Protein Expression Examples

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Protein Expression: Capabilities and Successes at BTR

BTR has extensive experience expressing single and multiple, homologous and heterologous, genes for the production of proteins in Gram positive and Gram negative bacteria, yeast, and filamentous fungal expression hosts, including:

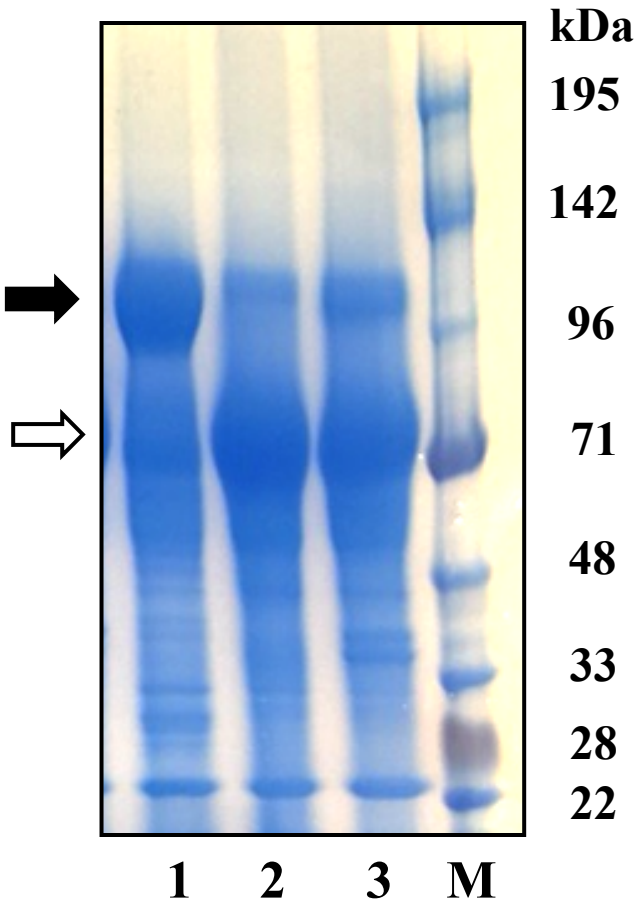
- ***Escherichia coli***
- ***Lactobacillus lactis***
- ***Bacillus subtilis***
- ***Bacillus licheniformis***
- ***Saccharomyces cerevisiae***
- ***Pichia pastoris***
- ***Hansenula polymorpha***
- ***Trichoderma reesei***
- ***Myceliophthora thermophila,***
  (previously known as *Chrysosporium lucknowense* C1)

BTR provides protein expression strain development services complemented by integrated fermentation process development, optimization, scale-up, and technology transfer services. A few examples are highlighted below

Example 1: Protein Expression, Secretion and Processing in *Trichoderma reesei*

- *T. reesei* is an attractive expression host:
  - Production of secreted protein as high as 100 g/Lwas reported in high cell density fermentation using inexpensive medium;
  - Protein in secreted form provides opportunity for low-cost down-stream processing
- BTR has demonstrated high expression and efficient secretion of a heterologous protein of interest (POI) as a functional enzyme using the promoter and secretion signal peptide from the cellobiohydrolase I gene (*cbh1*) and an non-antibiotic selection marker
- Compared to secreted protein samples of the untransformed host strain, density of the predominant band on SDS-PAGE (mostly CBH1) was dramatically diminished while the band of fusion protein CBH1 core-POI become the major band (Fig. 1)
- Insertion of a specific cleavage site between POI and its fusion partner allowed complete *in vivo* processing of the fusion protein into POI
- High level POI production was demonstrated in fed batch fermentation at 14-liter scale (Fig. 2)

Fig. 1 Secreted Protein in *T. reesei* Cultures

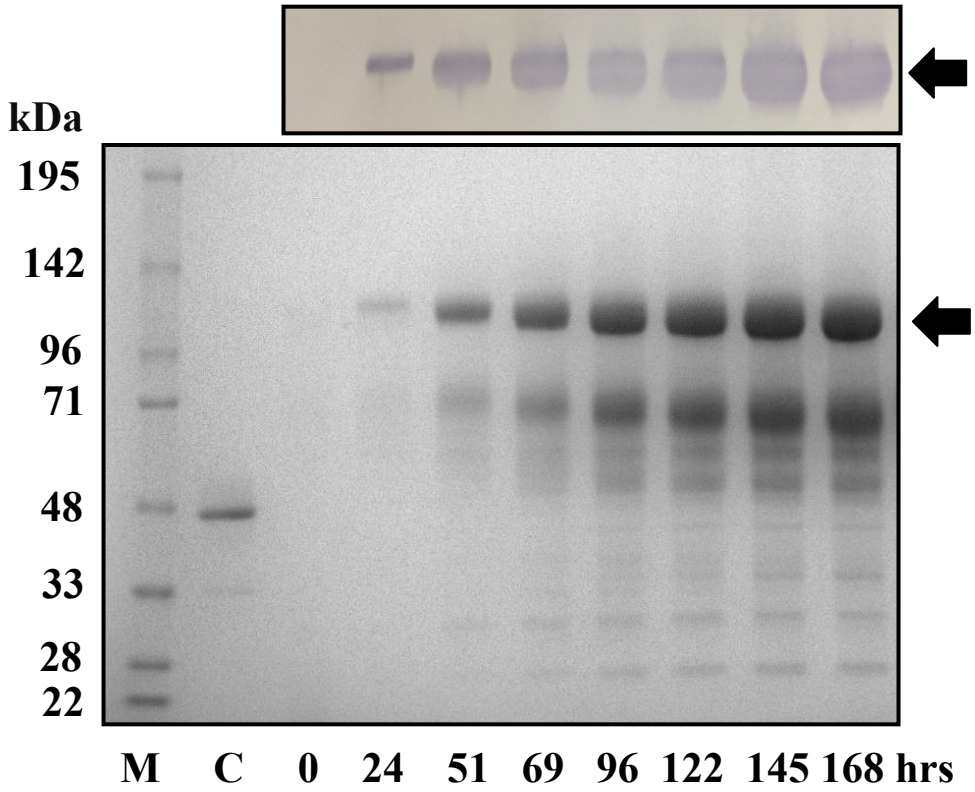


T. reesei strains were grown in a cellulose induction medium in shake flasks for 120 hrs.

Lane 1: BTR strain expressing a heterologous POI.
Lane 2: Untransformed host.
Lane 3: Control strain of significant POI expression.
M: RunBlue Prestained Markers (Expedeon).

- White arrow: Major secreted protein band in the medium of the untransformed host strain
- Black arrow: Major band corresponding to the POI fusion protein

Fig. 2 Production of a Secreted POI in Fed Batch Fermentation (14-Liter Scale)

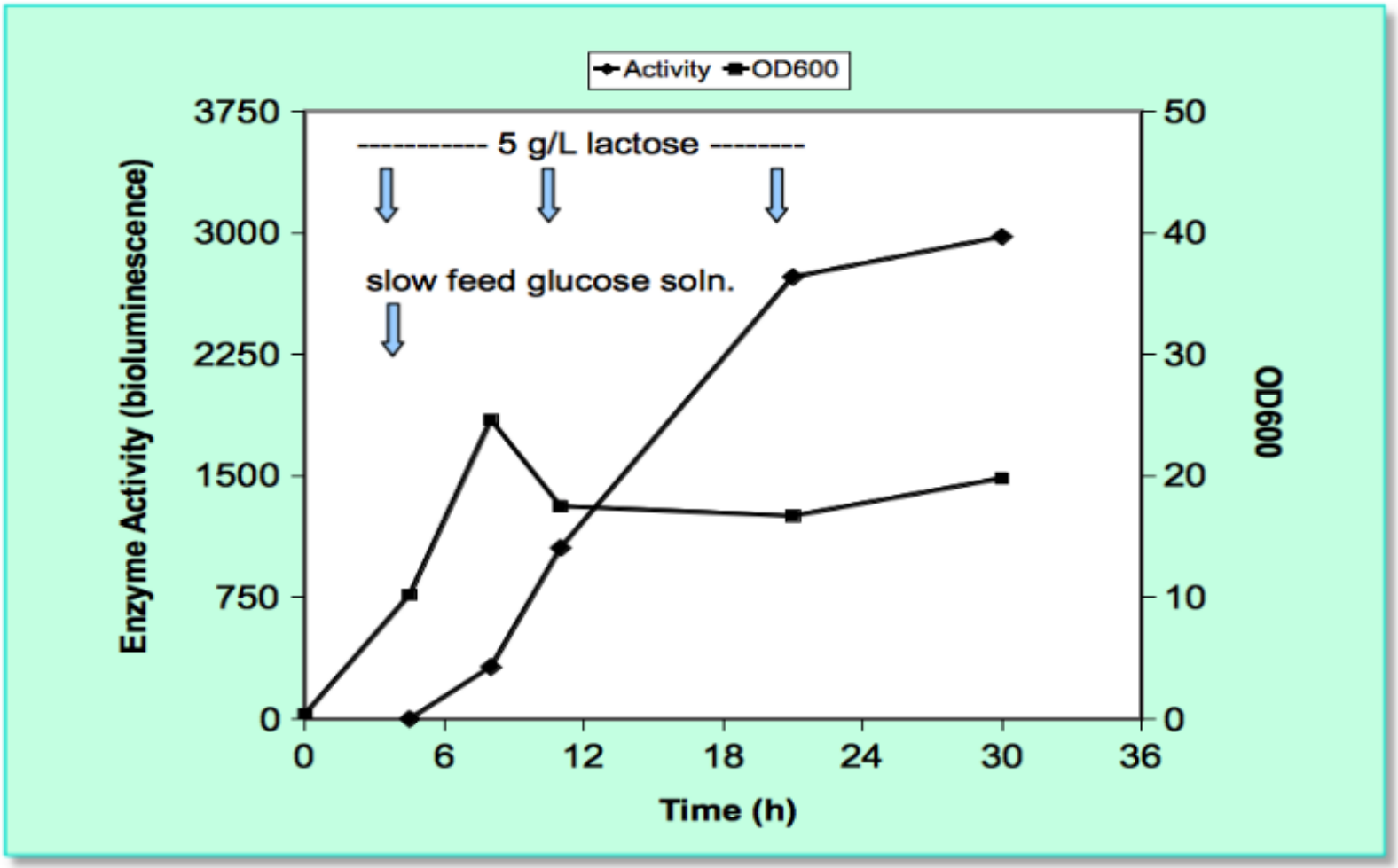


- 14-liter fed batch fermentation, secreted protein on SDS-PAGE (bottom panel), on Western blot (top panel). C: Protein control. M. Prestained Markers
- Fusion protein with a C-terminal FLAG tag produced as the major protein band (indicated by arrow).
- Only this band recognized by anti-FLAG antibody, implying no detectable proteolytic degradation of the fusion protein

Example 2: Protein Expression & Process Development in *E. coli*

- **Client needs**
  - A proprietary process to make a non-microbial bioluminescent protein
  - Develop process with titer goal ≥ 1 g/L
- **Development program**
  - Expression host: *E. coli*
  - Design and build vectors and transform, express and secrete a protein from an integrated synthetic gene
  - Protein expression measured by bioluminescence, SDS-PAGE and HPLC
  - Process development at 1- and 14-L scales
  - Final process: 2.1 g/L active protein (current commercial production at 8 g/L active protein)
  - Developed partial purification process
- **Development time: 9 months (in three phases)**

Fig. 3 Lactose-Induced Bioluminescent Protein Production



Example 3: Protein Expression/Process Development in Methanophilic Yeast *Hansenula polymorpha*

- Hercules contacted BTR to do process development program in native organism
- Process development program saw improvements in productivity and stability of enzyme
- BTR suggested switching to production in *Hansenula polymorpha* expression system
- BTR stably integrated the gene into this yeast system
- Introduced the transformants into process development and recovery program
- Price of catalyst decreased from $140 to $0.77/ MMIU

Fig. 4 Galactose Oxidase Production: Hercules