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Trypanosoma evansi possesses a gene similar to putative *Trypanosoma brucei rhodesiense* oligosaccharyl transferase clone I

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ABSTRACT

Recent data has shown that there are strong indications that the putative oligosaccharyl transferase genes from *Trypanosoma brucei rhodesiense* were conserved within the family Trypanosomatidae. Based on these findings, the study endeavored to determine if *Trypanosoma evansi* also possessed putative oligosaccharyl transferase (OST) clone I previously documented in *Trypanosoma brucei rhodesiense*. Using the DNA hybridization method (Southern blot analyses), genomic DNAs of *Trypanosoma brucei rhodesiense* and *Trypanosoma evansi* were processed using the same set of restriction enzymes and subsequently hybridized by the same set of DNA probes designed from the reported nucleotide sequence of *Trypanosoma brucei rhodesiense* putative oligosaccharyl transferase clone I. The results exhibited that *Trypanosoma evansi* also contains a gene similar to the reported *Trypanosoma brucei rhodesiense* putative OST gene clone I, as shown by the successful hybridization of the DNA probes to their complementary nucleotide sequences in the genome of the *Trypanosoma evansi* species. In addition, the data also showed that *Trypanosoma brucei rhodesiense* and *Trypanosoma evansi* genomes shared some common restriction sites and loci within the genome of each individual parasite species.

Key words: *Trypanosoma evansi*, oligosaccharyl transferase, N-glycosylation, DNA hybridization

Introduction

Raising livestock is considered as one of the major industries worldwide, wherein cattle, water buffaloes and horses are raised not just for meat supply but as important draft

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and transport animals (MANUEL, 1998). There are several diseases in livestock animals and one of major economic importance is trypanosomosis. The first trypanosome found to be widely distributed and pathogenic for mammalian species is *Trypanosoma evansi (T. evansi)* (VENTURA et al., 2002; SOULSBY, 1982). Just like other trypanosome species, the surface coat of *T. evansi* bloodstream forms in the animal host is endowed with a dense monolayer of variable surface glycoprotein (VSG) homodimers (FERGUSON, 1997). There are hundreds of VSG genes that are sequentially transcribed in the VSG expression sites. These glycoproteins undergo a complex protein modification process known as N-glycosylation, which in general contributes to the antigenic properties of the protein (LISOWSKA, 2002; IMPERIALI and HENDRICKSON, 1995).

The fundamental and salient step towards the production of glycoproteins through the N-linked glycosylation process is catalyzed by a single enzyme known as oligosaccharyl transferase (OST) (KAPLAN et al., 1987). It involves the co-translational attachment of pre-assembled high mannose oligosaccharide complexes (Dol-PP-GlcNAc2Man9Glc3) to asparagine residues in an Asn-Xaa-Ser/Thr consensus sequence (Xaa can be any amino acid excluding proline) of the growing nascent polypeptide chain being translocated into the endoplasmic reticulum (BRODSKY, 1998; IMPERIALI and HENDRICKSON, 1995; KAPLAN et al., 1987). In the process, these glycoproteins are sorted by the Golgi apparatus for distribution to the lysosome, secretory vesicle and mostly plasma membrane (SPIRO, 2002). N-linked glycosylation has a profound role in the biological function and physicochemical properties of many secreted and integral membranes. It was found to be involved in signal transduction (ZHONG et al., 2004), receptor expression (KELLEY and KINSELLA, 2003), protein folding and protein orientation (GODER et al., 1999) and the macromolecular barrier, as well as intracellular transport for most VSGs (PAYS and NOLAN, 1998).

The partial nucleotide sequence (BATICADOS et al., 2010a) and eventually the full-length DNA sequence (BATICADOS et al., 2010e) of putative oligosaccharyl transferase gene clone I from the anthropozoonotic trypanosome species, *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) were cloned and sequenced. The results of the nucleotide homology search indicated that the nucleotide sequence of the *T. b. rhodesiense* putative OST clone I gene has several homologues in the genome of different trypanosomatid species. It was also reported that the percentage similarity was higher between species of the same genus than of the same family of trypanosomes. In addition, it was molecularly shown that the primer pairs used for the molecular cloning of *T. b. rhodesiense* putative oligosaccharyl transferase (OST) were able to successfully amplify a matching amplicon size of ~2,000 bp in *Trypanosoma congolense* and *Trypanosoma evansi* species (BATICADOS et al., 2010a, 2010d). The data further indicated that the putative OST gene from *T. b. rhodesiense* was evolutionarily conserved between species of the same genus (BATICADOS et al., 2010d). The significant role played by the N-glycosylation enzyme

in the biogenesis of the N-linked VSG prompted us to investigate if the newly cloned potential oligosaccharyl transferase gene in *T. b. rhodesiense* (clone I) is also conserved in other species, particularly in the livestock trypanosome, *T. evansi*.

The results exhibited that *T. evansi* also possessed a putative OST clone I with a probable significant sequence similarity to the reported gene, as shown by the successful hybridization of the DNA probes to their complementary nucleotide sequences in the genome of the *Trypanosoma evansi* species. In addition, the data also showed that the *Trypanosoma brucei rhodesiense* and *Trypanosoma evansi* genomes shared some common restrictions sites and loci within the genome of each individual parasite species.

Materials and methods

Reagents. Unless otherwise stated, reagents of analytical grade from Sigma (USA), Perkin Elmer Life Sciences (USA), Gibco BRL (UK), Roche Molecular System Inc. (USA), ICN (USA) and Wako Pure Chemical Industries Ltd. (Japan) were used in the study. Components of the medium for *In vitro* cultivation were purchased primarily from Sigma (USA) and other supplements from the following companies: Gibco BRL (UK), Wako Pure Chemical Industries (Japan), Biosource International (USA), Boehringer Mannheim (Germany) and Cayla (France). PCR primers for cloning were from Sigma-Genosys Japan (Japan).

In vitro cultivation of Trypanosoma evansi. The *Trypanosoma evansi* Tansui strain was cultured as previously described (HIRUMI and HIRUMI, 1991). *T. evansi* Tansui strain stabilates were maintained in BALB/c mice. After reaching high parasitemia (~100 BSFs/field at magnification of ×400), infected blood was collected by intracardiac puncture, cultured and maintained *In vitro* in HMI-9 medium supplemented with 10% fetal bovine serum (Biosource, USA). The HMI-9 *In vitro* cultivation medium was prepared using the following compositions: Iscove's modified dulbecco's medium (IMDM) (Sigma, St. Louis, USA), 10 mM bathocuproine disulfonic acid (Sigma, St. Louis, USA), 100 mM pyruvic acid sodium salt (Sigma, St. Louis, USA), 16 mM thymidine (Sigma, St. Louis, USA), 40 mg/mL bovine serum albumin (Sigma, St. Louis, USA), 1 mg/mL bovine holo-transferrin, 100 mM hypoxanthine (Calbiochem, La Jolla, USA), 150 mM L-cysteine hydrochloride (Sigma, St. Louis, USA), 14 mM 2-mercaptoethanol (BDH Chemicals, Poole, England, 25 mM HEPES pH 7.2 (Sigma, St. Louis, USA), 200 mM L-glutamine (Invitrogen Co., Carlsbad, CA, USA), 10% v/v heat inactivated fetal bovine serum (BIOSOURCE International Inc., Camarillo, CA, USA), 100 IU/mL penicillin (Meiji Seika Kaisha, Ltd., Tokyo, Japan) and 100 ug/mL streptomycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan).

Genomic DNA extraction. Trypanosoma evansi genomic DNA was extracted as previously described (BATICADOS et al., 2010a, 2010b; BATICADOS et al., 2005). *In vitro*

cultured parasites with a cell density of 1-5 × 10^6 cells/mL were pooled and centrifuged for 5-10 min at 15000 rpm. Nine volumes of DNA extraction buffer (0.2 M NaCl, 10 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS) and proteinase K (Invitrogen Life Technologies, USA) to a final concentration of 100 µg/mL was added to the pooled parasites and followed by 6 hrs incubation at 55 °C with gentle agitation. Subsequent overnight incubation was also performed after mixing additional amounts of proteinase K per sample. The genomic DNAs were twice extracted using phenol-chloroform-isoamyl alcohol, ethanol precipitated and resuspended in Tris-EDTA buffer, pH 8.0 or deionized water. The concentration of the sample DNA was determined by a SmartSpec 3000 (Bio-Rad, USA) spectrophotometer.

Southern blot analysis. *Trypanosoma evansi* Tansui and *T. brucei rhodesiense* IL2343 genomic DNAs were digested using the same set of non-cutter (*Eco*RI, *Hind*III, *Xba*I) and single cutter (*Mlu*I, *Dra*II) restriction enzymes. The determination of the non-cutter and single cutter enzymes were based on the number of a particular enzyme restriction site in the nucleotide sequence of a gene after sequence analysis. Restriction enzymes with single and without restriction sites in the DNA sequence of a gene under study were classified as single cutter and non-cutter enzymes respectively. The first DNA probe (916 bp) used to hybridized the membrane was amplified from 603-1,518 TbrOST I partial nucleotide sequence using the forward 5'-GGA GCA ACT GAC AGC ATT GTT TG-3' and reverse 5'-GAT TTG ATA CCC GTA GTC CCA CC-3' primer pair. Another Southern blot analysis based on previous report (BATICADOS et al., 2010c) employing a different set of restriction enzymes (single cutter: *Alw*441 and *Avi*II; double cutter: *Bse*AI and *Hind*II) and DNA probe (333 bp; amplified by forward 5'-CGT GTC GTG CTT TTT CCA TTG-3' and reverse 5'-AAC TTA CCA ACT ACC AGG CGG G-3' primer pair) was carried out. The gels were processed and transferred onto nylon membrane (Hybond-N$^+$, Amersham Pharmacia Biotech, UK) as previously reported (SAMBROOK and RUSSELL, 2001). Pre-hybridization, hybridization and probe labeling were performed using Gene Images AlkPhos Direct Labelling and Detection System Kit and CDP-star (Amersham Pharmacia Biotech, UK) as chemiluminescent substrate. Imaging and analysis were performed after exposing the membrane to an X-ray film (Kodak, USA).

Results

The Southern blot analyses of *T. b. rhodesiense* and *T. evansi* genomic DNA using the same set of *T. b. rhodesiense* DNA probes, hybridization and film exposure conditions, exhibited that the DNA probes (Fig. 1. 916 bp; Fig. 2. 333 bp) successfully annealed to their complementary sequences in *T. b. rhodesiense* and *T. evansi* genomic DNAs.

The hybridization results using a 916 bp *T. b. rhodesiense* DNA probe revealed that T. b. *rhodesiense* and *T. evansi* displayed the same DNA band pattern except for samples digested with a *Dra*II enzyme (Fig. 1). It was shown that *T. b. rhodesiense* and *T. evansi*,

digested using *Eco*RI, *Hind*III and *Xba*I non-cutter enzymes, displayed the same DNA bands with molecular sizes of 9,491 bp, 9,112 bp and 10,297 bp respectively. Likewise, a similar band pattern was exhibited by a single-cutter enzyme, *Mlu*I (3 bands: 7741 bp, 3357 bp and 1976 bp) (Fig. 1A and 1B). A slightly different band pattern was however displayed using a *Dra*II single cutter enzyme. Three bands (5820 bp, 3289 bp and 279 bp) with the same molecular weights were shown in both trypanosome species after hybridization of *Dra*II digested trypanosome genomic DNA samples. The only difference was the additional DNA band (2473 bp) detected in *T. evansi* sample after Southern blot analyses (Fig. 1B). In addition, the data obtained suggests greater complexity using *Mlu*I and *Dra*II single-cutter restriction enzymes as shown by the three bands in *T. b. rhodesiense* and three and four bands in *T. evansi* respectively. These most likely resulted under conditions wherein the gene under study displayed an allelic *Mlu*I and *Dra*II restriction fragment polymorphism.

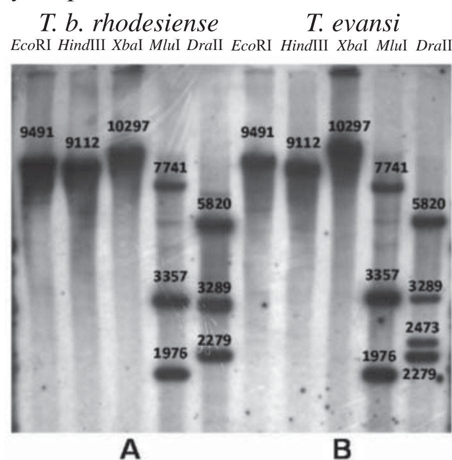

Fig. 1. Southern hybridization analysis of *Trypanosoma evansi* putative oligosaccharyl transferase. (A) *T. brucei rhodesiense* and (B) *T. evansi* DNA band patterns using single-cutter (*Mlu*I and *Dra*II) and non-cutter restriction (*Eco*RI, *Hind*II and *Xba*I) enzymes. The blot was probed using the 916 bp nucleotide fragment amplified from the nucleotide sequence of *T. brucei rhodesiense* putative oligosaccharyl transferase clone I.

Southern blot hybridization utilizing a *Trypanosoma brucei rhodesiense* 333 bp DNA probe (BATICADOS et al., 2010c), revealed that *T. b. rhodesiense* and *T. evansi* genomic DNAs digested using a *Alw*441 single-cutter enzyme had the same band pattern (7325 bp and 3303 bp.). A totally different band pattern was however displayed by *T. b. rhodesiense* (1854 bp and 1610 bp) and *T. evansi* (3885 bp and 3303 bp) genomic DNAs digested

using single-cutter restriction endonuclease *Avi*II. Consequently, it was observed that only *T. evansi* genomic DNA digested using *Avi*II and *Alw*441 shared a DNA band with the same molecular weight (3303 bp) (Fig. 2A and 2B).

A similar DNA band pattern was also observed between *T. b. rhodesiense* and *T. evansi* genomic DNAs digested using a *Hind*II double-cutter restriction enzyme (1899 bp and 1399 bp) and hybridized by a 333 bp DNA probe. However, two additional bands (1007 bp and 916 bp) were observed in *T. evansi* genomic DNA subjected to *Hind*II digestion. In the case of *T. b. rhodesiense* and *T. evansi* genomic DNAs restricted using a double-cutter enzyme (*Bse*AI), a similar band pattern (7860 bp, 3258 bp and 2880 bp) was also noticed. The only difference was the extra DNA band (6362 bp) spotted on the *T. evansi* blot after *Bse*AI incubation. The result of the Southern blot analysis strongly signifies that the gene under study has an allelic *Hind*II and *Bse*AI restriction fragment polymorphism.

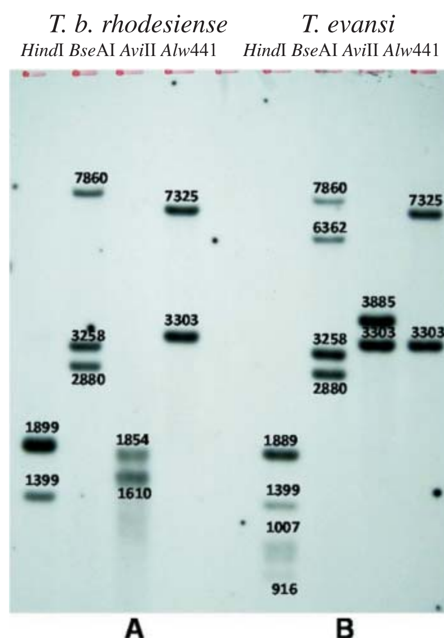

Fig. 2. Southern blot analysis of *Trypanosoma evansi* putative oligosaccharyl transferase (A) *T. brucei rhodesiense* and (B) *T. evansi* DNA band patterns using single-cutter (*Avi*II and *Alw44*I) and double-cutter (*Hind*II and *Bse*AI) restriction enzymes. The membrane was probed using the 333 bp nucleotide fragment amplified from the nucleotide sequence of *T. brucei rhodesiense* putative oligosaccharyl transferase clone I. The permission to use Fig. 2A (BATICADOS et al., 2010c) was obtained from the publisher of the Online Journal of Veterinary Research©.

Discussion

The Southern blot hybridization method is satisfactorily sensitive to detect DNA with complementary sequences to a specific DNA probe. This method can be a useful tool to identify nucleotide sequences that are either similar or identical to the sequence of the DNA probe. Furthermore, it can also be used to identify genes that are related but not identical in the sequence (REECE, 2004). Southern blot analysis can therefore be used to determine members of a gene family coming from a single species or orthologous genes of different origin (SAMBROOK and RUSSELL, 2001; SOUTHERN, 1975 as cited by REECE, 2004). In this paper, Southern transfer and hybridization were used to study how the reported gene, *T. b. rhodesiense* putative OST clone I, was organized within the *T. evansi* genome by mapping restriction sites in and around segments of *T. evansi* genomic DNA, using specific probes designed from the partial nucleotide sequence of *T. b. rhodesiense* putative OST clone I. It was recently demonstrated that the OST gene reported in *T. b. rhodesiense* was evolutionarily conserved in the Trypanosomatidae family. Similarly, in this study, an amplicon of ~2000 bp was amplified from *T. evansi* using primers for cloning *T. b. rhodesiense* putative OST. The PCR band was akin to the reported amplicon size of *T. b. rhodesiense* putative OST. In addition, the results also suggested that even in the absence of the nucleotide sequence, the putative *T. evansi* oligosaccharyl transferase probably shares significant similarity within the genome of other *Trypanosoma* species (BATICADOS et al., 2010d).

The present data, displaying almost 100% similarity in the DNA band pattern between *T. b. rhodesiense* and *T. evansi,* illustrate that the 916 bp and 333bp DNA probes designed from *T. b. rhodesiense* putative oligosaccharyl transferase clone I annealed to its complementary nucleotide sequences in the genome of *T. evansi.* As previously advocated, it is possible to place the DNA under study within a context of restriction enzymes and thus after enzyme digestion of genomic DNA, bands complementary to the used probe can be located using the Southern blot hybridization method (SAMBROOK and RUSSELL, 2001; REECE, 2004). Although strong hybridization signals were obtained, it cannot however be determined at this point whether the entire length of the DNA probe completely annealed in the digested genomic DNA of *T. evansi.* According to REECE (2004), bands obtained after X-ray film exposure will be either identical or highly related to the designed probe. In addition, since non-radioactive labeling of DNA probes was performed, it should also be pointed out that the intensity of the signal is not proportional to the amount of nucleic acid bound to the membrane. Furthermore, the strength of the hybridization signal is however proportional to the specific activity of the probe and inversely proportional to the length of the DNA fragment of the probe (SAMBROOK and RUSSELL, 2001).

The results of the study determined that almost all of the enzyme sites share a common loci or position in the genome of *T. b. rhodesiense* and *T. evansi*. These data therefore imply that *T. evansi* most likely possessed a putative oligosaccharyl transferase gene with a significant sequence similarity to the reported gene of *T. b. rhodesiense* clone I. It was formerly reported that although the DNA sequence of the putative *T. evansi* oligosaccharyl transferase gene candidate is still unknown, evidence indicates that it perhaps shares a significant percentage similarity within the genome of other *Trypanosoma* species (BATICADOS et al., 2010d). Furthermore, it can also be hypothesized that just like the putative OST recently cloned from *T. b. rhodesiense*, the coding sequence of *T. evansi* putatative OST gene candidate will also not exceed 3000 bp (BATICADOS et al., 2010a). The data also agrees with the previous study that indicated that this putative gene is conserved in the Trypanosomatidae family; specifically the *Trypanosoma* genus (BATICADOS et al., 2010d). It also further supports the ubiquitous characteristic nature of oligosaccharyl transferase in other organisms as previously documented (YAN and LENNARZ, 2002; ZUFFEREY et al., 1995).

Another important application of Southern blot analysis is to determine the number of gene copies in the genome of the organism under study. A recent report on the hybridization analyses of *T. b. rhodesiense* putative OST clone I revealed that it was a single-copy gene (BATICADOS et al., 2010c). The present Southern blot analysis data using restriction enzymes based on the annotated sequence of *T. b. rhodesiense* clone I also strongly indicated that the *T. evansi* gene, which probably shares significant sequence similarity to the reported gene, was also a single-copy gene.

In summary, the paper provided additional evidence that further support the previous claim that *Trypanosoma evansi* possesses a gene similar to *T. b. rhodesiense* putative oligosaccharyl transferase. Likewise, the paper further supported the evidence that the putative OST genes from *T. b. rhodesiense* were conserved within the Trypanosomatidae family, specifically *T. evansi*. Lastly, the data provided strong indications that a *Trypanosoma evansi* gene, believed to share significant sequence similarity with the reported gene of *T. b. rhodesiense* clone I, is also a single-copy gene.

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References

BATICADOS, W. N., N. INOUE, C. SUGIMOTO, H. NAGASAWA, A. M. BATICADOS (2010a): Molecular cloning and sequencing of *Trypanosoma brucei rhodesiense* putative oligosaccharyl transferase. Online J. Vet. Res. 14, 56-65.

BATICADOS, W. N., N. INOUE, C. SUGIMOTO, H. NAGASAWA, A. M. BATICADOS (2010b): Genomic cloning and sequence analyses of *Trypanosoma brucei rhodesiense* gene encoding Putative N-glycosylation enzyme". Acta Scientiae Vet. 38, 254-261.

BATICADOS, W. N., N. INOUE, C. SUGIMOTO, H. NAGASAWA, A. M. BATICADOS (2010c): Molecular characterization of *Trypanosoma brucei rhodesiense* putative oligosaccharyl transferase STT3 subunit (TbOST I). Online J. Vet. Res. 14, 163-175.

BATICADOS, W. N., N. INOUE, C. SUGIMOTO, H. NAGASAWA, A. M. BATICADOS (2010d): Putative oligosacharyl transferase from *Trypanosoma brucei rhodesiense* is conserved in family Trypanosomatidae. Online J. Vet. Res. 14, 253-259.

BATICADOS, W. N., N. INOUE, C. SUGIMOTO, H. NAGASAWA, A. M. BATICADOS (2010e): Full-length DNA cloning, sequence analyses and transmembrane topology modelling of putative *Trypanosoma brucei rhodesiense* oligosaccharyl transferase (TbOST I). Online J. Bioinform. 11, 162-176.

BATICADOS, W. N., W. H. WITOLA, N. INOUE, J. KIM, N. KUBOKI, X. XUAN, N. YOKOHAMA (2005): Expression of a gene encoding *Trypanosoma congolense* putative Abc1 family protein is developmentally regulated. J. Vet. Med. Sci. 67, 157-164.

BRODSKY, J. L. (1998): Translocation of proteins across the endoplasmic reticulum membrane. Int. Rev. Cytol. 178, 277-328.

FERGUSON, M. A. J. (1997): The structure and biosynthesis of trypanosomatid glycosylphosphatidylinositols. In: Trypanosomiasis and Leishmaniasis, (Hide, G., J. C. Mottram, Eds.), CAB International, U. K. pp. 65-77.

GODER, V., C. BIERI, M. SPIESS (1999): Glycosylation can influence topogenesis of membrane proteins and reveals dynamic reorientation of nascent polypeptides within the translocon. J. Cell Biol. 147, 257-265.

HIRUMI, H., K. HIRUMI (1991). *In vitro* cultivation of *Trypanosoma congolense* bloodstream forms in the absence of feeder cell layers. Parasitology 102, 225-236.

IMPERIALI, B., L. HENDRICKSON (1995): Asparagine-linked glycosylation: Specificity and function of oligosaccharyl transferase. Bioorg. Med. Chem. 3, 1565-1578.

KAPLAN, H. A., J. K. WELPLY, W. J. LENNARZ (1987): Oligosaccharyl transferase: the central enzyme in the pathway of glycoprotein assembly. Biochem. Biophys. Acta. 906, 161-173.

KELLEY, L. P., T. B. KINSELLA (2003): The role of N-linked glycosylation in determining the surface expression, G protein interaction and effector coupling of the alpha (α) isoform of human thromboxane A2 receptor. Biochim. Biophys. Acta 1621, 192-203.

LISOWSKA, E. (2002): The role of glycosylation in protein antigenic properties. Cell. Mol. Life Sci. 59, 445-455.

MANUEL, M. F. (1998): Sporadic outbreaks of Surra in the Philippines and its economic impact. J. Protozool. Res. 8, 131-138.

PAYS, E., P. NOLAN (1998): Expression and function of surface proteins in *Trypanosoma brucei*. Mol. Biochem. Parasitol. 91, 3-36.

REECE, R. J. (2004): Nucleic Acid Blotting. In: Analysis of Genes and Genomes. John Wiley and Sons Ltd., England. pp. 98-102.

SAMBROOK, J., D. W. RUSSELL (2001): Molecular cloning. In: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 6.33-7.26.

SOULSBY E,. J. L. (1982): Phylum: Sarcomastigophora Honigberg and Balamuth, 1963, Subphylum: Mastigophora Diesing, 1866. In: Helminths, Arthropods and Protozoa of Domesticated Animals, 7th ed., (Soulsby, E. J. L., Ed.). Baillière Tindall, London. pp. 514-582.

SPIRO, R. G. (2002): Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology 12, 43R-56R.

VENTURA, R. M., G. F. TAKEDA, R. A. M. S. SILVA, V. L. B. NUNES, G. A. BUCK, M. M. G. TEIXEIRA (2002): Genetic relatedness among *Trypanosoma evansi* stocks by random amplification of polymorphic DNA and evaluation of a synapomorphic DNA fragment for species-specific diagnosis. Int. J. Parasitol. 32, 53-63.

YAN, Q., W. J. LENNARZ (2002): Studies on the function of oligosaccharyl transferase subunit Biol. Chem. 277, 47692-47700.

ZHONG, X., R. KRIZ, J. SEEHRA, R. KUMAR (2004): N-linked glycosylation of platelet P2Y12 ADP receptor is essential for signal transdcution but not for ligand binding or cell surface expression. FEBS Lett. 562, 111-117.

ZUFFEREY, R., R. KNAUER, P. BURDA, S. STAGLJARI, S. HEESEN, L. LEHLE, M. AEBI (1995): STT3, a highly conserved protein required for yeast oligosaccharyl transferase activity *In vivo*. EMBO J. 14, 4949-4960.

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SAŽETAK

Nedavna istraživanja pokazala su da su geni za oligosaharil-transferazu protozoona *Trypanosoma brucei rhodesiense* vrlo dobro sačuvani unutar porodice *Trypanosomatidae*. Cilj istraživanja bio je otkriti je li ista pojava karakteristična za gen za oligosaharil-transferazu klona I protozoona *Trypanosoma evansi*. Genomske DNA vrste *Trypanosoma brucei rhodesiense* i vrste *Trypanosoma evansi* bile su pretražene hibridizacijom DNA (Southern Blot analizom) rabeći istu skupinu restrikcijskih enzima kao i iste probe za hibridizaciju DNA pripravljene na temelju objavljenog slijeda nukleotida za oligosaharil-transferazu klona I protozoona *Trypanosoma brucei rhodesiense*. Rezultati su pokazali da *Trypanosoma evansi* također sadrži gen koji je vrlo sličan genu za oligosaharil-transferazu protozoona *Trypanosoma brucei rhodesiense*, što je dokazano uspješnom hibridizacijom DNA proba s komplementarnim nukleotidnim slijedovima u genomu vrste *Trypanosoma evansi*. Istraživanje je pokazalo da *Trypanosoma brucei rhodesiense* i *Trypanosoma evansi* dijele i zajednička restrikcijska mjesta.

Ključne riječi: *Trypanosoma evansi*, oligosaharil-transferaza, N-glikoziliranje, DNA hibridizacija