BIOPROCESS ENGINEERING OF INSECT CELLS FOR ACCELERATING VACCINES DEVELOPMENT

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Insect cells emerged as a powerful and versatile platform for vaccines production, mostly using the lytic baculovirus expression vector system (BEVS). Stable expression in such hosts has been increasingly explored to circumvent BEVS-related drawbacks, but protein titers achieved to date are still seemingly low. The design of new or improved cell factories and bioprocess intensification strategies are therefore necessary to increase productivities and thus accelerate implementation of stable insect cell lines as a fast, cost-effective platform for vaccines manufacturing.

In this work, we implemented an innovative site-specific recombination strategy based on flipase-mediated cassette exchange technology to establish reusable insect (*Sf*-9 and High Five) cell platforms for fast production of enveloped virus-like particles (VLPs). Influenza M1 and HIV Gag proteins were evaluated as scaffolds, and proof-of-concept demonstrated using two membrane proteins: the influenza HA protein (for vaccines) and the human beta-2 adrenergic receptor (for drug screening or antibody discovery).

Aiming to improve production yields in developed stable cell lines, two bioprocess engineering schemes were evaluated (either individually or in combination): (i) adaptive laboratory evolution of insect cells to hypothermic culture conditions, and (ii) supplementation of insect cell cultures with productivity enhancers. The stable cell line expressing HIV Gag-VLPs was used as model. Under hypothermic culture conditions, adapted *Sf*-9 cells expressed up to 30-fold more HIV Gag-VLPs than non-adapted cells. Noteworthy, the element driving such increase in productivity is the adaptation process and not the temperature shift as the latter alone leads to lower production yields. A more modest increase in productivity (up to 7-fold) was observed when supplementing non-adapted cell cultures with productivity enhancers NaBu and DMSO. No synergistic effect was observed when combining adapted cells and supplementation with productivity enhancers. Production of HIV Gag-VLPs was successfully scaled-up to stirred-tank bioreactors.

The adapted cell line was then pseudo-typed with influenza HA protein for production of Gag-HA VLPs, and their performance benchmarked against (i) parental *Sf*-9 cells stably expressing Gag-HA VLPs and (ii) insect cells-BEVS, both cultured under standard temperature conditions (27C). Adapted cells showed increased production of Gag-HA VLPs when compared to parental/stable cells, corroborating previously obtained data, but still lower when compared to insect cells-BEVS. Bioprocess intensification strategies are currently under in-house testing to further improve yields of adapted cells and thus shorten the gap between stable insect cells and IC-BEVS.

Overall, the insect cell platforms and bioprocess engineering strategies herein assembled have the potential to assist and accelerate vaccines development.

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