CLONING AND EXPRESSION OF PROTEINS IDENTIFIED IN THE GENOME OF *Leptospira interrogans* serovar *Copenhageni* FOR POTENCIAL VACCINE AND DIAGNOSTIC USE

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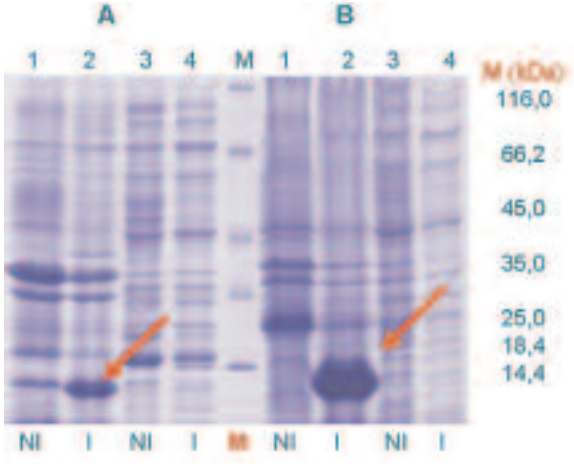
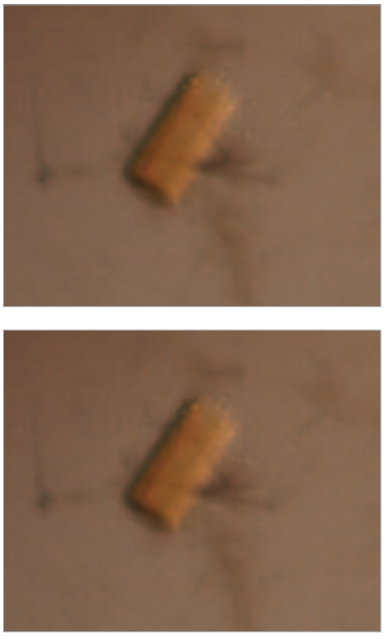


Purification of recombinant proteins. SDS-PAGE (15%) of purified recombinant proteins obtained by metal affinity chromatography. Proteins encoded by (1) LIC10009, (2) LIC10191, (3) LIC11947, (4) LIC12730, (5) LIC10494, (6) LIC12906

Leptospirosis is considered the most widespread zoonotic disease caused by the spirochaete from the *Leptospira* genus. In Brazil, 80% of all the diagnosed cases are due to *Leptospira interrogans* serovar *Copenhageni*. Rats are the main reservoir in urban centers and the bacteria are disseminated through animal urines. Around 10,000 cases of leptospirosis are annually notified to FUNASA (Health Ministry, Brazil), epidemically associated to the rainy seasons (http://www.funasa.gov.br). A promising way to treat this health problem would be a vaccination program, since the control of rats is a difficult task. Thus, the development of a suitable vaccine against leptospirosis is of seminal importance, since up to date, there is no licensed human vaccine against leptospirosis. Furthermore, the general clinical signs of this disease are often confounded with those of other diseases, like dengue and flu symptoms, reinforcing the need for an efficient diagnose assay for leptospirosis, not yet available in the market. Recently, the genome of the *Leptospira interrogans* serovar *Copenhageni* was described. The information of the genome would help to identify potential *Leptospira* vaccine antigen candidates or antigens for diagnose applications. The present project is divided in 3 subprojects, aiming: 1) cloning of 8 selected hemolysins. Recombinant proteins will be expressed in *E. coli* and further evaluated for their potential use as vaccine antigens and for potential use in diagnose. Biochemical and immunological characterization will also be performed; 2) cloning of 5 selected lipoproteins. Recombinant proteins will be expressed in *E. coli* and further evaluated for their potential use as vaccine antigens and for potential use in diagnose. Biochemical and immunological characterization will also be performed; 3) cloning and expression of 5 of the above antigens in *Salmonella thyphimurium*. The recombinant *Salmonella* will be evaluated as live vaccine against *Leptospira*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have accumulated data from dozens of potential antigens of *Leptospira*. Though we have initially proposed the study of 8 hemolysins and 10 lipoproteins, being 10 of them also expressed as live vaccine in *Salmonella thyphimurium*, we have expanded this universe. For instance, in the case of the subproject dealing with hemolysins, we started with 8 ORFs, but we ended up with 20 ORFs. All these were cloned and evaluated for expression in *E. coli*. This represents our first step in our experimental approach to uncover new antigens for vaccine or diagnostic kit development for leptospirosis. If these ORFs were expressed in *E. coli*, the study progress to step 2. If not, we try to express them using other *E. coli* strains, conditions. If they are still not expressed, we look for new ORFs to replace them. For those that we were able to express, the proteins are purified and antiserum is produced against each of them. These sera are immunocharacterized and used to screen a panel of different *Leptospira* serovars to define if they are conserved antigens among them. Those that are conserved, they are selected for the next steps, since we are searching for antigens that would compose a universal vaccine against all the *Leptospira* serovars but not a vaccine against a specific serovar. These ORFs are biologically characterized, for reactivity against patient sera as well as assayed as protective antigens in a hamster model of immunization and challenge with pathogenic virulent *Leptospira*. At least one antigen we presented in *Salmonella* was able to elicit partial protection in animal model. We were able to identify several conserved antigens among the serovars that would be useful for leptospira diagnosis, new adhesins that would mediate leptospira attachment to host cells, new antigens that mediate the inflammatory response observed during leptospira infection, besides other findings. We can anticipate that the project will at least increase our comprehension of this bacteria and the disease it causes that will allow us in the future to interfere with the progress of the disease.



Leptospira *antigen crystals for determining three-dimensional structures*

MAIN PUBLICATIONS

Hauk P, Negrotto S, Romero EC, Vasconcellos SA, et al. 2005. Expression and characterization of HlyX hemolysin from *Leptospira interrogans* serovar *Copenhageni*: Potentiation of hemolytic activity by LipL32. *Biochemical and Biophysical Research Communication*. **333**:1341-1347.

Barbosa AS, Abreu PA, Neves FO, Atzingen MV, et al. 2006. A newly identified leptospiral adhesin mediates attachment to laminin. *Infect Immun*. **74**: 6356-6364.

Nascimento ALTO. 2006. ?Leptospira interrogans: Genomics and "Immunomics" in Molecular Biology of Spirochetes (Cabello FC, Hulinska D, Godfrey HP, eds), **373**:115-123. *NATO Science Series: Life and Behavioural Sciences* ,416pp. IOSPress, ISBN: 1-58603-665-3.

Neves FO, Abreu PA, Vasconcellos SA, de Morais ZM, Romero EC, Nascimento ALTO. 2007. Identification of a novel potential antigen for early-phase serodiagnosis of leptospirosis. *Arch Microbiol*. **188**: 523-532.

Vieira ML, D'Atri LP, Schattner M, Habarta AM, et al. 2007. A novel leptospiral protein increases ICAM-1 and E-selectin expression in human umbilical vein endothelial cells. *FEMS Microbiol Lett*. **276**:172-180.

Hauk P, Macedo F, Romero EC, Vasconcellos SA, Morais ZM, Barbosa AS, Ho PL. 2008. LipL32, the Major Leptospiral Lipoprotein: the C-terminus is the Primary Immunogenic Domain and Mediates Interaction with Collagen IV and Plasma Fibronectin. *Infection and Immunity*, in press.

Atzingen MV, Barbosa AS, De Brito T, Vasconcellos SA, Morais ZM, Lima DCM, Abreu PAE, Nascimento ALTO. 2008. Lsa21, a novel leptospiral protein binding adhesive matrix molecules and present during human infection, submitted.

Gomez RM, Vieira ML, Schattner M, Malaver E, Watanabe MM, et al. 2008. Outer membrane proteins of Leptospira interrogans stimulate human umbilical vein endothelial cells (HUVECS) and express during infection, submitted.

Neves FO, Abreu PAE, Nascimento ALTO. 2006. Patent application pending. "Processo de clonagem, expressão e purificação de proteína de *Leptospira* sp para diagnostico", processo PI 0602669-9.

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