Title: INSIGHTS INTO BIOFILMS OF PATHOGENIC AND SAPROPHYTIC *LEPTOSPIRA* FROM DIFFERENTIAL PROTEIN EXPRESSION

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Abstract:

Leptospirosis is an infectious disease of public health impact, caused by pathogenic *Leptospira* spp.. *Leptospira* form biofilms, which may improve its survival in the environment and maintenance in host reservoirs. Molecular mechanisms of leptospiral biofilm are largely unknown. We investigated total protein expression of pathogenic *Leptospira interrogans* and saprophytic *Leptospira biflexa*, in two conditions: biofilm (*Biof)* and planktonic (*Plank*). For *L. biflexa*, we also compared protein expression in two time points: mature 48 h and late 120 h biofilms. Cells were grown in EMJH medium; *Biof* in glass tubes and *Plank* in polypropylene tubes under agitation. Analyses were done in 10% 1D SDS-PAGE, followed by densitometry. To predict candidates for differential expression, we jointly analysed proteomic data with previously obtained data from transcriptomic analysis of *L. biflexa* biofilms by RNA-Seq. For *L. interrogans*, protein bands with estimated molecular weight (MW) of 48, 103, 140 and 160 kDa were more expressed in *Biof* than *Plank*. Based on RNA-Seq, we hypothesized that these molecules could correspond to: (1) succinyl-CoA synthetase subunit, implicated in carbohydrate metabolism in biofilms of *Mycobacterium avium*; (2) hemin degradation protein HemS; (3) hemolysin putative signal peptide; (4) TonB-dependent iron receptor FecA; (5) putative TonB-dependent iron receptor. Iron uptake systems are often up regulated in biofilms, due to sessile lifestyle. For *L. biflexa Biof vs*. *Plank*, different expression was observed for proteins with MW of 28, 69 and 90 kDa. Based on RNA-Seq, we hypothesized that these molecules could correspond to: (1) OmpA-like protein, (2) apolipoprotein N-acyltransferase, (3) TonB-dependent iron receptor FecA. For *L. biflexa* mature *vs.* late biofilms, we found different expression for bands with estimated MW of 55, 60, 75, 139 and 187 kDa. Based on RNA-Seq, we hypothesized that these molecules could correspond to: (1) histidine kinases; (2) helicase; and (3) PNPase; all involved in biofilm regulation. Also, candidates could correspond to: (4) glycosyl transferase; (5) MdoG homolog; (6) alginate O-acetyltranseferase; which are all related to carbohydrate synthesis of the matrix. Our results suggest that genes for iron uptake, carbohydrate and lipoprotein metabolisms, adhesion and gene regulation are up-regulated in leptospiral biofilms. We expect this study to help to unravel the biology of this important bacterial pathogen.

Keywords: *Leptospira* biofilm, molecular mechanisms, proteome

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