Molecular Cloning and Sequence Analysis of Outer Membrane Lipoprotein-Encoding Gene LipL32 of Leptospira Interrogans Vaccinal Serovars in Iran

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Background & Objectives: Leptospirosis is an emerging infectious disease and is considered to be the most widespread zoonotic disease in the world. The outer membrane proteins play a crucial role in the process of adaptation by facilitating interactions between bacterial cells and its host. One such outer membrane proteins, lipl32 has been observed to be highly immunodominant antigen during human leptospirosis. The LipL32 is the major leptospiral outer membrane lipoprotein expressed during leptospiral infections. Antigenic characterization of the members of the species Leptospira interrogans is a necessary step towards to understanding the interactions between leptospires and the immune system.The aim of this study was molecular cloning and sequence analysis of the gene encoding LipL32 of Leptospira interrogans vaccinal Serovars in Iran.

Methods: *Leptospira interrogans* vaccinal serovars canicola (RTCC2805) grippotyphosa (RTCC2808), serjoe hardjo(RTCC2821) were used in this study which obtained from the Leptospira Reference Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran. The bacteria were subcultured into the selective culture medium EMJH. The genomic DNA was extracted by standard Phenol-Chlorophorm methods. The specific primers for proliferation of lipl32 gene were designed. The lipl32 gene was amplified and cloned into a cloning vector plasmid and transformed in competent E. coli JM 107cells. Recombinant plasmid was isolated from cells by kit (Roche).

Results: PCR amplification of the lipl32 gene using the designed primers resulted in an 835bp lipl32 gene product. The amplified gene was cloned in pTZ57R/T vector and transformed into E.coli (JM107) cells. The confirmation of the recombinants was made by picking the white colonies and carrying out colony PCR amplification of the gene. The sequence was deposited in the Gen Bank database. The percentage identity and divergence among different leptospiral serovars was deduced using the Blast programme. In our study, nucleotide sequencing results showed that gene encoding LipL32 was highly conserved among various pathogenic Leptospira strains (>94% Identity)

Conclusion: In conclusion, the cloned gene could be further used for expression of recombinant protein for serodiagnosis and also can be a good candidate for recombinant vaccine against leptospirosis.

Keywords: Leptospirosis; Pathogenic Leptospires; Cloning; Lipl32 Gene