FUNCTIONAL GENOMICS OF WOOD DEGRADATION – A PROJECT SUMMARY

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ABSTRACT

Modified wood can provide protection against a range of wood deteriorating organisms. Several hypotheses have been put forward for the mode of action against wood decaying fungi, including inhibition of action of specific enzymes, but they still need further testing. This paper summarizes results from a project focusing on molecular studies of fungal colonization in modified wood. The focus has been on furfurylated wood, but also thermally modified and acetylated wood has been studied.
Among the main finding was that wood modifications have an effect on the exploitation face of both brown and white rot colonization, but not on the exploration face. As already reported in a range of papers wood modification effects the wood moisture content, and this was confirmed within this project. New information was gathered about the effect on gene expression. Even before any mass loss was detected, differences in gene expression were measured. Within an eight week period, genes related to oxidative metabolic activity of *P. placenta* generally was higher in furfurylated wood compared to untreated Scots pine. Carbohydrate metabolism related expression varied. A similar comparison was done, but with longer incubation time and also including thermal modification and acetylation. In the beginning of the incubation of all treated wood samples, the genes coding for oxidative metabolic activity had higher expression levels than the untreated control. In the end of the incubation most of these genes were less expressed than in the untreated control. The genes used for carbohydrate metabolism and the alcohol oxidase showed a significant decrease after 14 weeks of incubation. At the same time an increase in gene expression of an enzyme putative involved in lignin decomposition was detected. It was also shown that the use of molecular methods in field trial evaluation can contribute with important additional information to the standard evaluation methods.

Key words: fungal colonization, gene expression, mode of action, quantitative real time PCR, wood modification,

INTRODUCTION

Environmentally more benign methods are warranted for wood protection. A range of studies the last decade have shown that modified wood can provide protection against a range of wood deteriorating organisms, including decay fungi. Wood modification involves the action of a chemical, biological or physical agent upon the material,

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resulting in a desired property enhancement during the service life of the modified wood (Hill 2006). An understanding of the mechanisms utilized by decay fungi when exposed to modified wood is important for further optimisation of new modified wood products. The mode of action of modified wood systems can be explained by three hypotheses put forward by Hill (2006): (1) The first scenario is the inhibition of action of specific fungal enzymes. The hydroxyl groups in the cell wall and/or in the lumen are substituted with other groups, causing the enzymes to no longer recognize the substrate. (2) Secondly, the equilibrium moisture content is lowered in modified wood, and therefore it is harder for fungi to access the moisture required for decay. (3) Thirdly, modification could cause blocking of cell wall micropores, and this fact lowers the substrate accessibility for decay fungi.

Application of the new molecular procedures to questions concerning the decay of wood and biocide breakdown by the wood decay fungi and associated microbial communities lags behind many fields of biology (Diehl et al. 2008). The use and new possibilities of molecular tools within the field of wood protection has been summarized by several authors (e.g. Diehl et al. 2008, Gelhaye and Morel 2009). To improve laboratory tests, biomarkers of wood degradation need to be developed taking into account the complexity of the wood composition and of the degradation mechanisms (Gelhaye and Morel 2009). Another challenge is that the exact mechanism of brown rot decay still is hypothetical and controversial (Kang et al. 2009a). The molecular tools developed within microbiology allow us to study gene expression, protein presence and enzyme activity. Few studies have been published so far, but one example is Kang et al. (2009b). They studied gene expression of selected decay enzymes produced during biodeterioration of three wood types. Among the findings was that it appears that ACQ-treated wood do not repress the production of the decay enzymes by the fungus but does inhibit the effectiveness/access of these enzymes on the modified substrate. Results from the study indicate that different resistant woods have different effects on the microbial communities and its enzymatic activities during decay.
It is worth to keep in mind that no single research technique can answer all questions about the decay of wood, we need to gather small pieces of the puzzle using different approaches (Diehl et al. 2008).

The aim of this paper is to summarize the results related to wood protection from the project ‘Functional genomics of wood degradation: strategies used by decay fungi against wood protection systems and natural host defence compounds’.

SUMMARY OF RESULTS

Comparing DNA content in modified wood

In Pilgård et al. (2010) quantitative real-time polymerase chain reaction (qPCR) was used to profile the DNA amounts of *T. versicolor* (L.) Lloyd (strain CTB 863A) during colonization of treated *Pinus sylvestris* (L.) sapwood. The wood modifications used were acetylation, furfurylation, and thermal modification, samples were harvested after 2, 4, 6 and 8 weeks. The traditional wood preservatives Cu-HDO and CCA were used as references. The maximum levels of fungal DNA in control specimens occurred after 8 weeks. For all wood treatments, the maximum fungal DNA level was recorded after an incubation period of 2 weeks, followed by a decline until the end of the trial at 8 weeks.

The observed decline in fungal DNA amount after 2 weeks of incubation probably reflected the inability of the mycelia to establish a wood exploitation phase on the treated wood. Fungal colonies have been found to exhibit autolysis of older parts of the mycelia, particularly when growing on nutrient-poor media (Olsson 2001). A longer incubation time was suggested for new studies to be able to learn more about colonization in modified wood. Thermally modified wood had the highest and furfurylated wood the lowest levels of total *T. versicolor* DNA throughout the test period. This trend was, however, not significantly proved. The possible lower amount of fungal DNA in furfurylated wood after 8 weeks might be owing to polymerization of the furfuryl alcohol in wood and this led to a physical blocking within the wood cell wall. Venås (2008) hypothesized that the reduced accessibility of carbohydrates in furfurylated wood is most probably owing to cell wall bulking. The observed low colonization level in furfurylated wood might lead to the conclusion that *T. versicolor* is not able to utilize the furfuryl alcohol polymer as an alternative carbon source. The most probable explanation of the levels of fungal DNA in thermally modified wood is the heat inflicted destructions in the wood cell walls. The heat also degrades hemicelluloses to a greater extent than other macromolecular components (Shafizadeh and Chin 1977), resulting in easier access to lignin for the white-rot fungus *T. versicolor*. Acetylation is a modification of the OH groups in the cell wall without polymerization and the treatment does not damage the cell wall. Acetylation falls between the destructive thermal modification and the blocking furfurylation concerning the severity of various wood modifications. Consistently, the colonization level of *T. versicolor* in acetylated wood was more pronounced than that observed in furfurylated wood. Other factors that might contribute to differences between the different modifications include pH and moisture content, but also possible differences in virulence in the different Petri dishes in the test. For the preservative-treated woods, Cu-HDO showed the lowest level of fungal DNA throughout the experiment, indicating that exploratory hyphal growth is limited owing to the phytotoxicity of the treatment. The other treatments did not inhibit the exploratory hyphal growth phase.

In Schmöllerl et al. (2011) data from mass loss, qPCR and qRT-PCR were used for profiling growth dynamics and gene expression of *Postia placenta* (Fr.) M.J. Larsen & Lombard (strain FPRL 280) in different wood substrates through different stages of decay. *P. sylvestris* sapwood was used for the following treatments and modifications: CCA, furfurylation, thermal modification and acetylation. The paper presents results from different time intervals, 2, 14 and 26 weeks. As already reported in a range of papers wood modification effect the wood moisture content, and this was confirmed within this study. The highest mass loss and the highest fungal DNA content were found in the control samples while acetylated wood had the lowest mass loss and fungal DNA content. The data from all treatments reflected a close relation of mass loss and fungal DNA content. This confirms earlier finding, e.g. Eikenes et al. (2005). Except for the CCA treated wood, the DNA content decreased after 14 weeks of incubation, this emphasizes the hypothesis of autolysis and/or reallocation within the hyphae of the fungus (Olsson 2001, Pilgård et al. 2010) after incubation on a nutrient poor substrate. In the CCA treated reference, the growth of the fungus seemed to start after a lag phase. This could be a consequence of buildup of tolerance to the preservative, but this hypothesis has to be proven by longer incubation. Anyway, it is no surprise that the maximal DNA content in the treated samples is lower than in the untreated control samples, confirming a protective function of all investigated wood treatments. In acetylated wood the DNA content decreased already after 2 weeks of incubation,

indicating a low availability of nutrients in the wood for *P. placenta*. This is consistent with the findings in Pilgård et al. (2010) using *T. versicolor* and 8 weeks of incubation.

Gene expression in modified wood

In Alfredsen and Fossdal (2010) gene expression of the brown rot fungus *P. placenta* was monitored after 2, 4 and 8 weeks of colonization in furfurylated *P. sylvestris* sapwood and in untreated control samples. The main finding was that genes related to oxidative metabolic activity generally was higher in furfurylated wood compared to untreated Scots pine. Carbohydrate metabolism related expression varied. For one endo-glucanase and two β-glucosidases the expression was lower in furfurylated wood compared to untreated control, while for one glucoamylase and one glucan 1,3b glucosidase the expression was higher in furfurylated wood. The four cytochrome P450 tested, involved in breakdown of toxic compounds, gave inconsistent results between furfurylated and untreated control samples. Phenylalanine ammonia lyase and cytosolic oxaloacetase gave higher expression in control than in furfurylated samples.

Generally, the increase in gene expression of all investigated *P. placenta* genes in Schmöllerl et al. (2011) was highest in CCA treated wood, and this suggest that the fungus is transcriptionally active despite not actively growing during the first 2 to 14 weeks on CCA. In the beginning of the incubation period, the genes coding for oxidative metabolic activity (Lac1 and AlOx) in treated wood samples had higher or similar expression rates compared to untreated control. The need for oxidative enzymes for the degradation of wood modifications and a stress response are possible interpretations for higher gene expression which were also described by Alfredsen and Fossdal (2010). In the end of the incubation, most of these genes tended to be less expressed in modified wood than in the non-treated control. The need for oxidative enzymes for the degradation of wood modifications and a stress response are possible interpretations for higher gene expression which were also described by Alfredsen and Fossdal (2010). In this investigation, the analysed time period of decay was longer, showing a clear decrease in gene expression of alcohol oxidase (AlOx) in modified wood after 26 weeks of incubation. According to the stress response interpretation the following reduction of gene expression could be explained by an adaption of *P. placenta* to the modification. If the genes were used for the neutralization of modifications, the later decrease of the gene expression could account for an effective reduction of inhibitory wood modifications, which results in a better access to sugar containing nutrients. The genes used for carbohydrate metabolism (EGlu3, Gamy) and the alcohol oxidase (AlOx) showed a decrease after 14 weeks of incubation for the different wood modifications. At the same time an increase in gene expression of a putative lignin degrading enzyme (MPOX) was detected. The combination of these two effects could be interpreted as a shift towards another metabolic pathway or reflect stress associated with fungal cell death and failed colonization attempt on treated samples.

Basisiomycete colonization in field stakes using qPCR.

The aim of Pilgård et al. (2011) was to evaluate (qPCR) as a tool for investigating details of the colonization pattern of basidiomycete decay fungi in wood samples after 6 years of soil exposure. Samples of *P. sylvestris* (heartwood without treatment), furfurylated *P. sylvestris* sapwood and Cu-HDO treated *P. sylvestris* sapwood was used.

The qPCR method based on basidiomycete DNA content in the wood had the highest sensitivity, while the ergosterol assay was more sensitive than the chitin assay. Visual rating was compared with laboratory analyses and was found to be correlating well with qPCR. This study demonstrates that qPCR in combination with microscopy provides relevant data about basidiomycete colonization in wooden field test materials.

CONCLUSION

- The maximum *T. versicolor* DNA level was recorded after 2 weeks, followed by a decline until the end at week 8 when comparing furfurylated, thermally modified and acetylated wood with control and two wood preservatives. Control samples had a gradual increase throughout the test period. One interpretation is that the fungus is able to colonize, but not utilize the modified within this timeframe.
- *P. placenta* was able to start causing mass loss in thermally modified and furfurylated wood after 14 weeks.
- Within an eight week period, genes related to oxidative metabolic activity in *P. placenta* generally was higher in furfurylated wood compared to untreated Scots pine sapwood. Carbohydrate metabolism related expression varied.
- Generally, expression of the investigated *P. placenta* genes we highest in CCA treated wood. In the beginning of the incubation of all treated wood samples, the genes coding for oxidative metabolic activity had higher expression levels than the untreated control. In the end of the incubation most of these genes were less expressed than in the untreated control. The genes used for carbohydrate metabolism and the alcohol oxidase showed a significant decrease after 14 weeks of incubation. At the same time an increase in gene expression of an enzyme putative involved in lignin decomposition was detected.
- The use of molecular methods in field trial evaluation can contribute with important additional information to the standard evaluation methods.

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