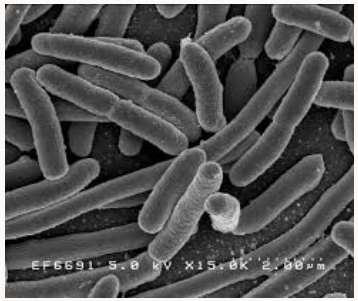
Protein Production and Purification

"Why the medium composition counts."

The *E. coli* System



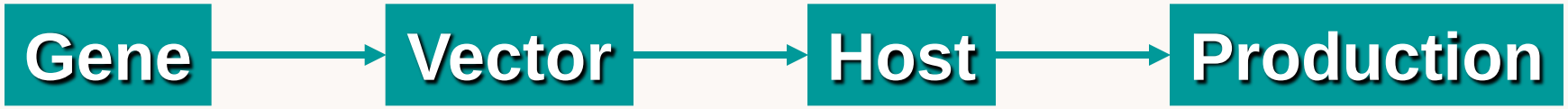
Advantages:

- Well developed science and tools with a 40 year history of successes
- Inexpensive
  - Short development timelines
  - Inexpensive tools
  - Low cost production systems

Disadvantages:

- Endotoxin contamination
- Heterologous proteins often accumulate as insoluble products in cytoplasm – "Inclusion bodies"
- Export to periplasm is possible but rare for secretion to the medium
- No post-translational glycosylation

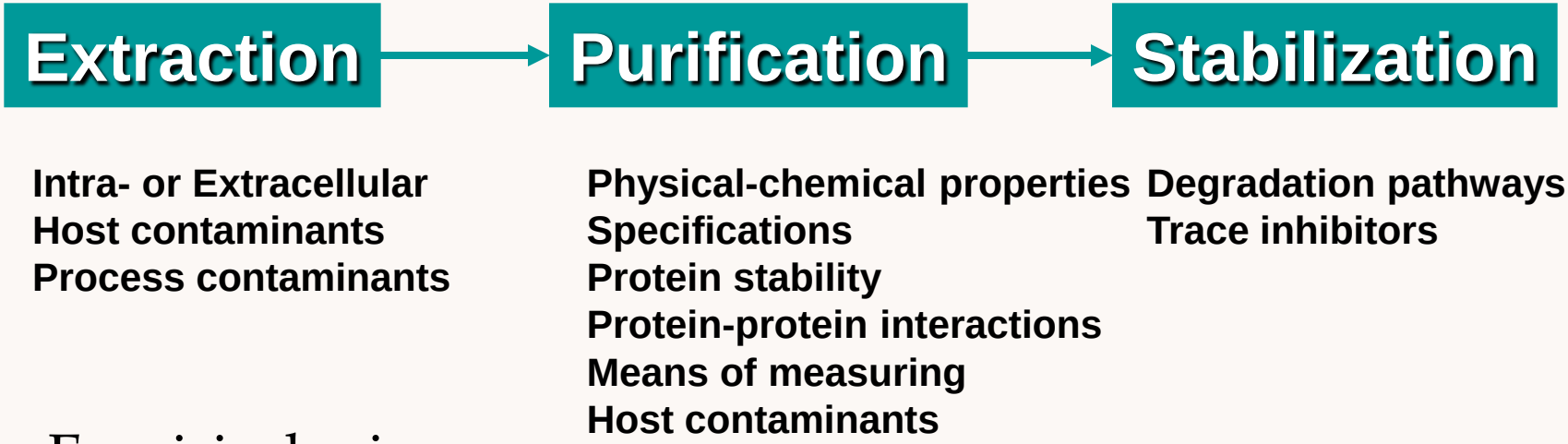
Variables in Protein Production



Gene	Vector	Host	Production
Codon Usage	Replication Origin	Drug Resistance	Temperature
mRNA Structure	Promoters	Protease Deficiency	Carbon Source
GC Content	Ribosome Binding	Redox Environment	Nitrogen Source
Regulatory Motifs	Regulatory Elements	Recombination	Micro-nutrients
Repeats	Terminators	Polymerases	Aeration
		Chaperonins	pH
			Metabolic Waste

Adapted from Gustafsson et al 2012. Protein Expression and Purification. 83:37-46.

Variables in Protein Purification



- Empirical science
- Highly interconnected
- Choice of host-vector system
  - Type of extraction process
  - Post-translational modifications

E. coli Expression Vectors

- Inducible, cytoplasmic accumulation
  - Lac Operon: promoters, T7, T5, *tac*, *trc*, *lacP*
  - Arabinose Operon
  - Tryptophan Operon
  - Lambda Repressor
- With Affinity Tags
  - His, MalE, GST, Trx, Flag, and others
- Periplasmic Expression
  - Signal Sequences: OmpA, PelB, SpA, PhoA, and others

The History of LB Broths

- Originally developed in the 1950's to cultivate *E. coli.*
- Comes in three variations
  - Miller, Lennox and Luria
  - Differ in NaCl content: 10, 5 and 0.5 g/L, respectively
- Period in time when optimum growth conditions were not known.
- Long before recombinant proteins were produced in *E. coli*.
- And they worked for the physiological and genetic experiments of the time.
- *However, ….*

The LB Broths

- Have no added carbon source.
- Are not buffered.
- No added phosphate, sulfate, or potassium

Composition of LB Broths	
Yeast Extract	5 g/L
Casein Hydrolysate	10 g/L
NaCl	10 g/L (Miller) 5 g/L (Lennox) 0.5 g/L (Luria)

Not designed or intended for production of recombinant proteins.

Better Media Formulations

Biomass yield of *E. coli* grown in six different medium.	
Medium	Biomass Yield (g/L)
LB (Miller) Broth	10
Glucose M9Y	16
Hyper Broth™	36
Power Broth™	24
Superior Broth™	18
Turbo Broth™	30
E. coli strain JM109 was grown in 100 ml shake-flask cultures in each medium at 37ºC for 16 h.	

- Not all proteins express well in *E. coli.*
- 20 years of helping clients overcome this limitation.
- Discovered that the carbon and nitrogen source can make a big difference.

Medium Composition *Makes a Difference*

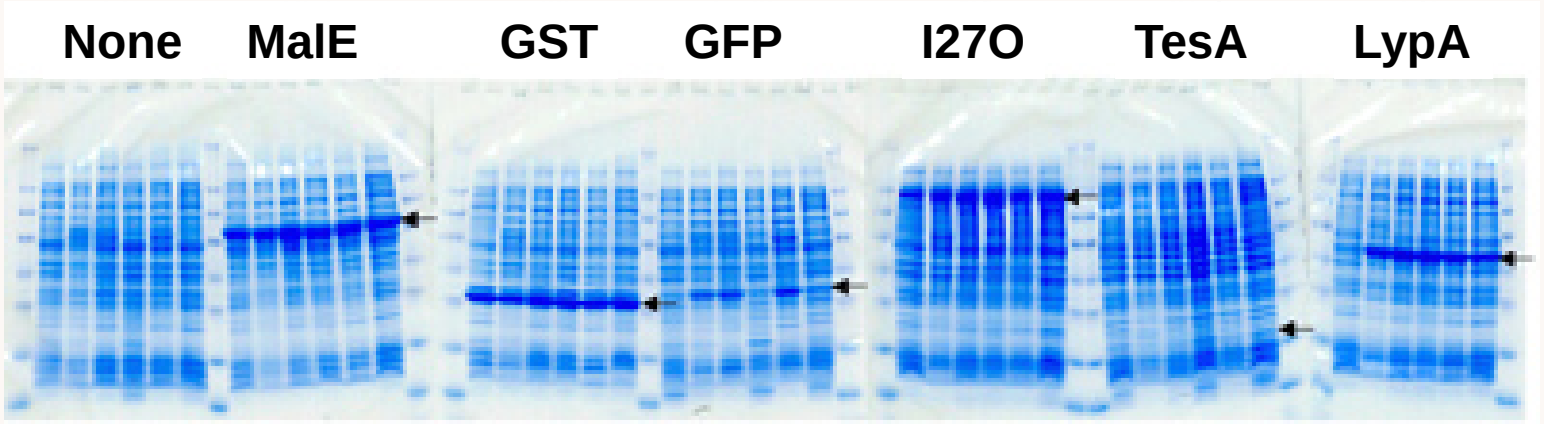
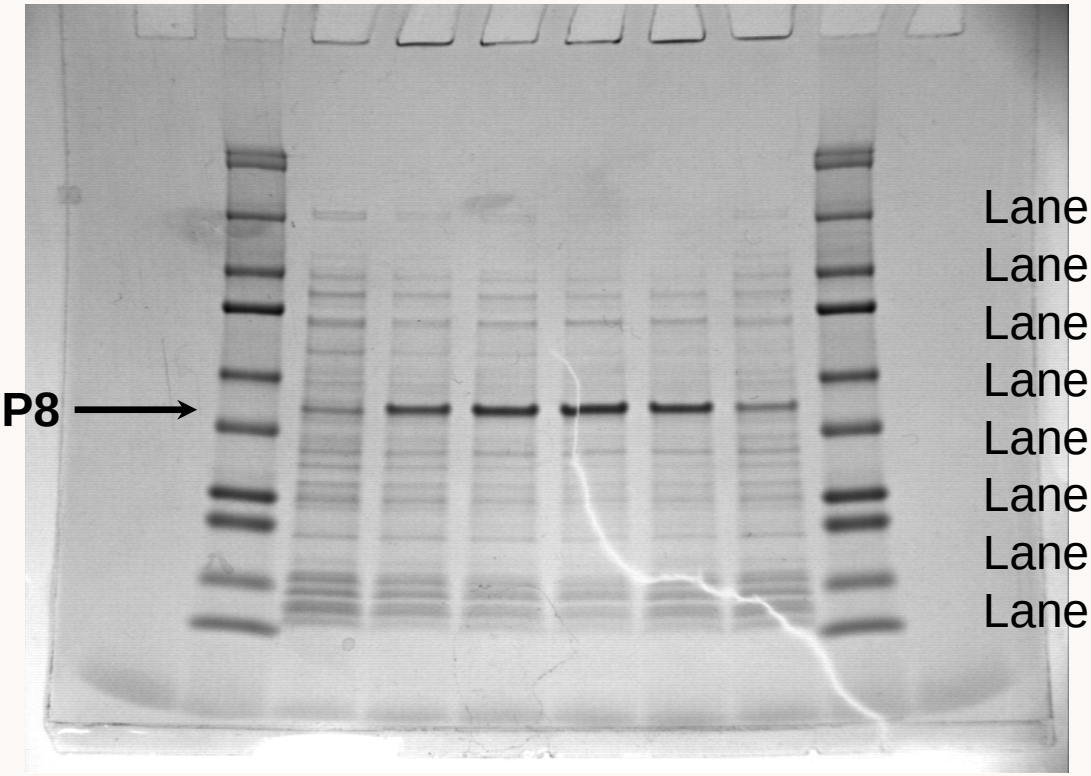


Figure. SDS-PAGE analysis of total protein from each strain in Table 1. Samples were prepared as described in the Materials and Methods section. Panel A - reference strain without a recombinant protein; Panel B to G - MalE, GST, GFP, I278, TesA, LypA, respectively. Arrows denote the location of the respective protein. Marker proteins are shown to the left and right of each set of cellular proteins. From left to right in each panel are samples from cells grown in LB (Miller), Glucose M9Y, Hyper Broth™, Power Broth™, Superior Broth™ and Turbo Broth™.

Medium Composition *Makes a Difference*

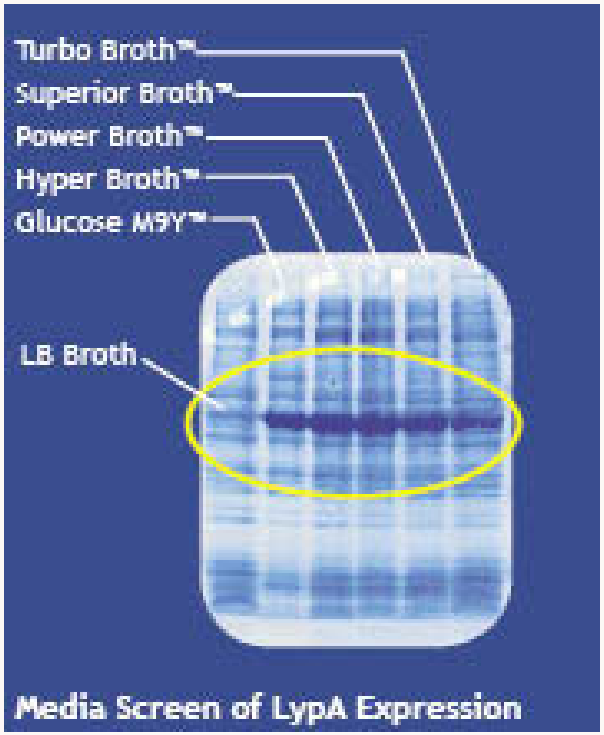
Mammalian protein produced in a 400 liter fementor

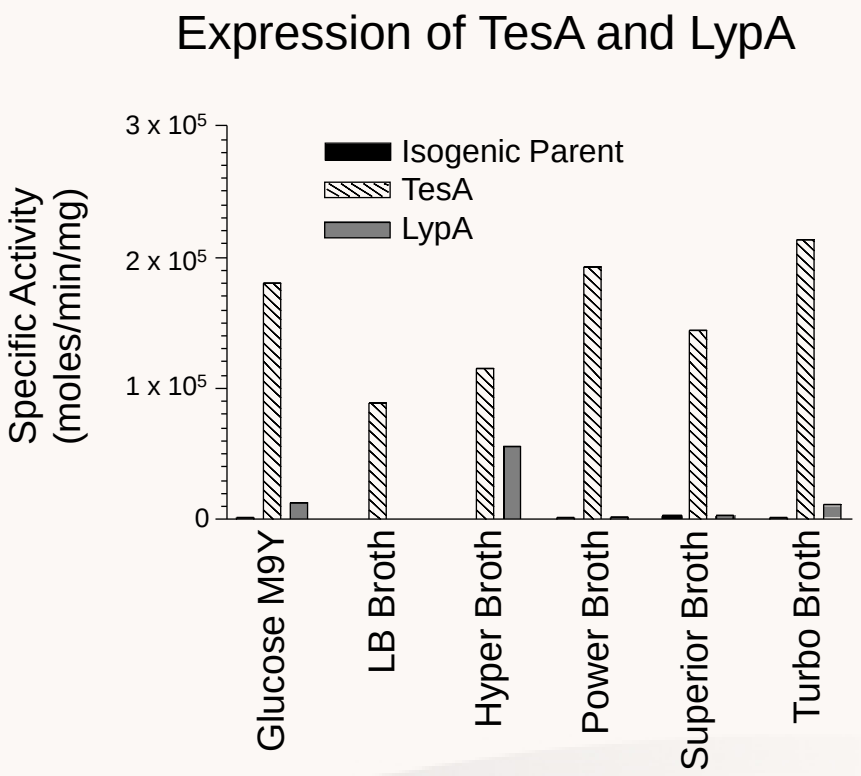


Lane 1 = MW marker
Lane 2 = Un-induced fermentor
Lane 3 = Fermentor harvest, IPTG
Lane 4 = UGA's Auto-induction
Lane 5 = Hyper Broth auto-induction
Lane 6 = Power Prime auto-induction
Lane 7 = Overnight Express™ auto-ind.
Lane 8 = MW marker.

Overnight Express™ Autoinduction Medium is a trademark of EMDMillipore.

Not Just More Protein, But More Active Protein





Recommendations

- Richer media formulations will yield more recombinant protein than LB Broths.
- The best medium to produce a protein in a given host-vector system should be empirically determined.
- A simple screen will make all the difference.

Where to Buy

- Screening Kits are available at Athena or one of our distributors.
- Visit www.athenaes.com/Expression.php
  - Look under “Protein Expression Media”

Media Optimization Kit

Available in standard and animal product free versions.

References

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