

RECOMBINANT *SALMONELLA* AS CARRIER OF *LEPTOSPIRA INTERROGANS*  
SEROVAR COPENHAGENI GENES FOR *IN VIVO* EXPRESSION. FROM GENE  
AMPLIFICATION TO *IN VITRO* PROTEIN EXPRESSION ESSAY.

Fraga, T.R.<sup>1,2</sup>, Nakajima, E.<sup>1,2</sup>, Martins, E.A.L.<sup>1,2</sup>

<sup>1</sup> Centro de Biotecnologia, Instituto Butantan, São Paulo, Brasil

<sup>2</sup> Pós-Graduação Interunidades em Biotecnologia, USP, São Paulo, Brasil

Leptospirosis is a widespread zoonosis whose causative agents are pathogenic bacteria from the genus *Leptospira*. The genome of the *Leptospira interrogans* serovar Copenhageni was analysed by bioinformatic tools and several proteins possibly related to its pathogenesis were identified. Seven leptospiral putative proteins were selected to be studied: three hemolysins (LIC11352, LIC11040 and LIC10657), two lipoproteins (LIC10508 and LIC10509), an outer membrane protein (LIC10537) and an ankyrin like protein (LIC12033). The genes were cloned for live vaccines studies using attenuated salmonella expressing the heterologous antigens *in vivo*. The system has the advantages of direct immunization and bacterial adjuvant effect. The aim of this work was to obtain plasmidial constructions containing the selected genes for *in vitro* and *in vivo* expression of the recombinant proteins. The genes were amplified from genomic DNA of *L. interrogans* Copenhageni by PCR, inserted in the vectors pDEST17 (Invitrogen) or pGEMT-easy (Promega), and subcloned in the expression vector pAEsox. Protein expression was tested in *E. coli* BI21(DE3) Star pLysS, from which the proteins are being purified, and in *Salmonella enterica* serovar Typhimurium SL3261, a vaccinal strain. All seven proteins were successfully expressed *in vitro*. The immunogenic properties of the proteins and the salmonella vaccines will be tested in hamster model.

Financial support: FAPESP.