

Cloning and sequencing of the virulent gene *LipL32* of *Leptospira interrogans* serovar Autumnalis

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Abstract

Aim: To clone the virulent gene *LipL32* of *Leptospira interrogans* serovar Autumnalis and to analyze the sequence with *LipL32* gene of other pathogenic serovars of *Leptospira*.

Materials and Methods: *Leptospira interrogans* serovar Autumnalis procured from *Leptospira* research laboratory, Chennai was used in the study. Polymerase chain reaction (PCR) was carried out for amplifying *LipL32* gene using the reported primers of *Leptospira Kirschnerii*. The PCR product was cloned into TA cloning vector and the vector was transformed into *E.Coli* DH5 cells. The plasmid was isolated from *E.Coli* and sent for sequencing with universal primers. The sequence was submitted in genbank with accession number JQ861883.

Results: The PCR product revealed an amplicon of 790 bp. The *LipL32* gene sequence of *Leptospira interrogans* serovar Autumnalis showed 99 % similarity with most of the pathogenic Leptospire.

Conclusions: *LipL32* gene of *Leptospira* is highly conserved in most of the pathogenic Leptospire. The study concludes that this gene could be used as a target for the diagnosis of leptospirosis in animals and humans and could be tested as an important candidate antigen for vaccine production.

Keywords: cloning, sequencing, *LipL32* gene, *Leptospira* Autumnalis

Introduction

Leptospirosis has re-emerged as an important zoonotic disease in India and is endemic in most of the southern states like Kerala, Tamil Nadu and certain parts of Andhra Pradesh [1-3]. Conventional methods of diagnosis like demonstration of organisms by dark field microscopy, isolation are less sensitive and Microscopic agglutination test, the gold standard test can be performed only in reference laboratories since it requires the maintenance of live cultures. The tests employing outer membrane proteins (OMPs) showed high sensitivity and specificity [4,5]. The results of recombinant OMP antigen based enzyme linked immunosorbant assay's (ELISA) are promising [6]. *LipL32* was consistently observed in all pathogenic leptospire and was observed to be the immunodominant protein antigen recognized by the human humoral response during natural infections. This protein could be useful as a marker of infection for laboratory case confirmation in the field and in differentiating leptospirosis from other causes of acute febrile illness [7]. Leptospiral protein p32 is the most immunodominant protein and could be employed for the development of novel diagnostic assays [8]. The present study was carried out to clone and sequence the *LipL32* gene of *Leptospira interrogans* serovar Autumnalis and to analyze the sequence relationship with other pathogenic leptospire.

Materials and Methods

Leptospira interrogans serovar Autumnalis,

procured from *Leptospira* research centre, Tamil Nadu Veterinary and Animal Sciences University, Chennai and maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy was used in the study. The organisms were maintained in Ellinghausen-McCullough-Johnson-Harris medium (EMJH) (Difco) supplemented with bovine albumin and sub cultured at seven days interval. *E.coli* DH5 was maintained in Luria-Bertani agar (HiMedia, Mumbai). The genomic DNA of *Leptospira* was extracted as per the method described [9]. The PCR was carried out using the reported primers of *LipL32* gene. The primer sequences were 5' - CGC GCT GCA GTT ACT TAG TCG CGT CA G AAG- 3' and 5'- CGC GGT CGA CGC TTT CGG TGG TCT CTG CCA AGC - 3' [11]. The primers were diluted in nuclease free water so as to have a final concentration of 20 pmol/μl. The reaction was carried out in a 25 μl reaction mixture, containing 10X PCR buffer with MgCl₂, 10 mM dNTP mix, 20 pmol of each primer, 1.0 U of *Taq* DNA polymerase, nuclease free water and template DNA. The PCR programme was standardized with one cycle of initial denaturation at 94° C for 4 min followed by 30 cycles each of denaturation (94° C for 45 sec, annealing (54° C for 45 sec) and extension (72° C for 45 sec) and final extension (72° C for 5 min). The PCR product thus obtained was analyzed by running on 1.0 % agarose gel. The gel was visualized under UV transilluminator and the results were documented in a gel documentation system (Alpha Innotech Corporation,

Table-1. Results of nucleotide Blast showing similarity of LipL32 gene of *Leptospira interrogans* serovar Autumnalis with other pathogenic serovars

S.No	Accession number	Description	Max score	Total score	Query coverage	Max indent
1	EU526391	<i>Leptospira interrogans</i> serovar Autumnalis strain N2 outer membrane lipoprotein LipL32 gene, partial cds	1322	1322	96%	100%
2	AY568679	<i>Leptospira interrogans</i> strain Lai major outer membrane protein (lipL32) gene, complete cds	1336	1336	96%	100%
3	JQ013520	<i>Leptospira interrogans</i> serovar Balico major outer membrane lipoprotein LipL32 (lipL32) gene, partial cds	1341	1341	98%	99%
4	AY609327	<i>Leptospira interrogans</i> serovar Grippytyphosa major outer membrane protein (lipL32) gene, complete cds	1339	1339	97%	99%
5	AY609325	<i>Leptospira interrogans</i> serovar Australis major outer membrane protein (lipL32) gene, complete cds	1339	1339	97%	99%
6	AY609321	<i>Leptospira interrogans</i> serovar Canicola major outer membrane protein (lipL32) gene, complete cds	1339	1339	97%	99%
7	AE016823	<i>Leptospira interrogans</i> serovar Copenhageni str. Fiocruz L1-130, chromosome I, complete sequence	1339	1339	97%	99%
8	GU592525	<i>Leptospira interrogans</i> serovar Hardjo hemolysis-associated protein I (hapI) gene, complete cds	1334	1334	97%	99%
9	GU183106	<i>Leptospira interrogans</i> serovar Icterohaemorrhagiae strain RGA hemolysis associated protein I (hapI) gene, complete cds	1334	1334	97%	99%
10	AY609328	<i>Leptospira interrogans</i> serovar Hebdomadis major outer membrane protein (lipL32) gene, complete cds	1334	1334	97%	99%
11	AY609326	<i>Leptospira noguchii</i> serovar Pomona major outer membrane protein (lipL32) gene, complete cds	1334	1334	97%	99%
12	AY609333	<i>Leptospira borgpetersenii</i> serovar Mini major outer membrane protein (lipL32) gene, complete cds	1328	1328	97%	99%
13	AY461917	<i>Leptospira kirschneri</i> strain 5621 LipL32 (lipL32) gene, partial cds	1317	1317	97%	99%

USA). The PCR product was gel eluted using GeneAll DNA purification kit as per the manufacturer protocol (GeneAll, Korea). The cloning vector pTZ57R/T was used for cloning the purified DNA as per the method described by the manufacturer (Fermentas). The cloning vector was transformed into *E. coli* DH5 cells and the culture was plated on Luria-Bertani (LB) agar plates containing ampicillin (100 µg/ml), with the help of sterile L-spreader and incubated at 37° C overnight. The obtained colonies were subjected to PCR and the confirmed colonies were inoculated into LB broth containing ampicillin at 37° C overnight in a shaker incubator for plasmid isolation. The plasmid was extracted by alkaline lysis method using GeneAll plasmid extraction kit as per the manufacturers protocol (GeneAll, Korea). The plasmid was reconfirmed by PCR before sending for sequencing. The plasmid was sequenced using universal M13 forward and reverse primers (SciGenom labs Pvt. Ltd, Cochin, Kerala). The vector sequence was removed using Vecscreen tool and the trimmed sequence was submitted to NCBI GenBank (accession no. JQ861883). Further, the nucleotide sequence was subjected to nucleotide BLAST to compare for sequence similarities with other sequences available in the NCBI database (<http://blast.ncbi.nlm.nih.gov>).

Results and Discussion

The extracted genomic DNA from *Leptospira interrogans* serovar Autumnalis showed an Optical Density of 1.7 indicating the purity of isolated DNA. The PCR amplification of *LipL32* gene revealed an amplicon of 790 bp on 1.0 % agarose gel (Fig-1). The sequence was deposited in the nucleotide GenBank under the accession no. JQ861883. The sequence when subjected to nucleotide BLAST search revealed a high

degree of similarity with other pathogenic serovars of *Leptospira* (Table-1). The *LipL32* gene is highly conserved in most of the pathogenic serovars of *Leptospira* as evidenced from the sequence analysis. The obtained sequences shown 100 % similarity with *LipL32* gene sequences of *Leptospira interrogans* serovar Autumnalis (EU526391) and *Leptospira interrogans* serovar Lai (AY568679) and 99% similarity with *Leptospira interrogans* serovars Balico (JQ013520), Grippytyphosa (AY609327), Australis (AY609325), Canicola (AY609321), Copenhageni (AE016823), hardjo (GU592525), Icterohaemorrhagiae (GU183106) and Hebdomadis (AY609328), *Leptospira noguchii* serovar Pomona (AY609326), *Leptospira borgpetersenii* serovar Mini (AY609333) and *Leptospira kirschneri* (AY461917). *LipL32* protein is the most abundant and an important surface protein present only in pathogenic *Leptospira* and found to play a major role in host immunogenic response and in infection [10]. The *LipL32* gene of *Leptospira interrogans* serovar Sejroe was found to be conserved in many pathogenic serovars [11] which is in agreement with the present study. Similar findings were reported by several other workers [12]. The utility of *LipL32* for diagnosis of leptospirosis in animals and humans has been documented widely [13-15].

Conclusion

The present study concludes that the similarity in the sequences of the cloned product with other pathogenic serovars, suggests its importance in the utility of *LipL32* gene as an important target for the diagnosis of leptospirosis in animals and humans as well as promising vaccine candidate as the protein is reported to be immunodominant.

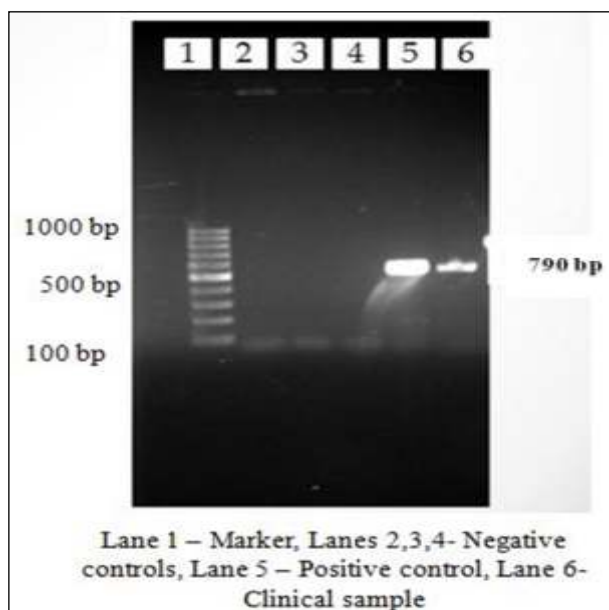


Figure-1. Amplification of LipL 32 gene by Polymerase chain reaction

Author's contribution

SVK, SJ and RA implemented the study design and carried out the work. All other authors were involved in scientific discussion and analysis of the data. All authors drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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