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MOLECULAR CHARACTERISATION OF TRANS-SIALIDASE GENE OF *Trypanosoma evansi* ISOLATED FROM INDIAN DROMEDARIES

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ABSTRACT

The present study was carried out to isolate the Trans-sialidase (ts) gene of *Trypanosoma evansi* by polymerase chain reaction, clone the amplicons in a suitable bacterial plasmid vector and characterise the gene through sequencing. The desired amplicon of ts gene of *T. evansi* was amplified by PCR using gene specific primers and identified on the basis of size of the gene. The amplicon of expected size was purified from the 1% low melting agarose gel. The DNA fragment of interest was then ligated to the pGEM- T Easy vector and ligated mixture was transformed into *Escherichia coli* JM109 strains for cloning. After cloning, screening of recombinants was done by Restriction Enzyme digestion of plasmid DNA and by colony PCR. After confirmation of clone, the plasmid DNA was sequenced and coding sequence of ts gene according to the results obtained was of 2241 bp. Tree topology of ts gene is based on the Neighbour-Joining method with 100% bootstrap values and identified ts gene sequence showed a close homology with other *Trypanosoma* spp. gene sequences.

Key words: Camel, cloning, sequence analysis, trans-sialidase gene, *Trypanosoma evansi*

Trans-sialidase (ts) is a GPI-anchored (Glycosyl-phosphatidylinositol) cell surface enzyme that was first described in *Trypanosoma cruzi* and is used to acquire sialic acid from host glycol-conjugates for sialylation of parasites plasma membrane glycoproteins. The sialic acid is transferred to mucin-like glycoproteins that are the most abundant molecules on the surface of trypomastigotes (Koliwer-Brandl *et al,* 2011). Besides, trans-sialidase plays a key role in the recognition of protein and induces the expression of adhesion molecules in the host cells, stimulates parasite entry and blocks apoptosis generated by growth factor depletion (Dias *et al,* 2008). Trans-sialidase may be involved in parasite survival from the complement-mediated host immune response, host cell invasion and pathogenesis due to its capacity of sialylating the mucin molecules that cover the parasite surface with a dense protective layer (Paris *et al,* 2005). Trans-sialidase is a functionally important structure that is present in pathogenic trypanosomatids but absent from their mammalian hosts thus trans-sialidase acts as a potential biological target that may be useful for drug design and gene product (Buschiazzo *et al,* 2012). Thus, the present study was undertaken to isolate Trans-sialidase gene of *Trypanosoma evansi* from camel by polymerase chain reaction, to clone the amplicon and to characterise the trans-sialidase gene through sequencing.

Materials and Methods

After confirmation of *Trypanosoma evansi* infection by blood smear examination, blood from infected host was collected and inoculated intra-peritoneally into Swiss albino mice (maintained at Small Animal Laboratory, NRC on Camel, Bikaner). DNA isolation from collected pellet of *T. evansi* was done as per the protocols given by ready to use kit with slight modifications. The trans-sialidase gene of *T. evansi* was amplified from genomic DNA using gene specific forward 5′ATGGAGGAACTCCACCAACAAAT3′ and reverse 5′TATAGATCTTCAAATCGCCAACACATACAT3′ primer sequences (Designed from published sequence Accession No. AF310232). Cycling condition for PCR were initial denaturation at 94°C for 4 minute, 35 cycles of 30 seconds at 94°C, 60 second at annealing temperatures of 51°C and 60 second at 72°C, followed by a final extension for 10 minute at 72°C. The PCR amplified products were checked with 1.5 kb DNA molecular weight marker in 1.2% agarose gel.

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Cloning and sequencing of trans-sialidase gene

The PCR product from Low melting point agarose slices were purified using Illustra GFX PCR DNA and Gel Band Purification Kit. The DNA fragments of ts gene and the pGEM- T Easy vector in which it is to be cloned were digested with T4 DNA ligase enzyme to generate compatible ends for ligation. The ligation was done (as per the Promega protocol with slight modification) in the reaction volume of 20 µl containing 10 µl of 2X Rapid ligation T4 DNA Ligase buffer [400mM Tris-HCl, 100mM MgCl2, 100mM DTT, 5mM ATP (pH 7.8 at 25°C)], 6 µl PCR product, 2 µl pGEM- T Easy vector and 2 µl of T4 DNA ligase. The contents were vortexed, spun down in a micro centrifuge for 3-5 seconds and incubated for overnight at 4°C. The ligation mix was used directly for transformation in JM109 high efficiency competent cells. After incubation of transformation culture 100 µl of each transformation culture were plated on to antibiotic agar plates in duplicate and incubated at 37°C for overnight (16-20 hours). Colonies harbouring recombinant plasmids were inoculated into LB broth and incubated at 37°C overnight with horizontal shaking. The plasmids DNA were extracted from culture using Illustra plasmid prep mini spin kit. The positive clone was confirmed by Restriction Enzyme digestion of plasmid DNAs with EcoR1 and colony PCR of plasmid colonies. Purified plasmid of ts gene was sequenced in both directions at Eurofins Genomics India Pvt. Ltd., Whitefield, Bengaluru.

Sequence analysis

After getting the accession number of gene sequence phylogenetic and sequence analysis of the ts gene of *T. evansi* was done. The phylogenetic and sequence analysis was done by use of Clustal X, MEGA5 and Praline sequence alignment softwares. Phylogenetic tree analysis of ts gene was done by using Neighbour-Joining (NJ) method and

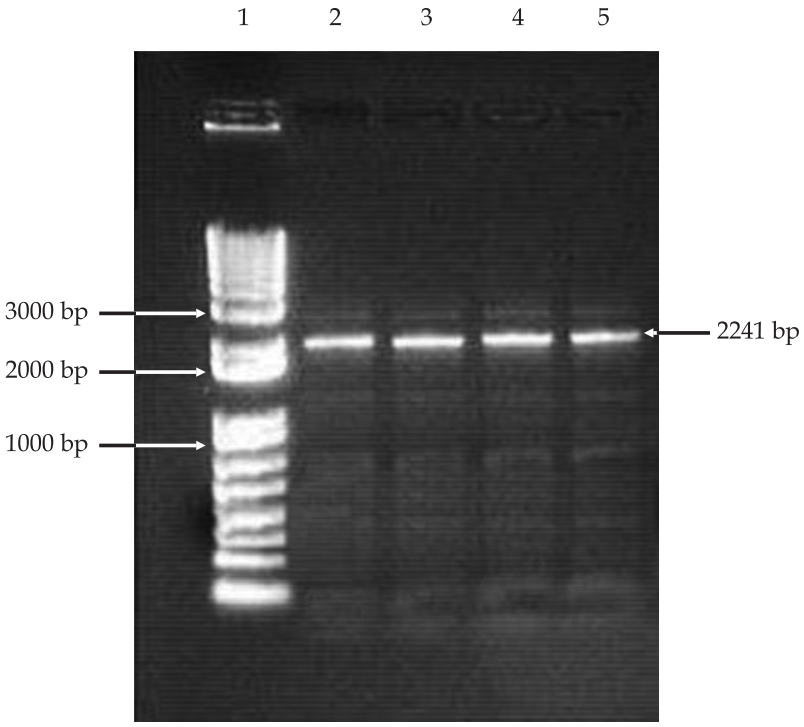

Fig 1. Amplification of Trans-sialidase gene of *Trypanosoma evansi* by PCR 1.1Kb plus DNA Ladder. 2-5. Amplicons

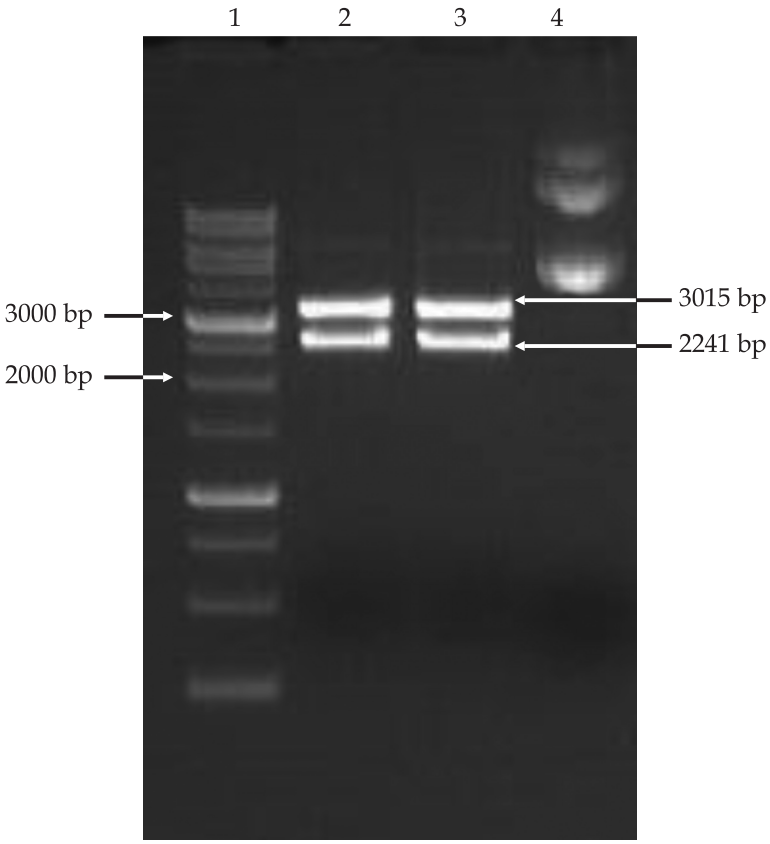

Fig 2. Trans-sialidase gene fragments of *Trypanosoma evansi* after restriction digestion of ts gene plasmid. 1. 1Kb plus DNA Ladder (2 Log DNA). 2-3. Trans-sialidase gene clone. 4. Uncut plasmid.

Maximum Parsimony (MP) method and implemented with bootstrap test involving simple stepwise addition.

Results and Discussion

The amplification band of ts gene was obtained in between 3000 bp and 2000 bp and the size of the amplicons was deduced from the standard log molecular sizes of the marker bands against their respective mobility (Fig 1). After cloning, the white colonies were screened for the presence of vector with insert. Two well separated DNA bands were seen in case of plasmids isolated from positive colonies upon digestion with EcoR1, the less intense lower band may correspond to the insert (Fig 2). Release of DNA fragments of around 2241 bp for ts gene was found after restriction enzyme digestion. Colony PCR was done for quick screening of plasmid inserts directly from *E. coli* colonies and amplification was found in wells of white colonies (Fig 3)

The coding sequence of ts gene according to the result obtained was of 2241 bp. After confirmation of the ts gene nucleotide sequence of *T. evansi* isolated from the host camel, the sequence was submitted to GenBank, NCBI database to which the assigned accession number is KF686337. For phylogenetic analysis of ts gene sequences of other Trypanosomatidae species already available in the genebank database were retrieved. Tree topology was based on the Neighbour-Joining (NJ) method showed a close homology with other *Trypanosomatidae* species sequences with 100% bootstrap values (Fig 4). The NJ, bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analysed.

In the present study, the Trans-sialidase gene sequence showed 99% homology with *T. evansi* (KF686337), 99% with *T. brucei* (XM841212). 99% sequence similarity was found between obtained ts gene sequence and *T. evansi* (FJ597949). Slightly lower homology was documented between the obtained ts gene sequence and *T. brucei* (AF310232, AF181287 and XM841210). The comparison with *T. brucei* (XM842470), *T. brucei,* (XM842471) and *T. brucei* (XM946607) a member belonging to Trypanosomatidae family showed only 49%, 48%, and 28% homology, respectively.

Based on the above homology, *T. brucei* (XM842470 and XM842471) are placed as two sub cluster of one mega cluster. *T. brucei* (XM841212, AF310232, XM841210 and XM839645) as four sub cluster of one mega cluster and the other mega cluster comprising of rest of the species. Exactly same type of sequence similarities was observed between *T. evansi,* (KF686337) and other documented species. The earlier reports of *T. evansi* by Yakubu *et al* (2009) showed 99% homology with obtained ts sequence and sequence of *T. evansi*, (FJ597949) and very few differences in sequence similarities were observed between these sequences when compared with other documented

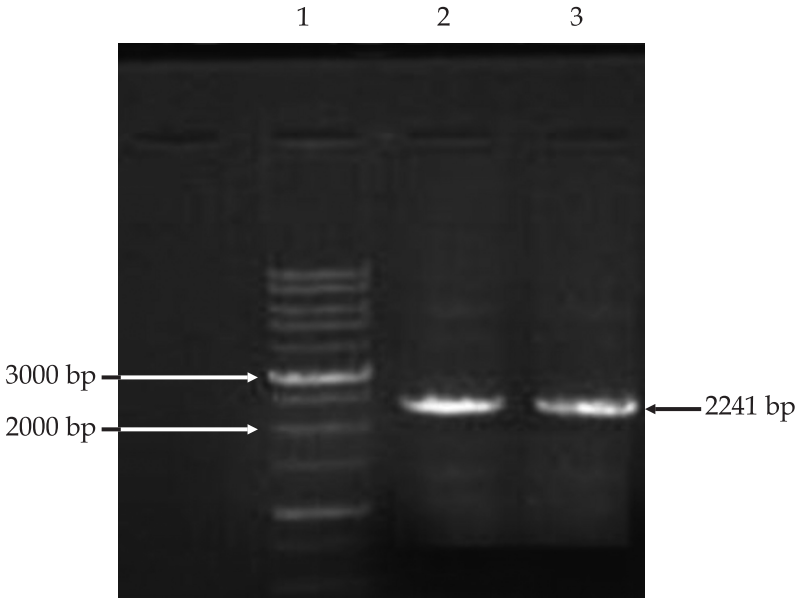

Fig 3. Amplification of Trans-sialidase gene of *Trypanosoma evansi* by Colony-PCR. 1.1Kb plus DNA Ladder. 2-3. PCR reaction with white colony shows amplification.

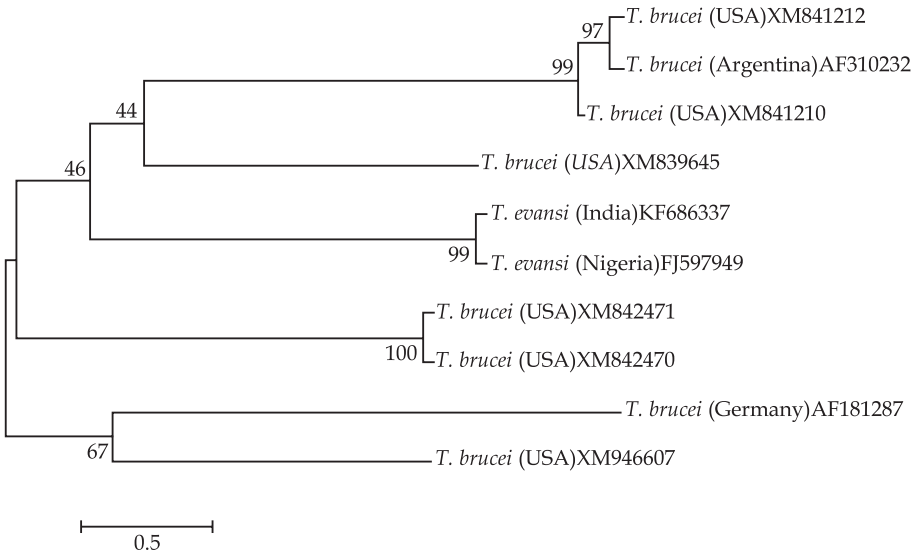

Fig 4. Phylogenetic tree analysis of Trans-sialidase gene using the Neighbour-Joining method.

species. In open reading frame of nucleotide sequence of ts gene in *Trypanosoma brucei* by (Montagna *et al*, 2002) revealed 99% homology with the 2316 bp encoding a polypeptide of 372 amino acids.

The sequence identity of obtained ts nucleotide sequence of *T. evansi* with other Trypanosomes species indicating that ts gene is highly conserved in the kinetoplastid species. It could therefore, be suggested that vaccine with Ts protein of trypanosomatidae parasite as the antigen could be effective against not only different strains within one trypanosomes species but also against other species of the same genus.

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