Novel glucuronoyl esterases for wood processing

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ABSTRACT

Wood tissue constitutes of a tight network of biopolymers. Covalent linkages between lignin and carbohydrates hamper efficient extraction of intact wood polymers. Selective cleavage of lignin-carbohydrate (LC) bonds by specific enzymes provides an aid for wood processing using mild conditions. Glucuronoyl esterases (GE) are presumed to target LC ester bonds between lignin alcohols and glucuronic acid residues of xylan. In this study we recombinantly produced novel fungal GE enzymes and characterized them using model substrates. The application of these esterases on native LCC fractions will provide insight into their biological function and their potential use in extraction processes.

KEYWORDS

Carbohydrate esterase family 15 (CE15), enzymes, forest products, lignin-carbohydrate complexes (LCC), xylan

INTRODUCTION

The forest industry experiences a shift from cellulose fibres for pulp and paper as the main product towards other wood-derived products and therefore has a great interest in the development of novel materials of improved performance and functionality. The Wallenberg Wood Science Center (WWSC) is a Swedish multidisciplinary research and competence platform with a long-term perspective on the development of more advanced materials based on new technologies.

Wood is viewed as a source of well-defined material components of higher value. Wood tissue consists of a composite containing cellulose, hemicelluloses and lignin (Figure 1). Covalent bonds between lignin and different polysaccharides form closely associated structures known as lignin-carbohydrate complexes (LCCs). As a result, the successful extraction of native wood polymers poses a major challenge for material biorefinery concepts. WWSC aims to develop process schemes that allow separation and fractionation of wood components in the form of well-defined wood polymers (Figure 1). The processing steps need to be mild enough to largely preserve the native wood polymer structure and LCCs need to be disintegrated.

Enzymes that target lignin-carbohydrate (LC) bonds are especially useful for biorefinery applications as they can facilitate the isolation of individual wood components (e.g. xylan) in combination with mild chemical treatments.

The main LC-bonds present in wood are believed to be esters, benzyl ethers and phenyl glycosides. [1,2] Glucuronoyl esterases (GEs) have been proposed to degrade ester bonds between glucuronic acid residues present as side chains on wood xylans and lignin alcohols. GEs were first described in 2006 by Špániková and Biely [3] and belong to the carbohydrate esterase (CE) 15 family. CE15 representatives are present in the genomes of a wide range of fungi and bacteria.

The majority of the GEs reported in the literature are bimodular and consist of a catalytic domain, a linker region and an N–terminal family 1 carbohydrate binding module (CBM). [4] The major function of CBM is to enhance the enzymatic activity by binding towards insoluble substrates. [5] CBM1s are exclusively found in fungal cellulases and hemicellulases and help the enzymes bind to cellulose surface. Furthermore, it has been reported that CBM1s can contribute to enhance synergistic effects during hydrolysis of lignocelluloses. [6]

The overall aims of our study were to characterize new GE enzymes, to investigate their capacity in disconnecting hemicellulose from lignin and to apply them in the extraction process.

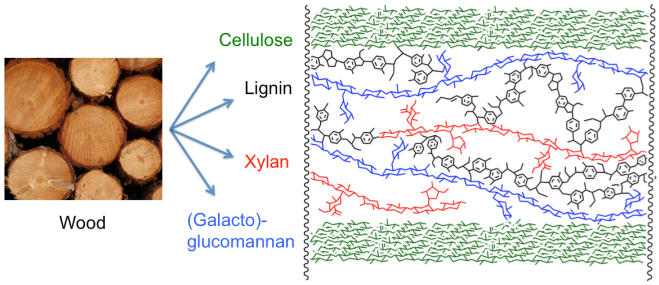

Figure 1. Schematic representation of secondary cell wall polymers in wood. For the general aim of WWSC, which is to generate new materials from trees, the efficient extraction of wood polymers is of great importance.

EXPERIMENTAL

Candidate genes homologous to known GEs were selected based on their phylogenetic position and habitat of source organisms. The selected candidate genes were codon optimized for the production system and obtained by synthesis. Putative GEs were cloned into pPICZα vector (Invitrogen) and expressed and secreted by the eukaryotic enzyme production host *Pichia pastoris*. Shake flask cultures were grown for 1 week at 30°C and 130 rpm in baffled flasks and expression was induced by addition of methanol to a concentration of 0.5% (v/v) every day. A HisPrep FF 16/10 column (GE Healthcare) was used with the ÄKTA system to

purify proteins containing a C-terminal polyhistidine (6xHis) tag from culture supernatants.

Purified enzymes were tested on model substrates (Figure 2), for which microtiter assays were established.

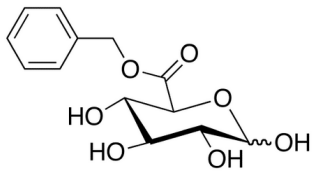

Figure 2. Benzyl-D-glucuronate used as model substrate for GE activity.

RESULTS AND DISCUSSION

Seven candidate genes encoding novel putative GEs from a diverse range of filamentous fungi were produced in *P. pastoris*. Expression of functional GEs was successful for three candidates as evidenced by esterase activity on the model substrate benzyl-D-glucuronate (Table 1). For those GEs that did not show activity, several bands were detected on a protein gel and three of the four candidates could also be detected by western blot using antibodies against the His-tag (data not shown). This indicates that three GEs were expressed and secreted, but were inactive under our conditions when using the model substrate. One GE was most likely not produced. No purification attempts were undertaken for inactive GEs.

The active GE enzymes were purified using a nickel column. Only one GE was purified in high yield (Table 1), while the two other enzymes remained mainly in the flow through. Alternative chromatographic purification methods (such as ion exchange chromatography or hydrophobic interaction chromatography) will be applied to those candidates.

Purified enzymes are tested on model substrates as well as LCC fractions and their applicability in wood processing is investigated.

Table 1. Glucuronoyl esterases (GEs) expressed and analysed in this study.

GE	CBM1[#]	active	purified
A2	+	+	+
L1	-	-	-
L3	-	-	-
L4	-	-	-
P8	+	+	-
S1	-	-	-
W8	-	+	-

[#]CBM: carbohydrate binding module;
+: present; -: absent

CONCLUSIONS

The current work shows that novel GE enzymes originating from a diverse range of microorganisms can be successfully produced using standard cloning techniques and secretion by the eukaryotic host *P. pastoris*. Screening for functional expression and characterization of novel enzymes requires the availability of model substrates. However, this can be problematic as novel enzymes, even though related to enzymes with known function, might differ in their substrate specificity and therefore might not be active on the available model substrates.

It also became obvious that standard His-tag purification can pose a challenge for certain enzymes and successful purification depends on the individual enzyme candidate.

One of the seven candidates represents a GE, which can be produced and purified in sufficient amounts for further characterization. This promising candidate is further investigated for its action on LCC fractions and will be evaluated for its potential applicability in wood processing.

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REFERENCES

[1] Balakshin, M. Y.; Capanema, E. A.; Chang, H.-M. *Holzforschung*, 2007, **61**, 1–7.

[2] Watanabe, T. *Trends in Glycoscience and Glycotechnology*, 1995, **7**, 57–68.

[3] Špániková, S.; Biely P. *FEBS Letters*, 2006, **580**, 4597–4601.

[4] d'Errico, C.; Jørgensen, J. O.; Krogh, K. B. R. M.; Spodsberg, N.; Madsen, R.; Monrad R. N. *Biotechnology and bioengineering*, 2015, **112**(5), 914-22.

[5] Várnai, A.; Mäkelä, M. R.; Djajadi, D. T.; Rahikainen, J.; Hatakka, A.; Viikari, L. In: *Advances in Applied Microbiology*, Chapter Four - Carbohydrate-Binding Modules of Fungal Cellulases: Occurrence in Nature, Function, and Relevance in Industrial Biomass Conversion, Volume 88, Editors Sima Sariaslani and Geoffrey Michael Gadd, Academic Press, 2014.

[6] Inoue, H.; Kishishita, S.; Kumagai, A.; Kataoka, M.; Fujii, T.; Ishikawa, K. *Biotechnology for Biofuels*, 2015, **8**, 77.