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Effect of wood modification on gene expression during incipient *Postia placenta* decay

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ABSTRACT

The mode of action of nontoxic wood modifications and the fungal response to modified wood are not fully elucidated. The aim of this study was to investigate the reaction of *Postia placenta* in terms of gene expression of selected genes upon the encounter of acetylated, DMDHEU-treated and thermally modified wood. Quantitative PCR (qPCR) showed that the investigated *P. placenta* genes involved in oxidative degradation were expressed at higher levels in modified wood than in untreated wood, while expression levels in modified wood for the investigated genes involved in enzymatic degradation were slightly lower than those in untreated wood. The results indicate that the response of *P. placenta* upon the encounter of modified wood is to up-regulate the expression of the oxidative degradation machinery. In addition, our results support the theory that the decay resistance of the herein studied modified woods is due to inhibition of fungal molecules, needed for oxidative degradation of wood polymers, to penetrate the wood cell wall.

1. Introduction

Modified wood materials have an improved resistance against wood decaying fungi (Hill, 2006). In contrast to traditional wood preservation, where the durability is achieved from the toxicity of the added chemicals and possibly non-recognition of substrate, very little is known about the mode of action of the nontoxic wood modifications and even less about fungal response to modified wood. Elucidating the response of the fungi towards modified wood may indicate the mechanisms behind the decay resistance and in what way wood modification can be improved.

Wood modification involves the action of a chemical, biological or physical agent upon the material, resulting in increased durability and/or other desired enhancements. Acetylation of wood is commonly done by reacting wood with acetic anhydride, which results in esterification of the accessible hydroxyl groups in the wood cell wall (Rowell et al., 1994). 1,3-dimethylol-4,5-dihydroxy ethylene urea (DMDHEU) modification is a wood modification with a water solution of DMDHEU (Militz, 1993). Indications of cross linking within the cell wall are found but the mechanisms of the reaction between DMDHEU and the wood cell wall are unknown (Yasuda et al., 1994; Krause et al., 2003). Thermal modification is a heat treatment of wood using mild pyrolysis, (Militz, 2002; Hakkou et al., 2006). During the process, hemicelluloses are degraded and autocondensation of lignin and lignin cross-linking with polysaccharides occur (Hakkou et al., 2006; Mohareb et al., 2011; Tjeerdsma et al., 1998).

Today, four established theories of mode of action of modified wood are prevalent: fungal enzyme inefficiency due to non-recognition (Rowell, 2005); fungal enzyme inefficiency due to lack of water at glycosidic bonds (Rowell, 2005; Rowell et al., 2009); reduced flow of fungal molecules into the wood cell wall due to micropore blocking (Hill et al., 2005); and inhibition of diffusion of fungal molecules due to insufficient amounts of moisture in the wood cell wall (Papadopoulos and Hill, 2002; Boonstra and Tjeerdsma, 2006). It has been shown that fungal enzymes are less efficient at degrading modified wood than untreated wood (Lekounougou et al., 2008; Venås, 2008; Verma and Mai, 2010), that acetylation and DMDHEU-treatment decreases micropore size (Hill et al., 2004; Dieste et al. 2009), that the size of the anhydride used in acetylation is more important than the number of OH-groups

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blocked (Papadopoulos and Hill, 2002; Hill, 2009) and that cross-linking in thermally modified wood is important for decay resistance (Rapp et al., 2008). To what extent the different modes of action contribute to decay resistance is unknown; however, the biochemical mechanisms of brown rot decay support the theory of inhibition of diffusion due to moisture exclusion (Ringman et al., 2013).

Among wood decaying fungi, brown-rotting basidiomycetes contribute to the most destructive type of decay in wooden buildings (Gilbertson and Ryvarden, 1986; Zabel and Morell, 1992). Brown rot fungi degrade wood through oxidative and enzymatic action (Goodell et al., 1997; Arantes et al., 2012). First, the fungus induces production of hydroxyl radicals inside the wood cell wall through secreting reductants and hydrogen peroxide (Goodell et al., 1997). The reductants reduce ferric iron (Fe^{3+}) in the wood cell wall to ferrous iron (Fe^{2+}) (Goodell et al., 1997, 2006), which react with the hydrogen peroxide and produce hydroxyl radicals in the Fenton reaction (Fenton, 1894). The hydroxyl radicals depolymerise hemicellulose and cellulose, modify lignin and generate sufficient rearrangements so that hydrolysing enzymes, which are too big to penetrate the intact wood cell wall, can diffuse into the cell wall and continue the degradation of the polysaccharides (Goodell et al., 1997; Baldrian and Valaskova, 2008; Arantes et al., 2012).

The most studied type of fungal reductants is hydroquinones (Baldrian and Valaskova, 2008; Arantes et al., 2012). After reduction of Fe^{3+}, the hydroquinones are recovered by a quinone reductase (Paszczynski et al., 1999; Jensen et al., 2002; Qi and Jellison, 2004). A protein model in *Postia placenta* (Ppl124517) is identified as a putative quinone reductase (Martinez et al., 2009) and transcription of this gene is significantly up-regulated in cellulose and lignocellulose medium (Martinez et al., 2009; Vanden Wymelenberg et al., 2010). Alcohol oxidase may contribute to extracellular production of hydrogen peroxide, since it has a preference for methanol which potentially is available from demethylation of lignin. A putative alcohol oxidase (Ppl118723) in *P. placenta* is transcribed at high levels and is highly up-regulated in cellulose medium compared to glucose medium (Martinez et al., 2009).

Following oxidative degradation, wood polysaccharides are further degraded enzymatically (Goodell et al., 1997; Fackler et al., 2007; Arantes et al., 2012). Enzymatic degradation by cellulose degrading fungi generally happens as follows: Endoglucanases cut the cellulose chains internally, providing ends for cellobiohydrolases to bind to and cleave off cellobiose units; and β-glucosidases subsequently hydrolyse cellobiose to glucose (Aro et al., 2005). However, brown rot fungi lack cellobiohydrolases (Baldrian and Valaskova, 2008). Processive endoglucanases, which both cleave cellulose internally and release oligosaccharides before detaching from the polysaccharide, could potentially substitute for their absence (Baldrian and Valaskova, 2008). Brown rot β-glucosidases are either intracellular, membrane bound or extracellular (Baldrian and Valaskova, 2008). They are relatively nonspecific and cleave xylose, mannose and galactose in addition to cellobiose (Baldrian and Valaskova, 2008). Sequencing of the genome of *P. placenta*, showed that this fungus possesses only two potential endoglucanases (Ppl115648 and Ppl103675) and several β-glucosidases (Martinez et al., 2009).

Previous studies have shown that genes involved in oxidative degradation of holocellulose in general are up-regulated during brown rot decay in modified wood, while genes involved in enzymatic degradation are not (Alfredsen and Fossdal, 2010; Schmöllerl et al., 2011; Pilgård et al., 2012).

To the knowledge of the authors, it is not known which regulatory mechanisms of the fungal wood degradation machinery are prevalent during brown rot degradation in modified wood. In order to improve the decay resistance of modified wood, it is of great importance to understand the decay mechanisms of incipient decay; however, fungal gene expression during this time-frame has not been extensively studied. Furthermore, the exact mechanisms for decay resistance in modified wood have still to be elucidated.

The aim of this study was to investigate the reaction of *P. placenta*, in terms of gene expression of alcohol oxidase Ppl124517, quinone reductase Ppl124517, endoglucanase Ppl103675 and β-glucosidase Ppl112501, upon the encounter of acetylated, DMDHEU-treated and thermally modified wood and to use these results for discussion of possible modes of action of modified wood.

Table 1
Primer sequences of target genes and endogenous control (previously used by Alfredsen and Fossdal (2010)).

Gene	JGI number	Primer sequences
β-tubulin	113,871	CAGGATCTTGTCGCCGAGTAC/ CCTCATACTCGCCCTCCTCTT
Alcohol oxidase	118723	CATCAAGAGCGCCAATCCAT/ GGCGCAAAGTCAGCCTTGT
Quinone oxidoreductase	124517	CGACGACAAGCCCAACAAG/ GATGACGATGGCGATTTTAGG
Endo-β-1,4-glucanases	103675	GTTCAGGCCGCATTGTCCT/ TTCCACCTGGCGTAATTGTG
Putative β-glucosidase	112501	TGCGCACGAATGAGTTGATAG/ CGCCTGCACACACACAACA

2. Experimental methods

2.1. Wood material and sample preparation

Boards of *Pinus sylvestris* sapwood were acetylated to 21 weight per cent gain, treated with 1.3 M DMDHEU using 5% MgCl2 as catalyst, or thermally modified according to the ThermoWood D scheme (212 °C). Miniblock samples ($10 \times 5 \times 30$ mm^3) (Bravery, 1979) were prepared from untreated and modified wood and leached according to EN 84 (1996). The samples were acclimatised in 20 °C and 65% RH for two weeks and subsequently sterilised with gamma radiation. To ensure similar conditions for fungal colonisation of all wood samples within each treatment (Junga and Militz, 2005), the samples were placed in pairs, one for each analysis, in Petri dishes containing 4% malt agar medium inoculated with *P. placenta* (strain FPRL 280). The exposed samples ($n = 4$) were harvested after 2, 6, 10, 14 and 56 days of incubation at 22 °C and 70% relative humidity. After harvesting, the mycelia covering the wood samples was manually removed and the samples were either dried and weighed for mass loss or frozen at −80 °C. Wood powder from the frozen samples was provided using a drill (3 mm bit diameter) (Jasalavich et al., 2000) followed by grinding with mortar and pestle.

2.2. RNA analysis

Total RNA was isolated from the wood samples and DNA was removed using MasterPure™ RNA Purification Kit (Epicenter), according to the manufacturer's instructions. RNA from each sample was converted into cDNA using TaqMan Reverse Transcription Reagents (using Oligo d (T)16) (Applied Biosystems) with 10 times the standard dNTP concentration. The samples were subsequently amplified with PCR (Rotor-gene, Qiagen). qPCR (Rotor-gene, Qiagen) was performed on cDNA samples ($n = 3$) using Rotor-gene SYBRGreen PCR kit (Qiagen). Primer sequences for target genes and endogenous control are listed in Table 1 (previously used by Alfredsen and Fossdal (2010)). As endogenous control, the house keeping gene β-tubulin was used. No PCR product was formed