Rapid Strain and Early Process Development for Recombinant Erwinia Asparaginase (Rylaze™)

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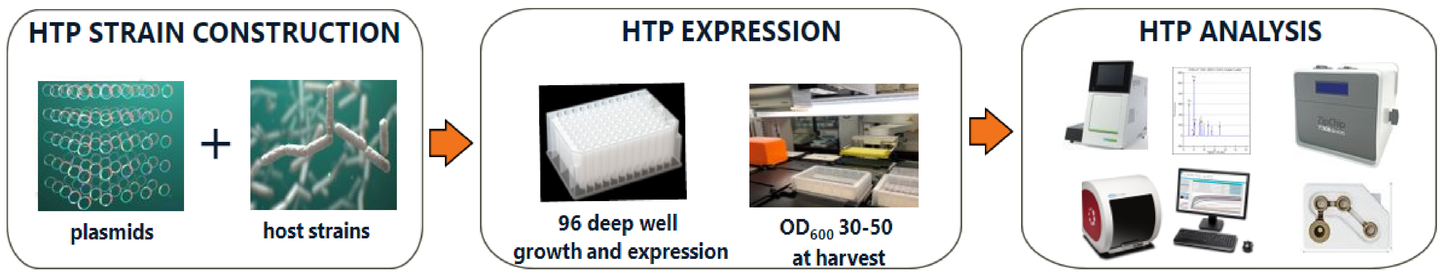
Rapid and comprehensive strain selection and an early process development program utilizing the Pelican Expression Technology™ were key to establishing the foundation for late-stage success for Rylaze™, a recombinant *Erwinia chrysanthemi* asparaginase for the treatment of acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL) in patients who have developed hypersensitivity to *E. coli*-derived asparaginase. Pelican Expression Technology™, a *Pseudomonas fluorescens*- based protein expression system is a robust and scalable platform for recombinant protein production.

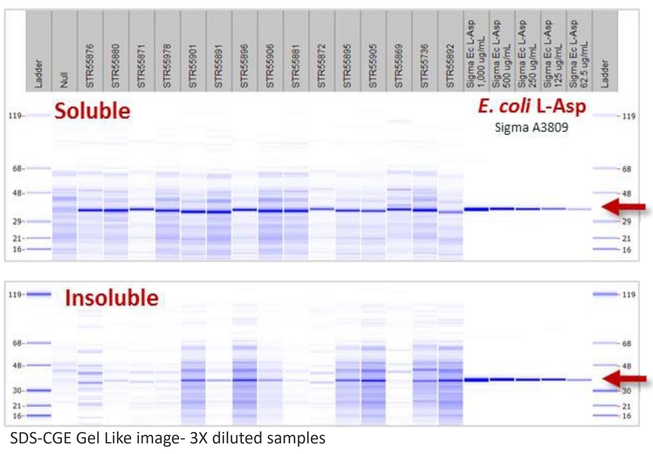
Rapid Strain Construction, Expression, and Analysis



2	Codon Optimized Genes
2	Plasmids
4	Transcription Promoters
3	RBS/ Translation Initiation Strengths
53	P. Fluorescens Native Signal Peptides
60	Folding Helper Overexpression Host Strains
150	Protease Deletion Host Strains

- Toolbox components combined to produce ***thousands of unique expression strains*** constructed and screened in parallel using automated workflows
- Interactions between toolbox components are explored to quickly identify production strain candidates using high throughput analysis to assess target quantity and quality
- Parallel automated processing enables rapid strain screening in 9 weeks

Unique high titer crisantaspase expression strains identified at 96-well scale

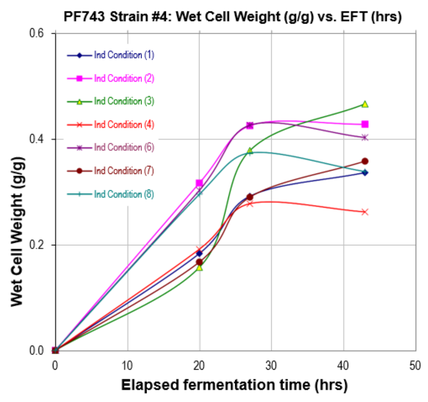


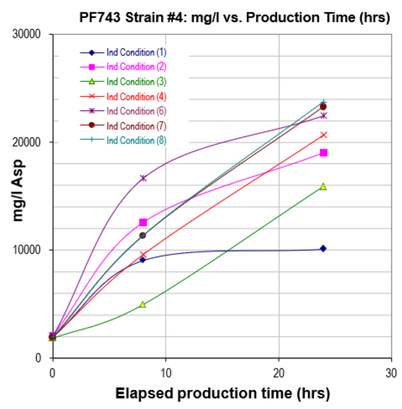
SDS-CGE Gel Like image- 3X diluted samples

- HTP 96-well strain screening identified strains producing active crisantaspase (or L-asparaginase) greater than 1 g/L soluble monomer was observed
- Construction of *P.fluorescens* host strains deficient in native L-asparaginase completed (chromosomal KOs)
- 5 total strains, representing a diversity of expression strategies, scaled to 2L fermentations for evaluation under multiple induction conditions
  - 4 periplasmic and 1 cytoplasmic localization strains

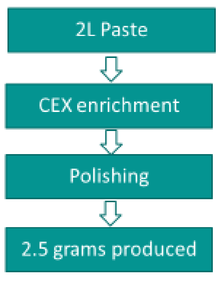
Fermentation Assessment

- Cytoplasmically localized PF743 (Strain #4) showed highest overall soluble titers of monomer ranging from 19 to 23 mg/L
- PF743 Strain #4 was cultivated under 8 different induction conditions (factors: wet cell weight at induction, IPTG concentration, pH, temperature)





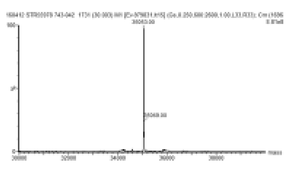
High Quality Material for Preclinical Development



Methods used for evaluation:

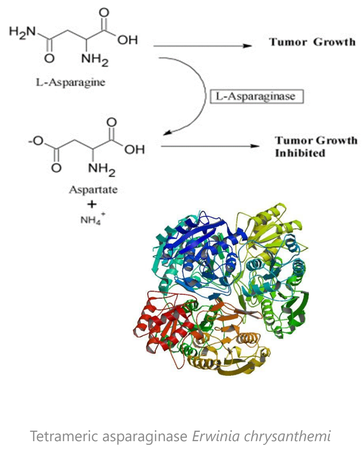
I. Activity using Nessler-based method
II. Tetramer by SEC
III. Western/SDS-PAGE
IV. Expected mass by LC-MS
V. Purity by RP-HPLC
VI. CD/IF

LC-MS: Intact Mass



- ✓ Small-scale expression and purification of crisantaspase using the Pfenex Expression Technology successfully demonstrated
- ✓ Greater than 2.5 grams of *P.fluorescens* produced crisantaspase meeting initial purity and potency targets was produced
- ✓ Chosen production strain moved forward into process development

ALL/LBL & Role of Asparaginases in Treatment



Tetrameric asparaginase *Erwinia chrysanthemi*

Acute Lymphoblastic Leukemia (ALL) is the most common form of childhood cancer affecting nearly 6000 patients per year in the US but also among the most curable[1]

Lymphoblastic Lymphoma (LBL) is a rare, fast-growing, aggressive sub-type of Non-Hodgkin's lymphoma most often seen in teenagers and young adults

Asparaginases are a key component of multi-agent chemotherapeutic regimen for the treatment of ALL and LBL and full and effective asparaginase administration is essential to best outcomes in patients

Until recently and due to world-wide supply challenges, patients with hypersensitivity to an E. coli-derived asparaginase had a critical unmet need for an immunologically distinct asparaginase product that would allow them to continue and complete their optimal treatment plan

1. National Cancer Institute, https://www.cancer.gov/types/childhood-cancers/child-adolescent-cancers-fact-sheet

Upstream Process Development

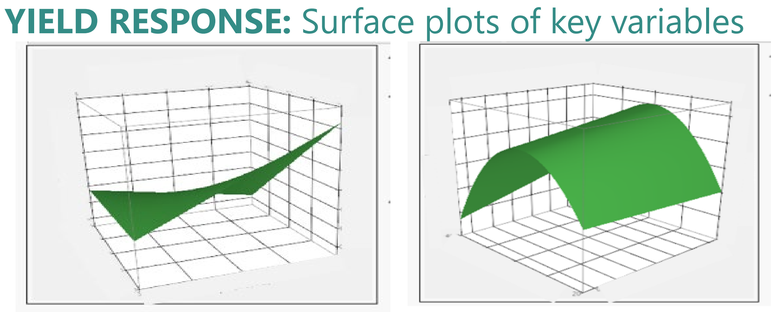
Fermentation → Lysis and Recovery → Capture Chromatography → Polishing Chromatography → Final DS

- 5 strains advanced
- Up to 9 variable induction conditions screened using DoE approach

  Typical factors tested
  - Temp.
  - pH
  - Inducer level
  - Wet cell weight
- Selection of robust production strain for further development

134 x 2L fermentations in 6 Design-of-experiment rounds to optimize conditions

YIELD RESPONSE: Surface plots of key variables



28 x 20L fermentations to optimize conditions and support Downstream Processing Development

Phase 3 ready process enabled transfer directly to the intended commercial scale

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