

Development of the filamentous fungus C1 as host for recombinant protein production

Theo Verwoerd^a, Vivi Joosten^a, Jurian Bronkhof^a, Jeffrey Bartels^a, Rob Joosten^a, Peter Punt^b, Hans Visser^a and Jan Wery^a

^aDyadic Netherlands, Nieuwe Kanaal 7-5, 6709 PA Wageningen, The Netherlands. ^bTNO Quality of Life, Utrechtseweg 48, 3700 AJ Zeist, The Netherlands

Introduction

Dyadic owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the production of a broad variety of enzymes. Here we describe the development of 1) strains that produce very high levels of cellulases and hemicellulases and 2) strains devoid of a cellulase background. The latter proved in particular to be useful for the production of specific single homologous and heterologous enzymes of interest in relatively pure form.

Development of (hemi-) cellulose hyper-producing strains

The C1 wild type strain was subjected to a strain development process in order to elevate the neutral cellulase productivity. A primary strain lineage was obtained (Fig. 1) yielding a high cellulase producing strain, HC (High Cellulase) that showed very low viscosity during fermentation due to a fragmented mycelia morphology. This strain produced up to 100 g/L of protein (Fig. 2) and was fermented at the industrial scale (150 m³) for commercial enzyme production.

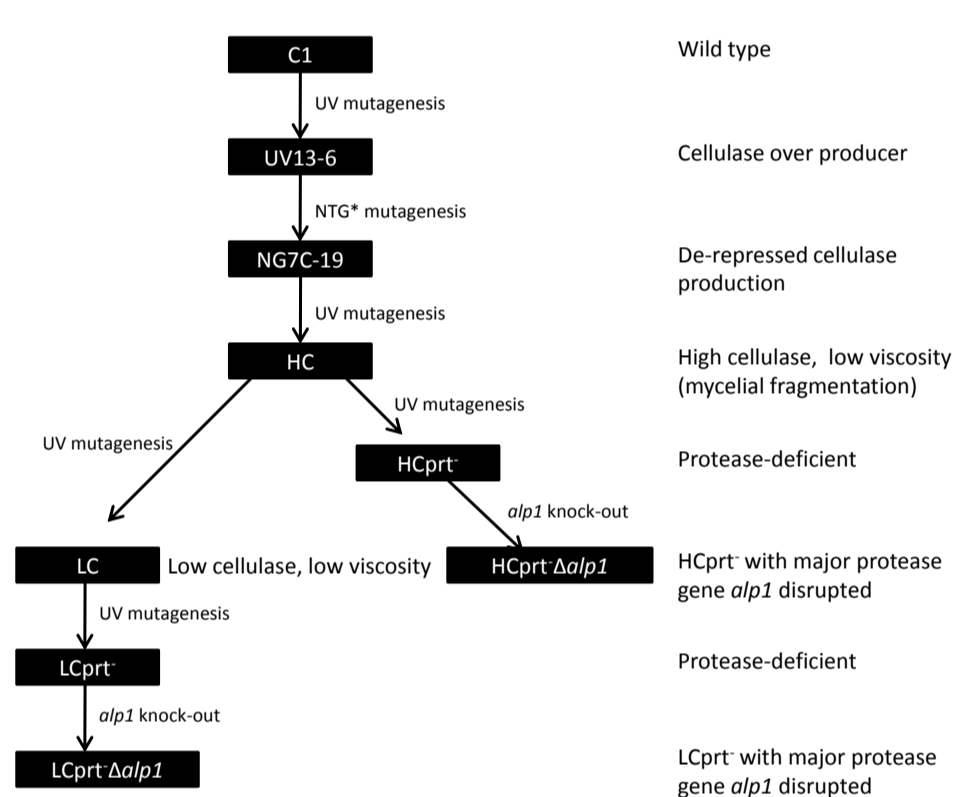


Figure 1. Partial C1 strain lineage. The wild type C1 strain was modified using random mutagenesis and recombinant DNA technology, yielding two types of strains, HC (High Cellulase) and LC (Low Cellulase). * NTG, N-methyl-N'-nitro-N-nitrosoguanidine.

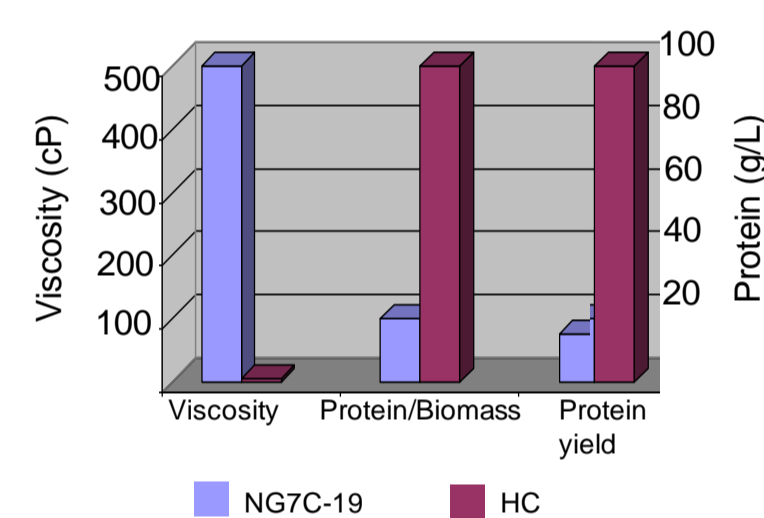


Figure 2. Comparison viscosity (in cP), protein/biomass and protein yields of the NG7C-19 and the HC strain.

Production specific homologous and heterologous proteins in C1

The C1 HCprt- strain was shown to be an excellent host for the production of a number of homologous and heterologous proteins. For example the expression of recombinant human IgG antibody against TNF α was assessed. Both the heavy and light chain encoding genes were expressed under control of the C1 *cbh1* promoter in HCprt-. Expression of biologically active IgG was achieved at the g/L level in the growth medium (Fig. 5). Furthermore, the recombinant antibody was not hyperglycosylated¹.

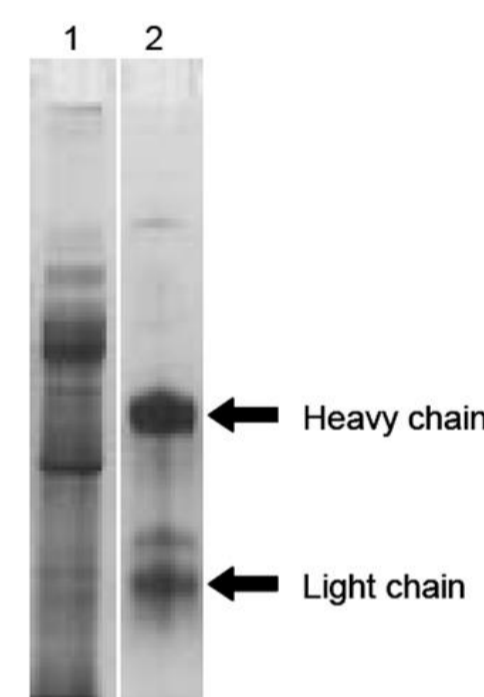


Figure 5. SDS-PAGE analysis of culture filtrate from a full length antibody expressing C1 strain. Lane 1 = Unpurified culture filtrate. Lane 2 = Protein A purified culture filtrate.

LC strains were used as host for the over expression of homologous proteins, in a relatively pure form, for functional characterization of these enzymes. A collection of over 75 functional homologous enzymes and several heterologous proteins have been successfully produced (Table 1) with production levels reaching up to 32 g/L of the desired protein and purity levels of up to 80% (Fig. 6).

Isolation of a low-cellulase background C1 strain

The HC strain was subjected to UV mutagenesis. Extensive screening yielded a mutant (LC; Low Cellulase background strain) devoid of its cellulase enzyme spectrum (Fig. 3). Culture filtrates of LC showed only about 1 - 2% of the cellulase activity level of its parental strain HC (from 278 - 294 to 4 - 6 Az-CMCase in U/ml), making it a very useful host for the production of relatively pure single enzymes of interest.

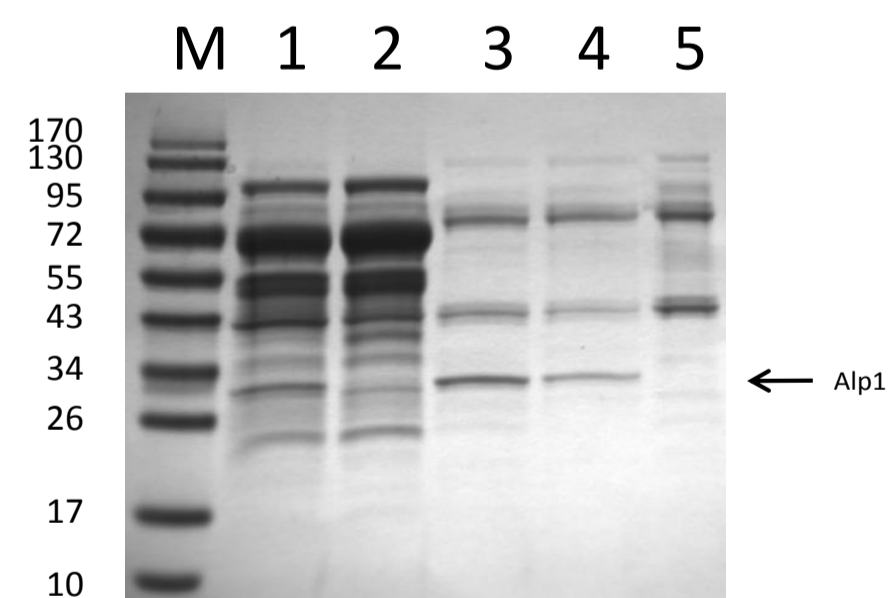


Figure 3. SDS-PAGE analysis of extracellular protein content of different C1 strains. 1, HC (high cellulase) 2, HCprt- 3, LC (low cellulase) 4, LCprt- 5, LCprt- Δ alp1 Molecular mass standard (kDa) Alp1, alkaline protease

Reduction of protease activity

Protease activity is a common aspect of fungal protein production and it may negatively affect the stability of enzymes. Both HC and LC strains were subjected to random and targeted approaches to reduce protease activity. C1 strains with greatly reduced overall protease activity levels (prt-) were obtained from both HC and LC strains (Fig. 3 and Fig. 4A and B).

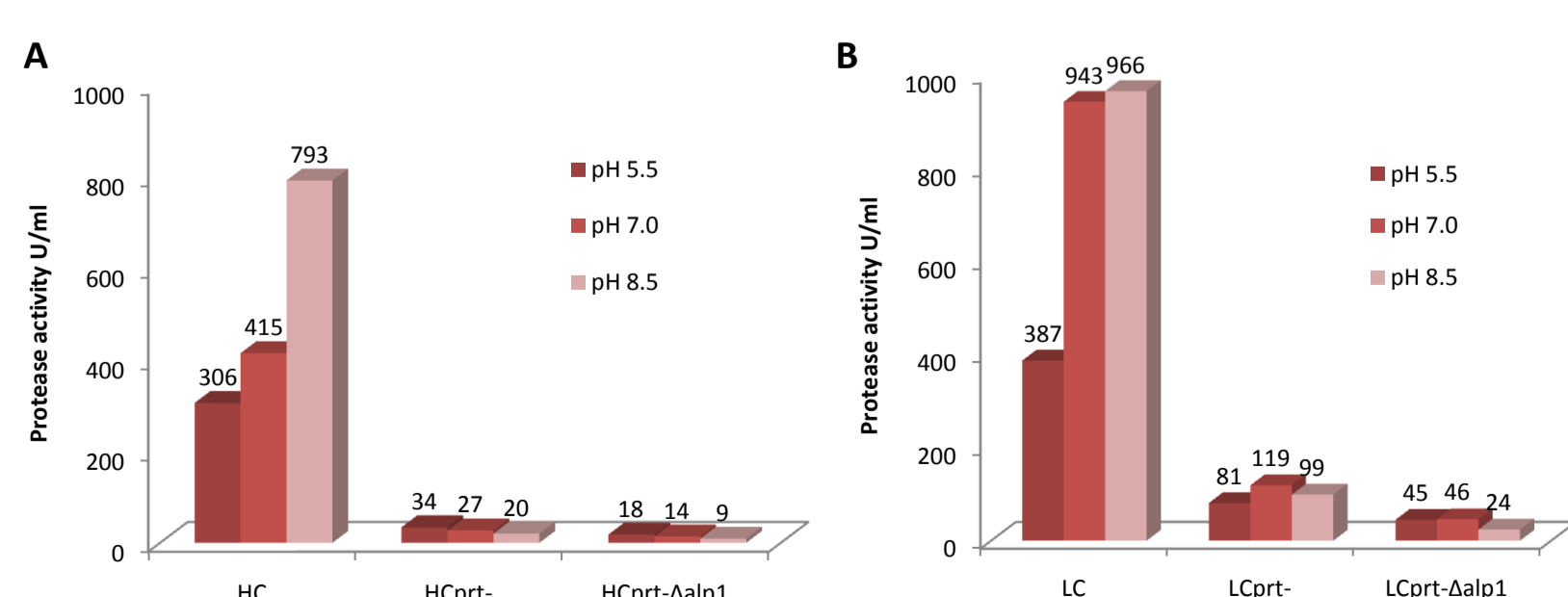


Figure 4. Protease activity (U/ml) in C1 HC (A) and LC (B) strains, assayed at different pH values. The activity was measured in end of fermentation samples. Δ alp1 refers to gene disruption of the major secreted protease in C1. Prt- refers to protease deficient.

Table 1. Examples of enzymes/proteins successfully produced in C1. Homologous enzymes are classified by their activities. Heterologous proteins were produced at the g/L scale.

Enzymes produced		Number
Homologous		
Cellulose degrading enzymes		23
Xylan degrading enzymes		34
Mannan degrading enzymes		4
Pectin degrading enzymes		14
Others		1
Heterologous		
Fungal	Xylanase, amylase, cellulase, endo-polygalacturonase, oxidase, phytase	
Bacterial	Xylosidase, cellulase	
Bacterial-directed evolution	Confidential	
Human	Immunoglobulin IgG1	

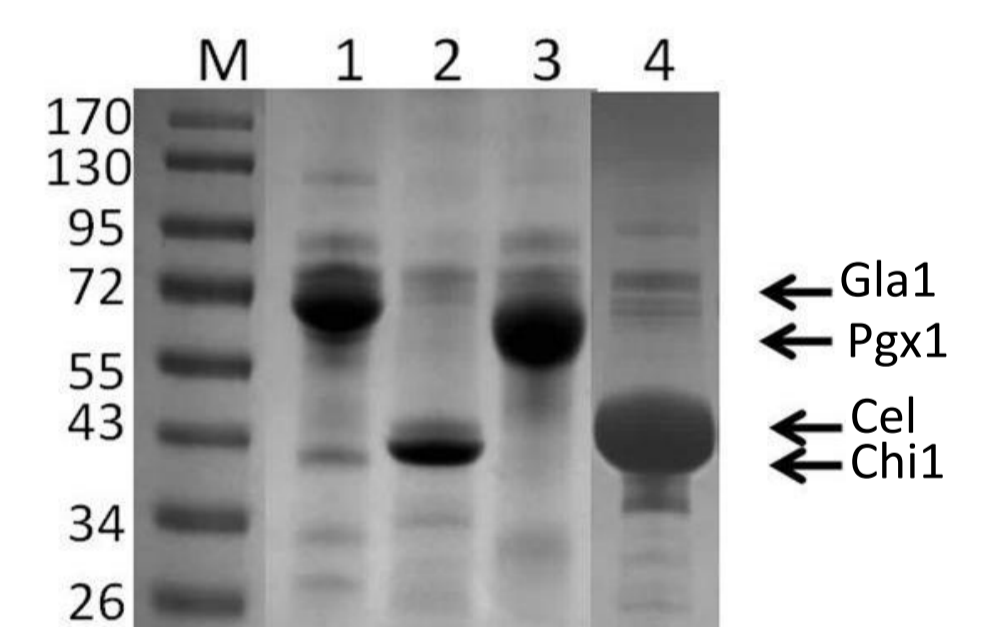


Figure 6. Secreted protein profiles for C1 LCprt- strains produced during fed-batch fermentation: M. molecular mass standard (kDa). 1. 70 kDa C1 glucoamylase (Glu1; 5.3 g/L) 2. 45 kDa chitinase (Chi1; 7.5 g/L) 3. 60 kDa exo-polygalacturonase (Pgx1; 7.4 g/L) 4. 44 kDa cellulase (Cel; 32 g/L)

Conclusions

- C1 was developed as a mature and versatile host for protein production.
- (hemi-) cellulase hyper-producing C1 strains (HC) have been developed and used for the commercial production of enzymes.
- Low-cellulase background C1 strains (LC) were developed for the production of single proteins in a relatively pure form.
- Low protease mutants of both HC and LC hosts have been generated, which has paved the way towards efficient production of heterologous proteins.

Contact: Dr. J. Wery (jwery@dyadic.nl)

References

¹Heerikhuisen et al. (2008) Poster presented by P. Punt during ECFG9 meeting (5 - 8th April 2008) Edinburgh, UK