

TITLE: *Leptospira* PRODUCE POLY-N-ACETYL-GLUCOSAMINE (PNAG) IN BOTH BIOFILM AND PLANKTONIC PHENOTYPES

AUTHORS: VASCONCELOS, L.¹; ALMEIDA, E. M. N.¹; VERÍSSIMO, G.¹; FIGUEIRA, C.P.²; RISTOW, P.¹.

INSTITUTION: ¹UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR, BA (INSTITUTO DE BIOLOGIA, CAMPUS ONDINA, LABORATÓRIO DE BACTERIOLOGIA E SAÚDE, RUA BARÃO DE JEREMOABO, 668, 1º ANDAR, CEP 40170-115, SALVADOR - BA, BRASIL). *E-mail:* paularistow3@gmail.com. ²INSTITUTO DE PESQUISA GONÇALO MONIZ - FUNDAÇÃO OSWALDO CRUZ, SALVADOR, BA (RUA WALDEMAR FALCÃO, 121, CEP 40296-710, CANDEAL, SALVADOR-BA, BRASIL).

ABSTRACT:

The polysaccharide poly-N-acetylglucosamine (PNAG) integrates capsules and the biofilm matrix of many pathogenic bacterial species, contributing to cell adhesion and immune evasion. Despite this conserved capsular polysaccharide is expressed by more than 35 bacterial genera, PNAG production by *Leptospira* has not been studied yet. Pathogenic *Leptospira* cause leptospirosis, a zoonosis that affects more than 1,000,000 people each year and bears impact in the livestock industry throughout the world. *Leptospira* form biofilms and are able to evade the host immune system. The objective of this work was to investigate PNAG expression by *Leptospira*. We cultivated pathogenic *Leptospira interrogans* serovar Canicola (planktonic) and saprophytic *Leptospira biflexa* serovar Patoc (planktonic and biofilm) in EMJH medium, at 29°C. Samples were fixed by ice-cold methanol and incubated with anti-PNAG polyclonal antibodies, followed by incubation with ALEXA Fluor 488 anti-rabbit secondary antibody. We detected the presence of PNAG in planktonic cells (*L. biflexa* and *L. interrogans*) and biofilms (*L. biflexa*). Although leptospiral capsule has never been demonstrated experimentally before, previous transcriptomic analysis of *L. biflexa* showed upregulation of LEPBI_I2021 during mature biofilm, a gene whose product is a putative capsule polysaccharide biosynthesis protein. On the other hand, LEPBI_II0131, which encodes the capsular polysaccharide biosynthesis protein CapD, was expressed in both biofilm and planktonic *Leptospira* phenotypes. Our experimental data demonstrated that *Leptospira* presents PNAG in its surface and PNAG is expressed in both pathogenic and saprophytic bacteria, and in planktonic and biofilm phenotypes. Whole transcriptomic data evidenced that *Leptospira* have functional capsular genes.

Keywords: capsule, polysaccharide, biofilm matrix.

Development Agency: CNPq.