TITLE: Study of virulence genes during biofilm formation in *Leptospira*

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Abstract:

Biofilms are the most common lifestyle of microorganisms. They protect bacteria against hostile environments. Biofilms are related with the chronicity of bacterial infections. Pathogenic leptospires cause leptospirosis, an important zoonosis. *Leptospira* form biofilms in natural environments, *in vivo* and *in vitro*. It is not known whether *Leptospira* remain virulent while forming biofilms. We aimed to investigate the role of virulence factors during *Leptospira* biofilm formation. For that, we performed a genomic and transcriptomic analysis using the model *L. biflexa*. We firstly performed a manual search for genes encoding virulence factors in *Leptospira* in NCBI PubMed. We then performed a BLASTp search of the virulence factors against *L. biflexa* genome (*e-value* < 1e-5 and identity > 40%). We analysed the transcriptional expression level of the genes in *L. biflexa* biofilm transcriptome dataset (BioProject PRJNA288909), comprised by the comparison between two phenotypes (biofilm and planktonic) and two growth periods (48 h and 120 h). We then performed domain analysis of the genes using InterProScan. We found 24 virulence factors in pathogenic *Leptospira*. From those, 17 presented homologs in *L. biflexa* genome. Four homologs presented differential expression in the transcriptome dataset. FcpA (flagellar sheath protein) was down regulated in the mature biofilm (48 h) in comparison with late stage (120 h), and up regulated in late biofilm comparing with late planktonic culture. HemO (heme oxygenase) was up regulated in mature biofilm in comparison with late biofilm. At 48 h, HemO was up regulated in biofilm comparing with planktonic cells. Loa22 (OmpA – like protein) was down regulated in late biofilm comparing with mature biofilm. FlaA2 (Flagellar filament sheath protein) was up regulated in mature biofilm comparing with late biofilm. Loa22 and its homolog LEPBI_I3149 (*L. biflexa*) share the same domain; HemO and its homolog (LEPBI_I0669) integrate the Haem oxygenase-like protein superfamily; FlaA2 and its homolog (LEPBI_I2336) integrate the flagellar filament outer layer protein family. Loa22 presents an OmpA domain, which is described to have a role in cell adhesion. It is well known that iron acquisition is essential for leptospiral growth and may have implications in biofilm formation. Flagella is important for biofilm formation in many bacteria. We believe this work will enlighten the possible roles of virulence factors in leptospiral biofilms.

Keywords: leptospirosis, virulence factors, bioinformatic analysis, transcriptome.

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