IMPROVED PRODUCTION OF VIRUS LIKE PARTICLES OF COWPEA CHLOROTIC MOTTLE VIRUS USING THE BACULOVIRUS EXPRESSION SYSTEM

Anayeli Martínez García
anayelli.martinez@ibt.unam.mx
Ruth Pastor, Laura Palomares, Octavio T. Ramírez,
Instituto de Biotecnología, Departamento de Medicina Molecular y Bioprocesos, Cuernavaca, Mor, 62210

Key words: CCMV, virus like particles, Insect –cell expression, nanoparticles, self-assembly.

Virus like particles (VLP) represent a powerful platform for drug and gene delivery due to their unique structural and biological properties. VLP are empty structures conformed by the assembly of viral capsid proteins. VLP of the cowpea chlorotic mottle virus (CCMV) are icosahedral structures of 28 nm in diameter and conformed by 180 capsomers, that have attracted interest because of their ability to reversibly self-assemble and disassemble *in vitro*, depending on pH and ionic conditions. Recombinant production of CCMV VLP has been reported using various expression systems, mostly *E. coli*, however poor results have been obtained, including low production yields, difficulty in refolding and recovery of inclusion bodies, and long and costly purification processes. In addition, the low stability of CCMV VLP at physiological pH has limited their applications in humans. To overcome such difficulties, in this work CCMV VLP were produced for the first time by the baculovirus-insect cells expression vector system (BEVS). BEVS was selected for its ease of scaling-up to industrial level, the high yields of protein expression, the possibility of performing complex post-translational modifications of secreted and correctly folded proteins, and its versatility for efficiently co-expressing several proteins. CCMV VLP produced in Sf-9 cells in 1L shake flasks (PSFM media, 27 °C, 110 rpm, 0.1MOI, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV)) were purified through saccharose gradients and identified by western blot. Electron micrographs and dynamic light scattering (DSL) measurements revealed that the recombinant capsomers self-assembled into empty icosahedral structures of 30nm diameter, similar to native CCMV. We showed that successful production of recombinant capsomers of CCMV by BEVS is possible, yielding high protein productivity, and offering the opportunity to design strategies to improve the CCMV as nanocarriers. In this work, nanocarriers produced by the BEVS and stabilized with several polymers will also be shown.

Acknowledgments: V. Hernández, M. A. Contreras.

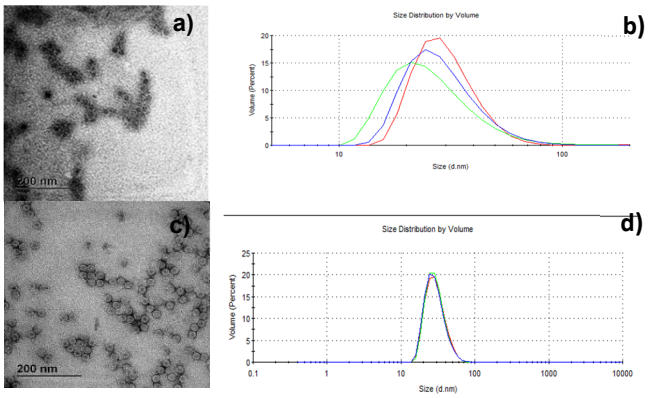

Fig 1. a) TEM of recombinant CCMV-VLP and b) Dynamic light scattering (DLS), volume distribution of the particles formed by recombinant VLP of CCMV (CCMV-bac). c) TEM of native CCMV. d) DLS, volume distribution of the particles formed by recombinant VLP of native CCMV (CCMV-wt). TEM images were obtained in a Zeiss Libra 120 transmission electron microscope at a magnification of 31,500x at 80 kV.

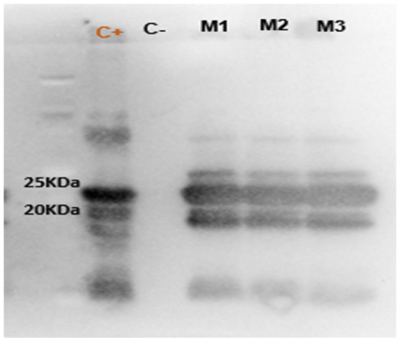

Fig 2. Western-Blot of recombinant capsid protein of CCMV, detected by polyclonal anti-CCMV, CCMV-wt as positive control

Table 1. size distribution by volume of VLP and percentage of population measured of recombinant (CCMV-bac) and native (CCMV-wt) CCMV.

ID	size (nm)	Distribution %
CCMV-bac	30.55	99.3
CCMV-wt	29.75	100