

## Development of a *Chrysosporium lucknowense* enzyme library

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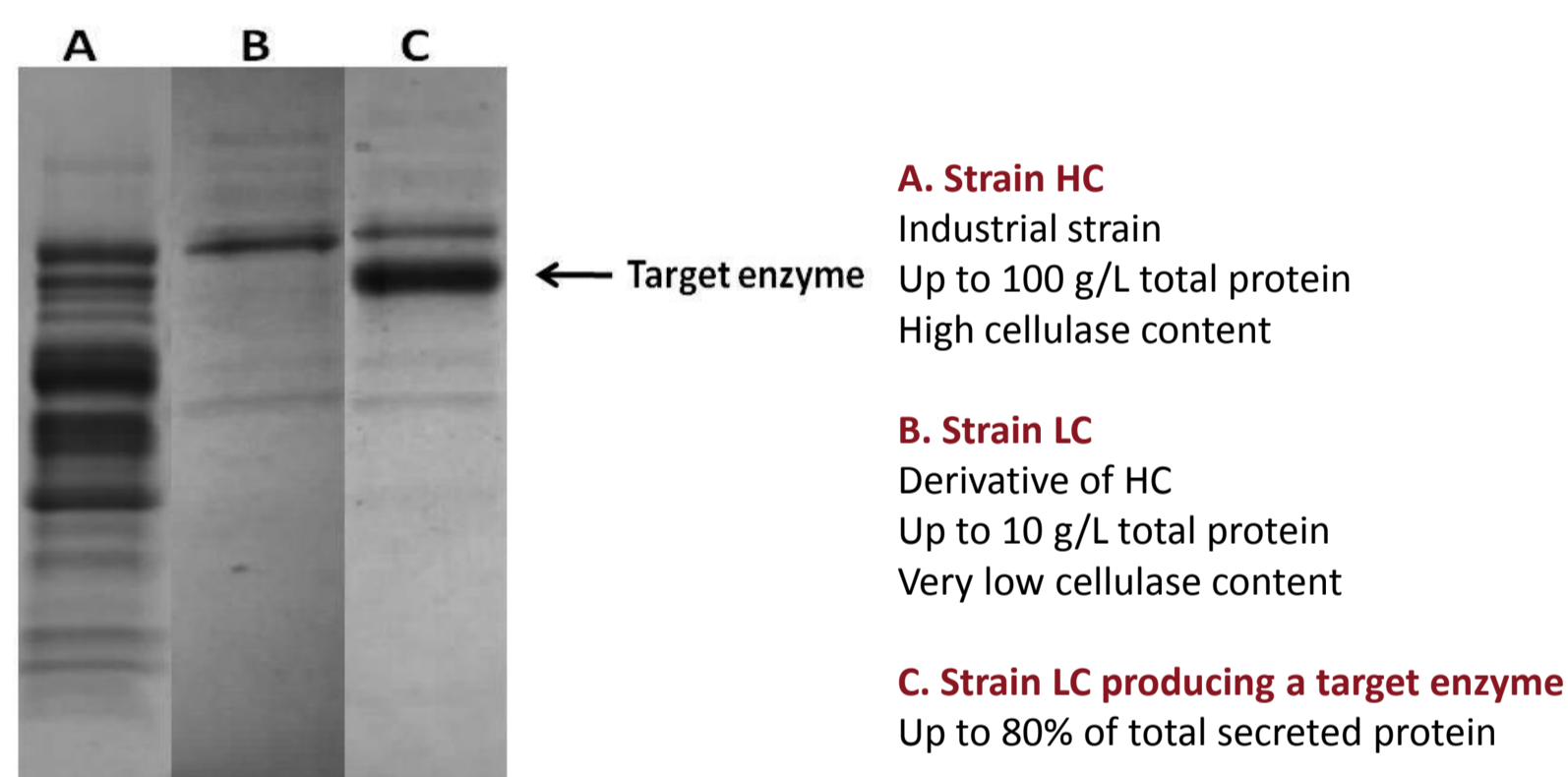
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### Introduction

Dyadic Netherlands owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the hyperproduction of a broad variety of enzymes. The C1 genome was sequenced and annotated, revealing numerous potential product opportunities. These include, amongst others, versatile enzymes for the food & feed industry and enzymes for the production of biofuels from renewable (non-starch) feed stocks. Here, we describe the production by one strain of relatively pure homologous and heterologous enzymes in the g/L range for characterization and development of tailored enzyme mixtures for various applications.

### C1 genome sequencing revealed many industrially relevant genes

Sanger sequencing of the 38 Mbp C1 genome and its automated annotation was performed in 2005. In 2009, C1 was re-sequenced (454-technology) yielding a higher quality sequence database. An impressive hydrolytic potential was revealed. Approximately 250 genes were assigned to encode carbohydrate-active enzymes belonging to a wide variety of glycoside hydrolase, carbohydrate esterase, polysaccharide lyase and glycosyl transferase families. Many of these putative enzymes can be used in a variety of industrial applications degrading or modifying plant material.



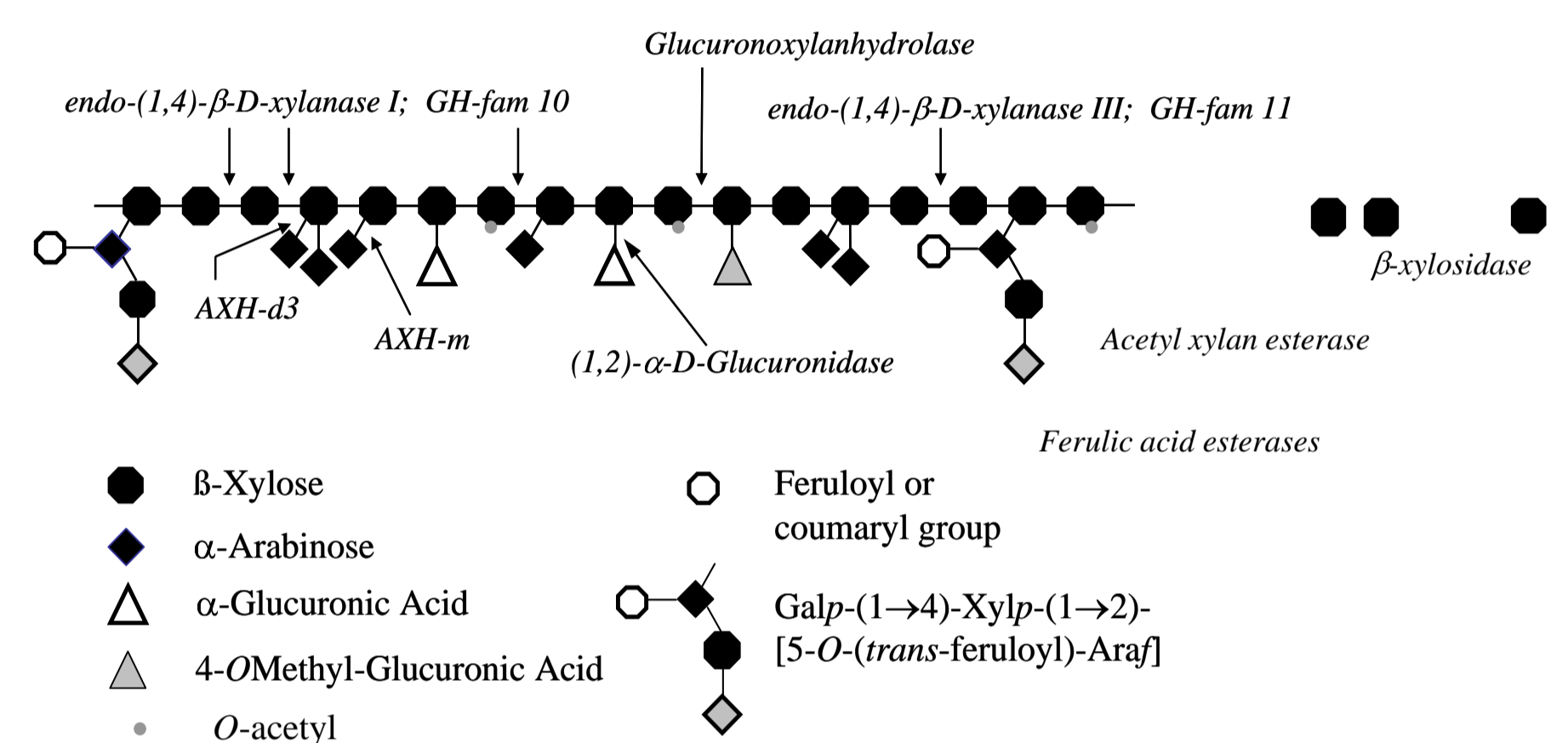
**Figure 1.** Comparison of the extracellular proteins profiles from C1 production strain HC (Lane A), low background strain LC (Lane B) and low background strain including a target enzyme (Lane C) by SDS-PAGE analysis of culture filtrates.

### Construction of enzyme library

For characterization purposes these genes are individually over-expressed in a special low cellulase background C1-strain, named LC (Fig. 1). This low protein background makes the LC strain an ideal host for the production of single enzymes, minimizing the downstream processing procedure. A collection of over 70 functional homologous enzymes and several heterologous proteins have been successfully produced (Table 1) with production levels reaching up to 8 g/L of the desired protein and purity levels up to 80% (Fig. 1).

**Table 1.** Number of enzymes successfully produced in LC strain classified by their activities.

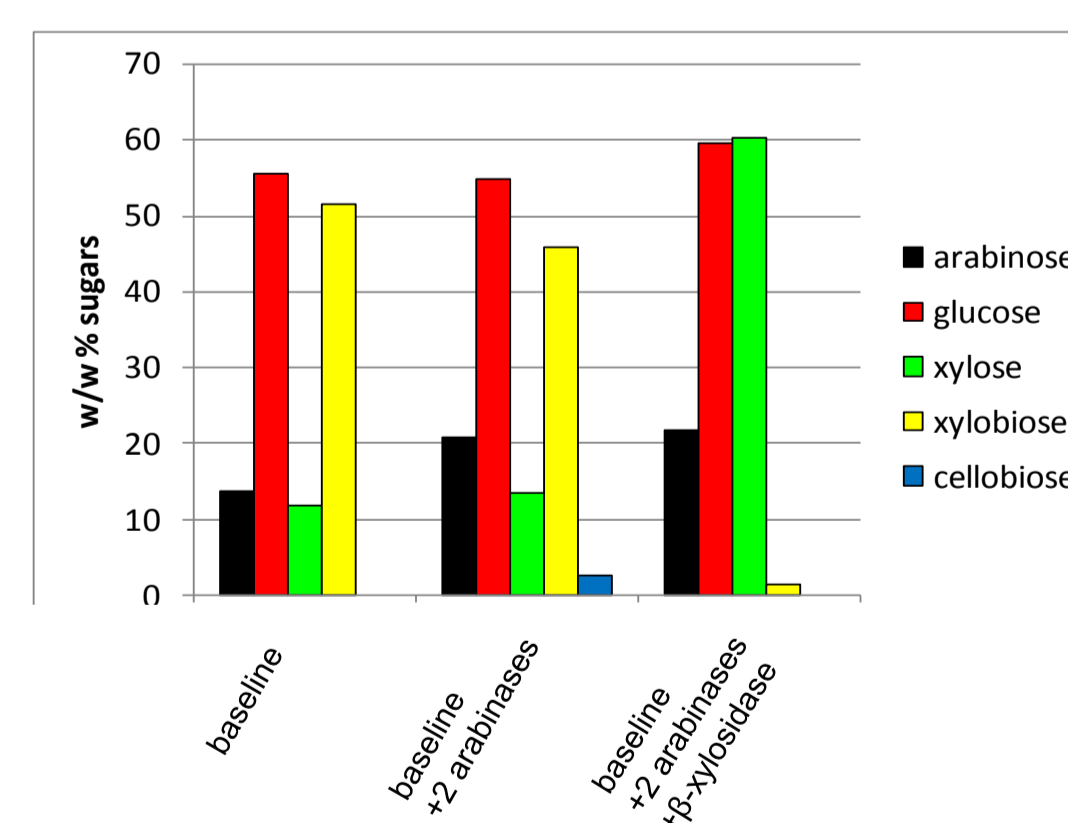
Enzymes produced	#
Cellulose degrading enzymes	19
Xylan degrading enzymes	34
Mannan degrading enzymes	4
Pectin degrading enzymes	16
Others	3



**Figure 2.** Model of arabino-xylan backbone as can be found in plant cell walls. Enzymes necessary for the complete hydrolysis of the backbone are indicated.

### Tailored enzyme mixtures

Many of these enzymes have been purified and characterized in detail making this enzyme library a unique tool for the development of tailored enzyme mixtures. For example, a diverse set of enzymes is needed to completely hydrolyze arabino-xylan from plant biomass (Fig. 2). If analysis of hydrolysis products reveals the presence of unaffected glycoside bonds then corresponding hydrolases are supplemented to the baseline enzyme mixture in order to increase the saccharification efficiency. This approach was applied for instance to the hydrolysis of pretreated wheat bran (Fig. 3). The baseline enzyme mix supplemented with the purified enzymes from the enzyme library show an increased release in glucose, xylose as well as arabinose. Thus an enzyme mixture was developed for second generation biofuel processes that very efficiently hydrolysed the arabino-xylan fraction of wheat-bran into fermentable sugars.



**Figure 3.** Graph showing the release of monosugars after 72h of saccharification on pretreated wheat-bran at 50°C. The baseline enzyme mix supplemented with the purified enzymes from the enzyme library show an increased release in glucose, xylose as well as arabinose.

### Conclusions

A library of over 70 functional enzymes has been constructed many of which have been purified and characterized in detail. Mixing these enzymes based on their characteristics provides a unique tool for the development of tailored enzyme mixtures for applications in various industrial processes, such as paper & pulp, textile, food & feed, brewing and second generation biofuel processes.

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Reference: Hinz, S. W. A. et al, 2009. Hemicellulase production in *Chrysosporium lucknowense* C1. *J. Cereal Sci.* doi:10-1016/j.jcs.2009.

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