ABSTRACT - APPLICATIONS OF BIOTECHNOLOGY IN HUMAN HEALTH (VACCINE AND DRUG DEVELOPMENT);

DEVELOPMENT OF VLPS FOR SARS-COV-2 USING INSECT CELL SYSTEM

Luis Giovani De Oliveira Guardalini (giovani_guardalini@hotmail.com)

Thaissa Consoni Bernardino (thaissa.bernardino@fundacaobutantan.org.br)

Wagner Quintilio (wagner.quintilio@butantan.gov.br)

Eutimio Gustavo Fernández Núñez (egfnunez@usp.br)

Soraia Attie Calil Jorge (soraia.jorge@butantan.gov.br)

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, has posed significant challenges to global public health. In response, the development of safe and effective vaccines has remained a key focus in biomedical research. This study explores the generation of Virus-Like Particles (VLPs) from SARS-CoV-2 through co-infection with recombinant baculoviruses carrying genetic information encoding the virus's structural proteins. VLPs offer a promising alternative, capable of eliciting a strong immune response without the risk of actual viral infection.

The main objective of this work is the production of SARS-CoV-2 VLPs as a potential vaccine candidate.

To achieve this, commercial plasmids were acquired and transformed into E. coli to allow site-specific transposition of genetic inserts into bacmids. Following transformation, alkaline lysis was performed to extract the bacmids, which

represent the condensed genetic material of the baculovirus. These bacmids were then used to transfect insect cells, leading to the generation of viral stocks of recombinant baculoviruses containing the genes encoding SARS-CoV-2 structural proteins. Bioreactor screening tests were also conducted to determine the optimal combination of baculoviruses for co-infection. This step was crucial to ensure efficient protein expression and to identify combinations most likely to assemble into virus-like structures. Transmission electron microscopy (TEM) was used to characterize the resulting VLPs, while dot blot assays were employed to assess protein expression.

After transformation and lysis, bacmid DNA concentrations were obtained in the range of 990 µg/mL for Bac-S, 870 µg/mL for Bac-M, 580 µg/mL for Bac-N, 830 µg/mL for Bac-RBD, and 980 µg/mL for Bac-E. Transfection resulted in baculovirus batches with titers of 10^6 pfu/mL for first-passage batches, 10^7 pfu/mL for second-passage, and 10^8 pfu/mL for third-passage batches.

Expression kinetics, measured by dot blot in coinfected samples within the bioreactor, showed that structural protein expression peaked around 63 hours post-coinfection, using the most effective combination identified through TEM analysis. These findings contribute to optimizing conditions for VLP production.

TEM micrographs confirmed that co-infection with the baculoviruses BVS:BVN:BVM, at multiplicities of infection of 3:2:1 respectively, yielded the most consistent formation of circular particles with an average diameter of approximately 100 nm.

This study demonstrates the feasibility of generating SARS-CoV-2 VLPs, and the results are promising for the development of a vaccine candidate. Efficient VLP production, coupled with the expression of the virus's structural proteins, represent a critical step toward the creation of an effective COVID-19 vaccine. The work conducted here contributes to the growing body of knowledge essential for rapid responses to future viral emergencies.

Palavras-chave: recombinant baculovirus; virus-like particles (vlps); insect cells; sars-cov-2.