CLONING OF BHALTERNIN FROM BOTHROPS ALTERNATUS VENOM FOR YEAST EXPRESSION

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Snake venoms act against bacteria and viruses. Besides, peptides and proteins are snake venom's biologic active compounds that are also of interest in the treatment and prevention of thrombotic disorders, in the effect of the arterial pressure and cancerous cells' contention. The characterization of the venom biomolecules is important for the development of new therapeutic alternatives. Bhalternin, a thrombin-like enzyme from *Bothrops alternatus* snake venom, may be of interest as a therapeutic agent in the treatment and prevention of thrombotic disorders. However, its isolation from crude venom is complex, involving three different chromatographic steps, and yielding approximately 1.8% from the initial venom. Thus, obtaining the recombinant form of Bhalternin can broaden its research, resulting in new therapeutic alternatives that expand the biotechnology perspective. This research aims at cloning of the Bhalternin for expression in yeast system. The Bhalternin gene was obtained from digestion of pPICBH 1 DNA with the restriction enzymes *Eco* RI and *Not* I. Cloning was performed with the vector pPICZα A. The transformation of the TOP10 competent bacteria was performed with the ligation reaction mixture containing the vector and gene of interest, as well as the mixture containing only the vector in parallel. The transformed cells were selected on the low salt LB medium with Zeocin. The plate obtained from the transformation with the DNA of interest originated 11 colonies and the control plate, two. The colonies were subjected to PCR and six out of the 11 colonies (54.5%) presented amplified sequence corresponding to the expected size. Two colonies were selected and their plasmid DNAs were analyzed with restriction enzymes, presenting two bands corresponding to the vector and the Bhalternin coding sequence. One of them was sequenced by the Sanger method and the sequence obtained was compared to the original sequence, showing 100% identity. The plasmid construction was successful and its DNA will be used for the transformation of *Pichia pastoris* cells for the Bhalternin expression.

Keywords: plasmid, yeast expression, Bhalternin, snake venom, antimicrobial.

Development Agency: FAPEMIG, PROPP-UFU, INCT-NANOBIOFAR, CNPq.