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Expression of *hsp15* gene of *Leptospira interrogans* under Stress Conditions

Upasna[1] and Satparkash Singh[2*]
[1]M.Sc. Scholar, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana (Punjab), India.
[2]Assistant Professor and In-charge Leptospira Lab, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana (Punjab), India.

(Corresponding author: Satparkash Singh)*
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ABSTRACT: Leptospires can adapt to different environmental as well as host stresses including change in temperature, pH, reactive oxidation species etc. The study was conducted to determine the change in mRNA expression of gene encoding heat shock protein 15 (Hsp15) of *Leptospira interrogans* under thermal and oxidative stress using qPCR which revealed that *hsp15* gene was not significantly upregulated or downregulated under specified stress. In addition, the *hsp15* gene was cloned and sequenced. To the best of our knowledge, this is the first study on effect of oxidative stress which is very important during infection, on expression of *hsp15 gene* of *L. interrogans*.

Keywords: Leptospirosis, Hsp15, qPCR, expression.

INTRODUCTION

Leptospirosis, a disease caused by bacteria belonging to genus *Leptospira*, is an economically important infection of humans and animals with a world-wide distribution (Choudhary *et al*., 2023). Like many other bacterial pathogens, leptospires have abilities to adapt themselves to survive in a wide range of environmental conditions outside & inside the infected hosts. Temperature change and oxidative stress represent two of the main external stresses which bacteria face during infection. Heat shock proteins (HSPs) found ubiquitously across the species are very important against stress (Singh *et al*., 2009). These proteins have been studied in leptospires also, demonstrating their diverse functions (Ho *et al*., 2021). Leptospires respond to environmental conditions through regulation of certain HSPs which are crucial for the survival of the bacteria and their ability to establish infection by stabilizing multi-chaperone network (Vinaiphat and Thongboonkerd 2017). The principal HSPs with chaperone activity belong to five conserved classes which include the small heat shock proteins (sHSPs). The sHSPs are small molecular chaperones found in almost all organisms with majority lying between 14 and 27 kDa and their number varies between species to species (Narberhaus, 2002). Hsp15, one of the sHSPs, is a highly conserved RNA binding protein. Presence of Hsp15 has been demonstrated in different serovars of the pathogenic *L. interrogans* while it was not detected in the saprophytic *L. biflexa* (Nally *et al*., 2001).

Understanding how HSPs are expressed during leptospiral infection is thus crucial to develop better understanding of pathogenesis of this bacterium (Davignon *et al*., 2024). The present work was planned with the objective of studying the expression of gene encoding Hsp15 of *L. interrogans* in response to stress using real time qPCR. Also, *hsp15* gene was cloned and sequenced. To the best of our knowledge, this is the first study to see the effect of oxidative stress which is very important during infection, on the expression of *hsp15* gene of *L. interrogans*.

MATERIALS AND METHODS

Bacterial cultures and plasmids. EMJH medium (BD Difco) was employed to maintain standard culture of *L. interrogans* grown at 29-30ºC. Top10 cells (strain of *Escherichia coli*) were used for transformation experiments. pGEMT-Easy vector (Promega, USA) was used for TA cloning of *hsp15* gene.

Cloning and sequence analysis of *hsp15* gene. Genomic DNA from bacteria was extracted by QIAamp Mini Kit (Qiagen, Germany). Using primer 3 plus software, primers were designed for polymerase chain reaction (PCR) amplification of gene encoding HSP15 along with some flanking regions (product size: 444bp). The primer sequences are given in Table 1. PCR reaction of 20 µl consisted of 2x Hot Star Master Mix (Qiagen, Germany), 1 µM of each primer; 1µl template and nuclease free water. The reaction was carried out with 35 cycles with annealing temperature of 55ºC for 30s. Analysis of PCR products was done by electrophoresis in 1.5% agarose (Sigma, Germany). Gel elution of gene product was done using gel elution kit (Qiagen).

For cloning of *hsp15* gene, gel purified PCR products were ligated with pGEMT-easy cloning vector (Promega, USA) following the manufacturer's recommendations. The transformed cells were plated into the Luria bertani agar plates containing ampicillin, X-gal and isopropyl β-D-1-thiogalactopyranoside (Cheema *et al*., 2007). Random white colonies were selected for plasmid isolation by QiAprep Spin Miniprep Kit (Qiagen). Restriction digestion of plasmids was done for confirmation of clones using enzyme NotI (Thermo Scientific, USA) as per manufacturer's instruction. The confirmed clone was sent to University of Delhi, India, for sequencing.

Nucleotide sequence representing complete open reading frame was translated into amino acid sequence of HSP15 using online tool (http://web.expasy.org/translate/). The tertiary structure of deduced protein sequence was predicted using the homology modeling (http://swissmodel.expasy.org/) (Arnold *et al*., 2006). Structure assessment and validation of the 3-D model was done by Ramachandran plot analysis.

Analysis of stress response. Response of bacteria against rise in temperature and hydrogen peroxide exposure was observed by real time qPCR, in the form of expression of *hsp15* mRNA. *L. interrogans* was grown in liquid EMJH medium incubated at 30ºC for 7 days. Grown culture was shifted to 37ºC for overnight and hydrogen peroxide (1mM) was added into overnight grown culture and kept further for 60 min. At the same time, a culture grown at normal 30ºC was also kept as control. RNA from cultures was isolated by TRIzol reagent (Sigma, Germany) as per manufacturer's instructions and stored at -80ºC until processed further. c-DNA was synthesized by Verso c-DNA synthesis kit (Invitrogen, USA) following the manufacturer's protocol.

SYBR Green qPCR master mix (Sigma) was used to amplify the *hsp15* gene. The *16S rRNA* gene was employed as an appropriate internal control (Fraser and Brown 2017). Sequences of the primers are as given in Table 1. PCR amplification efficiency of both genes was calculated before performing the actual experiment. Non-template control (NTC) was always employed to rule out non-specific amplification and reactions were put in duplicate. Dissociation curves were used to assess the specificity of amplified products, and the results were expressed as threshold cycle (Ct) value. After 39 cycles of amplification, the Ct values for the test and internal control genes were used to study expression analysis by fold change method (Livak and Schmittgen 2001).

RESULTS AND DISCUSSION

PCR amplification of *hsp15* gene produced specific product of size approximately 450 bp. Restriction digestion of the plasmids resulted in the release of insert with approximate size equal to the *hsp15* gene amplicon, thus confirming the clones (Fig. 1). A clone was sequenced and *hsp15* sequence was submitted to the BankIt, NCBI (Accession number: OL753434). Tertiary structure prediction with homology modelling created a model using HSPA protein (SMTL ID: 3gt6.1.A) as the template which was selected based on maximum homology. Ramachandran validation of the structure showed more than 95 % of the amino acids in the favorable region indicating the high accuracy of the model (Fig. 2) (Waterhouse *et al*., 2024).

The mRNA expression of *hsp15* gene of *L. interrogans* exposed to temperature shift from 30ºC to 37ºC and addition of H_2O_2 was studied using qPCR which is one of the common techniques for studying gene expression (Mishra *et al*., 2025). The amplification efficiencies of *hsp15* and *16S rRNA* were determined to be above 99 percent indicating exponential efficiency for these reactions. The melt curves revealed specific product amplification (Fig.3). The fold change in expression of *hsp15* mRNA under stress situations was found to be 1.0-1.1, which showed that its expression was not significantly changed during specified stress. In other words, the specified stress had no implication on its expression.

Heat shock proteins have been investigated in *E. coli* strains in terms of pressure dependent heat shock response (Coffin *et al*., 2024). The sHSPs are particularly important for bacterial colonization and intracellular survival in hosts and can have a variety of functions including endowing cells with thermo-tolerance *in vivo* or acting as molecular chaperones (Obuchowski *et al*., 2021; Sato *et al*., 2024). Unchanged HSP15 expression might indicate its role in adaptability to stress, which can be explained according to findings of similar studies on HSPs (Benjamin *et al*., 2024). For example, in a study, two highly conserved HSPs in leptospires namely DnaK and GroEL showed that their expression levels were not remarkably altered following temperature shift as compared to the basal state (Stamm *et al*., 1991). The genome of *L. interrogans* is larger than that of *L. biflexa* which presumably occur for the better survival of the former during transmission cycle from animal hosts to aquatic environments, in contrast to a lesser rearrangement of *L. biflexa* genome that has restricted transmission potential. Nevertheless, large genome does not lead to greater expression of HSPs in the pathogenic leptospires as these are chaperones that aid in stabilizing expression of downstream signaling cascade required for adaption in various conditions encountered upon infection. Zavala-Alvarado *et al*. (2020) observed that leptospires recruits heat shock response chaperones and DNA repair proteins to recover from oxidative damage. These findings suggested that bacterial factors impart in vivo resistance to reactive oxygen species produced by the host innate immune system. Similar experiments in cattle belonging to Indian native (Bos indicus) and exotic (Bos taurus) to study HSPs expression in response to heat stress have shown that Bos indicus which is known to have higher adaptability showed minimum change in expression of these molecular chaperons highlighting their role in adaptability (Kishore *et al*., 2014).

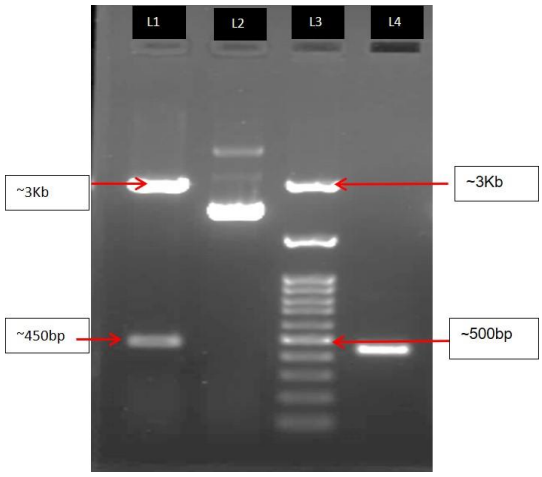

Fig. 1. RE digestion for confirmation of cloning: L1-*Not* I digested recombinant plasmid showing insert release, L2- undigested recombinant plasmid, L3-100 bp Ladder (Himedia, India), L4-*hsp15* gene amplicon for size comparison

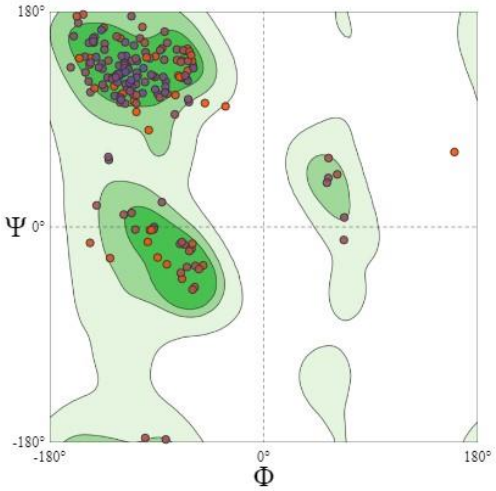

Fig. 2. Ramachandran Plot of the deduced protein structure showing spatial distribution of the amino acids.

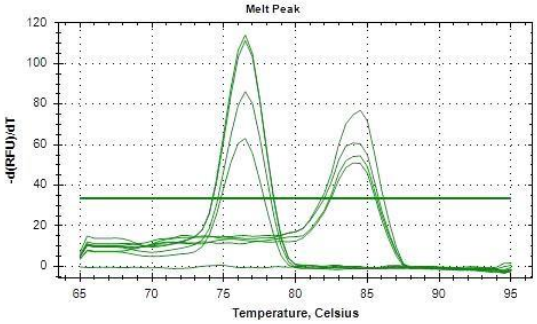

Fig. 3. Melt curves of *hsp15* and *16s rRNA* genes.

Table 1: Sequences of primers used in the study.

Sr. No.	Name	Sequence	Reference
1.	Hsp15-F:	5'-ACCAACTAACAACGATTAGGAGA-3'	GenBank: accession AF320330.1
2.	Hsp15-R:	5'-TGAACTCGCCGACGGATAAA-3'	-do-
3.	Hsp15RT-F:	5'-CCGTCACTTCTGAAGAAACCAA -3'	-do-
4	Hsp15RT-R:	5'-GGAAGATCGGCCAGTAGATAGA-3'	-do-
5	*16S rRNA*-F:	5'-GGCGGCGCGTCTTAAACATG-3'	Merien *et al*. (1995)
6.	*16S rRNA*-R:	5'-TTCCCCCCATTGAGCAAGATT-3'	-do-

CONCLUSIONS

The *hsp15* gene of *L. interrogans* was successfully cloned and sequenced which might shed light on its evolutionary significance. The deduced protein model was characterized and validated to be a stable prediction. Further, qPCR-based studies on *hsp15* gene expression in response to temperature and oxidative stress suggested its role in adaptability of this pathogen under these stress conditions.

FUTURE SCOPE

These findings might provide a basis for various avenues of explorations related to HSPs in their role in host-pathogen interactions.

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Conflict of Interest. None.

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