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Genomic Cloning and Sequence Analysis of *Trypanosoma brucei rhodesiense* Gene Encoding Putative N-glycosylation Enzyme

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ABSTRACT

Background: *Trypanosoma brucei rhodesiense* is a haemoflagellate parasite of zoonotic significance. Aside from its public health importance, this parasite subspecies gained notoriety because of their effective system to circumvent the immune response of vertebrate host. The parasite cell surface is covered with millions of VSG dimers, which serve as an almost infinite repertoire of biomolecules needed for evasion of host immune system. Around two decades ago, it was resolved that all trypanosome VSG is associated with one or more N-linked oligosaccharides, with a range of structures including high mannose and complex types. This complex process of protein modification known as N-linked glycosylation is catalyzed by oligosaccharyl transferase (OST). In general, the incorporation of glycan structures can alter protein's antigenic properties and recently it was established that glycan molecules associated with VSG were found to be important in several aspects of trypanosome-host interaction, especially during parasite evasion of the host defense mechanisms. Therefore, our major interest is to clone and characterize the trypanosome OST.

Material, Methods and Results: The template genomic DNA for PCR amplification was extracted as described previously. In an attempt to clone *Trypanosoma brucei rhodesiense* putative oligosaccharyl transferase, an amplicon of ~2000 bp was obtained having an open reading frame of 2057 bp and deduced primary structure composed of 685 amino acid residues (TbrOST II). Comparison of TbrOST II ORF with annotated putative oligosaccharyl transferase in the genome of other organisms revealed sequence identity to other kinetoplastid. TbrOST II had high nucleotide (Ns) and amino acid (As) sequence similarity with the genomes of *T. brucei gambiense* (Ns:99%; As:78%) and *T. brucei* (Ns:95-98%; As:77%-98%). There was also significant nucleotide and amino acid sequence identity in the genomes of *T. cruzi* (Ns:74%; As:63%), *Leishmania infantum* (Ns:70-83%; As:46-57%), *L. braziliensis* (Ns:69-81%; As:46-55%) and *L. major* (Ns:69-80%; As:46-57%). Sequence similarity (71-77%) from other origins was also exhibited. The nucleotide sequence alignments and analysis were performed using the Oxford University Mac Vector 6.5 sequence analysis software and CLC Workbench 5.6 software.

Discussion: The nucleotide BLAST results indicate that sequence identity is higher between species of the same genus rather than of the same family. It is known that *T. brucei, T. gambiense* and *T. rhodesiense* are members of the *Brucei*-complex or *Brucei* group. Although *T. brucei brucei* has more similarities with *T. brucei rhodesiense* than *T. brucei gambiense*, these parasites are morphologically indistinguishable. This is the probable reason why high sequence identity was displayed by other subspecies of the *Brucei* group. In addition, the high percent identity possessed by TbrOST II with other trypansomatids agrees with the evolutionarily conserved characteristics of the established OST. The DNA sequence data of TbrOST II showing similar sequences in the genome of other organisms further corroborate the previous reports regarding the ubiquitous nature of OST in other life forms. Based on the size of the amplicon and significant percentage of nucleotide and amino acid sequence identity to homologues within the genome of related species and various organisms, the results strongly indicate that TbrOST II is a trypanosome oligosaccharyl transferase gene candidate that should be fully characterized and subjected to functional genomic studies. The study reports the molecular cloning and sequencing of a potential oligosaccharyl transferase gene in *T. brucei rhodesiense* (TbrOST II). The sequence data has been deposited in the GenBank with accession number GU475126.

Keywords: *Trypanosoma brucei rhodesiense*, putative oligosaccharyl transferase, N-glycosylation.

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INTRODUCTION

Trypanosoma brucei rhodesiense is a haemoflagellate parasite of zoonotic significance. A wide range of mammalian fauna, especially domestic livestock and wild bovids, serves as reservoir host [23,26]. Aside from its public health importance, this parasite subspecies gained notoriety because of their effective system to circumvent the immune response of vertebrate host. The parasite cell surface is covered with millions of VSG dimers, which serve as an almost infinite repertoire of biomolecules needed for evasion of host immune system. Unique VSG are alternately produced by sequential expression of about a thousand trypanosome VSG gene reservoir per parasite; a phenomenon described as antigenic variation [5,6]. Around two decades ago, it was resolved that all trypanosome VSG is associated with one or more N-linked oligosaccharides, with a range of structures including high mannose and complex types [7,19,21, 24]. This complex process of protein modification is generally known as glycosylations, one of which is N-linked glycosylation.

The central event in the N-linked glycosylation process is catalyzed by oligosaccharyl transferase (OST) [11]. It catalyzes the co-translational addition of preassembled oligosaccharide complexes (Dol-PP-$GlcNAc_2Man_9Glc_3$) to an asparagine residue in an Asn-Xaa-Ser/Thr consensus sequon (Xaa can be any amino acid excluding proline) of the growing nascent polypeptide chain being translocated into the endoplasmic reticulum through a structure called translocon [4].

OST appears ubiquitous among eukaryotes and conserved throughout eukaryotic evolution. OST has been molecularly isolated and purified from mammalian sources, avian species and yeast *Saccharomyces cerevisiae* [3,11-14,22] as a multimeric enzyme.

In general, the incorporation of glycan structures to different protein moieties is precedent towards proper protein folding and stability, intracellular targeting, intercellular recognition, hormone synthesis, anti-apoptotic response, control of salt/osmotic stress, and cell surface expression of some glycoproteins [8,9,15,17]. Glycans can alter protein's antigenic properties and recently it was established that, glycan molecules associated with VSG were found to be important in several aspects of trypanosome-host interaction, especially during parasite evasion of the host defense mechanisms [20,21,16]. Therefore our major interest is to clone and characterize the trypanosome OST.

MATERIALS AND METHODS

Laboratory animals

Female 8-week-old BALB/c mice[1] were used in the study. The animal room was maintained at 22 ± 3^{0}C with a 12:12 hours of light-dark cycle. All experiments were conducted according to the guidelines for the care and use of laboratory animals, Obihiro University of Agriculture and Veterinary Medicine, Japan.

Trypanosomes and cultivation

Trypanosoma brucei rhodesiense IL2343 strain (Ivory Coast) stabilates were maintained in BALB/c mice. After reaching high parasitemia (~100 BSFs/field at magnification of x400), infected blood was collected by intracardiac puncture, cultured and maintained *in vitro* in HMI-9 medium supplemented with 10% fetal bovine serum[2] as previously described [10]. The HMI-9 *in vitro* cultivation medium was prepared using the following composition: Iscove's modified dulbecco's medium[3] (IMDM), 10 mM bathocuproine disulfonic acid[3], 100 mM pyruvic acid sodium salt[3], 16 mM thymidine[3], 40 mg/mL bovine serum albumin[3], 1 mg/mL bovine holo-transferrin, 100 mM hypoxanthine[4], 150 mM L-cysteine hydro-chloride[3], 14 mM 2-mercaptoethanol[5], 25 mM HE-PES[3] pH 7.2 , 200 mM L-glutamine[6], 10%v/v heat inactivated fetal bovine serum[2], 100 U/mL penicillin[7] and 100 µg/mL streptomycin[7].

DNA extraction

The template genomic DNA for PCR amplification was extracted as described previously [2]. Briefly, *T. brucei rhodesiense* IL2343 genomic DNA was extracted by adding 9 volumes of extraction buffer (0.2 M NaCl[8], 10 mM Tris-HCl[3,8] pH 8.0, 10 mM EDTA[3] pH 8.0 and 1% SDS), proteinase K[6] to a final concentration of 100 µg/mL and followed by 6 hours incubation at 55°C with gentle agitation. Overnight incubation was performed after additional proteinase K was placed. Genomic DNAs were phenol-chloroform-isoamyl alcohol[3] extracted, ethanol precipitated, and resuspended in Tris-EDTA buffer, pH 8.0 or deionized water. The concentration of the sample DNA was determined by spectrophotometry.

Polymerase chain reaction amplification of putative OST gene

The primers were designed from the nucleotide sequences of *T. brucei* genomic clones as guided by EMBL-EBI Parasite Genomes WU-Blast

2 database search (www.ebi.ac.uk/blast/parasites.html) for African trypanosomes with *L. major* putative OST STT3 subunit sequence as the query [EMBL Q9U5N8 (AJ251127.1)]. PCR amplication was performed using a forward primer[9] (5'-TGG TAC GAC TAC ATG AGC TGG TAC CCG CT-3') and a reverse primer[9] (5'-TGG ATC TCC TTC GCT GGC GGG TAC TG-3'). Distilled water was used as template for negative control reaction. The samples were programmed to a temperature-step cycle of 94°C at 10 min, 94°C at 30 sec, 60°C at 30 sec for a total of 30 cycles followed by 4 min extension at 72°C. The PCR products were analyzed by electrophoresis on 1% TAE (Tris-acetate-EDTA) agarose gel. The PCR product was then processed for cloning after agarose gel extraction using a commercial kit[10] according to the manufacturer's instructions.

Cloning and sequencing of PCR products

The PCR product was ligated into *EcoR* V site of pT7 bluescript plasmid vector[11] using Takara solution I ligation kit[12]. Ligation reaction was transformed into DH5 α competent *E.coli* cells and plated on Luria Britani's-ampicillin (LB-amp) agar dishes. The presence of insert was confirmed by restriction digest against *Hind* III & *Xba* I site from the cloning site of the plasmid vectors flanking the PCR product.

Prior to sequencing, twenty-five cycles of Bigdye PCR was carried out in a total volume of 5 µl and were performed using the following standard condition: 96^0C at 2 min, 96^0C at 10 sec, 50^0C at 5 sec and 60^0C at 4 minutes. Sequencing was started by the single strand dideoxynucleotide-chain-termination method using a cycle sequencing kit[13], DNA sequence analyzer[13] and T7 promoter primer and pUC/M13 reverse primers[9]. The second set[9] [forward (5'-GAC ATA CAG CGT CAG TTT GC-3'); reverse (5'-GAT GAA TGT GAG TGA AGA GAG C-3')] and the third set[9] [forward (5'-CGT TCG GAT TCT TCA AAC CTA CAG-3') and reverse (5'-AAT ACG GGC ATC TTC AGG CG-3')] primers were used to obtain the partial nucleotide sequence. The nucleotide sequence alignments and analysis were performed using sequence analysis softwares[14,15].

RESULTS

A putative trypanosome oligosaccharyl transferase gene was successfully amplified using *T. brucei rhodesiense* crude DNA as the template. Genomic clone of approximately 2000 bp was obtained after PCR amplification (Figure 1). Nucleotide sequencing revealed that the amplified band was composed of 2057 bp partial nucleotide sequence (Figure 2). The deduced partial primary structure of the *T. brucei rhodesiense* putative oligosaccharyl transferase clone II (TbrOST II) was composed of 685 amino acid sequence (Figure 2). Determination of the sequence homology in other organisms was carried out using NCBI Basic Local Alignment Search Tool (***BLAST***) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [1].

Sequence analysis showed that TbrOST II had significant nucleotide (Ns) and amino acid sequence (As) percent identity to putative oligosaccharyl transferase subunit coming from other kinetoplastid genome in the NCBI public database.

When compared with the related trypanosome species, the DNA size of *T. brucei rhodesiense* putative oligosaccharyl transferase was 200 bp than *T. brucei gambiense* (1842 bp, FN554968.1). In addition, the sequence homologues from *T. brucei* (2466 bp, XM_839672.1) (2466 bp, XM_839671.1) (2406 bp, AC159432.1)(2406 bp, XM_839670.1), exceeded TbrOST II clone by >400 bp. The Latin American trypanosome species, *T. cruzi,* with percent identity to TbrOST II on the other hand had a molecular weight of 2397 bp. (XM_803446.1). Furthermore, the data showed that the annotated DNA size of the homologues under genus *Leishmania* was greater than that of genus *Trypanosoma* with

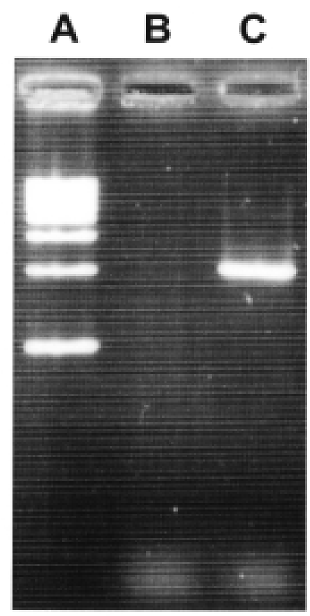

Figure 1. Polymerase Chain Reaction amplification of *Trypanosoma brucei rhodesiense* Putative Oligosaccharyl transferase clone II (TbrOST II). Lane (A), (B) & (C) represents 1 kbp DNA ladder, negative control reaction and ~ 2,000 bp PCR amplicon respectively.

a disparity ranging from ~300-500 bp (*L. infantum*) and ~200- 500bp (*L. major* & *L. braziliensis*).

Leishmania infantum displayed the greatest difference in terms of DNA molecular weight to TbrOST II (2592 bp, XM_001468890.1) (2385 bp, XM_001468892.1) (2355 bp, XM_001468891.1) followed by *L. braziliensis* (2580 bp, XM_0015 65753.1) (2319 bp, XM_001568165.1) (2565 bp, XM_001568167.1) (2472 bp, XM_001568166.1) and the least was *L. major* (2574 bp, XM_838130.1) (2322 bp, XM_838128.1) (2559 bp, XM_838127.1) (2373 bp, XM_838129.1) (2502 bp, AJ251127.1).

The molecular size of the amplicon showed akin to several homologues under order kinetoplastida. Taking into consideration the reported DNA size of the homologues from closely related species, the data suggest that the full-length nucleotide sequence of *T. brucei rhodesiense* putative oligosaccharyl transferase is also not more than 3000 bp.

Furthermore, the results using BLAST nucleotide (Ns) and amino acid (As) homology search showed that the *T. brucei rhodesiense* putative oligosaccharyl transferase had a very high percent sequence identity to the genome of *T. brucei* (Ns:98%,XM_839670.1; As:98%, XP_844763.1). Other homologues found in the genome of *T. brucei* (Ns:95%, XM_839672.1; As:78%, XP_844765.1) (Ns:96%, XM_839671.1; As:77%, XP_844764.1) and *T. brucei gambiense* (Ns:99%, FN554968.1; As:78%, CBH10989.1) also showed very high DNA sequence identity but with only fairly high amino acid sequence identity to TbrOST II. A moderate sequence similarity in the genome of *T. cruzi* (Ns:74%, XM_803446.1; As:63%, XP_808539.1) was also observed.

Subsequently, percent sequence identity hits in other members of family Trypanosomatidae, genus *Leishmania*, were also obtained (Figure 3). All the homologues from genus *Leishmania* displayed moderate and low nucleotide and amino acid sequence identity to TbrOST II respectively. The following are the different *Leishmania* clones from the GenBank with nucleotide and amino acid

A

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   1 TGGTACGACT ACATGAGCTG GTACCCGCTT GGCCGACCGG TGGGCACAAC CATCTTCCCC
  61 GGAATGCAGC TTACCGGTGT AGCCATTCAT CGTGTGCTGG AAATGCTCGG GCGAGGTATG
 121 TCCATCAACA ATATCTGTGT GTACATTCCT GCATGGTTCG GTAGTATTGC CACTGTGTTG
 181 GCTGCTCTCA TTGCGTACGA ATCATCTAAT TCGCTCAGTG TCATGGCGTT TACTGCGTAC
 241 TTTTTTTCCA TCGTACCTGC ACACCTGATG CGATCAATGG CTGGTGAATT TGACAATGAG
 301 TGTGTTGCAA TGGCGGCGAT GCTTCTGACG TTCTACATGT GGGTACGATC GTTACGCAGC
 361 TCAAGTTCGT GGCCCATTGG CGCTTTAGCT GGTGTGGCAT ACGGGTACAT GGTGTCCACG
 421 TGGGGTGGTT ATATTTTCGT GCTGAACATG GTAGCCTTCC ACGCTTCTGT ATGTGTACTG
 481 CTTGATTGGG CTCGTGGGAC ATACAGCGTC AGTTTGCTGA GGGCGTATTC ACTGTTTTTT
 541 GTCATTGGCA CTGCCCTTGC GATTTGCGTA CCGCCAGTGG AGTGGACGCC CTTCCGGTCG
 601 CTGGAGCAAC TGACAGCATT GTTTGTCTTC GTTTTCATGT GGGCACTCCA CTACTCGGAA
 661 TACCTGCGTG AGCGTGCCCG AGCGCCCATT CACTCTTCTA AAGCACTTCA GATCCGTGCC
 721 CGCATTTTCA TGGGCACACT CTCCTTGCTG TTGATTGTGG CAAGTTTGCT TGCCCCGTTC
 781 GGATTCTTCA AACCTACAGC GTACCGCGTC CGTGCGTTGT TCGTGAAACA TACGCGTACC
 841 GGAAATCCCC TCGTGGATTC TGTGGCCGAG CATCGGCCGA CGACTGCCGG GGCGTATCTG
 901 CGCTACTTTC ATGTTTGTTA CCCTCTTTGG GGGTGCGGTG GGCTCTCTAT GTTGGTATTC
 961 ATGAAAAAGG ACCGCTGGCG CGCCATTGTT TTTCTTGCTT CACTTTCCAC TGTTACGATG
1021 TATTTCAGCG CCCGTATGTC GCGATTACTT CTGTTAGCGG GTCCCGCAGC AACGGCTTGC
1081 GCCGGCATGT TCATAGGGGG GCTTTTTGAT CTGGCGCTGT CACAGTTTGG TGATTTGCGT
1141 AGCCCAAAAG ATGCCTCCGG CGATTCCGAT CCCGCGGGAG GGTCGAAGCG GGCAAAGGGC
1201 AAAGTTGTTA ATGAGCCTCC CAAAAGAGCC ATCTTTAGTC ACCGCTGGTT TCAACGTTTA
1261 GTGCAATCGT TGCCCGTCCC GCTACGACGT GGTATCGCGG TTGTGGTGCT CGTATGTCTC
1321 TTCGCCAATC CCATGAGACA CTCATTCGAA AAGTCCTGCG AGAAAATGGC ACATGCACTT
1381 TCATCTCCAA GGATCATTGC CGTGACTGAT CTACCCAATG GAGAGAGAGT CCTCGCCGAT
1441 GATTACTACG TGTCGTACTT GTGGCTGCGA AACAATACGC CTGAAGATGC CCGTATTCTC
1501 TCATGGTGGG ACTACGGGTA TCAAATCACT GGAATTGGCA ATCGCACAAC CCTTGCGGAT
1561 GGTAACACAT GGAGTCACAA GCACATAGCA ACTATTGGAA AGATGCTTAC ATCCCCTGTG
1621 AAGGAGTCAC ATGCTCTTAT ACGCCATCTC GCTGATTATG TGCTGATATG GTCTGGTCAA
1681 GATGGCAGCG ATTTACTTAA ATCGCCACAC ATGGCTCGGA TAGGCAACAG TGTATATCGC
1741 GATATGTGTT CAGAAGACGA TCCGCTGTGT AGACAGTTCG GGTTTTATAG TGGTGACCTC
1801 AATAAGCCTA CGCCTATGAT GCAGCGGTCC CTATTATACA ATCTGCACAG GTTTGGTACG
1861 GATGGCGGGA AGACACAACT GGATAAGAAC ATGTTTCAGC TCGCCTACGT GTCAAAGTAT
1921 GGTTTGGTGA AGATCTACAA GGTGATGAAT GTGAGTGAAG AGAGCAAGGC GTGGGTTGCA
1981 GACCCAAAGA ACCGCGTATG CGACCCGCCC GGATCTTGGA TATGCGCCGG CCAGTACCCG
2041 CCAGCGAAGG AGATCCA
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B

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   1 WYDYMSWYPL GRPVGTTIFP GMQLTGVAIH RVLEMLGRGM SINNICVYIP AWFGSIATVL
  61 AALIAYESSN SLSVMAFTAY FFSIVPAHLM RSMAGEFDNE CVAMAAMLLT FYMWVRSLRS
 121 SSSWPIGALA GVAYGYMVST WGGYIFVLNM VAFHASVCVL LDWARGTYSV SLLRAYSLFF
 181 VIGTALAICV PPVEWTPFRS LEQLTALFVF VFMWALHYSE YLRERARAPI HSSKALQIRA
 241 RIFMGTLSLL LIVASLLAPF GFFKPTAYRV RALFVKHTRT GNPLVDSVAE HRPTTAGAYL
 301 RYFHVCYPLW GCGGLSMLVF MKKDRWRAIV FLASLSTVTM YFSARMSRLL LLAGPAATAC
 361 AGMFIGGLFD LALSQFGDLR SPKDASGDSD PAGGSKRAKG KVVNEPPKRA IFSHRWFQRL
 421 VQSLPVPLRR GIAVVVLVCL FANPMRHSFE KSCEKMAHAL SSPRIIAVTD LPNGERVLAD
 481 DYYVSYLWLR NNTPEDARIL SWWDYGYQIT GIGNRTTLAD GNTWSHKHIA TIGKMLTSPV
 541 KESHALIRHL ADYVLIWSGQ DGSDLLKSPH MARIGNSVYR DMCSEDDPLC RQFGFYSGDL
 601 NKPTPMMQRS LLYNLHRFGT DGGKTQLDKN MFQLAYVSKY GLVKIYKVMN VSEESKAWVA
 661 DPKNRVCDPP GSWICAGQYP PAKEI
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Figure 2. Partial Nucleotide (A) and Deduced Amino Acid (A) Sequence of *Trypanosoma brucei rhodesiense* Putative Oligosaccharyl transferase (TbrOST II) (GenBank: GU475126).

sequenced regions having local similarity to TbrOST II: *L. infantum* (Ns:70%, XM_001468891.1; As:57%, XP_001468928.1) (Ns:70%, XM_001468890.1; As:56%, XP_001468927.1) (Ns:83%, XM_001468 892.1; As:46%, XP_001468929.1); *L. braziliensis* (Ns:69%, XM_001565753.1; As:54%, XP_00156 5803.1) (Ns:69%, XM_001568165.1; 55%,As:XP _001568215.1) (Ns:70%, XM_00156 8167.1; As:55%, XP_001568217.1) (Ns:81%, XM_0015681 66.1; As:46%, XP_001568216.1); *L. major* (Ns:69%, XM_838130.1; As:55%, XP_843223.1) (Ns:70%, XM_838128.1;As:57%, XP_843221.1) (Ns:70%, XM_838127.1; As:55%, XP_843220.1) (Ns:80%, XM_838129.1; As:46%, XP_843222.1) (Ns:80%,

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TbrOSTII ------------------------------------------------------------
Tbrucei  MTKGGKVAVTKGSAQSDGAGEGGMSKAKSSTTFVATGGGSLPAWALKAVSTIVSAVILIY
Tbgam    MTKGGKVAVTKGSAQSDGAGEGGMSKAKSSTTFVATGGGSLPAWALKAVSTIVSAVILIY

TbrOSTII ----------------------------------------------WYDYMSWYPLGRPVG
Tbrucei  SVHRAYDIRLTSVRLYGELIHEFDPWFNYRATQYLSDNGWRAFFQWYDYMSWYPLGRPVG
Tbgam    SVHRAYDIRLTSVRLYGELIHEFDPWFNYRATQYLSDNGWRAFFQWYDYMSWYPLGRPVG

TbrOSTII TTIFPGMQLTGVAIHRVLEMLGRGMSINNICVYIPAWFGSIATVLAALIAYESSNSLSVM
Tbrucei  TTIFPGMQLTGVAIHRVLEMLGRGMSINNICVYIPAWFGSIATVLAALIAYESSNSLSVM
Tbgam    TTIFPGMQLTGVAIHRVLEMLGRGMSINNICVYIPAWFGSIATVLAALIAYESSNSLSVM

TbrOSTII AFTAYFFSIVPAHLMRSMAGEFDNECVAMAAMLLTFYMWVRSLRSSSSWPIGALAGVAYG
Tbrucei  AFTAYFFSIVPAHLMRSMAGEFDNECVAMAAMLLTFYMWVRSLRSSSSWPIGALAGVAYG
Tbgam    AFTAYFFSIVPAHLMRSMAGEFDNECVAMAAMLLTFYMWVRSLRSSSSWPIGALAGVAYG

TbrOSTII YMVSTWGGYIFVLNMVAFHASVCVLLDWARGTYSVSLLRAYSLFFVIGTALAICVPPVEW
Tbrucei  YMVSTWGGYIFVLNMVAFHASVCVLLDWARGIYSVSLLRAYSLFFVIGTALAICVPPVEW
Tbgam    YMVSTWGGYIFVLNMVAFHASVCVLLDWARGTYSVSLLRAYSLFFVIGTALAICVPPVEW

TbrOSTII TPFRSLEQLTALFVFVFMWALHYSEYLRERARAPIHSSKALQIRARIFMGTLSLLLIVAS
Tbrucei  TPFRSLEQLTALFVFVFMWALHYSEYLRERARAPIHSSKALQIRARIFMGTLSLLLIVAS
Tbgam    TPFRSLEQLTALFVFVFMWALHYSEYLRERARAPIHSSKALQIRARIFMGTLSLLLIVAI

TbrOSTII LLAPFGFFKPTAYRVRALFVKHTRTGNPLVDSVAEHRPTTAGAYLRYFHVCYPLWGCGGL
Tbrucei  LLAPFGFFKPTAYRVRALFVKHTRTGNPLVDSVAEHRPTTAGAYLRYFHVCYPLWGCGGL
Tbgam    YLFSTGYFRSFSSRVRALFVKHTRTGNPLVDSVAEHRPTTAGAFLRHLHVCYNGWIIGFF

TbrOSTII SMLVFMKKDRWRAIVFLASLSTVTMYFSARMSRLLLLAGPAATACAGMFIGGLFDLALSQ
Tbrucei  SMLVFMKKDRWRAIVFLASLSTVTMYFSARMSRLLLLAGPAATACAGMFIGGLFDLALSQ
Tbgam    FMSVSCFFHCTPGMSFLLLYSILAYYFSLKMSRLLLLSAPVASILTGYVVGSIVDLAADC

TbrOSTII FGDLRSPKDASGDSDPAGGSKRAKGKVVNEPPKRAIF----SHRWFQRL-VQSLPVPLRR
Tbrucei  FGDLHSPKDASGDSDPAGGSKRAKGKVVNEPSKRAIF----SHRWFQRL-VQSLPVPLRR
Tbgam    FA-------ASGTEH--ADSKEHQGKARGKGQKRQITVECGCHNPFYKLWCNSFSSRLVV

TbrOSTII GIAVVVLVCLFANP--MRHSFEKSCEKMAHALSSPRIIAVTDLPNGERVLADDYYVSYLW
Tbrucei  GIAVVVLVCLFANP--MRHSFEKSCEKMAHALSSPRIIAVTDLPNGERVLADDYYVSYLW
Tbgam    GKFFVVVVLAICGPTFLGSEFRAHCERFSLSVANPRIIS-SIRHSGKLVLADDYYVSYLW

TbrOSTII LRNNTPEDARILSWWDYGYQITGIGNRTTLADGNTWSHKHIATIGKMLTSPVKESHALIR
Tbrucei  LRNNTPEDARILSWWDYGYQITGIGNRTTLADGNTWSHKHIATIGKMLTSPVKESHALIR
Tbgam    LRNNTPEDARILSWWDYGYQITGIGNRTTLADGNTWNHEHIATIGKMLTSPVKESHALIR

TbrOSTII HLADYVLIWSGQDGSDLLKSPHMARIGNSVYRDMCSEDDPLCRQFGFYSGDLNKPTPMMQ
Tbrucei  HLADYVLIWAGEDRGDLLKSPHMARIGNSVYRDMCSEDDPRCRQFGFEGGDLNKPTPMMQ
Tbgam    HLADYVLIWSGQDRGDLRKSRHMARIGNSVYRDMCSEDDPLCRQFGFYSGDLSKPTPMMQ

TbrOSTII RSLLYNLHRFGTDGGKTQLDKNMFQLAYVSKYGLVKIYKVMNVSEESKAWVADPKNRVCD
Tbrucei  RSLLYNLHRFGTDGGKTQLDKNMFQLAYVSKYGLVKIYKVVNVSEESKAWVADPKNRVCD
Tbgam    RSLLYNLHRFGTDGGKTQLDKNMFQLAYVSKYGLVKIYKVMNVSEESKAWVADPKNRKCD

TbrOSTII PPGSWICAGQYPPAKEI-----------------------------------
Tbrucei  PPGSWICAGQYPPAKEIQDMLAKRFHYE------------------------
Tbgam    APGSWICAGQYPPAKEIQDMLAKRIDYEQLEDFNRRNRSDAHYRAYMRQMG
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Figure 3. Amino Acid sequence identity of *Trypanosoma brucei rhodesiense* Putative Oligosaccharyl transferase (TbrOST II) within genome of genus *Trypanosoma*, family Trypanosomatidae. Alinged sequences were from *T. brucei* -Tbrucei (98%, XP_844763.1) and *T. brucei gambiense* -Tbgam (78%, CBH10989.1) species.

AJ251127.1; As:46% , CAB61 569.1).

DISCUSSION

The surface coat of trypanosome species were previously reported to have marked diversities in N-glycosylation [20]. This prompted us to hypothesize that trypanosomes also possess N-glycosylation enzyme. During the attempt to clone *T. brucei rhodesiense* putative oligosaccharyl transferase, a genomic clone of ~2000 bp was acquired.

Notably, *L. major* putative OST STT3 subunit sequence was used as the query during the primer design for PCR amplification of TbrOST II. The efficient amplification of the gene in genus *T. brucei rhodesiense* using primers designed from *L. major* stt3 gene as the query strongly indicate that TbrOST II seems to be conserved within the family Trypanosomatidae. The nucleotide BLAST results indicates that sequence identity is higher between species of the same genus rather than of the same family. It is known that *T. brucei*, *T. gambiense* and *T. rhodesiense* are members of the *Brucei*-complex or *Brucei* group. Although *T. brucei* brucei has more similarities with *T. brucei rhodesiense* than *T. brucei gambiense*, these parasites are morphologically indistinguishable [18,23]. This is the probable reason why high sequence identity was displayed by other subspecies of the *Brucei* group. This also indicates that the functional unit of this putative oligosaccharyl transferase is conserved within the *Brucei* complex trypanosomes. In addition, the high percent identity possessed by TbrOST II with other trypansomatids agrees with the evolutionarily conserved characteristics of the established OST [27, 25]. Consequently, DNA identity searches within the public databases obtained homologues in the genome of mammalian, nematode, arthropod and algae species. Selected organisms include *Mus musculus* (Ns:71%, NM_024222.2; As:29%, NP_077184.2), S*chistosoma mansoni* (Ns:73%, XM_002577919.1; As:29%, XP_002577965.1), *Drosophila pseudoobscura pseudoobscura* (Ns:77%, XM_002134519.1; As:31%, XP_002134555.1), *Thalassiosira pseudonana* (Ns:77%, XM_002288187.1; As:46%, XP_002288223.1) and *Phaeodactylum tricornutum* (Ns:73%, XM_002185331.1; As:46%, XP_ 002185367.1). Moreover, obtained amino acid sequence identity in the genome of fly, nematode, mouse and algae homologues also exhibited lower sequence similarity (29-46%) than the previously documented percent identity (~50%) of reported oligosaccharyl transferase in other eukaryotic species [25].

CONCLUSIONS

Finally, even currently regarded as putative, DNA sequence data of TbrOST II showing similar sequences in the genome of other organisms further corroborate the previous reports regarding the ubiquitous nature of OST in other life forms. Based on the size of the amplicon and significant percentage of nucleotide and amino acid sequence identity to homologues within the genome of related species and various organisms, the results strongly indicate that TbrOST II is a trypanosome oligosaccharyl transferase gene candidate that should be fully characterized and subjected to functional genomic studies. The study reports the molecular cloning and sequencing of a potential oligosaccharyl transferase gene in *T. brucei rhodesiense* (TbrOST II). The sequence data has been deposited in the GenBank with accession number of GU475126.

SOURCES AND MANUFACTURERS

[1]CLEA Japan, Inc., Tokyo, Japan.
[2]Biosource International Inc., Camarillo, CA, USA.
[3]Sigma, St. Louis, USA.
[4]Calbiochem, La Jolla, CA, USA.
[5]BDH Chemicals, Poole, England.
[6]Invitrogen Co., Carlsbad, CA, USA.
[7]Meiji Seika Kaisha, Ltd., Tokyo, Japan.
[8]Wako Pure Chemical Industries Ltd., Osaka, Japan.
[9]Sigma-Genosys, Hokkaido, Japan.
[10] GeneClean kit II, Q Biogene, Vista, CA, USA.
[11]Novagen, Madison, WI, USA.
[12]TAKARA Shuzo, Japan.
[13]Applied Biosystems, Forster City, CA, USA.
[14]Mac Vector 6.5 sequence analysis software, Oxford University, England.
[15]CLC Workbench 5.6 software. USA.

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