

Chrysosporium lucknowense C1: A novel fungus for the commercial production of efficient cellulases and hemi-cellulases.

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Dyadic International, Inc. owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the hyper production of a broad variety of enzymes. These include versatile enzymes for the production of biofuels from alternative (non-starch) renewable feedstocks.

Chrysosporium lucknowense C1

Production strains of *Chrysosporium lucknowense* (C1) have been developed that produce very large amounts of protein. These strains display a compact mycelium morphology during fermentation, referred to as propagules (Fig. 1).

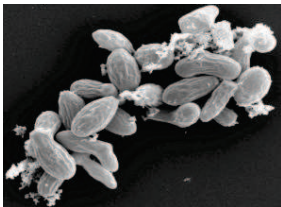


Fig. 1. C1 propagules by scanning microscopy.

C1 Fermentation

The propagule morphology allows for robust controlled fermentation with high protein yields, low energy input, short cycle times (Fig. 2, Table 1). C1 can be applied in large scale industrial fermentations up to 150,000 litres using relatively inexpensive and simple media.



Fig. 2. Labscale fermentation of C1 strains.

C1-tailored genetic tools

- Transformation system developed: high efficiency, stable integration.
- Gene expression: strong (inducible) expression signals.
- Self cloning (no foreign DNA).
- Protein production: efficient secretion signals.
- Targeted gene disruption possible.

C1-strain optimization

- Auxotrophic markers introduced.
- Low protease strains constructed.

Table 1. Influence of morphology on protein production. Values for parental strain NG7C-19 were set at 100%.

Strain	Viscosity (%)	Protein: Biomass ratio (%)	Protein yield (%)
NG7C-19	100	100	100
UV18-25	2	400	200
UV18-25 optimized	2	400	600

C1 genome sequencing project.

The sequencing and automated annotation of the C1-genome has been finished (Fig. 3). The obtained knowledge facilitates additional host strain improvements and revealed numerous potential product opportunities.

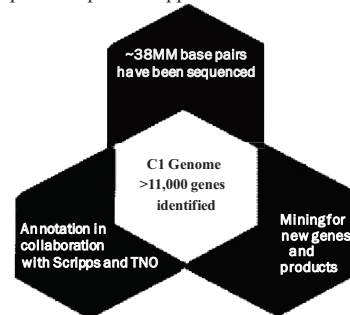


Fig. 3. C1 genome sequencing project.

C1's biomass converting potential.

C1-genome mining revealed an impressive hydrolytic potential. For example a large set of putative (hemi-)cellulase encoding genes have been discovered. Remarkable differences were observed in their number when compared to other fungi. An example of a subset of these genes is given in Table 2.

Table 2. Comparison of the C1 and the *Aspergillus niger* genome with respect to the number of a subset of putative (hemi-)cellulase encoding genes. *From The CAZy database.

Feature	<i>C. lucknowense</i>	<i>A. niger</i> *
Xylanases	11	5
Family GH61 endoglucanases	24	7
Cellulose Binding Modules (CBM1)	45	8

The molecular tools developed and the isolation of low protease C1 strains has created the opportunity to produce high amounts of individual (hemi-) cellulases (Fig. 4).

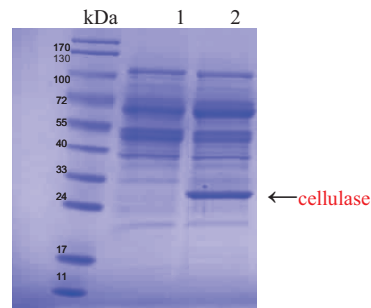


Fig. 4. An optimized C1 strain for the production of (hemi)cellulases. SDS-PAGE analysis of medium samples of a C1 host (lane 1) and a cellulase gene transformed C1 host (lane 2).

C1-cellulases obtained by (selective) over-expression and by purification have been tested for saccharification performance on cellulosic substrates.

Characterization of individual C1-cellulases.

Four cellobiohydrolases (CBHs) from C1 were purified and compared with purified CBH1 and CBH2 from *T. reesei* (Fig. 5). An almost 60% conversion of cellulose was achieved with only C1-CBH4 present.

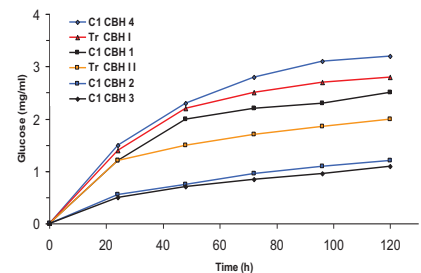


Fig. 5. Progress kinetics of Avicel (5 mg ml⁻¹) hydrolysis by purified cellobiohydrolases (0.1 mg/ml) in the presence of purified *Aspergillus japonicus* BGL (0.5 U ml⁻¹), 40°C, pH 5.0.

Hydrolysis by commercial and experimental enzyme mixtures.

Mixtures of C1 (hemi-) cellulases were composed (Table 3).

Table 3. Composition (mg/mL) of artificial multi-enzyme mixtures of purified C1 enzymes. All samples contained 0.5mg protein/mL.

Combination:	#1	#2	#3	#4
CBH1	0.2	0.2	0.2	0.1
CBH2	0	0	0	0.1
CBH4	0.2	0.2	0.2	0.2
EG2	0.08	0.07	0.04	0.03
EG5	0	0	0.04	0.04
BGL	0.02	0.02	0.02	0.02
XYL2	0	0.01	0	0.01

These mixtures were much more effective on cellulosic substrates than the commercial enzyme mixtures (Fig. 6).

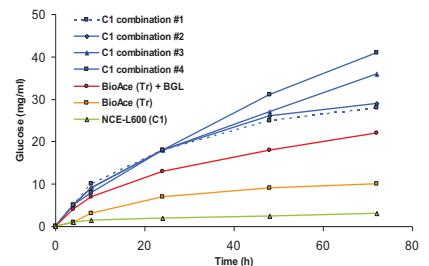


Fig. 6. Kinetics of hydrolysis of pretreated Douglas fir wood (50 mg/ml) by combinations of purified *C. lucknowense* enzymes (Table 3) and commercial crude cellulase preparations from C1 and *Trichoderma reesei* (Tr) each at protein loading of 0.5 mg/ml, 50°C, pH 5.0.

Conclusion

C. lucknowense C1 is a source of numerous (new) enzymatic activities, including enzymes for the conversion of (hemi-) cellulosic materials. C1 has been developed into a mature, robust and versatile production platform for these enzymes.

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