.A., Collins, C.D., Murphy, R.J., Lester, J.N. (2001) Leaching of chromate copper arsenated wood preservatives: a review. Environ. Pollut. 111:53–66.

Huh, N., Jang, Y., Lee, J., Kim, G.-H., Kim, J.-J. (2011) Phylogenetic analysis of major molds inhabiting woods and their discoloration characteristics. Part 1. Genus *Trichoderma*. Holzforschung 65:257–263.

Kim, G.-H., Kim, J.-J., Lim, Y.W., Colette, B. (2005a) Ophiostomatoid fungi isolated from *Pinus radiata* logs imported from new Zealand to Korea. Can. J. Bot. 83:272–278.

Kim, G.-H., Lim, Y.W., Song, Y.-S., Kim, J.-J. (2005b) Decay fungi from playground wood products in service using 28S rDNA sequence analysis. Holzforschung 59:459–466.

Kubicek, C.P., Harman, G.E. *Trichoderma* and *Gliocladium*. Vol. 1. Basic Biology, Taxonomy and Genetics. Taylor & Francis, London, 1998.

Lebow, S.T. Leaching of wood preservative components and their mobility in the environment. Summary of pertinent literature (general technical report, FPL; GTR-93). USDA Forest Service, Forest Products Laboratory, Madison, WI, USA, 1996.

Lee, M.J., Cooper, P. (2010) Copper monoethanolamine adsorption in wood and its relation with cation exchange capacity (CEC). Holzforschung 64:653–658.

Murmanis, L., Highley, T.L., Ricard, J. (1988) Hyphal interaction of *Trichoderma harzianum* and *Trichodema polysporum* with wood decay fungi. Material Organismen 23:271–279.

Namyslo, J.C., Kaufmann, D.E. (2009) Chemical improvement of surfaces. Part 1: novel functional modification of wood with covalently bound organoboron compounds. Holzforschung 63:627–632.

Ordentlich, A., Migheli, Q., Chet, I. (1991) Biological control activity of three *Trichoderma* isolates against Fusarium wilts of Cotton and muskmelon and lack of correlation with their lytic enzymes. J. Phytopathol. 133:177–186.

Pankras, S., Cooper, P.A. (2011) Effect of ammonia addition to alkaline copper quaternary wood preservative solution on the distribution of copper complexes and leaching. Holzforschung DOI: 10.1515/HF.2011.155.

Phillips-Laing, E.M., Staines, H.J., Palfreyman, J.W. (2003) The isolation of specific bio-control agents for the dry rot fungus *Serpula lacrymans*. Holzforschung 57:574–578.

Pilgård, A., Alfredsen, G., Hietala, A. (2010) Quantification of fungal colonization in modified wood: quantitative real-time PCR as a tool for studies on *Trametes versicolor*. Holzforschung 64:645–651.

Robinson, S.C., Laks, P.E. (2010) The effects of subthreshold loadings of tebuconazole, DDAC, and boric acid on wood decay by *Postia placenta*. Holzforschung 64:537–543.

Rossman, A.Y. (1996) Morphological and molecular perspectives on systematic of Hypocreales. Mycologia 88:1–19.

Rowell, R.M. (1975) Chemical modification of wood: advantages and disadvantages. Proc. Am. Wood Preserver's Assoc. 71:41–51.

Samuels, G.J., Dodd, S.L., Lu, B.-S., Petrini, O., Schroers, H.-J., Druzhinina, I.S. (2006) The *Trichoderma koningii* aggregate species. Stud. Mycol. 56:67–133.

Schmidt, O. (1977) Einflüsse auf die Hemmstofftoleranz von Pilzen. Holz Roh- Werkst. 35:109–112.

Schultz, T.P., Militz, H., Freeman, M.H., Goodell, B., Nicholas, D.D. Eds. (2008) Development of Commercial Wood Preservatives. Efficacy, Environmental, and Health Issues. ACS Symposium Series 982, pp. 655, American Chemical Society, Washington, DC.

Schultz, T.P., Nicholas, D.D. (2011) Relative fungal efficacy results from the soil block test with a long incubation period of three commercial copper wood preservatives. Holzforschung DOI: 10.1515/HF.2011.139.

Stasz, T.E., Harman, G.E., Weeden, N.F. (1988) Protoplast preparation and fusion on two biocontrol strains of *Trichoderma harzianum*. Mycologia 80:141–150.

Tascioglu, C., Tsunoda, K. (2010) Biological performance of copper azole-treated wood and wood-based composites. Holzforschung 64:399–406.

Tronsmo, A., Harman, G.E. (1993) Detection and quantification of *N*-acetyl-β-D-glucosaminidase, chitobiosidase, and endochitinase in solutions and on gels. Anal. Biochem. 208:74–79.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., Valéro, J.R. (2007) Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochem. Eng. J. 37:1–20.

Verma, P., Junga, U., Militz, H., Mai, C. (2009) Protection mechanisms of DMDHEU treated wood against white and brown rot fungi. Holzforschung 63:371–378.

Weindling, R. (1934) Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology 24:1153–1179.

Received October 29, 2011. Accepted December 8, 2011.