

CHARACTERIZATION OF PROTEOMIC PROFILE OF THE REPLICATION COMPLEX ASSOCIATED WITH THE YELLOW FEVER VIRUS

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Background: The yellow fever virus is an RNA virus from the Flaviviridae genus, which includes over 70 pathogenic species. This research aims to describe how to characterize the proteomic composition of the yellow fever virus replication system. The YFV genome encodes a polyprotein made of structural and non-structural (NS) proteins; the latter are essential for starting the replication complex.

Materials and methods: The yellow fever virus (YFV) replication complex was modified and labeled with green fluorescence protein (GFP) to help identify proteins involved in virus replication and their compartments. Afterwards, the YFV-GFP construct was used to infect mammalian cells, which were then cultivated to collect a biomass of 0.5-1 g infected cells in total. Intercellular fractions were extracted using ultracentrifugation in a sucrose density gradient fractionation. Anti-GFP immunoaffinity chromatography was conducted using sorbents containing anti-GFP antibodies, following the proteolytic digestion and LC-MS/MS tandem mass spectrometry analysis in the Impact II (Bruker Daltonics) mass spectrometry.

Results: Protein profiles of the construction searched in the Mascot server via the NCBI database and SwissProt. Identified the proteins involved in the replication process, such as genomic polyprotein (POLG), non-structural protein 1 (NS-1), non-structural protein (NS-2), as well as GFP. Be-

sides, host hamster proteins were extracted and identified among the YFV proteins.

Conclusion: The described approach, represented using the yellow fever virus, shows an adaptable method which can be applied to various viruses and plays a significant role in the development of effective target therapies against viruses.

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Key words: Yellow fever virus, genetic engineering, proteomics, mass-spectrometry

References:

1. Chu, P. W. G., Westaway, E. G. & Coia, G. Comparison of centrifugation methods for molecular and morphological analysis of membranes associated with RNA replication of the flavivirus Kunjin. *J. Virol. Methods* **37**, 219–234 (1992).
2. Uchil, P. D., Kumar, A. V. A. & Satchidanandam, V. Nuclear Localization of Flavivirus RNA Synthesis in Infected Cells. *J. Virol.* **80**, 5451–5464 (2006).
3. Uchil, P. D. & Satchidanandam, V. Architecture of the flaviviral replication complex: Protease, nuclease, and detergents reveal encasement within double-layered membrane compartments. *J. Biol. Chem.* **278**, 24388–24398 (2003).