



DIFFERENTIAL EXPRESSION IN *E. COLI* AND IMMUNODETECTION OF DENGUE 2 VIRUS NS3 PROTEIN

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Introduction: Identification of target proteins of dengue virus (DENV) specific T cells constitutes an appropriate approach for development of a vaccine safe and effective against dengue viruses (1).

The multifunctional NS3 protein has been identified as target of the cellular immune response. It has been demonstrated the predominance of cross-reactive inducing cytotoxic T lymphocytes (CTL) epitopes on dengue virus NS3 protein. These CTL may play a protective role in limiting virus replication (2). Previously, DENV2 NS3 protein has been cloned and expressed as insoluble protein using pQE vectors (3, 4). In this study, the full length cDNA of ns3 gene was cloned and expressed with N-terminal histidine tag in *Escherichia coli* (*E. coli*) as soluble protein. In order to evaluate the immune response elicited in BALB/c mice by the recombinant NS3 protein, a study of *E. coli* expression and antigenical evaluation of the NS3 protein was made.

Methods

Construction of NS3 plasmids

The RNA was isolated and amplified by PCR from D2 58/97 2P C6/36 HT (strain isolated from patient serum infected during Santiago de Cuba municipality Cuba outbreak in 1997 with DHF). The NS3 cDNA was digested with BamHI/HindIII and cloned into the pET-21d vector (Novagen) previously digested with the same restriction enzymes. Transformed clones were obtained by transformation of the ligation reaction in DH5 α cells. Recombinant clones were determined by BamHI/HindIII restriction analysis and colony PCR. Afterwards, positives clones were sequenced by using Sequenase 2.0 kit (Amersham-USB, USA).

Expression and Immunodetection of Recombinant NS3 protein

NS3 Expression: pET21d-NS3 expression plasmids were transformed into *E. coli* strain BL21 cells. In order to achieve the best expression conditions and a soluble NS3 protein an *E. coli* expression study was carried out. Parameters such as: temperature, IPTG concentration, induction time, additives addition were evaluated by using six recombinant clones. Different cellular disruption methods were applied to achieve the recombinant NS3 protein in the soluble fraction. All fractions were analyzed by SDS-PAGE.

NS3 Immunodetection: Protein was recognized by Western blot using NS3 monoclonal antibody (Mab), anti-NS3 protease, Mab anti-His 6x tag.

Results

Eight pET21d-NS3 clones were positives by colony PCR, restriction analysis and nucleotide sequencing (Figure 1). Six clones were expressed in BL21 cells, and all of them

expressed the NS3 protein. The best NS3 expression levels were obtained at 20°C, 0.5mM IPTG, 22h post-induction, and the use of glucose. NS3 was obtained at soluble fraction after cell disruption by using 0.1% Triton X-100, pH 7.5 in buffer lyses and 6 cycles of sonication. The recombinant NS3 protein exhibited recognition by homologous anti-NS3 Mab, anti-NS3 protease, and anti-Histidine Mab (Figure 2).

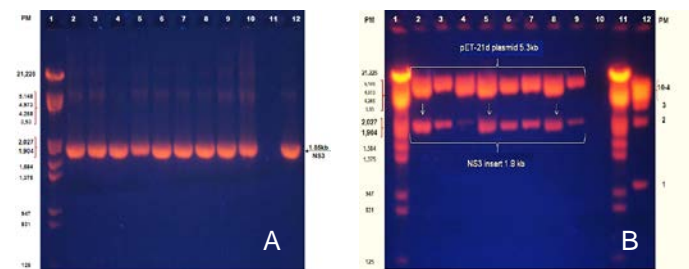


Fig 1. Selection of pET21d-NS3 recombinant plasmids. Lane 1: λ DNA EcoRI/HindIII. Lane 2-lane 8: recombinant clones 1-8, a band correspondent to ns3 gene is observed at 1.85kb. **A:** Colony PCR. Lane 10 and 12: positive controls (ns3), lane 11: negative control. **B:** BamHI/HindIII restriction analysis. Lane 11: λ DNA EcoRI/HindIII and lane 12: 1kb DNA marker. All DNA markers are from Promega.

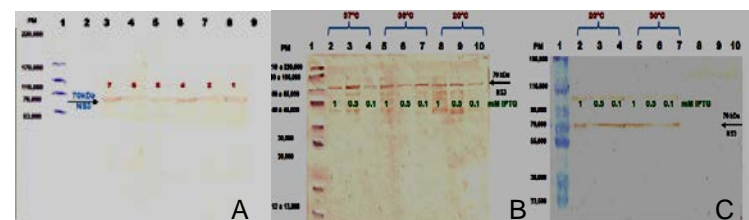


Fig 2. Immunoblotting of the recombinant NS3 protein. **A:** reactivity against anti-His Mab, expression of six recombinant clones. Expression of one recombinant clone at different temperature, IPTG induction, **B:** reactivity against anti-NS3 Mab, **C:** reactivity against anti-NS3 protease.

Conclusions

1. The recombinant NS3 protein was expressed in soluble form by using the pET expression system and BL21 cells.
2. The high solubility of NS3 protein was achieved by the use of no ionic detergent in the lysis buffer and sonication as cellular disruption method.
3. The recombinant NS3 soluble protein exhibited recognition by homologous anti-NS3 Mab, anti-NS3 protease and anti-Histidine Mab.

References

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