TITLE: RECOMBINANT EXPRESSION OF NON-STRUCTURAL PROTEINS 1 (NS1) OF DENGUE VIRUS TYPE 1 (DENV1) AND DENGUE TYPE 3 (DENV3) IN YEAST *PICHIA PASTORIS*

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ABSTACT:

Pichia pastoris yeast is of great interest to the biotechnology industry as it is an excellent host for the production of recombinant proteins. It has the ability to produce proteins in a stable manner and with post-translational modifications typical of eukaryotes. Pichia pastoris is a yeast that metabolizes methanol as a carbon source and presents two genes, AOX1 and AOX2, which encode enzymes with alcohol oxidase function. The AOX1 promoter is used for the expression of recombinant proteins under the induction of methanol in yeast cultures. The dengue virus belongs to the *Flaviviridae* family and has 4 antigenically distinct serotypes, named DENV1, DENV2, DENV3, and DENV4. The viral genome consists of single-stranded positive RNA strand encoding 10 final proteins, of which 7 are non-structural (NS) proteins that are related to replication, translation of the proteins themselves, and virulence of the serotypes. NS1 protein is used to detect early anti-NS1 antibodies in the suspected patient's serum, contributing to treatment in the early stages. In this context, the focus of this work was to use *Pichia pastoris* (KM71H) to produce large amounts of the NS1 protein of the DENV1 and DENV3 viruses for diagnostic applications. Yeasts were transformed, independently, by electroporation with the pPICZαA expression vectors cloned with the NS1 protein genes from each of the viruses (pPICZαA_NS1DENV1 and pPICZαA_NS1DENV3). The resulting colonies were selected through the resistance gene. Non-transformed yeasts were used as negative control of expression. Then, they were grown and submitted for genomic DNA extraction for PCR confirmation of transformation. After confirmation, the transformed yeast were cultured for 8 days and induced with methanol, every 12 hours, for expression of NS1 proteins in casamino acid enriched medium. The proteins produced in the soluble form were purified from the culture supernatant by affinity chromatography through the histidine tail (His-tag). Next, SDS-PAGE and Western Blot were performed for confirmation of the induced expression in the yeast. The results corroborate the efficient transformation and production of heterologous proteins. The next steps will be to carry out an ELISA immunological test to evaluate the diagnostic potential of recombinant proteins.

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

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