# *Chrysosporium lucknowense*
# a versatile fungal host for gene discovery and protein production.

**Hans Visser[1], Peter Punt [2] and Jan Verdoes [1]**

[1] Dyadic Nederland BV, Wageningen, The Netherlands and [2] TNO Quality of Life, Zeist, The Netherlands

Researchers of Dyadic International, Inc. isolated the ascomycetous fungus *Chrysosporium lucknowense* (C1), which we present as a versatile and attractive alternative to other (fungal) protein production systems.

## C1 key features

### C1 morphology

A mutant C1 strain was obtained, which showed a changed morphology. The hyphal mycelium is fragmented during fermentation resulting in spore-like structures referred to as propagules (Fig. 1). This offers some benefits using C1.

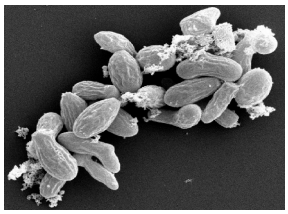

*Fig. 1. C1 propagules by scanning microscopy.*

C1 propagule cultures are compatible to liquid handling systems allowing high-throughput robotic screening of complex gene libraries for gene discovery purposes (Verdoes *et al*. 2007 Ind. Biotech. 3:48-57).

### Fermentation

The presence of propagules results in a low viscosity of the fermentation broth. As result, less energy is needed to obtain the required mixing and transfer of nutrients and oxygen.

The viscosity in fermentors of the low-viscosity C1 strain UV18-25 is 50-fold lower than its high-viscosity parent strain NG7C-19 (Table 1). Furthermore, the amount of protein produced per unit of biomass is 4-fold higher in strain UV18-25 than NG7C-19 under similar fermentation conditions (Table 1).

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

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

The protein yield of strain UV18-25 is twice the amount of strain NG7C-19 when grown under identical fermentation conditions. The lower viscosity of UV18-25 allowed the use of richer media and higher oxygen transfer rates, which resulted in a further 3-fold increase of the protein yield (Table 1).

C1 distinguishes itself also from other fungal systems by: (i) being able to produce and secrete proteins (e.g. cellulases) at neutral pH instead of the acidic conditions used in fermentations, and (ii) by shorter fermentation cycle times.

C1 is applied in large scale industrial fermentations up to 150,000 litres using relatively inexpensive and simple media.

## C1: a host for homologous and heterologous gene expression.

### Homologous gene expression

An efficient transformation system was developed based on homologous genes that complement auxotrophic mutants. Genes of interest are expressed using either strong constitutive or inducible promoters.

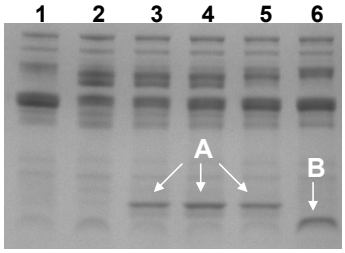


*Fig. 2. High g/L recombinant protein production yields in high protein background C1 strains. Lanes 1-2, empty strains. Lanes 3-5, overproduction of protein "A". Lane 6, overproduction of protein "B".*

By overproducing key components such as enzyme B (Fig. 2, lane 6), which is part of a multiple enzyme preparation, a significant increase in overall activity of the total enzyme preparation as well as better application performance per unit of activity is obtained (data not shown). The combined multiplicative effect results in significantly better production economics and performance characteristics.

### Heterologous gene expression: human full-length antibody production

Human heavy and light chains antibody genes, fused to a fungal glucoamylase encoding carrier gene for efficient secretion of the corresponding gene product, were expressed in C1 under control of the strong P*cbh1* promoter. The produced antibodies were purified using a protein A column (Fig. 3) and found to be of full-length and biologically active (data not shown). No obvious hyperglycosylation was observed; further glyco-analysis is in progress. Initial protein levels were 0.2 g/l.

### Heterologous gene expression: reduced protease activity.

In addition to the improved morphology and increased protein production yields, mutagenesis

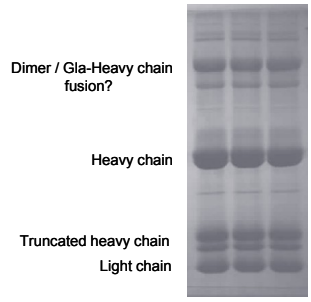


*Fig. 3. SDS-PAGE analysis of the protein A bound fraction from culture supernatant of a controlled fermentation of the antibody producing C1 strain.*

and recombinant DNA technology of C1 also resulted in a set of host strains with reduced protease activity. The goal of this set is to prevent proteolytic degradation of the target protein, which is illustrated in figure 4.

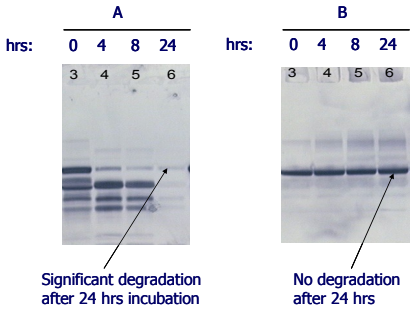


*Fig. 4. Incubation (24 hrs) of a commercial antibody with culture supernatant of a low protease C1 strain in which 1 (A) or 3 (B) protease encoding genes have been disrupted.*

Dyadic's C1 technology is a proprietary and patent-protected technology.

## Summary of C1 characteristics

- An integrated platform for screening, expression and production.
- Completely developed genetic toolbox.
- A large collection of proprietary strains as potential production hosts.
- High level of protein production and secretion at acidic to neutral pH.
- Relatively fast and low-viscosity fermentation.
- The C1 system is used on an industrial scale.
- Genes of different origins have been over-expressed.
- The C1 genome has been sequenced.
- Patent protected.

**For further information please contact:**
jverdoes@dyadic.com
gnedwin@dyadic.com