G.73- High Homology in the Gene for TevSTIB805.3.100 Protein in Trypanosoma evansi, T. brucei and T. equiperdum

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Surra is the most widespread trypanosomiasis worldwide, occurring in nearly 50 countries. It is caused by Trypanosoma evansi infections and results in significant economic and ecological damage. Recent proteomic studies have identified potential molecules that could offer alternatives for the diagnosis and treatment of this disease, notably the Variant Surface Glycoprotein (VSG) TevSTIB805.3.100. The objective of this study was to design PCR primers that flank the gene encoding TevSTIB805.3.100 and to evaluate the genetic similarities between this gene and the genomes of T. brucei and T. equiperdum. The present research was approved by the Ethics Committee on Animal Use (CEUA) of the State University of Santa Catarina - UDESC, under process number 5054240423. The primers (3.100Fw and 3.100Rv) were designed through an analysis of the T. evansi genome in TriTrypDB® to encompass the entirety of the TevSTIB805.3.100 gene. These primers were utilized to produce amplicons with genomic DNA from T. evansi, T. brucei, and T. equiperdum in PCR reactions. Subsequently, the PCR products were purified, followed by a Nested PCR using the 21-22mer primers. The resulting amplicons were then subjected to sequencing and analysis, including the generation of similarity scores, dendrograms, and dot plot graphs. Amplicons of approximately 1,800 bp were produced with the 3.100Fw/Rv primers and approximately 210 bp in Nested PCR for all Trypanosoma species. Sequencing data revealed high similarity (>97% identity; >94% query cover) between the amplified fragments of T. evansi, T. brucei, and T. equiperdum for both primers (21-22mer). Dot plot graphs and dendrograms illustrated a higher homology between the fragments generated for T. evansi and T. equiperdum with the 21mer primer, and higher between T. brucei and T. equiperdum for the 22mer primer. Specific regions of VSG TevSTIB805.3.100 exhibit a high degree of conservation across various Trypanosoma isolates, suggesting their potential utility as biomarker or for immunogenicity investigation. Keywords: Variant Surface Glycoprotein, Trypanosomatid, Trypanosomes

G.74- Recombinant production of the catalytic sites of the ACE1 enzyme and the ACE2 binding region peptide with SARS-CoV.

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Angiotensin-converting enzymes 1 and 2 (ACE 1 and 2) act decisively in the Renin-Angiotensin System. ACE 1 participates in blood pressure control and brain protection, among others. ACE2 is currently one of the entry points for the SARS-CoV-2 virus, which causes COVID-19. Peptides of ACE1 (catalytic sites) and ACE2 (binding SARS-CoV) can be interesting tools to solve and detect human dysfunctions. In this project, the objectives are: 1) obtain the catalytic sites of pure ACE1 (N and C domains), and 2) obtain the ACE 2 region that binds to the SARS-CoV virus in sufficient quantities for activity studies. The expression of catalytic sites linked to the ELP/Int sequence was at 16ºC, and the ELP/Int~capACE2 were at 16ºC, 20ºC, and 25ºC. The purification by precipitation of the three peptides was the only step with 0,8M of ammonium sulfate. The Intein self-cleavage in the three peptides was evaluated in four buffers with acid pH, namely MES pH 6.3; Tris-HCl pH 6.8; Cacodylate pH 6.5; and Bis-Tris pH6.2 in the incubation temperatures at 20ºC. Expression and purification by salt precipitation of the three peptides showed good production. The catalytic sites linked to ELP/Int yielded 47% and 70% for N and C in the precipitation step, respectively, and 39% for capACE2 at both temperatures. The expression of these was for N of 0.54 mg/mL.A600 and C of 0.61 mg/mL.A600, while for ELP/Int~capACE2 at 16ºC was not observed, and at temperatures of 20ºC and 25ºC at expression was the same, 0.01 mg/mL.A600. The preliminary results of evaluating the buffers and conditions for self-cleavage of Intein to the removal of ELP showed that the buffers MES and Cacodylate for csACEN, Tris-HCl for csACEC, and Bis-Tris for capACE2, all with incubation at 20ºC, had a yield below 10% in the obtaining of the pure peptides. In this present work, the expression of ELP/Int~capACE2 was higher at 25ºC, and the purification process improved the yield of all ELP/Int peptides and with lower bacterial contaminants. However, the conditions of Intein self-cleavage need more studies, especially regarding the temperature and incubation time of the sample. Keywords: ACE1 and ACE2, catalytic site, SARS-CoV-2