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Hemicellulase production in *Chrysosporium lucknowense* C1

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ABSTRACT

Filamentous fungi are widely used for enzyme production for the biofuel industry. The ascomycetous fungus *Chrysosporium lucknowense* C1 was isolated as a natural producer of neutral cellulases. It is at present an attractive alternative to well known fungi like *Aspergillus* sp. and *Trichoderma reesei* for protein production on a commercial scale. Besides many cellulases, a large number of hemicellulases (particularly xylanases and arabinofuranosidases) and esterases (acetyl xylan esterases and ferulic acid esterases) encoding genes have also been identified in the C1 genome. Many of these extracellular enzymes have been selectively expressed in C1 and then purified and characterized. Four arabinofuranosidases, two acetyl xylan esterases, two ferulic acid esterases, an α -glucuronidase and four xylanases have been purified and characterized. All these enzymes were found to be active towards arabinoxylans, demonstrating the high potential of C1 as a producer of hemicellulolytic enzymes.

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1. Introduction

Currently, a strong drive is manifest towards decreasing the CO₂ emissions into the atmosphere. A strategy to achieve this is the substitution of fossil fuels by alternatives derived from biomass, of which the production of bioethanol is an example. Leading countries in that area are Brazil and the USA where bioethanol is produced from sugar cane and corn, respectively (Budney and Sotero, 2007; Renewable Fuels Association, 2008). In Europe, wheat is mostly used as a feedstock for the bioethanol production. The processes used for both corn and wheat are based on the hydrolysis of the starch present in the crops, for sugar cane the sucrose is used. These so-called first generation bioethanol processes are interfering with human consumption of these crops. Therefore, alternative sources of biomass would be needed, such as the use of the 'by-products' of starch or sugar production. Ethanol produced from

residual plant materials is also referred to as second generation bioethanol.

These waste streams contain the fibrous material of the plants, which is left after removal of starch or sugar. The main compounds of this material are cellulose, hemicellulose and lignin (their amounts differ for each crop) besides some remaining starch or sucrose. The key challenge to use this biomass successfully for bioethanol production is to hydrolyze the polysaccharides into monomeric sugars in an economically feasible way. These sugars can then be fermented by yeast into ethanol.

The cellulose and hemicellulose are imbedded in the cell wall of plants and form a tight network with each other and with the lignin (Kabel et al., 2007a; Lam et al., 2001). To enzymatically hydrolyze the cellulose and/or hemicellulose a pretreatment is needed to open up the rigid structure of the cell wall (Kabel et al., 2007b; Lynd, 1996). Once opened up, the cellulose can be degraded by endoglucanases, cellobiohydrolases and β -glucosidases to glucose (Beldman et al., 1985), which can be fermented by yeast (*Saccharomyces cerevisiae*) to ethanol. Xylan has a much more diverse structure than cellulose and thus many more enzymes are needed to degrade the molecule completely to monosaccharides. The main enzymes needed for depolymerization are xylanases, assisted by accessory enzymes such as β -xylosidases and different arabinofuranosidases making the xylan backbone more accessible (Kormelink et al., 1993a; Sørensen et al., 2007; Van Laere et al., 1997). Other enzymes able to make the xylan backbone more

Abbreviations: AEC, anion exchange chromatography; Azo-WAX, azo-wheat arabinoxylan; C1, *Chrysosporium lucknowense*; CEC, cation exchange chromatography; GH, glycosyl hydrolase family; HIC, hydrophobic interaction chromatography; HPAEC, high performance anion exchange chromatography; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SEC, size exclusion chromatography.

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Table 1
Annotated hydrolase encoding genes from *Chrysosporium lucknowense* C1 with a possible activity towards cellulose and arabinoglucuronoxylans compared to the annotated genes in *Aspergillus niger* and *Trichoderma reesei*. GH: glycosyl hydrolases, CE: carbohydrate esterases.

Annotated enzyme	CAZY family ^a	Number of enzymes in C1	Number of enzymes in <i>A. niger</i> ^b	Number of enzymes in <i>T. reesei</i> ^c
β-Galactosidases	GH2	5	6	2
β-Glucosidases/β-xylosidases	GH3	11	17	12
Endoglucanases	GH5	8	10	2
Endoglucanases/cellobiohydrolases	GH6	3	2	2
Endoglucanases/cellobiohydrolases	GH7	6	2	3
Xylanases	GH10	4	1	1
Xylanases	GH11	7	4	4
Endoglucanases/xyloglucanases	GH12	1	4	1
1,3-Glucanases/xyloglucanases	GH16	8	13	11
1,3-Glucanases	GH17	4	5	–
α-Galactosidases	GH27	2	4	8
Arabinases/arabinofuranosidases/β-xylosidases	GH43	10	10	2
Arabinofuranosidases	GH51	2	2	–
Cellulases ^d	GH61	24	7	9
Arabinofuranosidases	GH62	2	1	1
α-Glucuronidase	GH67	1	1	1
Xyloglucanase	GH74	1	1	1
Exo-arabinases	GH93	2	–	–
Acetyl xylan esterases/ferulic acid esterases	CE1	6	3	n.i. ^e
Acetyl xylan esterases	CE4	4	7	n.i.
Acetyl xylan esterase/cutinase	CE5	3	5	2 ^f
Total		114	105	62

^a CAZY: carbohydrate-active enzymes (<http://www.cazy.org>).

^b Data were extracted from the CAZY database.

^c Data were extracted from the JGI database (<http://genome.jgi-psf.org/Trire2/Trire2.home.html>) and Martinez et al. (2008).

^d Function of these proteins is not completely clear.

^e Ferulic acid esterases were absent, acetyl xylan esterases were not indicated by Martinez et al. (2008), n.i.: not indicated.

^f Data extracted from Foreman et al. (2003).

degradable by xylanases are acetyl xylan esterases (Kormelink et al., 1993b; Poutanen et al., 1990), ferulic acid esterases (De Vries et al., 2002; Faulds and Williamson, 1995) and α-glucuronidases (De Vries et al., 1998; Golan et al., 2004; Heneghan et al., 2007). The structure of xylan differs highly for different plant materials and can be even more complex than described here (Allerdings et al., 2006; Saulnier et al., 1995). Complete hydrolysis of the xylan will result mainly in xylose and arabinose, which cannot be fermented by conventional yeasts. A genetically modified yeast or other C5 sugars fermenting micro-organisms is needed to produce ethanol from these monosaccharides (Kuyper et al., 2005; Watanabe et al., 2007; Wiedemann and Boles, 2008; Zhang et al., 1995).

Many enzyme mixtures for hydrolysis of (hemi)cellulose are currently produced by filamentous fungi, like *Trichoderma reesei* or *Aspergillus* sp. (Sørensen et al., 2005). In this research, a different fungus was used for the production of (hemi)cellulases, namely the ascomycetous fungus *Chrysosporium lucknowense* C1 (referred to as C1). This fungus was found to express by nature a broad range of cellulases and hemicellulases that degrade plant cell walls (Emalfarb et al., 2000, 2001, 2003). Industrial strains of C1 have been developed (Verdoes et al., 2007) and the full genome of C1 has been sequenced (unpublished data), which revealed many genes encoding cellulases, hemicellulases (like xylanases and arabinofuranosidases) and accessory enzymes (like acetyl xylan esterases and ferulic acid esterases). Many of these enzymes have been selectively expressed in C1 in order to purify and characterize them. In this publication an overview will be given of the hemicellulolytic potential of C1 regarding the degradation of plant cell wall material. Details of the described enzymes will be given elsewhere.

2. C1 as a production system

C1 was isolated from alkaline soil from the Far East of the Russian Federation. This original isolate was subjected to a strain and

process improvement program, in order to increase the cellulase production level. The resulting strains show, besides more efficient cellulase secretion, also a strong reduction in culture viscosity due to a morphology change. At a certain stage during the fermentation, the mycelium fragments into small elements, which are known as propagules resulting in a low culture viscosity. This results in better nutrient and oxygen transfer in fermentations and therewith in much higher protein production (Burlingame and Verdoes, 2006). Due to this morphology change it is also possible to use the strain for high throughput screening purposes (Verdoes et al., 2007). The fermentation conditions of this C1 strain are versatile. The fungus grows over a broad pH (4.5–8.8) and temperature range (25–44°C) and has short fermentation cycles. At present, a full genetic toolbox is available for C1, resulting in a range of different production strains, like for example strains with low protease background and strains with selectively enhanced productivity.

Since C1 was isolated as a natural cellulase producer, the initial research focused on the cellulase spectrum present in C1. Several endoglucanases and cellobiohydrolases have been isolated from the fermentation broth of the fungus and have been characterized in detail (Bukhtojarov et al., 2004; Gusakov et al., 2005). Nowadays, a great part of the research is also devoted to the hemicellulases present in C1, which resulted in the isolation of six xylanases (Ustinov et al., 2008). Complex mixtures of purified C1 enzymes have been developed (Gusakov et al., 2007) as well as several dedicated C1 strains, which produce a tailor-made mixture of enzymes designed to saccharify biomass. Both the mixtures of purified enzymes and the mixtures produced in genetically engineered C1 strains, demonstrate high saccharification performance on different cellulosic substrates.

3. C1 genome information

In 2005, the full genome of C1 has been sequenced, which revealed the presence of 11,000 genes. Due to confidentiality

Table 2
Enzymes overproduced in C1 with a proven activity towards glucuronoarabinoxylan.

Enzyme	CAZy family ^a	Enzyme code	Activity
Arabinofuranosidase	GH62	Abf1	Release of O-2 or O-3 substituted arabinose from mono-substituted xylose
Arabinofuranosidase	GH62	Abf2	Release of O-2 or O-3 linked arabinofuranosyl from mono-substituted xylose
Arabinofuranosidase	GH51	Abf3	Release of O-2 or O-3 linked arabinofuranosyl from mono-substituted xylose
Arabinofuranosidase	GH43	Abn7	Release of O-3 linked arabinofuranosyl residues from double-substituted xylose
α -Glucuronidase	GH67	Agu1	Release of 4-O-methyl-D-glucuronic acid from wheat arabinoxylan oligosaccharides, when located at the non-reducing end.
Acetyl xylan esterase	CE5	Axe2	Release of acetyl groups from acetylated arabinoxylan oligosaccharides. One acetyl ester remains on every oligosaccharide, at the non-reducing end.
Acetyl xylan esterase	CE1	Axe3	Release of acetyl groups from acetylated arabinoxylan oligosaccharides. One acetyl ester remains on every oligosaccharide, at the non-reducing end.
Ferulic acid esterase	CE1	FaeA1	Release of ferulic acid from wheat bran and corn AIS oligosaccharides. High affinity towards methyl ferulate and methyl sinapate, low affinity towards methyl caffeate.
Ferulic acid esterase	CE1	FaeB2	Release of ferulic acid from wheat bran and corn AIS oligosaccharides. High affinity towards methyl ferulate and methyl caffeate, low affinity towards methyl sinapate. Release of ferulic acid from sugar beet pulp AIS oligosaccharides.
Endo-xylanase	GH10	Xyl4	Release of arabinoxylan oligosaccharides from wheat arabinoxylan and oat spelt arabinoxylan, which resemble the ones released by known GH10 enzymes.
Endo-xylanase	GH11	Xyl6	Release of arabinoxylan oligosaccharides from wheat and oat spelt arabinoxylan, which resemble the ones released by known GH11 enzymes.
Endo-xylanase	GH11	Xyl7	Release of arabinoxylan oligosaccharides from wheat and oat spelt arabinoxylan, which resemble the ones released by known GH11 enzymes.
Exo-xylanase	GH11	Xyl10	Release of xylose and xylobiose from wheat and oat spelt arabinoxylan

^a CAZy: carbohydrate-active enzymes (<http://www.cazy.org>).

purposes, sequence information has not been published yet. Automated annotation showed that about 200 genes were putatively encoding carbohydrate-active enzymes, which indicated the high potential of C1 to degrade plant cell wall material. An overview of the putative enzymes involved in cellulose and/or hemicellulose degradation is indicated in Table 1. The enzymes are classified into families according to Henrissat (1991). The data for C1 were compared to other industrially used fungi, *Aspergillus niger* and *Trichoderma reesei*. These latter data were obtained from the CAZy (<http://www.cazy.org>; Cantarel et al., 2009) and JGI database (<http://genome.jgi-psf.org/Trire2/Trire2.home.html>; Martinez et al., 2008), respectively. Table 1 shows that C1 is very rich in (glucurono)arabinoxylan degrading enzymes compared to *Aspergillus* and *Trichoderma*, especially reflected by the number of xylanases and arabinofuranosidases. Eleven xylanases (families GH10 and GH11) were found in C1 compared to five in both *Aspergillus* and *Trichoderma*. Fourteen arabinofuranosidases (family GH43, GH51 and GH62) were found in C1 compared to thirteen in *Aspergillus* and three in *Trichoderma*. These data indicate that C1 is a promising source of hemicellulolytic enzymes. With regard to potential cellulases (families GH5, GH6, GH7, and GH12), C1 is comparable to *Aspergillus*. The large number of putative GH61 proteins in C1 is striking, although it is not completely clear what this function may be. They are presumed to stimulate other cellulases by preventing non-specific binding to the substrate (Karlsson et al., 2001; Merino and Cherry, 2007). It is remarkable that the number of cellulases in *Trichoderma* is lower than in C1 or *Aspergillus*.

A large number of the annotated genes have been cloned into a specially designed C1-expression host that allows production of relatively pure enzyme in lab scale fermentations. The strains were grown aerobically in 2 L fermenters in mineral medium under glucose limitation at pH 6 and 32°C. Many of the produced enzymes have been shown to be active towards (glucurono)arabinoxylan.

4. Characteristics of individual hemicellulases from C1

Table 2 shows a summary of the C1 enzymes that have been selectively produced in the described C1-expression host. They have been purified and were found to be active towards xylan.

4.1. Arabinofuranosidases

Arabinofuranosidases are enzymes that can hydrolyze arabinosyl linkages from various hemicelluloses such as arabinans, arabinoxylans, arabinogalactans and arabinose-substituted xyloglucans. The arabinoxylan, as present in the cell walls of grasses and cereals, consists of linear chains of β -D-(1,4)-linked D-xylopyranosyl residues, which can be substituted with α -L-arabinofuranosyl residue at the O-2 and/or O-3 position(s) (Brillouet et al., 1982; Gruppen et al., 1993). Currently, seven arabinofuranosidases have been selectively produced, purified and characterized. Four of them were found to release arabinose from wheat arabinoxylan polymers and oligomers, of which three (referred to as Abf1, Abf2 and Abf3) only removed arabinose residues linked to the O-2 or O-3 position of mono-substituted xylose residues. Fig. 1 shows the HPAEC profile after digestion of wheat arabinoxylan oligomers by Abf1. The oligosaccharides were prepared by enzyme incubation of wheat arabinoxylan (Megazyme) with a GH10 xylanase from *Aspergillus awamori* (Kormelink et al., 1993a). The HPAEC profiles of Abf2 and Abf3 activity were similar to that of Abf1 and were therefore not shown. Abf3 was found to have an optimal pH of 5.0, but is active in the pH range of 3.0–8.0. All three arabinofuranosidases were found to be different in molecular weight and iso-electric point, which will be elaborated in a future publication.

Arabinoxylans from cereals contain single and double-substituted xylose residues. The arabinofuranosidases described before, were not able to act on these double-substituted residues. However, to achieve a complete hydrolysis of arabinose residues, it

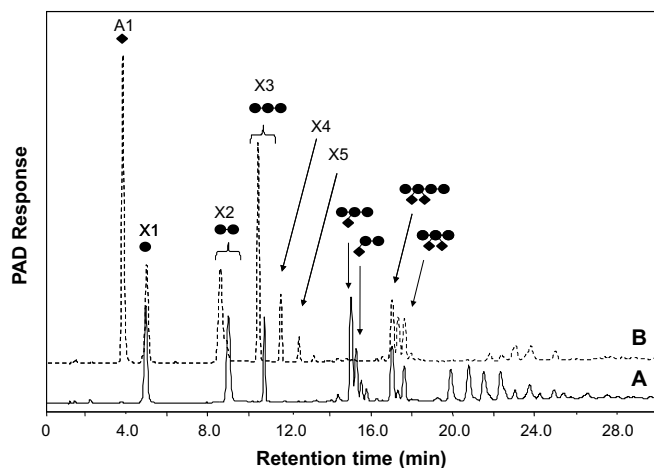


Fig. 1. HPAEC elution profile of wheat arabinoxylan hydrolyzed by a GH10 xylanase from *Aspergillus awamori* (Kormelink et al., 1993a) at 35°C during 24 h (A) and subsequently digested with arabinofuranosidase Abf1 from *Chrysosporium lucknowense* C1 at 35°C during 24 h (B). Arabinose is indicated by \blacklozenge and xylose by \bullet .

will be necessary to use an enzyme that is able to remove these arabinose residues that are double-substituted to one xylose residue. In the literature, two such enzymes have been described (Sørensen et al., 2006; Van Laere et al., 1997). It was found that C1 also expressed an arabinofuranosidase that is able to cleave only arabinose residues when attached to the O-3 position of a double-arabinose-substituted xylose residue (referred to as Abn7). Abn7 has an optimal pH of 5.0 and is active in a pH range of 4.0–8.0.

Three other arabinofuranosidases expressed in C1 (referred to as Abn1, Abn4 and Abn5) were found to be specifically active towards arabinan and not towards arabinoxylan. Because cereal biomass does not contain high amounts of arabinans, these enzymes will not be discussed in this paper.

4.2. Esterases

The annotated C1 genome indicated the presence of several putative esterase encoding genes, like acetyl xylan esterases and ferulic acid esterases (Table 1).

Acetyl xylan esterases are enzymes that are able to hydrolyze the ester linkage between acetyl and xylose residues in (arabino)xylans. Two purified acetyl xylan esterases (referred to as Axe2 and Axe3) were both found to release all acetyl groups except one from acetylated xylan oligosaccharides from Eucalyptus wood (Fig. 2), which were kindly provided by Prof. J.C. Parajo, University of Vigo, Spain. This resistant acetyl ester was found to be located at or near the non-reducing end of the oligosaccharide (evidence not shown). This suggests that the esterases are able to cleave all acetyl ester linkages as long as they are not close to the non-reducing end. However, it cannot be ruled out at this moment that the acetyl group of the xylose residue at the non-reducing end, migrated to the O-4 position (Kabel et al., 2003), which might not be attacked by the acetyl xylan esterases. The specific activity of these enzymes towards oligosaccharides was higher than towards polysaccharides, but 80% of the total amount of the acetyl esters is cleaved off in both cases for both enzymes. The optimum pH of both acetyl xylan esterases was found to be 7.0. They quickly lost their activity at pH values lower than 4.5. The optimum temperature was found to be 40°C. Both acetyl xylan esterases were found to have differences in molecular weight and iso-electric point, indicating that these are two different enzymes. More details of the enzyme characteristics will be published elsewhere.

Cereal arabinoglucuronoxylans contain ferulic acid residues, which are connected to the O-5 position of arabinose residues (Mueller-Harvey and Hartley, 1986; Saulnier et al., 1995; Smith and Hartley, 1983). Feruloyl esterases can release ferulic acid and to a lesser extent coumaric acid from xylans or pectins. These esterases can be divided into 4 groups, namely A–D. The main difference between groups A and D is their substrate specificity towards synthetic substrates and their capability of liberating diferuloyl bridges (Crepin et al., 2004). Benoit et al. (2008) introduced another classification of ferulic acid esterases, which is based on amino acid sequence homology. Characterization of two ferulic acid esterases from C1 indicated that one ferulic acid esterase (referred to as FaeA1) was a group A ferulic acid esterase, whereas the other (referred to as FaeB2) was a group B ferulic acid esterase. This was based on their activity towards methyl ferulate, methyl sinapate, and methyl caffeate. Both enzymes were active towards wheat bran AIS oligosaccharides, whereas only the group B esterase was also active towards sugar beet pulp AIS oligosaccharides. Fig. 3 shows the activity of FaeB2 towards both wheat bran AIS oligosaccharides and sugar beet pulp AIS oligosaccharides. Wheat bran AIS oligosaccharides were prepared by enzyme incubation of wheat bran Alcohol Insoluble Solids (AIS) with a GH10 xylanase from *A. awamori* (Kormelink et al., 1993a). Sugar beet pulp AIS oligosaccharides were prepared by enzyme incubation of sugar beet pulp AIS with Rapidase liq+ (DSM). So far, none of the enzymes has been tested on methyl coumarate or di-ferulic acid, therefore it is not possible to distinguish between groups A/D and group B/C. Based on the classification of Benoit et al. (2008), FaeA1 seems to have highest homology with ferulic acid esterases from subfamily 5 and FaeB2 with enzymes from subfamily 6. Both enzymes have a pH optimum of pH 7.0 and are not active at all at pH 4.0. Further characterization of these enzymes is in progress and will be published elsewhere.

4.3. α -Glucuronidase

Arabinoxylans from cereals or hard wood contain glucuronic acid residues, which are linked via an α -1,2-glycosidic linkage to the xylose residues of the backbone. These glucuronic acid residues can be methylated at their O-4 position. Several α -glucuronidases have been described to remove glucuronic acid or 4-O-methylglucuronic acid from (arabino)xylan oligosaccharides and these enzymes were highly specific (De Vries et al., 1998; Golan et al., 2004; Margolles-Clark et al., 1996).

A potential α -glucuronidase gene (encoded by *agu1*) was found in the genome sequence of C1. Expression of this gene, followed by enzyme purification and characterization, revealed that this enzyme was able to form unsubstituted xylo-oligosaccharides after digestion of Eucalyptus wood (glucurono)xylo-oligosaccharides and aldo-tetrauronic acid, suggesting that it was removing the 4-O-methylglucuronic acid residues. The aldo-tetrauronic acid used was not completely pure since besides two isomers, smaller xylo-oligomers containing a glucuronic acid residue were also shown to be present in the HPAEC profile (data not shown). The activity of the enzyme was judged from the release of monomeric glucuronic acid and unsubstituted xylo-oligomers. From the present results, it is not clear whether it is also able to release non-methylated glucuronic acid. Saponification of the substrate (removing the acetyl esters) before incubation with the α -glucuronidase increased the amount of glucuronic acid. This suggests that the activity of *Agu1* was hindered by the presence of acetyl esters located on or close to the uronic acid. The optimal pH of *Agu1* was found to be 6.0 and the optimal temperature was found to be 50°C. At pH 4.0 the activity was only 50% of the activity found at pH 6.0.

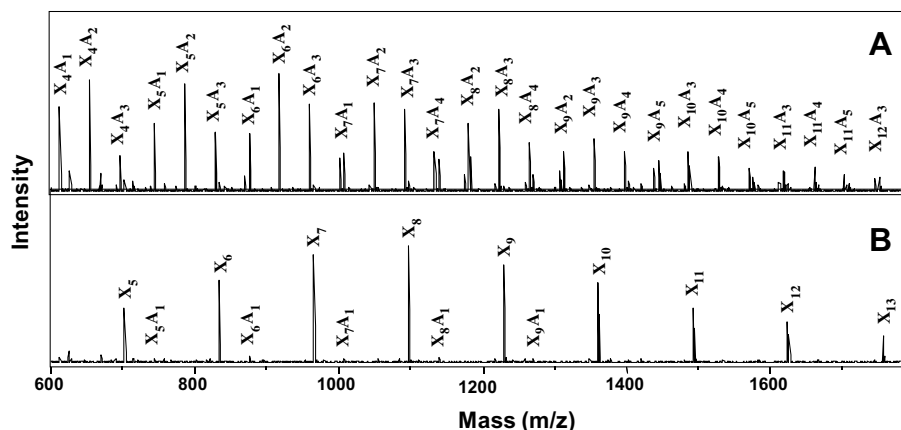


Fig. 2. MALDI-TOF mass spectrum of acetylated xylan oligomers from Eucalyptus wood before (A) and after (B) incubation with acetyl xylan esterase Axe2 at pH 7 and 40°C during 24 h. Xylose is indicated with X and acetyl esters are indicated with A. Only the oligosaccharides with the mass range of 600–1800 are indicated.

4.4. Xylanases

Genome sequence analysis and annotation revealed the presence of 11 xylanases. Six of these xylanases have been purified directly from the culture supernatant of C1. These have been characterized partly (Ustinov et al., 2008). All xylanases are also overexpressed selectively in C1 and are in the process of being purified and characterized in more detail. All crude xylanase preparations were found to be active towards the substrate Azo-WAX, except xylanase Xyl10. This latter enzyme has been classified as a GH11 endo-xylanase based on its amino acid sequence, but it contained only a very low activity towards Azo-WAX, which is a substrate commonly used to determine endo-xylanase activity.

Four out of the eleven xylanases have been tested for their activity towards oat spelt and wheat (arabino)xylan. The reaction products have been analyzed by HPAEC. Two of these xylanases, Xyl6 and Xyl7 were found to release different types of arabinoxylan oligosaccharides and a small amount of xylose. The degradation products formed were comparable to the degradation products formed by a known family GH11 enzyme from *A. awamori* (Kormelink et al., 1993a). After 24 h of incubation the amount of especially xylose and xylobiose had increased. The arabinoxylan oligosaccharides formed remained the same during the incubation. The same results were obtained when oat spelt xylan was used.

These results indicate that the enzymes possess endo-xylanase activity similar to family GH11 enzymes, which confirms the annotation of the genes based on amino acid sequence homology. Both Xyl6 and Xyl7 were found to differ in molecular weight and iso-electric point, indicating that these are really two different enzymes. More details will be given in a separate publication. A third xylanase, Xyl4, showed endo-xylanase activity which was similar to known family GH10 enzymes (Kormelink et al., 1993a). Xyl10, which had been classified as a GH11 xylanase, was found to release mainly xylose monomers after 1 h of incubation, instead of the expected oligomers related to other GH11 enzymes. A small amount of xylobiose was formed as well. After 24 h of incubation the amount of both xylose and xylobiose had increased, while still no other oligosaccharides had been formed. As discussed before, Xyl10 had a low activity towards Azo-WAX. Combining these results, it is suggested that this xylanase possesses exo-xylanase activity as will be further substantiated in ongoing research.

5. Future perspectives

Using the sequencing and annotation data as a lead to characterize individual (hemi)cellulases showed that C1 has a great potential as a producer of industrial enzyme cocktails. Currently several C1 preparations are available commercially, but these are particularly focusing on cellulase performance. The development of new products for use in biomass degradation with the aim to produce bioethanol is continuously ongoing. By studying the properties of individual enzymes expressed by C1, knowledge is being gathered to specifically improve enzyme cocktails and design new dedicated ones. The same development is going on for hemi-cellulases. However, the structure of arabinoxylan is very complex and diverse in different plant materials, which makes it difficult to develop one mixture for all arabinoxylan rich biomass. Our aim is to gain knowledge on the characteristics of the many enzymes expressed by C1 and combine these data to design an optimal mixture for the selected biomass. At the same time, the production system of the fungus is being developed in such a way that it will express the enzyme mixture needed in one fermentation run.

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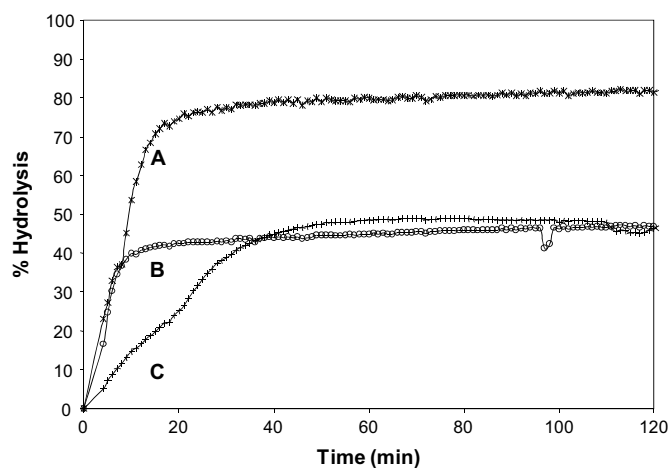


Fig. 3. Activity of the type B feruloyl esterase FaeB2 from C1 towards wheat bran AIS oligomers (A), corn AIS oligomers (B) and sugar beet pulp AIS oligomers (C) at pH 7 and 35°C during 120 min. The release of ferulic acid is expressed as a function of the total amount of ferulic acid present in solution according to Ralet et al. (1994).

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