TITLE: PRODUCTION OF ZIKA VIRUS' EDIII AND ΔN-NS1 USING A BIFUNCTIONAL VECTOR FROM GRAM-POSITVE BACTERIA IN ESCHERICHIA COLI

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ABSTRACT:

The arrival of the Zika virus (ZIKV) in Brazil, a flavivirus as well as the Dengue virus and yellow fever, and its consequent epidemic during the summer of 2015-2016 created a new scenario in the national control of arboviruses. Neurological complications associated with ZIKV infection, such as Guillain-Barré syndrome, in young and adult, and microcephaly in fetuses and newborns have raised concerns about ZIKV. Thus, the search for rapid and accurate diagnosis, as well as prophylactic control measures such as the development of a vaccine, have become a goal of real world importance, especially in tropical countries where the virus is transmitted by the endemic dipteran vectors of the genus Aedes. In the present work, sequences encoding the ZIKV EDIII and ΔN-NS1 protein subunits were inserted into a bifunctional plasmid pHCMC02 under the control of the constitutive promoter PlepA in order to obtain heterologous expression of the protein subunits in *Escherichia coli*. For this purpose, we used a vector whose functionality was previously thought to be exclusive to gram-positive bacteria (*Bacillus subtilis*), but our group found to be bifunctional in competent *Escherichia coli* Dh5-α. ZIKV EDIII and ΔN-NS1 were chosen by their lower similarity with Dengue Virus homologue proteins and synthetically obtained with BamHI and XbaI restriction sites on the 5' and 3' points for cloning into pHCMC02. Competent *E. coli* Dh5-α was then transformed with the new constructs containing either ZIKV EDIII or ZIKV ΔN-NS1 DNA sequences and the cellular extract was evaluated for their serum reactivity by indirect ELISA using serum from a patient whose infection by ZIKV was confirmed by qPCR and a control serum. Overall, the antibody titers from the ELISA assays using the serum form the infected patient were higher than the control serum. For the ΔN-NS1, the patient serum presented a antibody titer 3 fold higher than the control serum, while for the EDIII the patient serum presented a antibody titer 2 fold higher than the control serum. The plasmids constructs were proved to be suitable for *E. coli* heterologous expression of reactive recombinant antigens EDIII and ΔN-NS1 of ZIKV, representing a easy way to produce polypeptides that might contribute to the development of serological assays and vaccine strategies, aiming at the diagnosis and prevention of ZIKV infection.

KEYWORDS: Flaviviridae, Arboviruses, Vaccines, ELISA, Zika.

DEVELOPMENT AGENCY: MPT-BA, CAPES and CNPq