

EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF DENGUE VIRUS 2 IMMUNOGENIC NONSTRUCTURAL PROTEIN 1 (NS1) IN *PICHIA PASTORIS*

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Dengue virus is a member of *Flaviviridae* family. It causes dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue virus contains a single-strand RNA of about 10.7kb. This genome encodes three structural proteins (E, C and M) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5). NS1 is a 40-kDa glycoprotein that may form homodimers. Several reports have shown that NS1 protein is highly immunogenic and may induce protective antibodies with complement fixing activity, killing infected target cells. Our aim is to characterize it biochemically and to investigate its function during dengue virus infection. This protein was obtained from a plasmid containing the whole dengue virus 2 genome, cloned into a pPICZ α B plasmid specific to *Pichia pastoris* expression system. After transformation, we selected zeocin-resistant recombinant clones. Positive colonies were grown in minimal methanol and dextrose medium, to identify Mut⁺ transformants, and in high zeocin concentration complex medium, to screen for multi-copy integrants. The highest expression level occurred 96 hours after induction, with 1% methanol added every 24 hours to maintain induction. The expressed protein was identified by Western Blotting, using an acidic fluid polyclonal antibody. Currently, we are carrying out the structural and functional characterization of the purified protein.

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