

## Recent advances in developing *Myceliophthora thermophila* C1 enzymes for the degradation of (hemi-)cellulose

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### Introduction

Dyadic develops the filamentous fungus *Myceliophthora thermophila* C1, an ascomycete which was previously classified as *Chrysosporium lucknowense* as a proprietary protein expression platform for the efficient production of enzyme mixtures for the degradation or modification of lignocellulosic biomass. Originally this fungal strain was isolated as a producer of neutral cellulases, but genome sequencing revealed that the organism also has a high xylanolytic potential. Currently, the genomic knowledge of C1 is being exploited.

### Genome information of C1

The genome of C1 wild-type strain VKM F-3500-D was initially sequenced in 2005 by Sanger-sequencing and recently re-sequenced by a paired-end approach using the 454 pyro-sequencing technology. Both data sets were assembled into a new C1 draft genome. The genome size was approximately 38.5 Mbp. The data revealed a large number of plant cell wall degrading enzymes: more than 200 genes were identified encoding cellulases, hemicellulases and accessory enzymes (Table 1). C1 distinguishes itself from other industrial fungi (e.g. *Trichoderma reesei* and *Aspergillus niger*) by the presence of a relatively high number (26) of GH61 enzymes. Members of this family enhance the degradation of cellulosic materials and are proposed to be redox-active hydrolytic enzymes (oxidohydrolases), that require a reducing environment for optimal activity. A possible source of reducing equivalents is cellobiohydrolase (CDH) of which at least three types are present in C1. In addition, arabinan degrading enzymes<sup>1,2</sup> and (glucurono) xylan degrading enzymes<sup>4,5</sup> are abundantly present. Xylan degrading enzymes are needed for the efficient hydrolysis of the hemicellulose fraction of lignocellulosic feedstocks used for the production of biofuels and biochemicals.

**Table 1.** Overview of putative (hemi-)cellulase encoding genes that are present in the C1 genome, based on manual annotation.

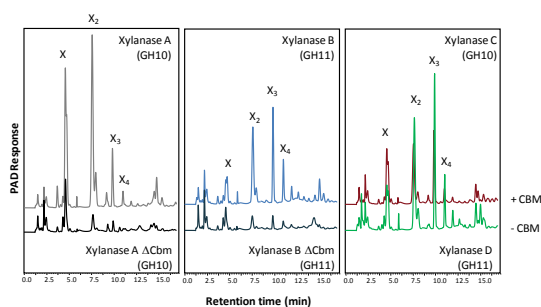
Putative activity	Number of genes in C1
β-Glucosidases	7
Endo-glucanases + CBH	13
GH61	26
CDH + CDH-like	9
β-Xylosidase	5
Xylanase	13
Arabinoxylan hydrolases (AXH)	7
Esterases (AXE + FAE)	13
α-Glucuronidases	2
Arabinases + arabinofuranosidases	7

### Production and characterization of mono component enzymes

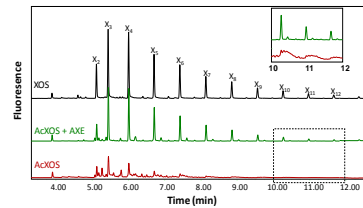
A library of over 70 single glycosyl hydrolases or carbohydrate esterases has been obtained by selective over-expression of each corresponding gene. Many of them have been tested for the modification or saccharification of different complex (hemi-)cellulosic substrates.

### Xylan degrading enzymes

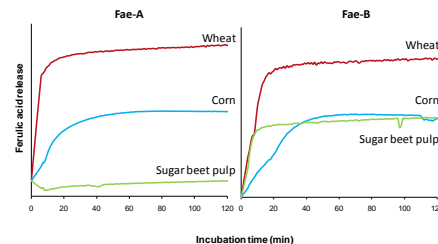
Xylan is, after cellulose, the most abundant biopolymer in the plant kingdom. Its chemical structure is complex and enzymatic degradation or modification of this structure requires many different enzymes. C1 produces the complete spectrum of enzymes to degrade glucurono(arabino)xylan, such as thirteen different xylanases that all have different substrate specificities or mode of action, of which an example is shown in Figure 1. Beside the xylanases also a large spectrum of accessory enzymes, such as arabinoxylan hydrolases, α-glucuronidases and esterases, is present in C1<sup>3-5</sup>. Examples of esterases are shown in Figure 2 and 3. A C1 acetyl xylan esterase (AXE) removes almost all acetyl groups from acetylated xylooligosaccharides (AcXOS), resulting in linear xylooligosaccharides (Figure 2). The action of two feruloyl esterase, Fae-A and Fae-B, is shown in Figure 4. Fae-A is able to release ferulic acid from oligosaccharides derived from wheat and corn, whereas Fae-B is also able to release ferulic acid from sugar beet pulp oligosaccharides.



**Figure 2** HPAEC diagrams of four different xylanases (of which two with and without CBM) after incubation on wheat straw at 50°C and pH 5 during 24 h. X = xylose, X<sub>2</sub> = xylobiose, X<sub>3</sub> = xylootriose, X<sub>4</sub> = xylootetraose



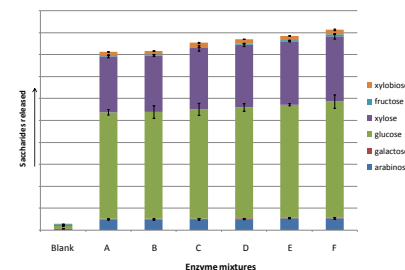
**Figure 3.** CE electropherograms of saponified xylooligosaccharides (XOS) and acetylated xylooligosaccharides (AcXOS) digested by C1 AXE. Experiments were performed at pH 7.0 and 40°C. The inset shows a magnification of the graph between 10 and 12 min. X<sub>n</sub> indicates a xylo-oligomer containing n xylosyl residues.



**Figure 3.** Ferulic acid release by two C1 feruloyl esterases on oligosaccharides derived from wheat (red line), corn (blue line) and sugar beet pulp (green line). Experiments were performed at pH 7.0 and 35°C.

### Development of complete enzyme mixtures

Insights obtained from the study of synergistic interaction between the different C1 (hemi-)cellulases allowed development of efficient enzyme mixtures tailored for the complete saccharification of ligno-cellulosic feedstock (Figure 4, as an example). This figure shows the saccharification efficiency of a C1 (hemi-)cellulase mixture alone (A) or in combination with three different mono component C1 xylanases. Addition of one or a combination of xylanases improve the amount of monosaccharides released. This is not only reflected in the xylose release, but also in the glucose release.



**Figure 4.** Addition of a (combination) of xylanase(s) improve the release of mono- and disaccharides after saccharification with a complete C1 cellulase/hemicellulase enzyme mixture. Experiments are carried out on mildly pretreated wheat straw at 50°C and pH5 during 24 h. A: C1 cellulase/hemicellulase mixture; B: C1 mixture + Xylanase E; C: C1 mixture + Xylanase A; D: C1 mixture + Xylanase A + E; E: C1 mixture + Xylanase A + Xylanase F; F: C1 mixture + Xylanase A + Xylanase E + Xylanase F.

### Conclusions

- C1 is rich in carbohydrate active enzymes, particularly hemicellulose degrading enzymes.
- All thirteen C1 xylanases act with different substrate specificity or mode of action.
- Different esterases are present in C1, which all differ in their specificity and mode of action.
- Tailor made enzyme mixtures are designed and produced in C1 that efficiently hydrolyse different lignocellulosic feedstocks.

### Acknowledgements

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### References

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