DOI : 10.5958/2277-8934.2017.00013.3 *Vol 24 No 1, p 85-88*

IDENTIFICATION AND MOLECULAR CLONING OF HEAT SHOCK PROTEIN-70 (*HSP*-70) GENE OF *Trypanosoma evansi* ISOLATED FROM CAMEL

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ABSTRACT

Present study was carried out to isolate the Heat Shock Protein-70 gene of *Trypanosoma evansi* using PCR. The desired amplicons of Heat Shock Protein-70 gene from genomic DNA of *T. evansi* were successfully amplified by PCR using gene specific primers at annealing temperature of 54°C. Amplified PCR product was identified on the basis of its size in agarose gel electrophoresis as 1956 bp. For cloning the purified DNA fragment was ligated to the pGEM- T Easy vector and ligated mixture was transformed into *Escherichia coli* JM109 strains. The cells containing recombinant plasmid were identified on the basis of white/blue colony selection on LB agar containing X-Gal, IPTG and ampicillin. Screening of recombinant was done by Restriction Enzyme digestion of plasmid DNAs using EcoRI and confirmed on the basis of gene size, i. e. 1956 bp for Heat Shock Protein-70 gene. Colony PCR was done for quick screening of plasmid inserts directly from *E. coli* colonies in the presence of insert specific primers.

Key words: Camel, cloning, heat shock protein-70, *Trypanosoma evansi*

Camel trypanosomosis is caused by blood protozoan parasite *Trypanosoma evansi*. It is the most pathogenic parasitic disease of camelids causing high morbidity and mortality (Luckins, 1992). Vaccine development against this organism *Trypanosoma evansi* is also hampered due to variation of surface proteins as antigen. Variant surface glycoprotein coat of *Trypanosoma evansi* is changeable when host forms antibody against it.

The major researches today are being focused on identifying various invariant protein components of trypanosome as potential drug target and basis for vaccines. Cloning and expression of mitochondrial heat shock protein 70 of *Trypanosoma congolense* and potential use as a diagnostic antigen has been studied (Bannai *et al,* 2003). Kumar *et al* (2015) studied production and preliminary evaluation of *Trypanosoma evansi* HSP 70 for antibody detection in equids. Heat shock proteins (HSPs) are a class of polypeptides powerfully induced by heat shock that mediate profound levels of stress resistance (Craig, 1985; Ellis, 2007). HSPs are molecular chaperones, binding to (holding) and refolding other cellular polypeptides (clients) with aberrant conformations (Ellis, 2007). There are a number of families of molecular chaperones (a–d), with members of class a (Hsp70, Hsp110, GRP170) and class c (Hsp90, Grp94/Gp96) thought to be of particular significance in tumour immunology (Murshid *et al,* 2011). These properties have been intensively studied for Hsp70 and Hsp90 and are largely inferred for the sibling proteins. Thus, present study was carried out to isolate the Heat Shock Protein-70 gene of *Trypanosoma evansi* using PCR and cloning of the gene.

Materials and Methods

Preparation of trypanosome strains, DNA isolation and PCR amplification

After confirmation of *T. evansi* isolates by blood smear examination, blood from infected camel was inoculated intraperitoneally in Swiss albino mice (maintained at Small Animal Laboratory, NRC on Camel, Bikaner) for propagation of trypanosomes. DEAE (Diethyl amino ethyl) cellulose column chromatography method was used for purification of trypanosomes (Lanham and Godfrey, 1970). DNA isolation from collected pellet of *T. evansi* was done as per the method utilised by Desquesnes and Davila (2002) for the preparation of animal Trypanosomes DNA from plane blood. The

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procedure for DNA isolation was same as the most commonly used procedure for DNA isolation from blood suggested by Sambrook and Russel (2001). Nucleotide primers for the amplification of HSP-70 gene of *T. evansi* were designed using the published sequence of HSP-70 gene of *Trypanosoma cruzi* (Accession No. FJ222459.1): forward primer 5′-ATGACGTACGAGGGAGCCAT-3′ and reverse primer 5′- CACTTCCTCCACCTTCGGTC-3′. PCR amplification of the HSP-70 gene was performed by cycling conditions as initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 60 sec, extension at 72°C for 1 min and 30 sec, and final extension for 10 min at 72°C. The PCR amplified products were checked with 1.5 kb DNA molecular weight marker in 1% agarose gel.

Cloning

The PCR products from low melting point agarose slices were purified by illustra GFX PCR DNA and Gel Band Purification Kit (GE healthcare, USA) using the manufacturer's protocol. The DNA fragment of HSP 70 gene and the pGEM-T Easy vector (Promega, USA) 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 $MgCl_2$, 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 then used directly for transformation in JM109 competent cells (Promega, USA). After incubation of transformation culture 100 µl of transformation culture was plated onto antibiotic agar plates and incubated at 37°C for overnight (16-20 hr). Colonies harbouring the recombinant plasmid 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 (GE healthcare, USA) according to the manufacturer's instructions. The positive clones were identified by Restriction Enzyme digestion of plasmid DNAs with *Eco*R1 and Colony PCR of plasmid colonies.

Results and Discussion

The genomic DNA was analysed in 0.8% analytical agarose gel and was found to be intact without much shearing. Gene specific forward and reverse primers were used for amplification of HSP-70 Gene and the amplicons were analysed by agarose gel electrophoresis. An intensely amplified DNA was seen in lanes B and C using genomic DNA (Fig 1). The size of the intense band was deduced from the standard curve drawn between the log molecular sizes of the marker bands against their respective mobility and found to be 1956 bp. The amplified product was purified from the LMP agarose gel and ligated with pGEM-T Easy vector (Promega). One hundred µl of transformation culture was plated onto X-gal-IPTG-Ampicillin agar plate. There were several white colonies along with a few blue colonies. The blue colonies represented the presence of vector alone but few of them contained vector with insert. The white colonies represent recombinant clones carrying insert in the plasmid. Two well separated DNA bands were seen in case of plasmid isolated from positive colonies upon digestion with *Eco*RI, the less intense lower band may correspond to the insert (Fig 2). Release of DNA fragments of around 1956 bp for heat shock protein 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 and also in blue colony (Fig 3).

Trypanosomes undergo antigenic variation that enables them to evade the host's immune system (Donelson *et al,* 1998). VSG covers the entire parasite including the flagellum presumably as a protective shield against host antibodies. This protective coat which determines the antigenic features of the parasite, is easily recognised by the host and is highly immunogenic. Heat shock proteins have been emerging as prospective drug targets (Shonhai, 2010). Drugs have been discovered to cause cellular stress resulting in the induction of heat shock proteins, ultimately improving cytoprotection (Burcham *et al,* 2012). In the present study, the amplicon size obtained was 1956 bp. Kumar *et al* (2015) identified the nucleotide sequence of 2116 bp Heat Shock Protein-70 (HSP70) from *T. evansi* proteome. Bannai *et al* (2003) examined the ability of mitochondrial heat shock protein 70 (MTP) of *Trypanosoma congolense* as a diagnostic antigen. The cDNA clone contained an open reading frame of 1,977 bp encoding a polypeptide consisting of 659 amino acids. Jose *et al* (2012) cloned, sequenced and expressed the HSP-60 gene of *Leishmania major*. Sequence analysis revealed an open reading frame of 1770 bp encoding a putative polypeptide of 589 amino acids. However, in this study, the DNA fragment amplified in the PCR

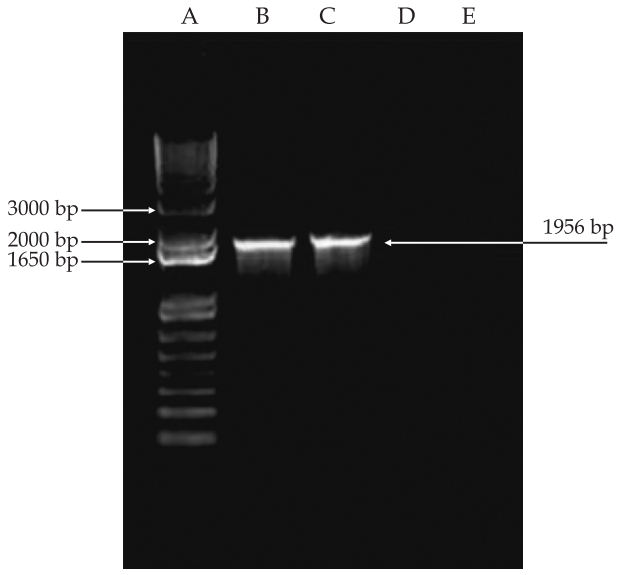

Fig 1. Amplification of *HSP*-70 gene of *T. evansi* by PCR. A. 1Kb plus DNA Ladder, B-C. Amplicons.

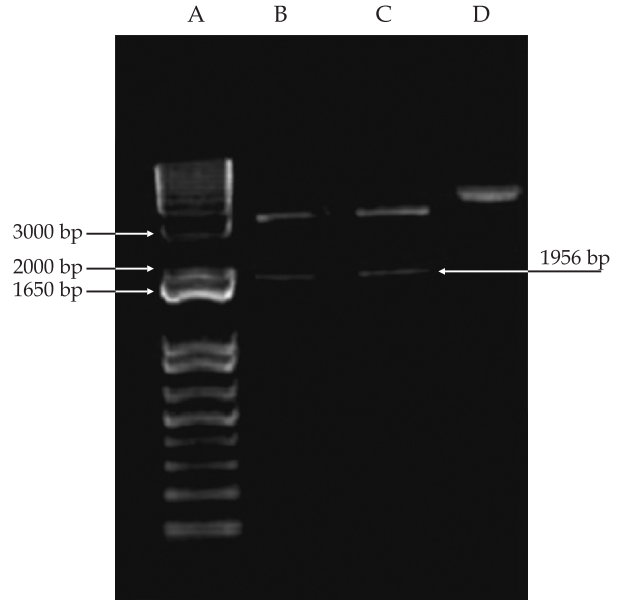

Fig 2. HSP-70 gene fragments of *T. evansi* after restriction digestion of HSP-70 gene plasmid Legends. A. 1Kb plus DNA Ladder, B – C. HSP-70 gene clone D. Uncut plasmid.

reaction was of expected size (1956bp) and highly target specific region of heat shock protein 70 gene of *T. evansi*. With the cloning and sequencing of heat shock protein gene of *T. evansi* and expression of this protein may be useful discovery of new protective antigen.

References

Bannai H, Sakurai T, Inoue N, Sugimoto C and Igarashi I (2003). Cloning and expression of mitochondrial heat shock protein 70 of *Trypanosoma congolense* and potential use as a diagnostic antigen. Clinical and Diagnostic Laboratory Immunology 10(5):926-933.

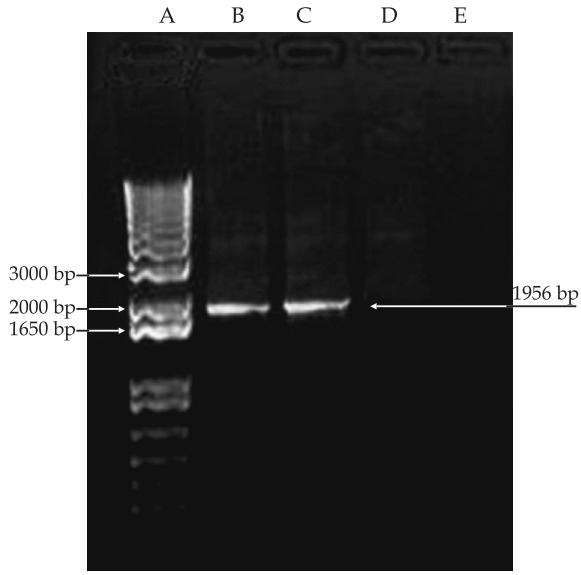

Fig 3. Amplification of HSP-70 gene of *T. evansi* by Colony-PCR. A. 1Kb plus DNA Ladder, B–C. PCR reaction with white colony shows Amplification, D-E. PCR reaction shows no amplification.

Burcham PC, Raso A and Kaminskas LM (2012). Chaperone Heat Shock Protein 90 Mobilisation and Hydralasine Cytoprotection against acrolein-induced carbonyl stress. Molecular Pharmacology 82:876-886.

Craig EA (1985). The heat shock response. CRC Critical Reviews in Biochemistry 18:239-280.

Desquesnes M and Davila AMR (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. Veterinary Parasitology 109:213-231.

Donelson JE, Hill KL and El-Sayed NM (1998). Multiple mechanisms of immune evasion by African trypanosomes. Molecular and Biochemical Parasitology 91(1):51-66.

Ellis RJ (2007). Protein misassembly: macromolecular crowding and molecular chaperones. Advances in Experimental Medicine and Biology 594:1-13.

Jose MR, Carmen C, Lineth G, Rudy P, Concepcion JP and Carmen C (2012). Sequence analysis of the 3'-untranslated region of HSP70 (type I) genes in the genus Leishmania: its usefulness as a molecular marker for species identification. Parasites and Vectors 5:87.

Kumar J, Chaudhury A, Bera BC, Kumar R, Kumar R, Tatu U and Yadav SC (2015). Production and preliminary evaluation of *Trypanosoma evansi* HSP70 for antibody detection in Equids. Acta Parasitologica 60(4):727-734.

Lanham SM and Godfrey DG (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Experimental Parasitology 28(3):521-34.

Luckins AG (1992). Protozoal disease of camel. Proc. Ist International Camel Conference, Feb. 2-6, held at Dubai. pp 23-27.

Murshid A, Gong J, Stevenson MA and Calderwood SK (2011). Heat shock proteins and cancer vaccines: developments

in the past decade and chaperoning in the decade to come. Expert Review of Vaccines 10(11):1553-68.

Pathak KML and Khanna ND (1995). Trypanosomosis in camel (*Camelus dromedaries*) with particular reference to Indian subcontinent: a review. International Journal of Animal Science 10:157-162.

Sambrook J and Russel DW (2001). Molecular Cloning- A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York. 3rd Edition. (Vol. I to III).

Shonhai A (2010). Plasmodial heat shock proteins: targets for chemotherapy. FEMS Immunology and Medical Microbiology 58:61-74.

FORM IV

(See Rule 8)

1. Place of Publication	:	Camel Publishing House, 67, Gandhi Nagar (West), Near Lalgarh Palace, Bikaner-334001, Rajasthan
2. Periodicity of its publication	:	Triannual
3. Printer's Name	:	Deep Chand Sankhla
(Whether citizen of India)	:	Yes
Address	:	Near Polytechnic College Shiv Bari Raod, Bikaner
4. Publisher's Name	:	Tarun Kumar Gahlot
(Whether citizen of India)	:	Yes
Address	:	67, Gandhi Nagar (West), Near Lalgarh Palace, Bikaner-334001, Rajasthan
5. Editor's Name	:	Tarun Kumar Gahlot
(Whether citizen of India)	:	Yes
Address	:	67, Gandhi Nagar (West), Near Lalgarh Palace, Bikaner-334001, Rajasthan
6. Names and address of individual who own the newspaper and partners or share holders holding more than one per cent of total capital.	:	Tarun Kumar Gahlot 67, Gandhi Nagar (West), Near Lalgarh Palace, Bikaner-334001, Rajasthan

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Dated : 01.04.2017

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