Introduction to Protein Expression Services Offered by Bio-Technical Resources

Bio-Technical Resources

www.biotechresources.com

1035 S 7th Street, Manitowoc, WI 54220

Phone: (920) 684-1158

Fax: (920) 684-5519

Email: info@biotechresources.com

Protein Expression Systems Offered at BTR (Non-Proprietary)

- *E. coli:* intracellular or secreted protein
- *Bacillus subtilis:* secreted protein in particular
- *Saccharomyces cerevisiae:* intracellular protein
- *Pichia pastoris:* especially secreted protein
- *Trichoderma reesei:* secreted protein

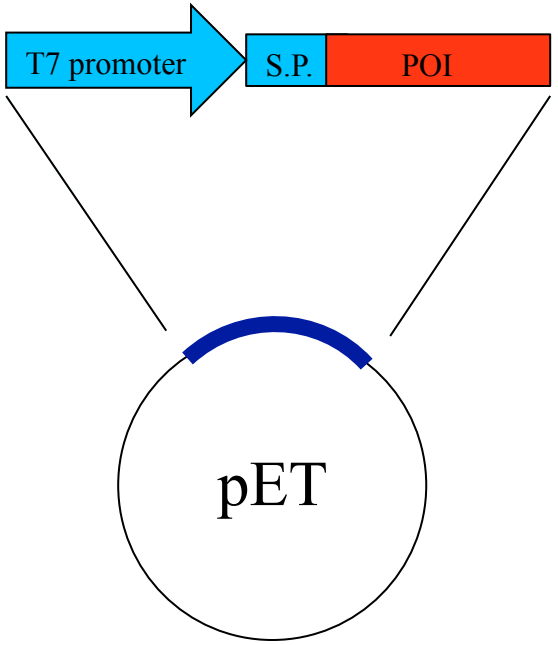
No single host system is the best option for all types of proteins and applications

BTR has the capacity and proven experience to help you to choose and develop the most efficient production system for your protein of interest (POI)

Protein Expression in *E. coli*

- **Host of choice for new POI targets, especially of prokaryotic origin**
- **Fast growth to high cell density in simple minimal media**
- **High levels of intracellular expression achievable for many proteins**

- **Challenges:**
  Protein misfolding, inclusion body formation, endotoxin
- **Option:**
  Periplasmic expression demonstrated for several proteins



Protein Expression in *Bacillus subtilis*

- Historically, the most widely used production hosts of enzymes for feed and food applications
- Nonpathogenic, nontoxigenic, lacks endotoxin
- GRAS (Generally Recognized as Safe) status with the US FDA
- Choice of multiple inducible or constitutive promoters and various secretion signal peptides for optimization
- Strains with multiple protease genes deleted to minimize proteolytic degradation of target POI
- Grow as fast as *E. coli* to high cell density in simple minimal media
- Secretes active enzymes at high levels

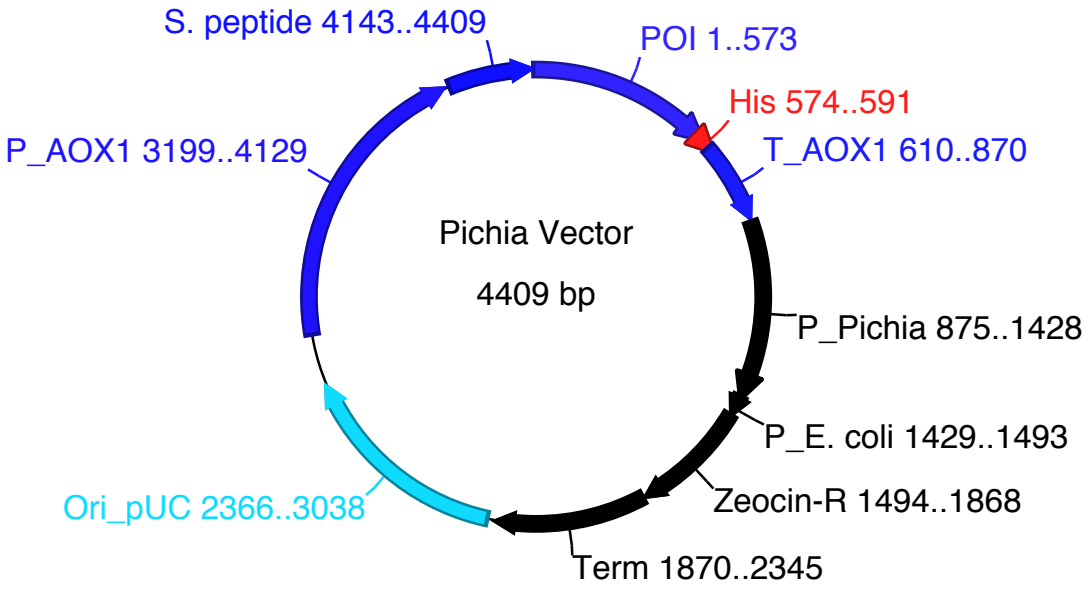
Protein Expression in *Saccharomyces cerevisiae*

- Circumvent complications associated with *E.* coli, e.g., endotoxin, inclusion body formation, absence of glycosylation
- Suitable for production of many proteins from human and other mamamals
- Most advanced gentic engineering system for eukaryotic cells
- GRAS status
- Choice of various promoters, option of replicating plasmids vs. chromosomal integration to optimize production of your POI

Protein Expression in *Pichia pastoris*

- Ability to perform higher eukaryotic protein modifications, such as glycosylation, disulphide bond formation and proteolytic processing
- Much higher capacity to produce secreted protein than *S. cerevisiae*
- Choice of various vectors, promoters and secretion signal peptides to optimize production of any target proteins
- Microtiter plate platform for fast screening for the best clones
- Reported production of intracellular protein: >20 g/liter of culture volume at high cell density of 100 g/L biomass
- GRAS status for several protein products for food and biopharmaceutical applications

Typical Expression Vectors for *Pichia*

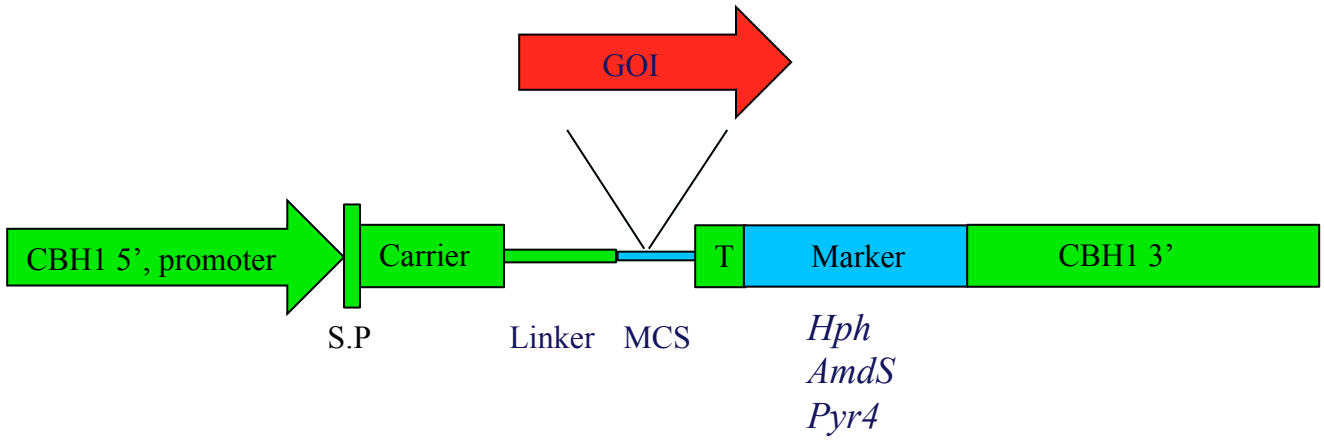


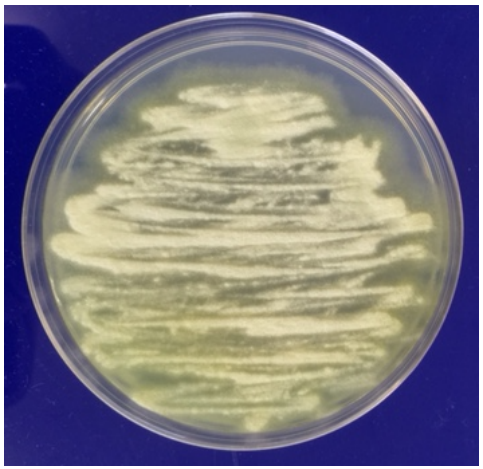
- **Methanol inducible AOX1 promoter**
- **α-factor signal peptide**
- **Chromosomal integration at the AOX1 locus**
- **Selection options**
  - Zeocin selection in both *E. coli* and *Pichia*
  - Complementation of histidine auxotroph in *his*$^{-}$ *Pichia* strains

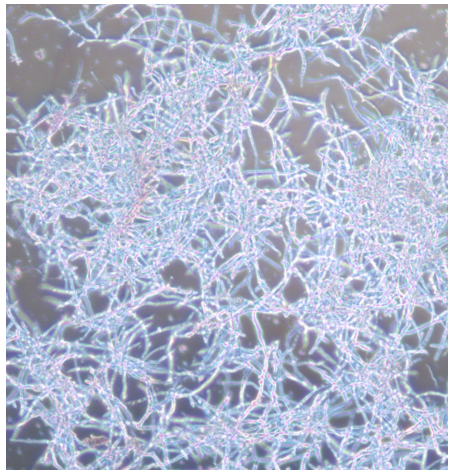
Protein Expression in *Trichoderma reesei*

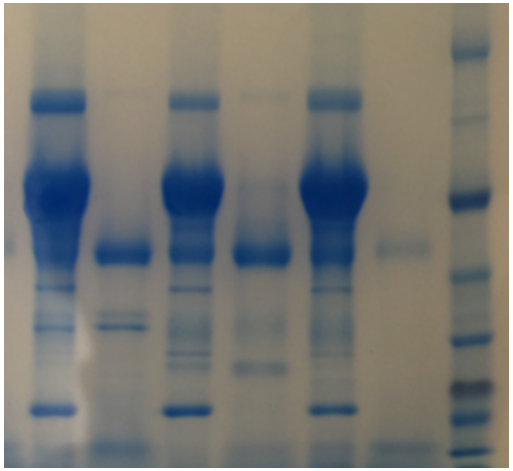
- Reported over 100 g per liter cellulase produced at commercial scale
- Well established genetic engineering tools and methodology
- Strong *cbh1* promoter, other inducible and constitutive promoters
- Single or multiple copies of expression cassette stably integrated in the chromosome
- Simple and cheap growth media
- Protein secreted into the medium, providing an opportunity for low-cost downstream processing

Expression and Integration Cassette for *Trichoderma*





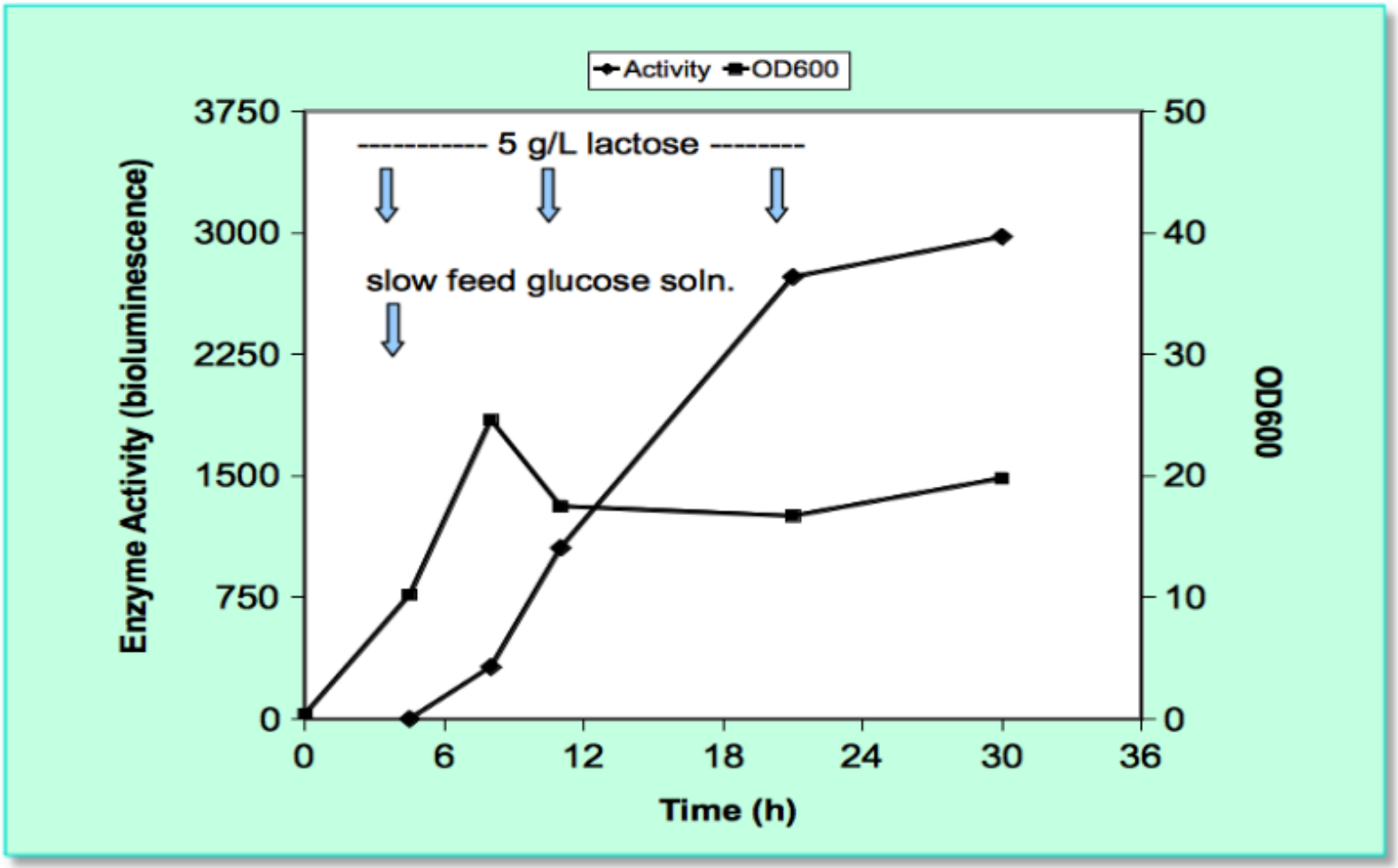




Example:
Protein Expression & Process Development

- **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 >5 g/L active protein)
  - Developed partial purification process
- **Development time = 9 months (in three phases)**

Lactose-Induced Bioluminescent Protein Production



Doing Contract Research with BTR

- **Confidential Disclosure Agreement to discuss research opportunity with client is implemented**
- **BTR prepares Cost Proposal including**
  - Details of research program objectives
  - Cost, staffing, duration, and deliverables of program
- **Client and BTR discuss the Cost Proposal and finalize a Work Plan**
- **Professional Services Agreement, incorporating the Work Plan, is prepared and signed**
- **Research program commences at BTR**
- **IP and technology developed belongs to client**