PROCESS INTENSIFICATION COMBINED WITH ADAPTIVE LABORATORY EVOLUTION ENHANCE VLP-BASED VACCINE CANDIDATES PRODUCTION IN INSECT CELLS

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Insect cells are excellent hosts for recombinant protein expression, however increasing their productivity is needed to make them more suitable alternatives for vaccine production. The work herein developed aims at solving this bottleneck by combining process intensification (i.e. perfusion at high-cell-density and continuous operation) and evolutionary engineering (i.e. high-producing insect cell lines established via adaptive laboratory evolution, ALE) approaches.

Stable insect cells producing influenza HA-Gag VLPs and adapted to hypothermic growth (22 °C) using ALE (up to 12-fold higher protein expression over non-adapted cells) were cultured in 1 L stirred-tank bioreactors under perfusion operation mode up to $100x10^6$ cell/mL. Cell-specific Gag and HA production rates were similar to a batch process, resulting in 8-fold increase in influenza HA-Gag VLPs volumetric titer. Aiming to further intensify the VLPs production cells were cultured in continuous operation mode at $20x10^6$ cell/mL (to avoid mass transfer limitations); the impact of cell retention device (ATF vs TFF) and cell specific perfusion rate (CSPR) on cell growth and protein expression kinetics was evaluated. Continuous production of Gag-HA VLPs was possible using both retention devices and CSPR of 0.04 nL/cell.d, TFF inducing higher cell lysis when compared to ATF at later stages of the process (kD = 0.009 vs 0.005 h^{-1}, for TFF and ATF respectively). Reducing CSPR to 0.01-0.02 nL/cell.d using ATF had negligible impact on specific production rates. Noteworthy, the space time yield (i.e. the ratio of p24 or HA formed per volume of culture medium consumed per process time) of the continuous process was 3-fold higher than that of batch and perfusion operation modes, resulting in lower medium consumption and higher product concentration (facilitates purification).

In another study, a continuous multi-stage bioreactor process was implemented to produce influenza HA-VLPs using the insect cell-baculovirus expression vector system and High Five insect cells adapted to neutral pH via ALE (3-fold higher cell-specific productivity over non-adapted cells). Specifically, a set-up composed of four bioreactors (one cell growth bioreactor simultaneously feeding cells to three production bioreactors with continuous product harvesting) was implemented to assess the impact of residence time (RT) on the process performance. RT was shown to be a key parameter with high impact on the kinetics of cell growth and HA-VLPs/baculovirus production and on virus passage effect.

Overall, combining the use of cell lines with improved fitness with intensified processes allows for higher recombinant protein production thus contributing for accelerating vaccine development.