INSECT CELL-BASED PRODUCTION PROCESSES INTENSIFIED VIA HIGH CELL DENSITY PERFUSION AND CONTINUOUS CULTURE

Bárbara Fernandes, iBET and ITQB-NOVA, Apartado 12, 2781-901 Oeiras, Portugal
bfernandes@ibet.pt
Ricardo Correia, iBET and ITQB-NOVA, Apartado 12, 2781-901 Oeiras, Portugal
Inês Isidro, iBET and ITQB-NOVA, Apartado 12, 2781-901 Oeiras, Portugal
Paula M. Alves, iBET and ITQB-NOVA, Apartado 12, 2781-901 Oeiras, Portugal
António Roldão, iBET and ITQB-NOVA, Apartado 12, 2781-901 Oeiras, Portugal

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Insect cells are excellent hosts for recombinant protein expression and increasing their productivity contributes to unlock their full potential for vaccine production. The work herein developed combined 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. In a follow-up study, cells were cultured in continuous operation mode at $20x10^6$ cell/mL (to avoid mass transfer limitations) using different cell retention devices (ATF vs TFF) and cell specific perfusion rates (CSPR, 0.01, 0.02 and 0.04 nL/cell.d); impact on cell growth and protein expression kinetics was evaluated. Continuous production of HA-Gag VLPs was possible using both retention devices, although TFF induced higher cell lysis when compared to ATF at later stages of the process (i.e. k_D = 0.009 vs 0.005 h^{-1}, for TFF and ATF respectively), and all CSPRs tested. Noteworthy, the space time yield (i.e. the ratio of HA-Gag VLPs 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.

In another study, a continuous multi-stage bioreactor process was implemented to produce influenza HA-VLPs using the insect cell-baculovirus expression vector system (IC-BEVS) 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, 18, 36, 54 h) on the process performance. RT was shown to be a key parameter with high impact on the kinetics of HA-VLPs production, with RT=54 h maximizing HA-VLPs titers.

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.