TITLE: CLONING OF NON-STRUCTURAL PROTEIN GENE 1 (NS1) OF DENGUE VIRUS TYPE 2 (DENV2) IN pPICZαA FOR HETEROLOGOUS EXPRESSION IN YEAST *PICHIA PASTORIS*

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ABSTRACT:

Dengue is one of the most important human infectious diseases transmitted by arthropods in the world. Annually, infections with the dengue virus cause more than 100 million cases of classic dengue fever and more than 500 thousand cases of dengue hemorrhagic fever. Brazil leads the ranking of countries where the disease is endemic with more than 1.5 million cases already reported, according to the World Health Organization (WHO). The dengue virus belongs to the *Flaviviridae* family and has 4 antigenically distinct serotypes, named DENV1, DENV2, DENV3, and DENV4. The flavivirus genome consists of a single RNA positive polarity tape averaging 11 kilobases (Kb). Translation of the genome forms a single polyprotein which upon proteolytic processing results in the final ten viral proteins. The proteins C, M and E are components of the viral structure and are related to recognition and cellular infection, as well as protection of the genetic material. The 7 non-structural proteins (NS) are related to viral replication, the expression of the viral proteins themselves and the virulence of the serotypes. The detection of early anti-NS1 antibodies in the patient's serum is used for rapid diagnosis of the disease contributing to treatment in the early stages. The objective of the present work was to clone the gene of NS1 protein of dengue virus type 2 (DENV2) in the expression vector pPICZαA. This cloned plasmid will be used for gene expression in *Pichia pastoris* yeast aiming at the heterologous production of antigens for immunological assays. To that end, the pUC57 cloning vector was obtained with the NS1 protein gene sequence optimized for expression in yeast. First, the plasmids pUC57_NS1DENV2 and pPICZαA were amplified in *E. coli* cells TOP10F and extracted by alkaline lysis method. Then, a double digestion was performed on both plasmids at the restriction sites for the EcoRI and KpnI enzymes. In an agarose gel, the bands pertaining to the NS1DENV2 insert and the digested pPICZαA vector were excised and purified by the Qiaquick Gel Extraction kit. Cloning was then performed by ligating the gene into the vector by the T4 ligase enzyme, and finally, the resulting plasmid pPICZαA_NS1DENV2 was used for genetic transformation of E. coli TOP10F. Confirmation was done by PCR and plasmidial digestion. The results confirm the success of gene cloning in the vector. That will be used in the next steps of yeast transformation.

KEYWORDS: dengue, NS1 protein, *Pichia pastoris*, heterologous expression

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