RAPID FERMENTATION OPTIZATION FOR VACCINE DEVELOPMENT

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Pichia pastoris is widely used to produce heterologous proteins including vaccines such as hepatitis B [1]. P. pastoris is capable of achieve high cell density during fermentation and has the ability to secrete recombinant proteins. Fermentation optimization can be a time consuming and laborious process that can cause delays in early vaccine development. To overcome this, we have made use of parallel automated scalable fermentation technology. Four single-use parallel small-scale fermenters (ambr250 modular) were used to rapidly screen and optimize fermentation parameters. A non-replicative rotavirus (NRRV, PATH) vaccine was expressed in P. pastoris. The methylotrophic yeast was grown in fed-batch mode using chemically defined media with glycerol as carbon source and induced with methanol. Intact mass spectrometry was used to determine the distribution of full length and truncated species at different induction times during fermentation. Zymogram analysis was used to detect potential proteases present in the fermentation supernatant.

The bioreactor operating parameters (pH, temperature, induction time and media composition) were optimized to improve cell growth and product yield. Resulting in product yields of ~ 1 g/L and purity levels of ~ 80% in the fermentation supernatant. Conditions were selected to reduce levels of truncation and increase production of full-length product. It was observed that temperature had little or no effect on mitigating product truncation; while fermentation pH and induction time had a greater effect, significantly reducing product truncation. In addition, zymogram analysis showed that levels of contaminating proteases in the supernatant were also affected by fermentation parameters and induction time. Cation exchange chromatography (CIEX) was used to purify the product directly from the fermentation supernatant showing that it is possible to integrate the up-stream process of fermentation with down-stream purification into a single procedure.

Single-use small-scale fermenters are useful for a rapid screening and optimization of fermentation parameters for vaccine development. These results show that the bioreactor operating parameters have a great effect on both product yield and quality and fermentation parameters can be optimized to reduce degradation of secreted products.

Reference