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# The Microbial Antibodies Secretion Expression Platform With Scale-Down Fermentors

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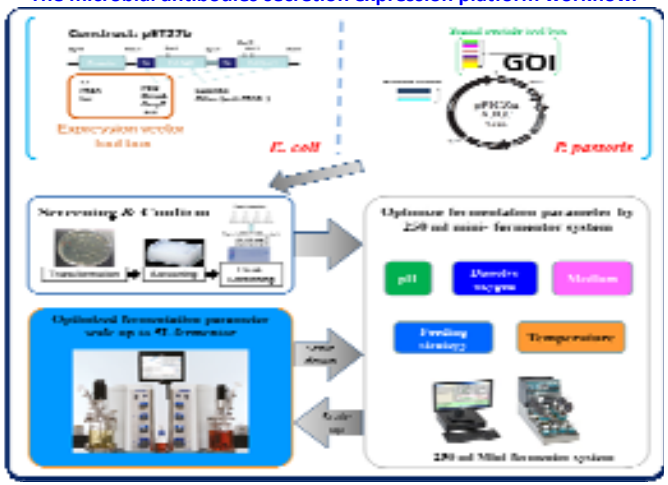
The production of antibody-based drugs using microbial expression systems is more cost effective with ease of gene manipulation compared to mammalian expression systems. In our team, antibody fragments (ex: BsAb, scFv and Fab) were produced from tow microbial expression system, the first one is yeast *Pichia pastoris* secretion expression system included with the AOX1 as driven promoter and driven methanol free promoter and second is *E. coli* secretion expression system.

The microbial antibodies secretion expression platforms are included expression vector construction, high expression strain screening, fermentation process development and scale-up & down process optimization. To achieve high yield expression from vector construction to fermentation process optimization have been manipulate in our team. The *Pichia pastoris* expression in 250 ml fermentor process AOX1 driven promotor can yielded over 500 mg/L scFv. After scale-up from 250 ml fermentor to 5L fermentor, the methanol fed-back control system also applied on the 5 L fermentor, can achieve 1.7 g/L scFv in 5 days. The *Pichia pastoris* methanol free system can achieve 50-100 mg/L yield in 250 ml and 5L fermentor.

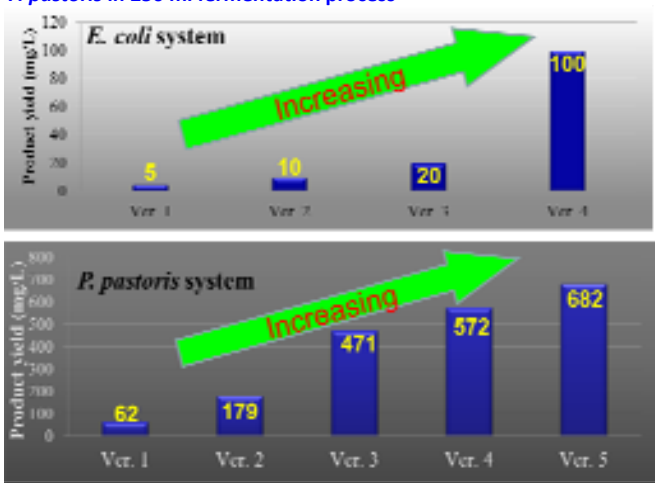
The *E. coli* expression process has passed through screening for high production yield clones in 2 ml deep-well then confirmed by using 250 ml flask scale. Feeding medium, DO, pH etc., parameters were investigated by parallel 250 ml-fermenter. The parameters from 250 ml fermentor were validated by using 5 L fermenter. Under this scale-up procedure, the antibody Fab was 100 folds production yield, production deep well stage at 1 mg/L, production from 250 ml fermentor stage is 50-100 mg/L and production 5 L fermentor stage is over 35-90 mg/L.

Although different antibodies will result in different production yield, building a reliable platform to predict production yield from antibody cell clones under deep well and shake flask stage serves a good scale-down model for future scale-up prediction.

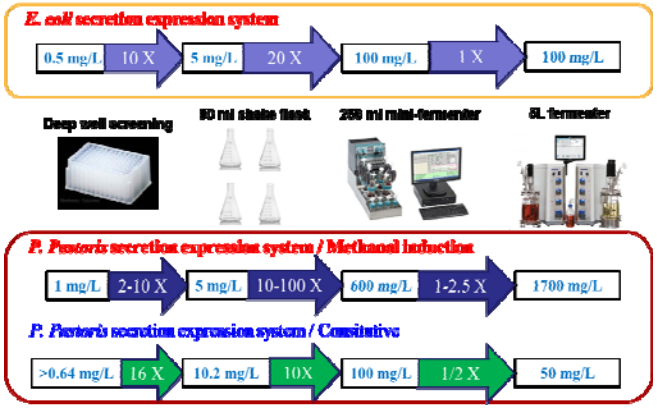
## The microbial antibodies secretion expression platform workflow.




## Capabilities of antibody fragments production by *E.coli* and *P. pastoris* in 250 ml fermentation process




## The improvement of production yield in *E. coli* and *P. pastoris*




## The ability of microbial production platform

| Scale | *E. coli* intracellular soluble | *E. coli* inclusion body/refolding | *E. coli* secretion | *Pichia* secretion (Methanol induction) | *Pichia* secretion (Methanol free) |
|---|---|---|---|---|---|
| 250 ml | √ | √ | √ | √ | √ |
| 5 L | √ | √ | √ | √ | √ |
| 30 L | √ | -- | -- | -- | -- |

## Fab, BsAb, scFv and light chain production yield improvement from deep well, shake flask, 250 ml mini-fermentor to 5L fermentor

| | The protein maxima concentration (ug/ml) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | The protein increase by each step | |
| | Deep well | Flask (50ml) | Dasgip | 5L Fer. | From Deep well to Flask | From flask to Dasgip |
| Fab | - | 2.9 | 52.40 | 52.13 | - | 18 |
| BsAb | 0.04~0.24 | 0.32~5.00 | 131 | 53.95 | 8~21 | 12 |
| scFv | 0.16~2.56 | 0.64~5.12 | 7.0~682.0 | 1754.6 | 2~8 | 7~133 |
| Light chain | 0.025 | 0.40 | 109.8 / 72 | N.A. | 16 | 275 |
| scFv-2/methanol free | 0.64 | 2.56 | 30 | 14 | 4 | 12 |
| scFv-2/methanol induction | 0.64 | 2.56 | 30 | 14 | 4 | 12 |
| scFv-1/methanol free | 0.64 | 10.2 | 107 | 58 | 16 | 10 |

## Conclusion

1. The microbial secretion expression platform are already to produce Fab, scFv and BsAb by *E. coli or P. pastoris*.
2. Due to the factory safety issue, methanol free system in *P. pastor* have more benefit then AOX1 system, especially in larger fermentation scale.

## Reference

1. Damasceno L. M. et. al., 2004, An optimized fermentation process for high-level production of a single-chain Fv antibody fragment in *Pichia pastoris*., Protein Expression and Purifcation (37), 18-26.
2. Felber M., et. Al., 2014, Strains and molecular tools for recombinant protein production in *Pichia pastoris*., Methods in Molecular biology (1152), 87-111.
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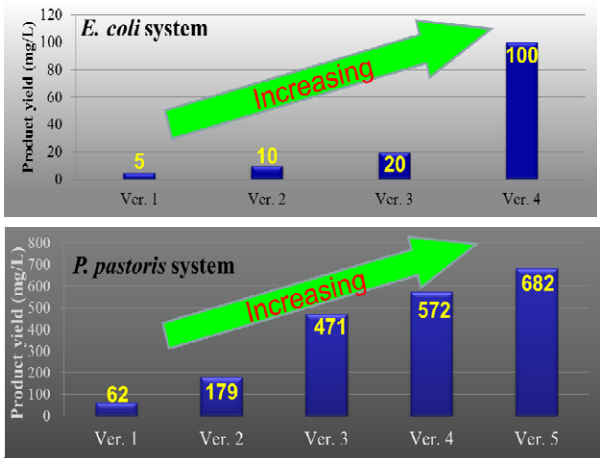
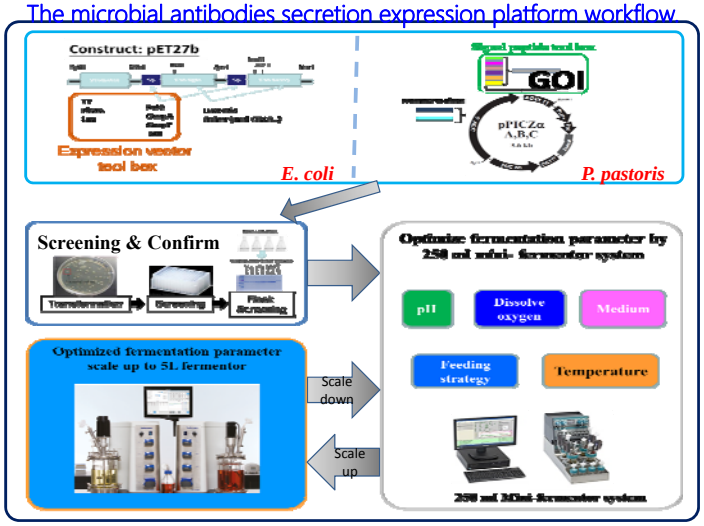



## The microbial antibodies secretion expression platform workflow

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|---|---|---|---|---|---|
| 250 ml | √ | √ | √ | √ | √ |
| 5 L | √ | √ | √ | √ | √ |
| 30 L | √ | -- | -- | -- | -- |

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