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INVESTIGATING AND SEQUENCING OF THIOL-SPECIFIC ANTIOXIDANT GENE IN A SYRIAN STRAIN OF *LEISHMANIA TROPICA*

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ABSTRACT: *Cutaneous leishmania* is a common medical problem in Syria, which has become yet more widespread in most of the Syrian governorates after the current war. An effective vaccine is needed to prevent a large scale spread of leishmaniasis in the country. In this regard, protein vaccination has shown promising prospects of creating this much-needed vaccine. Thiol Specific Antioxident (TSA) plays a fundamental role in the life of parasites. Its gene provides protective properties in the form of DNA vaccines against infections by many species of parasites and bacteria. So far, the presence of this gene in the genome of the Syrian strains of *L. tropica* has not been proven. This study aimed to investigate the presence of the gene in the Syrian strain of *Leishmania tropica* promastigotes, clone and compare with the TSA sequence in other Leishmaniaspp. We isolated genomic DNA from these parasites, designed primers for amplification of the gene encoding this protein, and standardized the PCR conditions.

INTRODUCTION: Parasites of the genus Leishmania cause leishmaniasis. This disease appears in three main clinical forms (visceral, cutaneous mucosal, and cutaneous) [1]. Although leishmaniasis is not a familiar name as malaria is, this disease significantly affects most of the world's population [2]. It is estimated that the disease is present in 98 countries and about 2 million new cases are reported each year [3]. Since, the beginning of the Syrian War, cases of *Cutaneous leishmania* (CL) have increased severely in Syria [4]. Transmission of the parasite occurs during the bite of infected sand flies on mammalian hosts [3].

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In the Cutaneous form of leishmaniasis, the patient usually presents with one or more ulcers in the skin, which can cause disfiguring scars [5]. Several chemical drugs have been applied to treat of CL. Many problems limit the use of these drugs (*e.g.*, high cost, high toxicity, and ineffectiveness in some endemic regions) [6]. There are only a few approved drugs, no preventative drugs, and no vaccine [7].

Numerous research in animal models and humans has evaluated the potential of live genetically modified attenuated vaccines, as well as a variety of recombinant antigens or their coding DNA [2]. Several studies have been conducted that have used rats, mice, and hamsters as experimental models for *Leishmania tropica* infection [8]. Among the vaccine candidates, thiol-specific antioxidant has been presented as one of the main vaccine candidates. It is a 22.1 kDa protein that is expressed in both

forms of the Leishmania (amastigote and promastigote) [6]. Thiol-specific antioxidant protein has been reported to be strongly antigenic for both mice and humans and to stimulatea T-helper1-dependent immune response in the host body, particularly by strong stimulation of cellular immunity [9]. Since, TSA protein has main roles in the life of the Leishmania parasite and it is able to induce an immune response when used as a DNA vaccine or as recombinant proteins against infection with Leishmania species, we can say that TSA protein and its gene represent good candidates for a vaccine. TSA gene of the Syrian *Leishmania tropics* strain has not been sequenced, so this study has chosen TSA to design a protein vaccine against the infection with *L. tropica*, which is the causative agent in the majority of cutaneous cases in Syria. The presence of TSA gene was not demonstrated in *L. tropica* Syrian strains. The present systematic study is the first to investigate whether this gene exists in the Leishmania Syrian strain genome, to prove if there is an expression of this gene in these parasites, and to sequence it.

MATERIALS AND METHODS:

Primers Design for PCR: In the absence of a published sequence of genes in the *L. tropica* genome, we used CLC free workbench 7 and Vector NTI Express programs and the clustalw2 and emboss needle tools to make an alignment for sequences of TSA gene in other leishmania species. Through <http://www.idtdna.com/calc/analyzer>, <http://www.geneinfinity.org/sms/primanalysis.html> tools primers were designed. High purified Primers were ordered from alpha-Biotech, Canada. Final sequences for primers were: [TSA gene primers; forward, : 5' - ATT CAT ATG TCC TGC GGT AAC GCC AAG- 3' ; reverse, 5'- ATT GGA TCC CAT GGA ATT CTT ACT GCT TGCTGA AGT ATCC-3'. Underlined are the NdeI and BamHI restriction sites included for direct cloning in the PET15-b expression vector [Promega, United States].

DNA Extraction: The Leishmania DNA was extracted from the promastigotes of a Syrian strain of *L. tropica* by DNA extraction kit [Promega, USA], according to the manufacturer's instructions. Then it was electrophoresed on 1% agarose gel. *L. tropica* Syrian strain, LCED-Syrian Strain 01, provided by the Leishmania Centre for Epidemiological and Biological Studies, Damascus University. Promastigotes were grown in RPMI - 1640 medium [Lonza-USA] supplemented with 10% fetal calf serum [Cytogen, GmbH, Germany].

PCR Amplification of TSA: A one-step green master mix [Genedirex, USA] and the designed primers mentioned above were used to amplify the DNA of the TSA gene by thermal cycler [Bio-rad, USA]. Gradient annealing temperature was used in order to optimize the PCR protocol. The PCR reaction was made in 25 µl of a mixture of the following components: 1 µl of 10 µM from each primer, 2.5 µl of template DNA, 12.5 of PCR master mix, and 8 µl of nuclease-free water. The conditions of thermal cycling were as follows: 95ºC for five minutes followed by 35 cycles of 95ºC for one minute, 57ºC for 45 sec for TSA gene, 72ºC for one minute, and finally 72ºC for five minutes. the PCR products for both extracted DNA were loaded on 1% agarose gel, stained with ethidium bromide, and visualized on UV transilluminator. British CLEAVER electrophoresis device was used to make electrophoresis. The current was 120 mA, and the voltage was 100 V.

Plasmid Construction and Purification: The Gene Jet PCR Purification kit [Thermoscientific, Lithuania] was used to purify the PCR product containing the TSA coding sequence, as per the manufacturer's instructions. Double digest with NdeI plus BamHI was applied on Purified the PCR product and the PET15-b expression vector [Promega, United States]. T4 DNA ligase [Thermoscientific, Lithuania] was used to ligate Digested DNA with PET15-b-TSA. Clone was transformed into E-coli BL21. GF-1 plasmid DNA extraction kit [Vivantis, Malaysia] was used to purify recombinant plasmid extracted from colony.

Sequence Analysis: Full-length double-stranded sequence analysis was performed on purified recombinant plasmid PET15-b-TSA by sequencing, using T7 [forward] and PCR 3'[reverse] vector primers also the full-length double-stranded sequence analysis was performed using designed primers mentioned above on automated sequencers [ABI -3730XL System, ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, South Korea].

RESULTS AND DISCUSSION:

Quality and Purity Evaluation of DNA: Extracted DNA electrophoresis on a 1% agarose gel showed only one band **Fig. 1,** which demonstrated that degradation of extracted DNA did not happen. The purity of the extracted DNA was 1.78, which revealed a high degree of purity of the extracted DNA.

Amplification of TSA Gene using Extracted DNA: PCR protocol was optimized using gradient annealing temperatures, and the most suitable annealing temperatures were selected, which were 57°C for TSA gene, PCR products showed by Gel electrophoresis, on the level of DNA and only one band belong to the gene, and its sizes were approximately 600 base pair, this present study has proven the presence of TSA gene in a Syrian strain of *L. tropica* genome **Fig. 2** and also in the strain.

Cloning and Sequencing of the TSA Gene: The PCR product from the DNA of Thio specific antioxidant gene was sequenced and identified in an attempt to clone and characterize the *L. tropica* TSA gene. **Fig. 3** shows the complete nucleotide sequence of TSA gene (600 pb) DNA insert, TSA 600 pb.

Ncbi nucleotide blast was used to compare these predicted sequences with the sequences of proteins coded by TSA DNA in other species of Leishmania, such as *L. donovani*, *L. major*, and *L. ifantum* [appendix1]. Interestingly, *L. tropica* TSA shows significant similarities with TSA of *L. Infantum* [94.97%], *L. major* [95.48%], *L. donovani* [95.98%], and *L. Mexicana* [99.67%]. *L. aethiopica* [96.48], *L.* chagasi [94.95].

TSA Protein Sequence:
MSCGNAKINSPAPSFEEMALMPNGSFKKISLS
AYKGKWVVLFFYPLDFTFVCPTEIIAFSDNVS
RFNELNCEVLACSMDSEYAHLQWTLQDRKK
GGLGAMAIPMLADKTKSIARSYGVLEESQGV
AYRGLFIIDPHGMVRQITVNDMPVGRNVEEV
LRLLEAFQFVEKHGEVCPANWKKGAPTMKP
EPKASVEGYFSKQ.

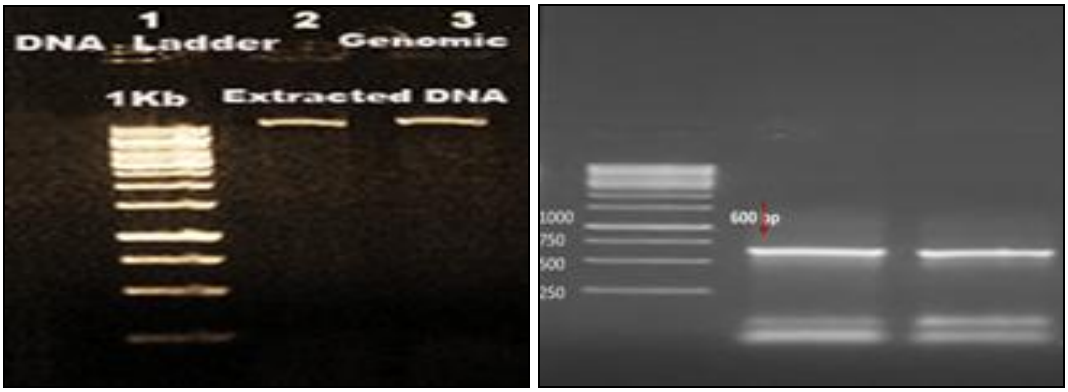

FIG. 1: ELECTROPHORESIS OF EXTRACTED *L. TROPICA* DNA: LANE 1 DNA LADDER 1KB, LANE 2,3 EXTRACTED *L. TROPICA* DNA

ATGTCCTGCGGTAACGCCAAGATCAACTCTCCCGCGCCGTCCTTCGAGGAGATGGCG
CTCATGCCCAACGGCAGCTTCAAGAAGATCAGCCTCTCCGCCTACAAGGGCAAGTGG
GTCGTGCTCTTCTTCTACCCGCTCGACTTCACCTTCGTGTGCCCGACAGAGATCATCGC
GTTCTCCGACAACGTGAGTCGCTTCAACGAGCTCAACTGCGAGGTCCTCGCGTGCTCG
ATGGACAGCGAGTACGCGCACCTGCAGTGGACGCTGCAGGACCGCAAGAAGGGCGG
CCTCGGGGCCATGGCGATCCCAATGCTGGCCGACAAGACCAAGAGCATCGCTCGTTC
CTACGGCGTGCTGGAGGAGAGCCAGGGCGTGGCCTACCGCGGTCTCTTCATCATCGA
CCCCCATGGCATGGTGCGTCAGATCACCGTCAACGACATGCCGGTGGGCCGCAACGT
GGAGGAGGTTCTGCGCCTGCTGGAGGCTTTTCAGTTCGTGGAGAAGCACGGCGAGGT
GTGCCCCGCGAACTGGAAGAAGGGCGCCCCCACGATGAAGCCGGAACCGAAGGCGT
CTGTCGAGGGATACTTCAGCAAGCAGTAA

FIG. 2: AMPLIFICATION PRODUCTS OF *L. TROPICA* THIOL SPECIFIC ANTIOXIDANT ON 1% AGAROSE GEL ELECTROPHORESIS STAINED WITH ETHIDIUM BROMIDE. LANE 1: DNA LADDER 1 KB, LANE 2AND 3: THIOL SPECIFIC ANTIOXIDANT AMPLIFICATION PRODUCT 600 BP

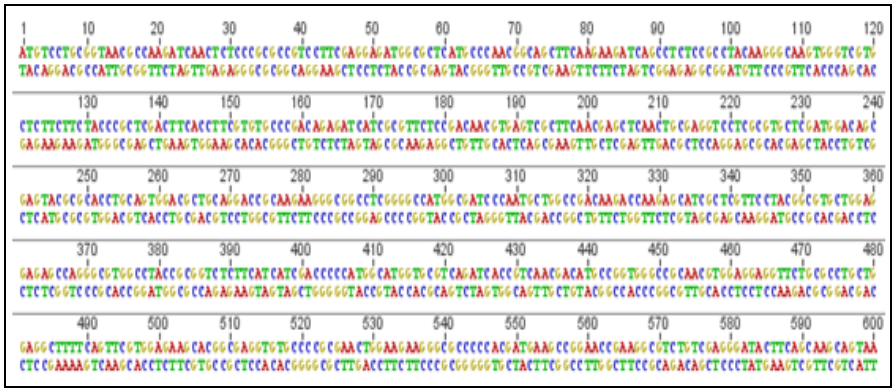

FIG. 3: THE COMPLETE NUCLEOTIDE SEQUENCE OF THIOL SPECIFIC ANTIOXIDANT GENE IN SYRIAN STRAIN OF *LEISHMANIA TROPICA*

CONCLUSION: This study has demonstrated that thiol specific antioxidant gene is a part of *L. tropica* genome. This study has proved the presence of that thiol-specific antioxidant gene in a Syrian *L. tropica*. For the first time, we can prove the presence of thiol specific antioxidant gene in *L. tropica*, define the sequence of the DNA of that thiol specific antioxidant gene and attempt to submit this gene to the Gene bank with an accession number. The immunogenicity of TSA protein can now be evaluated as DNA vaccine against *Leishmania species*.

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CONFLICTS OF INTEREST: Nil

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