GRAM LEVEL scFv EXPRESSION PLATFORM OF *PICHIA PASTORIS*

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The methylotrophic yeast *Pichia pastoris* secretion expression system has been developed for the antibody fragments (scFv) production platform. The platform includes three technology platforms, the first one is strain generation, the second is fermentation process development in 250 ml fermentor and the last is process scale up to 5 L. A recombinant scFv went through clone generation, include signal peptide tool box, normally yield 2.5 mg/L titer in deep well. Through the fermentation process development of induction medium composition and feeding strategy by Eppendorf Dasgip parallel 250 ml mini fermentor. During induction step, feeding 100% methanol as induction medium can only produce less than 50 mg/L scFv while feeding methanol-sorbitol mixture can significant increase the production yield to 306 mg/L in five days, about 6-folds increase in productivity. With the supply of additional nitrogen source during glycerol feeding step or at induction step, higher scFv production with 510 mg/L can be achieved. Thus, following the medium composition optimization, the production titer was improved 10 folds in 250 ml mini-fermentor stage. Moreover, when we switched the induction medium feeding strategy from DO-stat to the stepwise feeding, the titer increased form 510 mg/L to ~1000 mg/L and yielded another 2- folds improvement. During medium composition and feeding strategy optimization at 250 ml mini fermentor scale, the production titer could increase 20 folds. Overall, the production titer increased 400 folds from cell line generation to 250 ml fermentation parameter optimization. Furthermore, the process parameter can be scale-up to 5 L fernentor achieving > 1 g/L. Recent progress to include BIP in the expression vector gave at least 2 fold improvement in scFv titer in shake flask, the new clone will be optimized in our established 250 ml and 5 L fermentation platform.

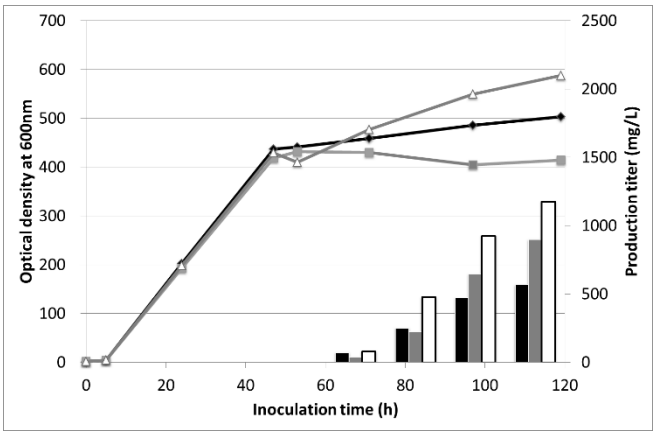

Figure 1. Biomass optical density profile and production titer using different feeding strategy. White column and triangle, methanol and sorbitol mixture feed by constant feeding rate. Gray column and squared, methanol and sorbitol i feed separately by using constant feeding rate and DO-stat feeding strategy, respectively; Black column and diamond, methanol and sorbitol mixture feed by using DO-stat feeding strategy;