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CLONING AND EXPRESSION OF CDNA SEQUENCES CODING TO RHIPICEPHALUS MICROPLUS PARAMYOSIN FRAGMENTS

Autor(es): BRUNA FERREIRA LEAL, ITABAJARA DA SILVA VAZ JR., CARLOS ALEXANDRE SANCHEZ FERREIRA

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» Instituição: PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL (PUCRS)

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Rhipicephalus microplus is a cattle tick that causes great damages to livestock. Conventional tick control use acaricides, resulting in contamination of milk and meat, selection of resistant tick populations, as well as raising costs. Therefore, the development of a vaccine has been considered an alternative to control tick populations and, for this, identification and characterization of protective antigens have been searched. Paramyosin is a protein present in invertebrates, able to modulate the host immune system during a parasitic infestation. Vaccination trials using this protein against different parasites have been reported with satisfactory results. Analyses of sera of infected patients with some parasites showed a humoral immune response mainly against specific regions of paramyosin (N-terminal or carboxi-terminal). Thus, this work aims the cloning and expression of cDNAs coding for paramyosin fragments (N-terminal, internal and carboxi-terminal) of R. microplus to characterize and determine the main immunogenic and protective region. Amplification of DNA sequences was performed by PCR using primers designed to include a sequence coding to six histidines. Cloning was performed using Champion pET Directional TOPO Expression kit (Invitrogen) and the vector with the different DNA sequences were inserted by electroporation in Escherichia coli TOP 10 strain. The construction containing the DNA sequence coding to carboxi-terminal fragment was inserted by heat shock in E. coli BL21 strain and expression was performed using IPTG. Cloning was confirmed by PCR and vector sequencing, whereas expression was verified by western-blot. Expression analyses are being conducted for the other recombinant fragments and, thereafter, all will be purified by affinity chromatography. ELISA and western-blot will be performed to analyze the recognition degree of the different paramyosin fragments by bovine sera infested and not-infested with ticks. These experiments can help the evaluation of paramyosin as a possible candidate antigen to integrate a vaccine against tick.