Characterization of the Recombinant Leptospiral Adhesin LIC10314 Identified by Proteomics Analysis in Virulent Strains.

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Leptospirosis is one of the most spread zoonosis worldwide, caused by spirochetes of the genus *Leptospira.* Preventive measures represent the best alternative to control the disease due to the difficulty to control the proliferation of rodents and to the absence of an efficient vaccine. Functional genomics strategies, such as proteomics, resulted in the identification of 134 novel proteins, many of them identified only in virulent strains and assigned as hypothetical proteins (Vieira et al., unpublished results). One of these proteins, LIC10314, identified in virulent *Leptospira* isolated from kidney of infected animals was selected for further studies. The gene LIC10314 was amplified from leptospiral genomic DNA and cloned into pAE, an *Escherichia coli* expression vector. The construction pAE-LIC10314 was transferred into *E. coli* BL21-SI competent bacteria and protein expression was achieved by addition of high salt concentration. The expected protein band of 63 kDa was observed in the soluble form, and the recombinant protein was purified by metal affinity chromatography. Purified rLIC10314 showed to be highly immunogenic in BALB/C mice, yielding titers higher than 1:80,000. Adhesion assays to ECM molecules showed that the rLIC10314 binds to laminin and collagen IV in a dose-dependent fashion. The LIC10314 protein proved to be located in the bacterial outer membrane as assessed by immunofluorescence technique. Our data suggest that LIC10314 is a novel adhesin of *Leptospira* with a possible role in the leptospiral pathogenesis.

Key words: Adhesin, Functional Genomics, Leptospira, Proteomics, Recombinant Protein

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