Cloning of Mayaro virus structural polyprotein sequence into pPICZα A expression vector in *Escherichia coli*

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The Mayaro virus (MAYV) is an arbovirus (*Togaviridae* family, *Alphavirus* genus) whose main vector is the *Haemagogus janthynomis* mosquito. In humans, it is responsible for causing a debilitating febrile illness. MAYV has been spreading rapidly throughout the Americas and this situation has caused concern for its emergence potential in urban environments. In addition, Mayaro fever has similar symptoms to other arboviruses, which makes diagnosis difficult. Therefore, there is a need to develop strategies to protect the population, since there are still no vaccines or specific treatments for Mayaro fever. The aim of this study was to evaluate the cloning of the MAYV structural polyprotein sequence into the pPICZα A expression vector, which contains the coding sequences for C, E1, E2, E3 and 6k proteins, in *Escherichia coli* bacteria. *E. coli* TOP10 competent cells were transformed by electroporation. The transformants were selected and grown in low salt LB medium (Luria-Bertani; 1% peptone, 0.5% yeast extract, 0.5% NaCl) supplemented with Zeocin™ (20 µg/mL). Then, to confirm the transformation, the colonies were cultured to obtain a larger volume of cells and plasmid DNA was extracted by alkaline lysis. The DNA was quantified by reading the absorbance at 260 nm on NanoDrop™ 2000 spectrophotometer. Transformation was confirmed by PCR with primers specific to AOX1 promotor. Bands were visualized by electrophoresis in 1% agarose gel. Cloning was confirmed by observing the expected size of the 4256 bp insert band. After confirmation, plasmid DNA was linearized with restriction enzyme EcoRI to be further used in yeast transformation. As a result, the plasmid DNA collected will be used in future experiments aimed at expressing the structural proteins of MAYV in the form of virus-like particles (VLPs) using the yeast *Komagataella phaffii*. The VLPs generated and characterized will be evaluated as an antigen for vaccines and diagnostic kits.

Key words: Mayaro virus, virus-like particles, vaccine.

Clonagem da sequência da poliproteína estrutural do vírus Mayaro no vetor de expressão pPICZα A em *Escherichia coli*

O vírus Mayaro é um arbovírus da família *Togaviridae* e gênero *Alphavirus*. Em humanos, causa febre aguda, vômito, diarreia e dores articulares que podem evoluir para um quadro crônico de artrite e miosite. Logo, é necessário desenvolver estratégias que protejam a população, visto que ainda não existem vacinas ou tratamentos específicos para a febre do Mayaro. Neste sentido, este trabalho teve como objetivo avaliar a eficiência da clonagem da sequência de poliproteína estrutural de MAYV em vetor de expressão pPICZα A, que contém as proteínas estruturais, do envelope viral E1, E2 e E3, e a proteína 6k em *Escherichia coli*.

Palavras-chave: Mayaro virus, *virus-like particles*, vaccine.

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