Amplification, Cloning, Expression and Purification of Enzyme Adenosine Deaminase (ADA) of *Trypanosoma evansi*

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INTRODUCTION: *T. evansi* causes a highly pathogenic disease in horses popularly known as "Surra" or "Mal das Cadeiras". The Pantanal (Brazil) outbreaks are recorded due to the large population of horses. To date there are no effective treatment methods causing major damage to livestock. Trypanosomes present themselves vulnerable in relation to the metabolism of purines as these organisms do not have the pathway "*De novo*" of meeting their requirements through the rescue of preformed bases and demonstrate complete dependence of purines of their hosts. Among the components of this system point out that adenosine has its concentration controlled by the enzyme adenosine deaminase (ADA). **OBJECTIVES:** The objectives of this study were to amplify, clone and sequence the ADA gene from *T. evansi* DNA samples and express the recombinant protein ADA. **RESULTS AND DISCUSSION:** The coding region of the ADA gene was amplified from genomic DNA of *T. evansi* and yielded a sequence of 1857 bp showed high degree of similarity (95%) ADA *T. brucei*. This region was cloned into the pGEM-T vector Easy®. After digestion of construct pGEM: ADA fragments were cloned into expression vector pET30. The expression analysis by SDS-PAGE demonstrated that the temperature of 18ºC for 24 hours and 0,05 mM IPTG induction was the most effective indicating molecular mass of approximately 68 kDa. The Western blot analysis detected the ADA enzyme in the protein extract of T. evansi only in the insoluble fraction. The protein was solubilized and purified by affinity chromatography. **CONCLUSIONS:** Through these results the presence of ADA in *T. evansi* was confirmed. Tests will be performed to detect the enzymatic activity of ADA. Their study can contribute to the development of new chemotherapeutic agents and the development of specific inhibitors for the ADA.

Keywords: T.evansi. Metalbolismo of purines. Adenosine deaminase (ADA).

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