

## Development of a *Chrysosporium lucknowense* C1 host for single enzyme production

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### Introduction

Dyadic owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the hyper production of a broad variety of enzymes. Cellulases are the main class of extracellular enzymes secreted by C1 production strains. Here, we describe the development of a low cellulase background *Chrysosporium lucknowense* C1 strain, which proved in particular to be useful for the targeted production of specific single enzymes at high yields.

### Facilitated enzyme purification

Filamentous fungi, particularly species such as *Aspergillus niger*, *A. oryzae*, *Trichoderma reesei* and recently also *Chrysosporium lucknowense* C1, can produce large quantities of extracellular enzyme mixtures. Purification of individual enzymes from these mixtures for enzyme studies is laborious and often results in low yields of pure enzyme. An alternate approach is to use an enzyme production host, which shows the capacity to secrete high levels of a specific protein in a low extracellular protein background level. This minimizes the downstream processing procedure and makes it relatively easy to obtain pure enzymes.

### Isolation of a low-cellulase background C1 strain

C1 strain HC, a hyper producer of cellulases and other carbohydrate active enzymes, was subjected to UV-mutagenesis. Extensive screening yielded a mutant (LC) devoid of its cellulase enzyme spectrum. Culture filtrates of HC and LC were compared for cellulase activity. It was shown that the remaining cellulase activity of LC was only about 1-2% of the level of its parental strain HC (Table 1). Profiling of the extracellular protein content by SDS-PAGE showed a strong reduction of the number of proteins present (Fig. 2).

Table 1. Comparison of cellulase activities of HC and low-cellulase background strain LC.

Strain	AzoCMCase (U/ml)
HC	278-294
LC	4-6

### Further optimization strain LC

In order to further reduce the extracellular protein background, some major proteins (i.e. a chitinase and an alkaline protease) were removed by means of targeted disruption of the corresponding genes (Fig. 1).

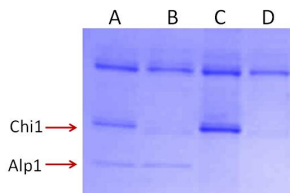


Figure 1. Reduction of extracellular protein content of LC strains by targeted gene disruptions. A, strain LCprt; B, strain LCprt  $\Delta$  *chi1*; C, strain LCprt  $\Delta$  *alp1*; D, strain LCprt  $\Delta$  *alp1*  $\Delta$  *chi1*. Chi1, chitinase. Alp1, alkaline protease.

### Production of single enzymes: functional characterization of C1's biomass converting potential

C1-genome sequencing and mining revealed an impressive hydrolytic enzyme potential. A large set of putative (hemi-) cellulase encoding genes have been discovered. Remarkable differences were observed when compared to other fungi. An example of a subset of these genes is given in Table 2. Strain LC was used as host for the production of high amounts of individual (hemi-) cellulases (Fig. 2) enabling the purification and functional characterization of these enzymes. As such, an extensive library of over 70 C1 carbohydrate active enzymes was constructed. This library represents a unique tool for the development of tailored enzyme mixtures.

Table 2. Comparison of the C1, *Aspergillus niger* and *Trichoderma reesei* genomes with respect to the number of a subset of putative (hemi-)cellulase encoding genes. GH, CAZY glycoside hydrolase family number. CE, CAZY carbohydrate esterase family number.

	C1	<i>A. niger</i> *	<i>T. reesei</i> **
Cellulases (GH3, 5, 6, 7, 12)	~ 29	~ 35	~ 26
Cellulases (GH61)	~ 24	~ 7	~ 9
Cellulose binding domains (CBM1)	~ 46	~ 8	~ 11
Xylanases	~ 11	~ 5	~ 5
Arabinofuranosidases/arabinases	~ 14	~ 13	~ 3
Esterases (Axe, Fae)	~ 10	~ 10	~ 2

\* From the CAZY database

\*\* From the JGI database and literature

### Heterologous proteins

Strain LC has also been successfully used in the production of certain heterologous proteins (data not shown). However, some heterologous proteins were degraded by proteases after being produced and secreted. An inventory of potential harmful proteases was made using genome mining and MS analyses. Proteases belonging to catalytically different type of peptidase families were identified. Similar to the background proteins, harmful proteases were disrupted by targeted gene knock-out techniques.

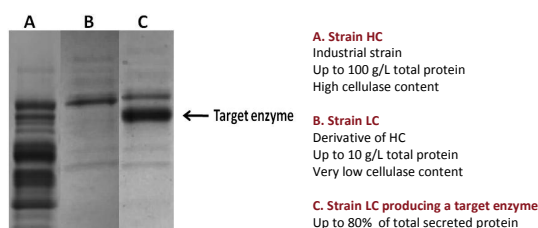


Figure 2. Protein profiles of HC and LC strains as analyzed by SDS-PAGE.

### Future work

We continue to improve strain LC by disruption of genes encoding (remaining) major background proteins and proteases. Furthermore, we focus on increasing the protein production levels and supplementing the enzyme library with new enzymes.

### Conclusions

- Low-cellulase background *Chrysosporium lucknowense* strains, LC, were developed for the production of single proteins in a relatively pure form.
- The LC strains provide a powerful tool for enzyme characterization studies and development of mono-component enzymes and tailored enzyme mixtures.

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