TITLE: DETERMINATION OF REGULON ZUR IN *L. INTERROGANS* SV. COPENHAGENI STR. A10

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ABSTRACT:
Leptospirosis is a zoonosis of worldwide relevance and one of its etiological agent is a bacterium called *Leptospira interrogans*. Definitive hosts are rodent mammals, cattle and dogs while humans are accidental or secondary hosts due to contamination usually related to sewage and flooding issues. There are several studies that try to elucidate the mechanisms of virulence of this bacterium and, still, little is known of the regulation and relevance of these factors during the infectious process. Metallic ions such as iron, for example, have already been described as relevant for the virulence of many pathogens. Zinc is one of the most important metal ion for bacteria due to their need for proteins (as cofactor and/or structurally). The zinc homeostasis in several bacteria is partially dependent of Zur (Zinc Uptake Regulator) proteins belonging to the family of transcriptional regulators named Fur (Ferric Uptake Regulator). In *L. interrogans* sv. Copenhageni str. L1-130 genome there are 4 Fur-family regulators annotated of which our analyzes suggest that LIC20147 paralog codes for the putative Zur regulator. Genomic analyzes and computational modeling trials have demonstrated that the Zur protein of *L. interrogans* resembles FurB (Zur) from *Mycobacterium tuberculosis* and, therefore, the Zur binding site from *M. tuberculosis* was used in an in silico array in order to find putative Zur binding sites in the *L. interrogans*. Some putative Zur-regulated regions were amplified by PCR with FAM-marked primers while the putative Zur encoding gene was cloned in pET28a, sequenced and used for expression of His-Zur recombinant protein. FAM-marked probes as well His-Zur purified were used in an EMSA, but preliminary results indicate that Hiz-Zur from *L. interrogans* do not bind to the putative Zur binding sites predicted. The purified protein is in use to obtain polyvalent serum in mouse which will be used in the future in a ChIP-Seq experiment for determination of the Zur regulon in *L. interrogans*. Growth curves were also conducted to determine the susceptibility of this bacterium to different regimes for zinc availability and metal deficiency (obtained by the use of EDDS and TPA chelators) measuring cultures absorbance at 420 nm. Our preliminary results indicate that *L. interrogans* tolerates other concentrations of zinc in addition to those predicted in the bloodstream, and has a Zur transcriptional regulator potentially involved in regulating metal uptake.

KEY WORDS: Leptospirosis, zinc, virulence, zur

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