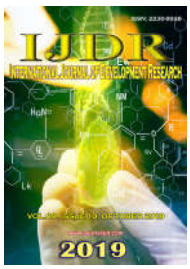
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 09, Issue, 10, pp. 30900-30904, October, 2019



RESEARCH ARTICLE OPEN ACCESS

EXPRESSION, REFOLDING AND PURIFICATION PROTOCOL FOR *Trypanosomaevansi* ADENOSINE DEAMINASE PROTEIN IN *E.COLI* ROSETTA GAMI

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ARTICLE INFO

ArticleHistory:
Received 29th July, 2019
Received in revised form
17th August, 2019
Accepted 03rd September, 2019
Published online 30th October, 2019

Key Words:

T.evansi; Purine metabolism; Adenosine deaminase (ADA); Refolding

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ABSTRACT

Trypanosomaevansi causes a highly pathogenic disease in equines popularly known as "Surra". In Brazilian Pantanal, outbreaks are recorded due to the large population of horses. Losses to livestock are due to inefficient treatments. Trypanosomes are vulnerable to purine metabolism because they do not have the *De novo*pathway and they satisfy their requirements by salvaging the preformed bases demonstrating acomplete purine dependence on their hosts. Among the components of this system, we highlight Adenosine,which has its concentration controlled by the enzyme Adenosine Deaminase (ADA). The objectives of this study were amplifying, cloning and sequencing*ADA*gene in*Trypanosomaevansi* (*TeADA*). The coding region of *TeADA*was amplified from the *T. evansi* genomic DNA and the 1857 bp sequence showed a high degree of similarity (95%) with *T. bruceiADA* gene (*TbADA*). The amplicon was cloned in apGEM-T Easy® vector and expressed using pET30 vector. Protein expression wasanalysed usingSDS-PAGE and the best condition to obtain a protein of approximately 68 kDa was 18 °C for 24 hours and induction with 0.05 mM IPTG.Protein was solubilized through refolding and it was purified by affinity chromatography.Expression of TeADAwas confirmed by Western blot.

Citation: Cristina Alves Ribeiro, Franciane Batista, Ketriane Mota de Souza *et al.* 2019. "Expression, refolding and purification protocol for Trypanosomaevansi adenosine deaminase protein in e.coli rosetta gami", *International Journal of Development Research*, 09, (10), 30900-30904.

INTRODUCTION

Trypanosomaevansi is the most widespread pathogenic trypanosome in Latin America, Asia and Africa, causing a disease called "Surra" (Gutierrez, 2010 and Rjeibi, 2015), that affects domestic and wild animals (Kurup, 2012). It is mechanically transmitted by hematophagous insects of *Tabanidae* and *Stomoxidae* genera and by bats (*Desmodusrotundus*) (Habila, 2012 and Wells, 1984). The first sign of infection is the swelling of the skin caused by the multiplication of the parasites (Habila, 2012). After this, the main symptomsare rapid weight loss, intermittent fever, pelvic edema and blindness (Rodrigues, 2005 and Colpo, 2005). The death of the animals usually occur within weeks or few months, however, there are reports of chronic infections with the progress of the diseasemoving forward several months (Rodrigues, 2005). Trypanosomes do not have the *De novo*pathway for purine synthesis (Rottenberg, 2005), then their purine requirements were acquired by Purine salvage pathway, showing complete dependence on purines from their hosts (Ogbunude, 1983 and Pérez-Aguilar, 2015). Despite the numerous studies, purine metabolism still requires a more detailed biochemical characterization (Da Silva, 2011 and Sanchez, 2002). In the salvage pathwayadenosine deaminase (ADA) catalyzes the deamination of adenosine generating inosine or, adenosine can undergo the action of a phosphorylase (PNP) which forms adenine (Pérez-Aguilar, 2015; Da Silva, 2011; Sanchez,, 2002 and El Kouni, 2003). In mammals, ADA plays different roles and its deficiency causes many disorders (Dalla Rosa, 2013). Previous studies indicate that all African trypanosomes, except *T. vivax*, do not have ADA activity (Ogbunude, 1983). A recent study detected ADA in *T. evansi* and it has adapted an assay to measure its enzymatic activity (DA SILVA, 2011). The aims of this study wereto express and to purify ADA enzyme of *T. evansi*.

MATERIAL AND METHODS

Amplification of the coding region of the ADA enzyme gene: The coding region of the ADA gene was amplified by

PCR with specific oligonucleotides: TeADAF forward (5'TGCGGACATATGATGCATGTGGATGTGCCTC3') and reverse TeADAR (5'TAAATTCTCGAGTCATGCAACGCG TTGCTCCC3') flanked by*Nde*I and *Xho*Icleavage sites, respectively. Briefly, PCR assay was performed in a total volume of 25 µl containing 80 ng of the genomic DNA and 8.5 pmoles of each primer pair, 1U Taq DNA polymerase, 0.2 mMdNTPs, 25 mM MgCl2, 5 µl buffer (5x Green GoTaq® Flexi buffer (Promega, Madison, USA) and ultrapure water to reach the final volume. PCRwas performed in a Maxygene H Thermal Cycler (Axygen, Union City, USA) with the following parameters: 5 min at 95˚C, followed by 30 cycles of 30s at 95˚C, 30s min at 68˚C, 30s min at 72˚C, and a final extension step of 10 min at 72˚C.Amplicons were analyzed by electrophoresis on 1.5% agarose gel stained with Gel Red dye (Biotium, Freemont, USA) with a 100 bp ladder standard (Ludwig Biotecnologia, Porto Alegre, Brazil), and visualized under UV light.Fragment of 1857 bp (corresponding to TeADA according http://tritrypdb.org) was cut and purified by "*Pure link quick gel extraction kit*" (Invitrogen, Carlsbad, USA), following the manufacturer's protocol.

Cloning vector TeADA enzyme gene into pGEM-T-Easy®
Purified DNA was cloned into apGEM-T-Easy® vector (Promega, Madison, USA) using T4 DNA ligase (Promega, Madison, USA) and transformation into *E.coli* DH10B electrocompetent cells and it was transferred to solid LB medium containing sodium ampicillin (100µg / ml), isopropyl ß-D-1-thiogalactopyranoside (IPTG 0.5mM) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal 20µM). Plates were incubated at 37 ° C for 12 hours. Positive clones were expanded in LB liquid containing ampicillin (100µg / ml). After 12 hours of growth, the recombinant plasmid was purifiedwith "*Pure Link Quik Plasmid Miniprep*" following the manufacturer's instructions.

Gene cloning into pET28a expression vector: The pGEM-T-Easy: ADA and pET28a expression vectorswere digested with *Nde* I and *Xho* I restriction enzymes. Samples were analyzed by electrophoresis and the 1.5% low melting agarose gel was visualized under UV light and the fragments of interest were cut and separately purified by the "*Pure Link quick gel extraction kit*". For the construction pET28a: ADA, it was performed in a binding reaction using the vector index calculation, indicated in the manufacturer's protocol. The ligation product was transformed into *E.coli* DH10B electro competent cells and transferred to LB plates containing kanamycin (50µg / ml). The clones were subjected to electrophoresis on 1.5% low melting agarose gel and visualized in UV light. Plasmid DNA extraction was performed through the "*Pure Link Quick Plasmid Miniprep kit*" following the manufacturer's instructions.

Gene cloning into expression vector pET30: The pET28a: ADA construction and the pET30 expression vector were digested with restriction enzymes *Nco*I and *Xho*I. This step enabled the products to maintain the His * Tag N-Terminal of the pET28a: ADA construction. Samples were resolved in electrophoresis on 1.5% low melting agarose gel and analyzed in UV light and the fragments of interest (ADA and pET30 gene) were cut and separately purified by the *"Pure link quick gel extraction kit*".For the pET30: ADA construction the same protocol above was used. All plasmid constructs and recombinants were confirmed by DNA sequencing by Ludwig Biotec (Brazil) using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) (Figure 1).

Expression of recombinant protein: PET30-ADA vector was transformed into electro competent*E. coli* bacteria of *Rosetta gammi* strain. After transformation, cells were grownin LB medium supplemented with kanamycin (50µg / ml) and chlorofenicol (35mg / ml).One colony was transferred in 250 ml LB medium supplemented with kanamycin (50 µg / ml) and chlorofenicol (35 mg/ml). Stirring was carried out at 200 rpm and 37 ° C until reaching the optical density ($OD_{.600nm}$) of 0.6 (~ 3 hours). For gene expression induction it as added IPTG at a concentration of 0.05 mM and the culture was maintained for a further 24 hours at 18 ° C. Bacteria were then concentrated by centrifugation at 3,000 g for 10 minutes at 4 °C. Supernatant was discarded and the pellet was resuspended in 12.5 ml of Buffer A (50nM Tris-HCl, 300mM NaCl, pH 8.0). The sample was sonicated in 4 cycles of 30 seconds at minimum power with intervals of 30 seconds in an ice bath. Further centrifugation was performed for 40 minutes at 4 ° C and 8,000g.

TeADArecombinant protein refolding: The pellet was resuspended in 5 ml of Buffer A containing 1M Urea. The sample was sonicated in 4 cycles of 30 seconds at minimum power with ice bath interval followed by centrifugation at 8,000 g for 40 minutes at 4 °C. This step was repeated three times. After the last wash the collected material was resuspended in 2.5 ml of Buffer A and 8M Urea was dropwise added until the sample became clear and homogeneous (approximately 10 ml). The first dialysis, at 1:10 ratio against Buffer A containing EDTA (0.1 mM), occurred for 4 hours at 4 ° C. The second dialysis was performed at a ratio of 1: 100 against Buffer A for 16 hours at 4 ° C. Solubilized protein sample was recovered by centrifugation at 8,000 g for 40 minutes at 4 ° C.

TeADArecombinant protein purification: Ni-NTA Superflow resin was pre-equilibrated with ten times the volume of the Buffer A column. Solubilized protein was applied and washed with ten column volumes of Buffer A and ten column volumes of Buffer B (50 mMTris-HCl, 300 mMNaCl, pH 8.0, 30 mM imidazole). Protein was then eluted with three column volumes of Buffer C column (50 mMTris-HCl, 300 mMNaCl, pH 8.0, 300 mM imidazole).SDS-PAGE (4.5% gel concentration and 12% gel separation) and Western Blot were performedto analyze the purification.

RESULTS AND DISCUSSION

Amplification, sequencing and cloning: For PCR standardization, a hybridization temperature gradient was tested with temperatures ranging from 63 °C to 69 °C. The temperature of 68.3 °C showed the best amplification. As expected *TeADA* gene presented a fragment of approximately 1857bp, corresponding to the size of the gene indicated in the database (http://tritrypdb.org). The PCR amplified fragment was sequenced and analyzed using BLAST tool in (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence obtained showed a 100% identity with T. *evansi ADA* gene (Figure 1) and 95% identitywith *T. brucei ADA* confirming that the amplified fragment of interest corresponds to the coding sequence of the ADA gene.

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TevADA      ATGCATGTGGATGTGCCTCACGACTGTTTCCCGGGTTGTGCCACTCCTCTTGAGGCATTT   60
pET30ADA    ATGCATGTGGATGTGCCTCACGACTGTTTCCCGGGTTGTGCCACTCCTCTTGAGGCATTT   60

TevADA      GCTGTAGCCCTCCGTTCTCAACCGCGTGGTTTTGGTTGGGACTGTAGTGCAATTAGTGCC   120
pET30ADA    GCTGTAGCCCTCCGTTCTCAACCGCGTGGTTTTGGTTGGGACTGTAGTGCAATTAGTGCC   120

TevADA      CTGGATGGTAGGGGGGAGGTGGTAGCAGGGTTTGTGCTGGGACTGCCCCGCGCCTCTCCC   180
pET30ADA    CTGGATGGTAGGGGGGAGGTGGTAGCAGGGTTTGTGCTGGGACTGCCCCGCGCCTCTCCC   180

TevADA      TGTGATAAAATTAGTGGTCCTAGTTTTGTGTGCGTCTCGTTGGGAAGTGGGACGCGTTGT   240
pET30ADA    TGTGATAAAATTAGTGGTCCTAGTTTTGTGTGCGTCTCGTTGGGAAGTGGGACGCGTTGT   240

TevADA      GTGGGTTACAAACCAGTGGAGTTTACTGTTGAGGCCGATTTGATGCTGAGAGACGGGCAC   300
pET30ADA    GTGGGTTACAAACCAGTGGAGTTTACTGTTGAGGCCGATTTGATGCTGAGAGACGGGCAC   300

TevADA      GCGGAGGTTATGGCCCGGCGTGGCCTCGTGGCATTCCTTCTGGATGCAGCAGCGTATCTT   360
pET30ADA    GCGGAGGTTATGGCCCGGCGTGGCCTCGTGGCATTCCTTCTGGATGCAGCAGCGTATCTT   360

TevADA      TCACGGGGTGACGACCGACTTCATTTTGCCGTTGAGCGTCACCATTGTTTTCTTCAGTTC   420
pET30ADA    TCACGGGGTGACGACCGACTTCATTTTGCCGTTGAGCGTCACCATTGTTTTCTTCAGTTC   420

TevADA      TCCGGCGGTGACGCAAGTTGTGATGCAGTGGGGGAGTGTTTCCGTCTTCGGCCCGGCGTG   480
pET30ADA    TCCGGCGGTGACGCAAGTTGTGATGCAGTGGGGGAGTGTTTCCGTCTTCGGCCCGGCGTG   480

TevADA      AGCGTGCACTTGGTGTGCACAGAGTATCCCTGCGGTGCGATGTCTACCCCATTCGGCGGG   540
pET30ADA    AGCGTGCACTTGGTGTGCACAGAGTATCCCTGCGGTGCGATGTCTACCCCATTCGGCGGG   540

TevADA      GCTCATGTGCTCCTGAGTACGCCTAGCGGCCGTTCCCTTTTCGACGCGGATTGGATAGCA   600
pET30ADA    GCTCATGTGCTCCTGAGTACGCCTAGCGGCCGTTCCCTTTTCGACGCGGATTGGATAGCA   600

TevADA      ACCCTTTGCTCGTTGGAGAGGAGCATGTATCTCGCCGCGATGGCAAGACCCTACCTGAGT   660
pET30ADA    ACCCTTTGCTCGTTGGAGAGGAGCATGTATCTCGCCGCGATGGCAAGACCCTACCTGAGT   660

TevADA      TTGTGGACGCTCGCGTGTTTGATGGTCCCGGCTCCTTTACCGTTTGCTATGGCCACCGGG   720
pET30ADA    TTGTGGACGCTCGCGTGTTTGATGGTCCCGGCTCCTTTACCGTTTGCTATGGCCACCGGG   720

TevADA      TAGCTGCTCACCGGTGCTGGCCGGTGGACGATATGCCGTTCGTAGCCCGTGTCAAACCGG   780
pET30ADA    TAGCTGCTCACCGGTGCTGGCCGGTGGACGATATGCCGTTCGTAGCCCGTGTCAAACCGG   780

TevADA      GGAAGGGTCGGCAAAACCTCTGCATGTCGTGTTCGGACAAATTGCTTCGCTGGCACTGCT   840
pET30ADA    GGAAGGGTCGGCAAAACCTCTGCATGTCGTGTTCGGACAAATTGCTTCGCTGGCACTGCT   840

TevADA      TGGGCATCCAGGGCCGAAGACGAATGCGACTGTTTCCGGAGCCCATCCGCCTGGCGGCGG   900
pET30ADA    TGGGCATCCAGGGCCGAAGACGAATGCGACTGTTTCCGGAGCCCATCCGCCTGGCGGCGG   900

TevADA      TGTGGCTTCCGCGAAAGGTTAGCGTTGTTAGTGACAACTGTGTAGCGGTTAGTGCGTTTT   960
pET30ADA    TGTGGCTTCCGCGAAAGGTTAGCGTTGTTAGTGACAACTGTGTAGCGGTTAGTGCGTTTT   960

TevADA      CCCCTCTAGAGAAAGCAAGAGAGGGCCTAAATAGCCGCTTGCATTGTTTTTGTCCTCGTC  1020
pET30ADA    CCCCTCTAGAGAAAGCAAGAGAGGGCCTAAATAGCCGCTTGCATTGTTTTTGTCCTCGTC  1020

TevADA      TTCGCCCTGAGGAACTAGGTGCCCCCTCTGAGGGAACAAATGAGCCTGTGCGAGGTGGTT  1080
pET30ADA    TTCGCCCTGAGGAACTAGGTGCCCCCTCTGAGGGAACAAATGAGCCTGTGCGAGGTGGTT  1080

TevADA      CGCCATTTCCGCAGGTGGATGTTTGTGATTTTGAATCGTCCGCCCTCCGTTTGATCCTGG  1140
pET30ADA    CGCCATTTCCGCAGGTGGATGTTTGTGATTTTGAATCGTCCGCCCTCCGTTTGATCCTGG  1140

TevADA      TGGGCATGAGTGGGGACGATACCCCTCTGGGAAACGGTGGCGCGCCCAAAAGAAAGGATG  1200
pET30ADA    TGGGCATGAGTGGGGACGATACCCCTCTGGGAAACGGTGGCGCGCCCAAAAGAAAGGATG  1200

TevADA      AATCATGCTGGTCCCGTGCTGCGTGGGCCACAGTGGAGGATGGAAATCAAGTTGTTCCCT  1260
pET30ADA    AATCATGCTGGTCCCGTGCTGCGTGGGCCACAGTGGAGGATGGAAATCAAGTTGTTCCCT  1260

TevADA      GTTCCGCGGGTGCAAAAAGGCGTAGGGGGGAACTTGAGGGCGTCGTCGTGCGGTGCGTTT  1320
pET30ADA    GTTCCGCGGGTGCAAAAAGGCGTAGGGGGGAACTTGAGGGCGTCGTCGTGCGGTGCGTTT  1320

TevADA      TGCGCTGGAACGAAGAGAGCACCGCAGCGTTGAACACCAAGGCTGGTGTTCCACGTGGCG  1380
pET30ADA    TGCGCTGGAACGAAGAGAGCACCGCAGCGTTGAACACCAAGGCTGGTGTTCCACGTGGCG  1380

TevADA      TAACGAAGCAAAGTATGAATCGCACAGTGCAACAATTGCTTCAGCTTCAATCGGACGTAT  1440
pET30ADA    TAACGAAGCAAAGTATGAATCGCACAGTGCAACAATTGCTTCAGCTTCAATCGGACGTAT  1440

TevADA      ATTCGGGAGCAAAACCTCCTGACCCTGATACCGCTGCGCTACTGGAGGGGATAGCGTCTC  1500
pET30ADA    ATTCGGGAGCAAAACCTCCTGACCCTGATACCGCTGCGCTACTGGAGGGGATAGCGTCTC  1500

TevADA      GGTTTCCACTCTCTAGGTTGTGGATGGCGTTAAGGCAGCGGGAAATTGCCCGGACGGTTT  1560
pET30ADA    GGTTTCCACTCTCTAGGTTGTGGATGGCGTTAAGGCAGCGGGAAATTGCCCGGACGGTTT  1560

TevADA      CGGGGAGATATCATCAAAGCTGTCGTAGGGTACAGGCCTTTCCTCCAACGGAGGGTGAAG  1620
pET30ADA    CGGGGAGATATCATCAAAGCTGTCGTAGGGTACAGGCCTTTCCTCCAACGGAGGGTGAAG  1620

TevADA      CTGATCACGAAACTAGGCGAGTTGGCAATTATTCGCTTTATATCCCTCAGCGGGTTGTGT  1680
pET30ADA    CTGATCACGAAACTAGGCGAGTTGGCAATTATTCGCTTTATATCCCTCAGCGGGTTGTGT  1680

TevADA      CGGTTGGTGACATGGCGGAAATAGGGAGGAACATCGCATCTGGTGACTCCGTGCATTGTT  1740
pET30ADA    CGGTTGGTGACATGGCGGAAATAGGGAGGAACATCGCATCTGGTGACTCCGTGCATTGTT  1740

TevADA      GGCTACAGGGGTCGCAGCGTGTCGGAGAGTCCGTCCCTGACGAGGTGTGGAAGGAGGGGC  1800
pET30ADA    GGCTACAGGGGTCGCAGCGTGTCGGAGAGTCCGTCCCTGACGAGGTGTGGAAGGAGGGGC  1800

TevADA      TAGGACGTAAACTTCCGTTGCTTTGGGTAGAAAAGCGGGAGCAACGCGTTGCATGA      1856
pET30ADA    TAGGACGTAAACTTCCGTTGCTTTGGGTAGAAAAGCGGGAGCAACGCGTTGCATGA      1856
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Figure 1. Alignment between ADA gene TevSTIB805.10.11400 from TriTrypdb (TevADA) and the cloned protein in pET30 (pET30ADA), showing identical sequence

Cloning in expression vector: The pGEM: ADA constructions were digested. Four samples were submitted to PCR and fragments of approximately 1857bp and 3015bp were released, consistent with the sizes of the *ADA* enzyme gene and the pGEM-T Easy® cloning vector (Promega).After the construction of pET28a: ADA, several attempts at expression and purification were performed without success. This result could indicate that the recombinant protein was not exposing His*Tag during its refolding, making impossible to purify the recombinant protein. As a strategy, pET28a: ADA was digested with the *Nco*I and *Xho*I enzymes to keep the His*tag of the N-terminal portion available. The expression vector pET30 (Novagen®) was digested with the same restriction enzymes to add a second His*Tag which would be exposed during the refolding of the protein thus enabling its purification.

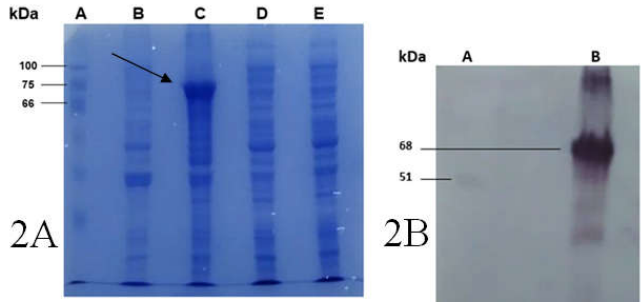

Figure 2. 2A- Analysis of ADA expression by SDS-PAGE. (A) Scienco Marker SMLW-1; (B) pET30 Insoluble; (C) ADA Insoluble with region with 75kDa highlighted byblack arrow; (D) pET30 Soluble; (E) Soluble ADA (E).2B - Western Blot of the *T. evansi*ADA protein. (A) BGLAProtein purified by LabHev; (B) Insoluble ADA

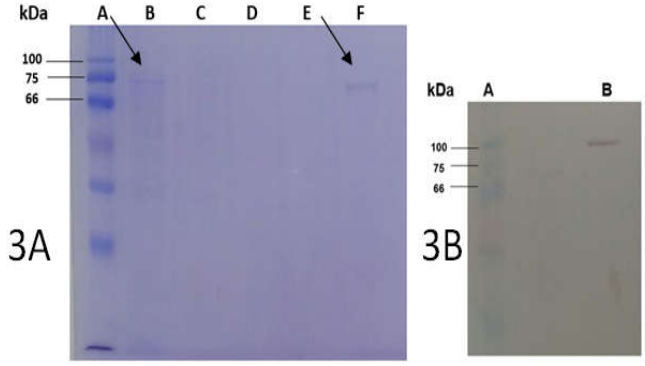

Figure 3. 3A - Analysis of the solubilization and purification of the ADA protein by SDS-PAGE. (A) Scienco Marker SMLW-1; (B) Ada after refoldinghighlighted byblack arrow; (C) Flow-through Ada after Ni-NTA Superflow resin; (D) Flow-through Buffer A; (E) Flow-through Buffer B; (F) ADA solubilized and purifiedhighlighted by black arrow.3B - Western blot of ADA protein. (A) Scienco Marker SMLW-1; (B) Solubilized and purified ADA

Recombinant protein expression: Rare Codon Calculator (RaCC) tool (*http://nihserver.mbi.ucla.edu/RACC*) was used to analyze the coding region of ADA geneand*E.coli Rosetta gammi* lineage (Novagem®) was chosen to expression assays. Several conditions for recombinant ADA enzyme expression were tested using different temperatures (18 ° C, 25 ° C and 37 ° C), times (3,6,9,12 and 24 hours) and concentrations of IPTG (ranging from 0.05mM to 0.1mM). To identify the expression of ADA recombinant protein, a polyacrylamide gel (SDS-PAGE) made it possible to separate the proteins by their size by electrophoresis (Rea, 2015). As a molecular weight marker, Scienco (Brazil) SMLW-1was used in addition to the expression vector pET30 (Novagen®). The temperature of 18ºC for 24 hours and induction with 0.05mM IPTG was the most efficient.In TriTrypDB database (*http://tritrypdb.org/tritrypdb/*), *T.evansi* shows only one *ADA* gene that is located on chromosome 10 and has a molecular mass of approximately 68kDa. The reaction showed an intense band with molecular mass of approximately 75 kDa (Figure 2A). This molecular mass corresponds to the 619 amino acids of the ADA sequence in addition to the two His*tags in the N-terminal portion added by the expression vectors pET28 and pET30a.Western blot analysis was used for the detection of the ADA enzyme in the total protein extract of *T. evansi*. After transferring the proteins to a nitrocellulose membrane, the same was treated with a blocking solution and then with probes through the primary and secondary antibody detecting the target protein (Rea, 2015). The reaction demonstrated an intense bandcorresponding to ADA enzyme (Figure 2B).

Solubilization and purification of the recombinant protein: For protein solubilizationit was used urea as a chemical denaturant and, after denaturation, the protein can be renatured to its 3D structure. There is no universal method and thus the search for an adequate and effective protocol becomes indispensable (Middelberg, 2002). Denatured protein solution was dialyzed against a renaturation buffer. Dialysis is based on the diffusion of molecules and ions across the membrane (Rottenberg, 2005) and because it is a slow process, it may provide the time needed for the protein to reach its 3D structure.The affinity chromatography separated the proteins based on the surface differences of the molecule (Rodrigues, 2005). The incorporation of His*Tag in the C or N-terminal portion of the target protein confers the same possibility of purification through this technique (Ogbunude, 1983). To confirm the success of the solubilization and purification the protein was subjected to SDS-PAGE (Figure 3A) and Western blot (Figure 3B) confirming the efficiency of the protocols used. ADA in mammals catalyzes important reactions such as purine metabolism and extracellular concentration of adenosine. In addition, ADA can act as a costimulatory regulating the immune system and acts as an allosteric modulator of ARs having physiological implications. Thus, it can be considered a protein that performs more than one function and its inhibition can lead to unexpected complications (Harris, 2012). However, the differences between ADA in the host and ADA of *T. evansi*can be explored so that the treatment of this trypanosomiasis does not interfere with the physiological functions of ADA in the host (Dalla Rosa, 2013). The main substrate of ADA, adenosine, is involved in the pathogenesis of anemia as well as in the functions of *T. evansi* (Bottari, 2014).

Conclusions

This is the first study to present a protocol for expression and purification of T. evansi ADA protein.It will allow follow-studies to evaluate the levels of enzymatic activity and to perform the production of monoclonal antibody.Should ADA demonstrate the vital importance for *T. evansi*, the study of this enzyme may contribute to the development of specific inhibitory agents that would aid in the development of new

hemotherapeutic agents more efficient than those currently used.

Acknowledgments

This study was supported by CNPq and FAPESC.Cristina Ribeiro was afellowshipfromFundo de Apoio à Manutenção e ao Desenvolvimento da Educação Superior– FUMDES.

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