Antonie van Leeuwenhoek (2009) 95:285–293
DOI 10.1007/s10482-009-9311-1

ORIGINAL PAPER

Characterization of a strong CCA-treated wood degrader, unknown *Crustoderma* species

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Received: 15 October 2008 / Accepted: 27 January 2009 / Published online: 11 February 2009

Abstract In this study, basidiomycete isolates that possessed a strong ability to degrade chromated copper arsenate (CCA)-treated wood were characterized. These fungal isolates, which were collected from CCA-treated pine log wastes, showed no recognizable morphological properties on culture media. Nucleotide sequence analysis of the large subunit rDNA of the isolates revealed that they were one species. Based on the high sequence similarity (>95%) and close phylogenetic relationship with several known species of *Crustoderma*, the fungal isolates characterized in this study were classified as a *Crustoderma* sp. In a wood degradation test, *Crustoderma* isolate KUC8611 produced a remarkably higher weight loss in CCA-treated *Pinus radiata* (68.7%), *Pseudotsuga menziesii* (39.7%), and *Tsuga heterophylla* (38.5%) wood than other evaluated basidiomycete species, including *Crustoderma flavescens* and *Crustoderma corneum.* In addition, extracellular enzymes for cellulose and protein degradation were detected when the isolates were cultured in chromogenic media, which supports the finding that isolate KUC8611 is a wood degrader. Furthermore, an in vitro test for metal tolerance revealed that isolate KUC8611 showed strong arsenic tolerance, but that it could not tolerate copper. Finally, isolate KUC8611 produced lower amounts of oxalic acid than copper-tolerant fungi such as *Fomitopsis palustris* and *Antrodia vaillantii*. To the best of our knowledge, this is the first study to report the degradation of CCA-treated wood by a *Crustoderma* species.

Keywords Biodegradation · Brown rot fungi · CCA-treated wood · *Crustoderma* species · Metal tolerance

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Introduction

Chromated copper arsenate (CCA) is a chemical wood preservative comprised of chromium, copper and arsenic compounds. CCA is used in pressure treated wood to protect against insects and microbial

decay (Eaton and Hale 1993). However, the use of CCA-treated wood has been banned due to its potential adverse effects against humans and environmental contamination (Clausen 2004; Green and Clausen 2005; Kartal et al. 2004). As a result, CCA-treated wood has been removed from the market in Korea (Choi et al. 2007, 2008). Despite this, it is estimated that, as of 2006, over one million cubic meters of CCA treated wood materials were in service in Korea (Choi et al. 2008). Due to an expected service-life of 20–50 years, a large quantity of CCA-treated wood will need to be continuously removed and remediated during this time frame (Choi et al. 2008; Clausen 2000; Sierra-Alvarez 2007). Consequently, the accumulation of waste products from CCA-treated wood will pose a major environmental concern in the future. CCA-treated wood in many other countries is currently disposed of in landfills. However, this method is not an acceptable means of disposal due to the potential for soil and groundwater contamination (Sierra-Alvarez 2007). Therefore, bioprocessing of waste wood through methods such as biodegradation and bioleaching is currently being evaluated to determine if these are feasible methods of remediation. Illman et al. (2000) investigated the use of wood decay fungi to reduce the volume of waste using an easily managed, cost-effective system and found that biodegradation of waste wood in compost or clean-up sites could be a suitable alternative to landfills.

In this study, a number of basidiomycetous fungi were screened for their ability to degrade CCA-treated wood. The results of this screening are discussed with regard to the identification and characterization of an unknown *Crustoderma* species that was found to be highly effective at degrading CCA-treated wood.

Materials and methods

Fungal isolation and molecular identification

A basidiomycete isolate, KUC8611, was isolated from CCA-treated wood wastes and identified based on analysis of the nucleotide sequence of its large subunit rDNA (LSU rDNA) region. The fungus was grown for 7 days at room temperature on 2% MEA plate with a layer of cellophane sheet. The mycelium was then scraped from the surface of the cellophane sheet with a sterile scalpel, after which the genomic DNA was extracted using the method described by Lim et al. (2005). The obtained genomic DNA was then used as a template for PCR amplification of the LSU rDNA regions using the LR0R-LR3 primer set (http://www.biology.duke.edu/fungi/mycolab/primers.htm) under the reaction conditions described by Kim et al. (2004). Next, the amplified PCR products were analyzed on an ABI 3700 automated sequencer (Perkin-Elmer Inc., Foster City, CA, USA) at the DNA Synthesis and Sequencing Facility of the MACROGEN Company (Seoul, Korea). The determined nucleotide sequence of isolate KUC8611 was searched through the GenBank DNA database for the closest matching LSU rDNA sequences of known fungi (Altschul et al. 1994). For phylogentic analysis, the LSU rDNA sequence of the isolate KUC8611 was aligned with LSU rDNA sequences of reference fungi using Clustal X program (Thompson et al. 1997). The nucleotide sequence alignments were then manually adjusted using the PHYDIT program, version 3.2 (http://plasza.snu.ac.kr/~jchun/phydit/). Next, phylogenetic trees were constructed with PAUP*4.0b10 (Swofford 2002) using the neighbor joining method. Branch stability was then assessed by 1,000 bootstrap replications implemented by PAUP*4.0b10. The determined nucleotide sequences of the isolate KUC8611 and a reference species, *Antrodia vaillantii* SEL8501, were deposited into the GenBank DNA sequence database under accession numbers EU024960 and EU024959, respectively.

Wood degradation test

The fungal cultures used in this test are shown in Table 1. Isolate KUC8611 was originally isolated from Korean pine (*Pinus koraiensis*) logs that had been treated with CCA Type C and then exposed to an outdoor environment for approximately 5 years during field testing (Choi et al. 2007) (Table 1). In addition, two isolates, *Crustoderma* sp. KUC8065 and *Gloeophyllum trabeum* KUC8607, were collected from CCA-treated wood in service in Korea (Kim et al. 2005). To test the wood degrading activity, four brown rot fungi that are known to be copper-tolerant, *Antrodia vaillantii* SEL8501, *Fomitopsis palustris* TYP 0507, *Meruliporia incrassata* Mad-563, and *Wolfiporia cocos* IFO30268, were evaluated concurrently

Table 1 Fungal cultures used in this study

Fungal species	Isolate No.	Host	Origin	Collection or supplier[a]
Antrodia vaillantii	SEL8501	–	Japan	RISH
Crustoderma sp.	KUC8611	*Pinus koraiensis* logs treated with CCA	Seoul, Korea	KUC
Crustoderma sp.	KUC8065	*Pinus radiata* logs treated with CCA	Seoul, Korea	KUC
Crustoderma flavescens	HHB-9359-Sp	*Quercus* sp.	Wisconsin, USA	CFMR
Crustoderma corneum	HHB-5695-Sp	*Pinus ponderosa*	Montana, USA	CFMR
Fomitopsis palustris	TYP0507	–	Japan	RISH
Gloeophyllum trabeum	KUC8067	*Pinus radiata* logs treated with CCA	Seoul, Korea	KUC
Meruliporia incrassata	Mad-563	*Pinus echinata*	Virginia, USA	CFMR
Wolfiporia cocos	IFO30268	–	Japan	RISH

[a] *RISH*, the Research Institute for Sustainable Humanosphere, Kyoto University, Japan; *KUC*, Korea University Culture Collection, Korea; and, *CFMR*, the Center for Forest Mycology Research, Forest Products Laboratory, Northern Research Station, Madison, WI, USA

(Clausen and Green 2003; Green and Clausen 2003). Two isolates, *Crustoderma flavescens* HHB-9359-Sp and *Crustoderma corneum* HHB-5659-Sp, which were not known to be copper-tolerant, were also included to check for the presence of interspecific variation in degradability.

For the wood decay test, sapwood blocks (19 × 19 × 19 mm) were prepared using the wood from three tree species, Douglas-fir (*Pseudotsuga menziesii*), radiata pine (*Pinus radiata*), and Western hemlock (*Tsuga heterophylla*), which were imported from New Zealand and/or North America. The sapwood blocks were vacuum-treated with CCA Type C (to approximately 3.5 kg/m^3). Following treatment, the wood blocks were incubated at 60°C for 2 days to induce accelerated fixation of the heavy metal components into the sapwood. Each test fungal isolate was then used to inoculate the treated wood blocks with eight replicates. Untreated wood blocks were included as a control. The inoculated wood blocks were then incubated at the optimum growth temperatures for each test isolate and screened using a modified soil block test without a feeder strip (Curling et al. 2002). Samples of treated and untreated wood blocks were harvested 12 weeks after inoculation. The percentage of weight loss was then calculated for each fungal isolate-treated wood block.

Physiological and biochemical characteristics of isolate KUC8611

The growth rate and optimum growth temperature of isolate KUC8611 was determined by culturing the fungi on 2% Difco MEA (20 g Difco malt extract, 15 g Difco agar, 1,000 ml distilled water). The inoculated plates were then incubated at 15, 20, 25, 30, and 35°C, respectively. Next, the fungal growth rate was determined by measuring the diameter of three replicate colonies along two perpendicular lines crossing the center of the colony. To determine the wood rot type produced by the isolate, oxidase reactions were rated on gallic or tannic acid media (Davidson et al. 1938).

The ability of the isolate to produce extracellular enzymes was evaluated by culturing the organism on chromogenic media supplemented with different polymeric carbon substrates (Yoon et al. 2007). Briefly, the fungal isolate was precultured on 2% MEA (Difco, USA) at 25°C for 5 days. The preculture was then transferred onto media containing 0.5% CM-cellulose (Sigma, USA), D-cellobiose (Sigma, USA), avicel (Fluka, Ireland), pectin (Sigma, USA) and starch (Sigma, USA), as well as an enzymatic carbon source, 0.1% yeast nitrogen base (Difco, USA) as a fundamental nitrogen source, 0.5% Congo Red dye (Sigma, USA) for chromogenic reaction, and 1.5% agar powder. To evaluate the lipase and protease production, spirit blue agar containing Tween 80 and olive oil and skim milk agar (Difco, USA) were used, respectively. After 5–7 days of culture at 25°C, the enzyme activity was evaluated based on the formation of a clear zone (plaque) around the fungal colony (Table 4). To ensure that the isolate possessed cellulase activity, *Trichoderma reesei* (ATCC56765), a known cellulolytic fungal isolate, was used as a positive control. In addition,

Saccharomyces cerevisiae was used as a negative control.

Test of metal tolerance and measurement of oxalic acid production with pH change

To evaluate the metal tolerance characteristics of the fungi, the minimum inhibitory concentrations (MIC) of copper and arsenic were determined using 2% MEA supplemented with 14.5, 58.0, and 289 ppm of copper ($CuSO_4 \cdot 5H_2O$, >99%, Showa Chemicals Inc., Tokyo, Japan) and 17.8, 35.5, and 71.0 ppm of arsenic ($Na_2AsO_4 \cdot 7H_2O$, >98%, Yakuri Pure Chemicals Co., Ltd, Osaka, Japan), respectively (Table 2). To prepare the supplemented plates, 1 l of MEA was autoclaved and then cooled to approximately 45°C, after which the metal compounds were added. Test fungi were then inoculated onto media amended with each metal compound and then incubated at their optimum growth temperatures. Three replicates were used, and the rate of growth was investigated by measuring the diameters of the colonies as described above after 5 days of incubation.

As shown in Table 2, six different isolates were cultivated for 1 week in a flask containing 100 ml of fermentation broth that contained 20 g of malt extract and 1,000 ml of deionized water. The amount of oxalic acid formed in the culture media was then determined by high performance liquid chromatography (Agilent 1100) and the pH value of the fermentation broth was measured using a pH meter (Fisher Scientific).

Results and discussion

Identification of isolate KUC8611

Analysis of the obtained LSU rDNA region sequence revealed that isolate KUC8611 shares high nucleotide sequence similarity with species belonging to the genus *Crustoderma*. The sequence of isolate KUC8611 was most similar (99.8% homology) to that of an unknown *Crustoderma* sp. (AY858355) that was previously isolated from CCA-treated *Pinus radiata* logs in our laboratory and identified as isolate KUC8065 (Table 1). In addition, the sequences of the ITS rDNA of isolates KUC8611 and KUC8065 were 100% homologous (unpublished data). Therefore,

Table 2 The growth, metal tolerance, and oxalic acid production of brown rot fungi on MEA amended with metal components after 5 days of incubation

Fungus	Oxalic acid production (g/l)	pH of medium after growth	Optimum growth temperature (°C)	Mean growth (mm) on MEA media amended with metal components						
				Control	Copper			Arsenic		
				0 ppm	14.5 ppm	58 ppm	289 ppm	17.8 ppm	35.5 ppm	71 ppm
Antrodia vaillantii SEL8501	0.8	2.64	25	50.9	55.4	52.9	–[a]	39.9	34.3	20.5
Crustoderma sp. KUC8611	0.1	3.60	30	63.4	3.9	–	–	56.8	32.0	9.3
Crustoderma sp. KUC8065	0.1	3.59	30	62.1	2.6	–	–	52.0	36.8	14.1
Crustoderma flavescens HHB-9359-Sp	0.0	3.66	30	68.7	62.8	–	–	6.7	–	–
Crustoderma corneum HHB-5695-Sp	0.0	4.73	25	11.6	–	–	–	–	–	–
Fomitopsis palustris TYP0507	0.9	2.23	35	82.0	70.5	12.6	–	42.3	20.4	–
Gloeophyllum trabeum KUC8067	0.0	3.93	35	58.4	29.2	–	–	53.5	48.1	26.8
Meruliporia incrassata Mad-563	0.1	3.12	30	24.2	21.7	20.5	–	14.7	11.5	–
Wolfiporia cocos IFO30268	0.1	3.63	30	60.2	71.8	62.6	–	–	–	–

[a] No growth

these two organisms, which were isolated from different substrata (one from CCA-treated radiata pine and another from Korean pine), can be considered as the same species. The next closest matches to the LSU rDNA region amplified in this study were to *C. corneum* (AY219386), *C. flavescens* (AY219387), and *C. longicystidium* (AY219388) with similarities of 95.0, 94.88, and 94.99%, respectively.

Phylogenetic analysis revealed that isolates KUC8611 and KUC8065 formed a group with *Crustoderma* species. In addition, all of the analyzed *Crustoderma* species formed a monophyletic group with a 100% bootstrap supporting value in a polyporoid clade (Fig. 1). These results demonstrate that isolate KUC8611 is phylogenetically positioned in the basidiomycete genus *Crustoderma.* Overall, based on the similarity and phylogenetic analyses, we identified isolate KUC8611 as a *Crustoderma* species similar to isolate KUC8065.

Although several *Crustoderma* species have previously been described (Ginns and Lefebvre 1993), we could not identify the KUC8611 and KUC8065 isolates to the species level. This is partially because these isolates are a failure to form fruiting bodies and morphological features on culture media, which are critical for taxonomic decisions. In addition, current information regarding comparable DNA sequence data from a wide range of *Crustoderma* species is not available in known DNA databases.

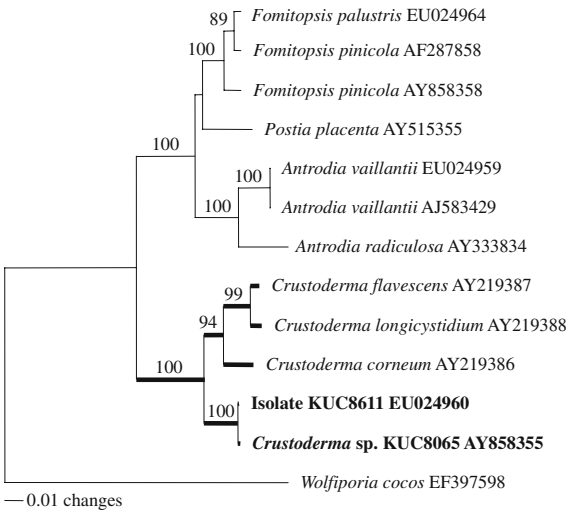

Fig. 1 Phylogenetic tree showing the relationship of *Crustoderma* species with several brown rot fungi based on the LSU rDNA sequences. *Wolfiporia cocos* was used as an outgroup. Bootstrap values from 1,000 replicates are shown on the branches. The branches of the *Crustoderma* clade are represented by bold lines. The GenBank accession numbers are given with the species names

Degradation of CCA-treated wood

Radiata pine, Douglas-fir, and Western hemlock are the primary trees used in landscape structures in Korea and many other countries. Therefore, wood of these trees were chosen in this study for degradation test. Among the three tree species, radiata pine wood showed lowest natural durability against decay, as indicated by the highest percent weight losses from untreated sapwood blocks (Table 3).

When the ability of the known copper-tolerant fungi to degrade the block was evaluated, only *F. palustris* could decrease the dry weight of CCA-treated sapwood blocks produced by each of the evaluated species by >20%. *A. vaillantii* was found to reduce the dry weight of CCA-treated radiata pine sapwood blocks by >20%; however, it reduced the dry weight of CCA-treated Douglas-fir and Western hemlock sapwood blocks by <7%. In addition, treatment with *M. incrassata* and *W. cocos* resulted in less than a 7% decrease in the dry weight of CCA-treated sapwood blocks of each of the tree species evaluated in this study, even though these fungi were found to decrease the dry weight of untreated radiata pine blocks by >55% (Table 3). Green and Clausen (2003) demonstrated that *M. incrassata* Mad-563 and *W. cocos* FP97438sp induced a 51 and 32% loss of weight in copper citrate treated southern yellow pine, respectively. These findings and the results of the present study suggest that the ability of fungi to decompose wood may differ among tree species (Table 3). As well, the biodegradability of the treated wood varies significantly with copper-based preservatives as shown in the previous studies on the isolates of the copper-tolerant fungus *W. cocos* (Clausen et al. 2000; De Groot and Woodward 1999).

In the present study, treatment with *C. flavescens* resulted in a 14% decrease in the dry weight of Douglas-fir wood blocks; however, it did not induce a decrease in the radiata pine and Western hemlock wood blocks (Table 3). Furthermore, *C. corneum* did not induce a decrease in the dry weight of any of the wood blocks, regardless of whether they were treated or not. Taken together, these findings indicate that *C. corneum* is not

Table 3 Mean dry weight loss (%) of CCA-treated sapwood blocks by *Crustoderma* species and other well-known basidiomycetes

Fungal species	Isolate No.	Mean day weight loss (%)					
		Radiata pine		Douglas fir		Western hemlock	
		Untreated	CCA-treated	Untreated	CCA-treated	Untreated	CCA-treated
Antrodia vaillantii	SEL8501	21.0 ± 5.2 e*	23.9 ± 1.1 c	12.8 ± 2.6 e	6.1 ± 0.9 e	10.2 ± 3.7 d	4.4 ± 0.6 d
Crustoderma sp.	KUC8611	66.7 ± 2.3 a	68.7 ± 1.6 a	36.9 ± 4.3 ab	39.7 ± 3.9 a	37.1 ± 3.7 b	38.5 ± 1.4 a
Crustoderma sp.	KUC8065	55.7 ± 5.2 c	58.0 ± 4.0 b	39.3 ± 7.3 a	34.9 ± 4.8 b	37.8 ± 6.1 b	34.0 ± 5.4 b
Crustoderma flavescens	HHB-9359-Sp	52.7 ± 8.0 cd	1.3 ± 0.2 e	24.1 ± 4.2 d	14.0 ± 2.3 d	17.4 ± 2.3 d	0.5 ± 0.5 ef
Crustoderma corneum	HHB-5695-Sp	0.2 ± 0.1 f	0.5 ± 0.2 e	3.3 ± 1.3 f	1.3 ± 0.4 f	0.2 ± 0.3 e	0.0 ± 0.0 f
Fomitopsis palustris	TYP0507	46.6 ± 1.9 d	26.8 ± 1.9 c	31.8 ± 7.9 bc	20.6 ± 3.2 c	37.9 ± 8.3 b	21.5 ± 5.8 c
Gloeophyllum trabeum	KUC8067	63.5 ± 5.1 ab	2.7 ± 1.6 e	29.6 ± 6.7 cd	3.4 ± 0.8 ef	27.8 ± 8.2 c	2.0 ± 1.0 def
Meruliporia incrassata	Mad-563	57.8 ± 6.7 bc	6.1 ± 2.2 d	39.0 ± 7.9 a	3.9 ± 2.6 ef	47.5 ± 6.1 a	3.6 ± 2.1 de
Wolfiporia cocos	IFO30268	59.5 ± 4.2 bc	1.0 ± 0.1 e	2.5 ± 1.6 f	0.6 ± 0.2 f	17.6 ± 1.9 d	0.1 ± 0.1 f

*Numbers followed by the same letter in each column are not significantly different ($\alpha = 0.05$) according to Duncan's method. Data were obtained 12 weeks after incubation

able to degrade wood. However, the two *Crustoderma* isolates (KUC8611 and KUC8065) evaluated in this study showed significantly higher levels of weight loss for each of the wood blocks when compared to the other organisms. Indeed, these isolates effectively degraded the untreated wood and the CCA-treated wood. Furthermore, of the fungi evaluated here, only these isolates were able to decay CCA-treated wood of radiata pine, Douglas-fir and Western hemlock. When the two *Crustoderma* isolates were compared, isolate KUC8611 induced a greater weight loss than isolate KUC8065, which indicates that it is a stronger degrader of CCA-treated wood. The highest reported degradation rate of CCA-treated wood to date was that of a brown rot fungus, *M. incrassata* TFFH-294 (Illman et al. 2000), which was recently re-identified as *Antrodia vaillantii* based on molecular methods (Hastrup et al. 2006). However, although this fungus degraded 36.8% of the dry weight of CCA-treated wood blocks, it only degraded Southern yellow pine. Therefore, the results of the present study suggest that the unknown *Crustoderma* isolates evaluated in this study have the potential for use in biodegradation projects designed to reduce the volume of CCA-treated wastes or as an agent for composting CCA-treated wood.

It is important to note that the two *Crustoderma* isolates, KUC8611 and KUC8065, degraded the CCA-treated sapwood blocks to the same degree as they degraded the untreated sapwood blocks. These findings indicate that the two *Crustoderma* isolates are CCA-tolerant fungi. The difference in the extent of wood weight loss between the two isolates indicates that intraspecific variation exists in their ability to degrade wood, which is similar to the results of studies conducted to evaluate other copper tolerant species such as *Wolfiporia cocos* (Clausen et al. 2000; De Groot and Woodward 1999) and *Antrodia vaillantii* (Collet 1992). Considering that these two isolates of very good wood degraders originated from the CCA-treated wood wastes of two different tree species, screening of CCA-treated wood wastes from a variety of trees species would be useful for the detection of new *Crustoderma* sp. that have good wood degrading ability.

Physiological characteristics, metal tolerance, and oxalic acid production

The *Crustoderma* isolate, KUC8611, was confirmed to be brown rot fungus and its optimum growth temperature and growth rate was determined to be 30°C and 14.2 mm/day, respectively. These results indicate that isolate KUC8611 grows relatively rapidly and favors higher temperatures. Because isolate KUC8611 showed a strong ability to degrade CCA-treated wood blocks, its tolerance to copper and arsenic was evaluated and compared to that of other *Crustoderma* species and brown rot fungi. Only *A. vaillantii, F. palustris*, and *M. incrassata* were tolerant to copper at concentrations of 58 ppm. When the *Crustoderma* species were evaluated, the two *Crustoderma* isolates (KUC8611 and KUC8065) and *C. corneum* were found to be sensitive to copper, and only *C. flavescens* tolerated copper at a concentration of 14.5 ppm. However, the two *Crustoderma* isolates were highly tolerant of arsenic (Table 2). It is interesting to note that, even though they were sensitive to copper, they could grow on and degrade CCA-treated wood blocks. In addition, the other two *Crustoderma* species, *C. flavescens* and *C. corneum*, were sensitive to arsenic. Taken together, these findings demonstrate that metal tolerance to copper and arsenic varies among species of *Crustoderma*, which demonstrates that they have a mechanism that enables them to overcome the toxicity of Cu in treated wood.

Certain copper-tolerant wood decay fungi can readily decay CCA-treated wood at levels intended to inhibit degradation by fungi (Illman and Highley 1996). These fungi are also known to produce large amounts of oxalic acid, which converts chrome and arsenic salts into water-soluble oxalates and to precipitate copper as insoluble copper oxalate (Clausen et al. 2000; Sierra-Alvarez 2007). Although tests for oxalic acid in liquid media do not necessarily indicate the products that a species of fungi will produce in a woody substrate (Clausen et al. 2000; Green and Clausen 2003), the production of oxalic acid in this study was measured in liquid media. The copper-tolerant fungi, *F. palustris* and *A. vaillantii,* produced higher amounts of oxalic acid (0.8–0.9 g/l) than the other tested fungi (Table 2). In addition, the other copper tolerant fungus, *M. incrassata*, produced a lower amount of oxalic acid (0.1 g/l), which was similar to the amount of oxalic acid produced by isolates KUC8611 and KUC8065. Furthermore, *C. flavescens* and *C. corneum* did not produce any oxalic acid. Taken together, these results suggest that there is a relationship between oxalic acid production and the ability of fungi to degrade

CCA-treated wood; however, this relationship was not thoroughly evaluated in this study. Therefore, future investigations should be conducted to elucidate this relationship (Clausen et al. 2000; De Groot and Woodward 1999; Green and Clausen 2003). Meanwhile, the wood decay fungus, *G. trabeum*, is known to be copper sensitive and a nonaccumulator of oxalic acid (Clausen et al. 2000; Green and Clausen 2003; Humar et al. 2006). Although the *G. trabeum* used in this study was collected from CCA-treated wood, the results observed in the present study were similar to the results of previous studies.

Cellulose is the major wood component of most trees; therefore, fungi must have the ability to produce extracellular cellulases to degrade wood. To confirm its ability to produce extracellular cellulases, the strong CCA-treated wood degrader, isolate KUC8611, was tested on chromogenic media. Isolate KUC8616 was found to produce cellulose degrading enzymes such as avicelase, carboxy-methyl cellulase, and β-glucosidase (Table 4), as well as protease. These findings indicate that the unknown *Crustoderma* sp., KUC8611, is a useful wood degrading fungus.

Crustoderma species have been recovered from a wide range of environmental habitats, including decaying wood, mine timber, cooling towers, fence posts, and play grounds (Ginns and Lefebvre 1993; Kim et al. 2005). However, it is not known if the degrading ability of CCA-treated wood is a common characteristic of all species of the genus *Crustoderma* or unique to isolate KUC8611. Because this is the first report of a *Crustoderma* fungus with the ability to degrade CCA-treated wood, additional studies should be conducted to determine if other species of *Crustoderma* can degrade CCA-treated wood. In addition, further study is necessary to elucidate the exact mechanism by which the degradation of CCA-protected wood by *Crustoderma* fungi occurs.

Table 4 Test of extracellular enzyme production by isolate KUC8611

Enzymes	Substrate	Production[a]
Amylase	Starch	Negative
Avicelase (cellulase)	Avicel	Positive
β-Glucosidase	D-cellobiose	Positive
CM-cellulase	CM-cellulose	Positive
Lipase	Tween 80, olive oil	Negative
Pectinase	Pectin	Negative
Protease	Skim milk	Positive
Xylanase	Xylan	Negative

[a] Positive: formation of a clear zone; Negative: no formation of a clear zone

Acknowledgments This work was supported by the KTOL grant funded by The National Instituted of Biological Resources, Korean Government.

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