DEFINING THE MULTIPLICITY AND TYPE OF INFECTION FOR THE PRODUCTION OF ZAIRE EBOLA VIRUS-LIKE PARTICLES IN THE INSECT CELL BACULOVIRUS EXPRESSION SYSTEM

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Ebola virus hemorrhagic fever affects thousands of people worldwide with high mortality rates. The Ebola virus has a short incubation time between 2-21 days and death usually occurs within 4-10 days[1]. Ebola virus disease is characterized by a sudden onset of fever, weakness, headache, diarrhea and vomiting, internal and external bleeding[2]. In the *Filovirus* family, Zaire Ebola virus (ZEBOV) is the most aggressive and virulent species, its fatality rates have been reported to be up to 90%[3]. Even when important advances in vaccine development have occurred, the need of safe and effective vaccines persists[4]. An alternative is the production of virus-like particles, which are formed by the recombinant virus structural proteins that self-assemble into highly immunogenic structures[5]. The ZEBOV contains three main structural proteins: the glycoprotein (GP), the viral matrix protein 40 (VP40) and the nucleoprotein (NP). GP induces humoral and cellular responses by itself but when VP40 is co-expressed, the immune response increases in a mouse model[6]. NP determines the structure of the resulting VLP. To our knowledge, there is no information about the production conditions that result in coexpression and assembly of ZEBOV recombinant proteins. In this work, a multifactorial experimental design was used to evaluate 32 different conditions for the production of the ZEBOV structural proteins utilizing the insect cell-baculovirus expression system technology (BEST). Multiplicity (MOI = 0.1 or 5 ufp/cell) and consecutive times of infection (0 or 6 hours after the first infection) were the principal factors, and the production of each recombinant protein and assembly of VLP were the evaluated responses. We observed that multiplicity of infection had an impact over expression of the recombinant proteins, higher multiplicities increased yield and VLP assembly. In contrast, later times of infection reduced the production of each protein. The initial presence of VP40 resulted in a higher concentration of NP. The conditions where the simultaneous expression of the three structural proteins and where VLP were detected were identified. The highest MOIs for bacVP40 and bacGP were needed. bacNP should be added during the initial infection with an MOI of 0.1, or at 6 hpi at MOI of 5. The obtained ZEBOV-VLPs were similar to native virus. The obtained VLP are a candidate vaccine under evaluation.

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References:

1. Shuaib F, Gunala R, Musa EO, Mahoney FJ, et al., 2014. Ebola virus disease outbreak-Nigeria, July–September 2014. Morb. Mortal. Wkly. Rep. 63 (39),867–872.
2. Qiu X, Audet J, Wong G. Fernando L, et al., 2013. Sustained protection virus infection following treatment of infected nonhuman primates with ZMAb. Sci. Rep. 3,
3. Richardson JS, Wong G, Pillet S, Schindle S, et al., 2011. Evaluation of different strategies for post-exposure treatment of Ebola virus infection in rodents. J.Bioterror. Biodef. S1, 007
4. Ige, Ohimain E, 2016. Recent advances in the development of vaccines for Ebola virus disease. Virus Research 211: 174-185.
5. Palomares LA, Ramírez OT, 2009. Challenges for the production of virus-like particles in insect cells: The case of rotavirus-like particles. Biochem. Eng. J. 45: 158-167.
6. Wahl-Jensen, V. et al (2005). Role of Ebola virus secreted glycoproteins and virus-like particles in activation of human macrophages. Journal of Virology, 79(4), 2413-2419