Scaling up the biotechnological process of the recombinant rabies virus glycoprotein

Monize C. Decarli[1], MSc; Diogo P. dos Santos[1], MSc; Renato M. Astray[2], PhD; Daniella C. Ventini-Monteiro[2], PhD; Soraia A. C. Jorge[2], PhD; Daniela M. Correia[1], PhD; Juliana S. da Silva[1], MSc; Mayra P. Rocca[3], MSc; Hélio Langoni[3], PhD; Benedito D. Menozzi[3], MSc; Carlos A. Pereira[2], PhD; Claudio A. T. Suazo[1], PhD.

[1]Department of Chemical Engineering, Federal University of São Carlos, SP, Brazil. [2]Laboratory of Viral Immunology, Butantan Institute, SP, Brazil. [3]Department of Veterinary Hygiene and Public Health, São Paulo State University, SP, Brazil.

Abstract

The rabies virus glycoprotein (RVGP) is the main antigen of vaccine formulations. A robust *Drosophila* S2 cell line (S2MtRVGPH-His) was engineered by our group for the expression of recombinant RVGP (rRVGP) using metal-inducible promoters. The objective of this work was to evaluate the potential of a WAVE Bioreactor™ in the initial steps of scaling-up the rRVGP production process by the S2MtRVGPH-His cell line to produce rRVGP in sufficient quantities for immunization and characterization studies. The WAVE bioreactor is an innovative approach for the cultivation of animal cells as it offers high process flexibility, as well as cost and time savings. For this purpose, we firstly established a Schott flasks procedure for culturing the S2MtRVGPH-His lineage. Using an inoculum of $5x10^5$ cells/mL in culture medium (Sf900-III) induced with $CuSO_4$, adequate pH range and parameter values such as time of induction (72 h) and temperature (28 °C) to optimize rRVGP production could be defined. In the sequence, the procedure was reproduced in culture experiments conducted in a WAVE bioreactor 2/10 using a 2 L Cellbag. The results in Schott flasks and WAVE bioreactor were very similar, yielding a maximum titer of rRVGP above of 1 mg/L. After the rRVGP production process, the animals were immunized with rRVGP and submitted to rabies virus challenge. The rRVGP assessed in the immune system of the vaccinated animals showed high levels of anti-RVGP antibodies, statistically not different from the levels induced by a commercial vaccine. The animals immunized with rRVGP also survived the rabies virus challenge, whereas two negative group controls did not. This bioprocess enables an efficient scale-up of the production with high quality immunoactive glycoprotein and may be promising in terms of obtaining rRVGP in the near future in the order of grams for use in immunological, preclinical or clinical assessments.

Image

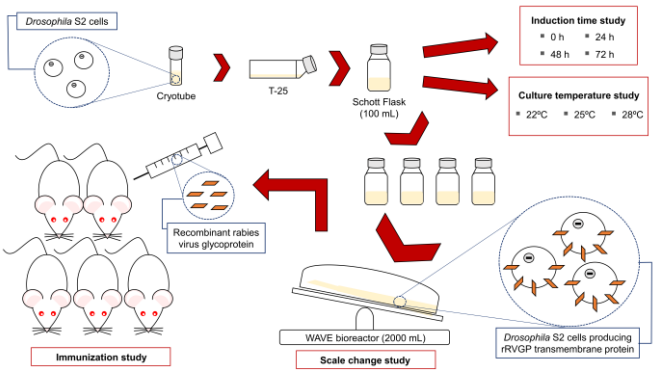


Figure 1: Schematic representation of *Drosophila melanogaster* S2MtRVGPH-His cell culture in Sf-900 III medium. In Schott flasks, batch cultures were carried out for 192 hours with an induction time at 0, 24 and 48 or 72 hours (induction time study) and at 22, 25 or 28°C (culture temperature study). In the WAVE bioreactor, batch cultures induced at 72 h and 28°C lasted 120 hours (scale change study). The quality of the rRVGP produced by the S2MtRVGPH-His cells taken at 120 h of culture from WAVE bioreactor was evaluated (immunization study).

Recent Publications

1. Swiech et al. (2008) Bioreactor culture of recombinant *Drosophila melanogaster* S2 cells: characterization of metabolic features related to cell growth and production of the rabies virus glycoprotein. *Cytotechnology. 57*:61-66.
2. Ventini et al. (2010) Recombinant rabies virus glycoprotein synthesis in bioreactor by transfected *Drosophila melanogaster* S2 cells carrying a constitutive or an inducible promoter. J. Biotechnol. 146:169-172.
3. Moraes et al. (2012) *Drosophila melanogaster* S2 cells for expression of heterologous genes: From gene cloning to bioprocess development. Biotechnol. Adv. 30:613-628
4. Loffelhotlz et al. (2013). Dynamic Single-Use Bioreactors Used in Modern Liter and m^3 Scale Biotechnological Processes: Engineering Characteristics and Scaling Up. Adv Biochem Eng Biotechnol. 138:1-44.
5. Astray et al. (2013) Kinetic studies of recombinant rabies virus glycoprotein (RVGP) cDNA transcription and mRNA translation in *Drosophila melanogaster* S2 cell populations. *Cytotechnology*. 65:829-838.



Biography

Monize Caiado Decarli is Biotechnologist (Bachelor's and Master's degrees at Federal University of São Carlos, UFSCar, SP, Brazil) and nowadays is developing her PhD Thesis in Chemical Engineering at the State University of Campinas (UNICAMP, SP, Brazil). She has expertise in bioprocess, biotechnology, animal cell culture and microbiology, and has been working in these fields since 2010. She has developed the bioprocess production of rRVGP in Wave Bioreactor during two years and half, in the course of her Master research work. This approach can be promising in terms of obtaining in the near future rRVGP in order of grams to use in preclinical assessments aiming the development of a recombinant rabies vaccine.

Email: monizedecarli@gmail.com

Notes/Comments: