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BT-P05

DEVELOPMENT OF A REVERSE GENETIC SYSTEM FOR THE STUDY OF BACULOVIRUS P74 PROTEIN

Turco CS, Belaich MN, Herrero-Sendra S, Ghiringhelli PD
LIGBCM. Dpto Ciencia y Tecnología. Universidad Nacional de Quilmes. E-mail: ceciliaturco@gmail.com

Baculoviridae is a virus family containing members that infect insects from different orders: Lepidoptera, Hymenoptera and Diptera. These pathogens are excellent candidates for biological control of agriculture pests because they specifically infect and kill the host. The large cccdsDNA genomes (80-180 kbp) encode between 90-180 proteins. During infection exist two phenotypes: BVs (Budded Viruses), responsible to systemic infection; and OBs (Occluded Bodies), containing a protective matrix and responsible of per os infection. Each phenotype has different proteins involved in specific host cell recognition: F or GP64 in BVs and PIFs (Per Os Infectivity Factors) in Obs.
P74 protein is one of the 5 recognized PIFs, a transmembrane polypeptide located in virions derived from OBs. With the aim to study its function we designed and developed a reverse genetic strategy based on Bac to Bac system. In particular, we modified the bacmid of AcMNPV by the replacement of p74 gene for polyhedrin; and then, we introduced gfp and 16 p74 chimeric genes constructed from different species viruses: AcMNPV, SeMNPV, HaMNPV and AgMNPV. On the other hand, we constructed fusions between p74 and dsRed to infer the P74 topology. The different recombinant viruses generated in this work were studied by microscopic observations, immune trials and bioassays highlighting the role of each structural P74 domain.

BT-P06

EFFICIENT PRODUCTION OF A KDEL-TAGGED DENGUE VIRUS PROTEIN IN PLANT CELL SUSPENSION CULTURES

Martínez CA, Giulietti AM, Rodríguez Talou J
Microbiología Industrial y Biotecnología, Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires. E-mail: carom@ffyb.uba.ar

Dengue virus envelope glycoprotein (DV-E) is the antigen associated with immunity induction and it is an effective candidate for the development of a subunit vaccine and a promising antigen for diagnostic kits. As a part of a project to develop a plant-made dengue virus vaccine, we explored the ability of plant cells to produce DV serotype 2 (DV-2) E protein in *Nicotiana tabacum* and *Morinda citrifolia* cell suspension cultures. DV-E cDNA was cloned with a signal peptide at its 5' end and with and without the addition of KDEL endoplasmic retention sequence at its 3' end to analyze its influence in recombinant protein accumulation levels. The expression cassette was sub-cloned into pCAMBIA 1305.2 binary vector and the cell suspension culture transformation was carried out using *A. tumefaciens* LBA4404. The maximum accumulation levels (0,71 ± 0,06 mg DV-E/L) were obtained by tobacco cells at 5 days of culture when KDEL tetrapeptide was fused. It represents 0,3% of the total soluble protein. Its integrity was confirmed by western blot. The recombinant protein was reactive with anti-E monoclonal and polyclonal antibodies. Our results demonstrate for the first time that plant cell *in vitro* cultures represents a low cost expression system suitable for the production of recombinant DV-E protein in which biosafety conditions are guaranteed.

BT-P07

HUMORAL IMMUNE RESPONSES AGAINST FOOT-AND-MOUTH DISEASE VIRUS INDUCED BY HETEROLOGOUS VIRAL VECTORS

Romanutti C[1], D'Antuono A[1], La Torre J[1], Malirat V[1], Zamorano P[2], Quattrocchi V[2], Mattion N[1]
[1]Instituto de Ciencia y Tecnología Dr. César Milstein-CONICET, [2]INTA Castelar. E-mail: cromanutti@yahoo.com.ar

Foot-and-mouth disease virus (FMDV) is the etiological agent of one of the most transmissible diseases of livestock, causing severe outbreaks and important economic losses worldwide. Herpes Simplex type 1 (HSV) amplicons and type 5 recombinant adenovirus (Ad) vectored vaccines were used for the induction of specific FMDV immune responses in mice. BALBc mice were vaccinated with Ad and HSV vaccines expressing FMDV structural proteins and the 3C viral protease. Three groups of mice received two sequential immunizations with HSV or Ad vectors, or 1μg of inactivated FMDV, respectively. Homologous HSV/HSV or Ad/Ad prime/boost immunizations induced long-lasting FMDV specific antibodies. Mice immunized with HSV amplicons generated mixed IgG1/IgG2a immune responses, while immunization with Ad induced IgG2a as the predominant isotype. Challenge of mice vaccinated with HSV amplicons with a high dose of live virus, resulted in partial protection, with a significant reduction of viremia when compared to mice immunized with Ad. Heterologous vaccination regimens, priming with Ad and boosting with HSV, induced higher levels of FMDV specific antibodies than homologous prime/boost regimes. We demonstrated that Ad and HSV vectors encoding FMDV structural proteins induce potent humoral immune responses when administered either in homologous or heterologous prime/boost immunizations.

BT-P08

OPTIMIZED LOVASTATIN PRODUCTION BY SOLID-STATE FERMENTATION WITH *Aspergillus terreus* WILDTYPE STRAIN

Cabral ME, Joya CM, Figueroa LIC, Fariña JI
PROIMI-CONICET. Tucumán, Argentina. E-mail: meucabral@hotmail.com

Statins can inhibit the *de novo* cholesterol biosynthesis at the rate limiting step of HMG-CoA reductase catalysis. For this reason, statins are clinically used as effective drugs for hypercholesterolemia treatment. Objective. This work was aimed at optimizing and simplifying medium composition and fermentation conditions at shake-flask scale. In a second stage, these results were subsequently applied for the massive lovastatin production by Solid Substrate Fermentation (SSF). Methodology. *Aspergillus terreus* MEC was cultured by submerged fermentation (SF) in lactose-yeast extract medium, in shake flasks at 250 rpm and 25°C during 14 days. Optimization included different C- and N- sources and the decrease of salts and trace elements concentration. Additionally, the influence of soy flour incorporation and the replacement of lactose by milk whey powder were evaluated. Different solid supports (sugarcane bagasse, wheat straw, soy flour) for SSF were assayed. Extracted lovastatin was analyzed by RP-HPLC with a Diode Array Detector. Results and conclusions. A lovastatin production of 63 mg/L could be reached with optimized liquid medium. Production was significantly increased, up to ~1000 mg/L, by SSF including cheap and readily available substrates such as whey milk adsorbed on textured soy flour, highlighting the relevance of fermentation strategies for secondary metabolite production.