

In silico identification of transcriptional regulatory pathways in *Leptospira biflexa* biofilms

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Abstract

Bacteria of the genus *Leptospira* comprehend 65 genomic species including pathogenic, intermediate and saprophytic groups. Pathogenic leptospires are the etiologic agent of leptospirosis, a disease of public health and veterinary public health impacts worldwide. Biofilms improve survival of microorganisms in hostile environments and are related to various medical conditions. *Leptospira* form biofilms in vitro and in vivo. Nevertheless, the regulatory mechanisms of biofilm formation in *Leptospira* are poorly known. Saprophytic *Leptospira biflexa* shares several genetic and functional similarities with pathogenic species and can be used as a model to study biofilms. In this study, we aimed to identify transcriptional regulators involved in biofilm formation in *Leptospira biflexa* and to describe the regulatory pathways of these regulators. Firstly, we selected transcriptional regulators predicted for *Leptospira biflexa* in P2TF database. Secondly, we conducted a similarity search using Protein BLAST with those regulators against previous data from a *Leptospira biflexa* transcriptome analysis of biofilm versus planktonic cells, in two time points: 48 h (mature biofilm) and 120 h (late biofilm). Transcriptomic data is publicly available under BioProject accession number PRJNA288909. After identifying biofilm regulatory genes in *L. biflexa* transcriptomic data, we checked for their expression levels to understand if a particular regulator was contributing positively, negatively or being neutral in the context of biofilm regulation. Finally, we performed a functional annotation, in order to classify all the regulators found using COG database. In total, we predicted 138 transcriptional regulators for *L. biflexa*, comprising sigma factors, two-component systems, response regulators and other DNA-binding proteins. Among those, we identified 38 (27.5%) regulators as participating in the biofilm phenotype. From the results analyzed so far, we found that the sigma factor LEPBI_II0101 integrate a network alongside with other sigma factors, response regulators, RNA-polymerase subunits genes and two-component systems in all transcriptomic comparisons, leading us to infer that this regulator is important to sense environmental changes and modify expression. We also found that the alternative sigma factor FliA positively regulates motility and chemotaxis, and interacts with other flagellar proteins in the mature biofilm. Motility and chemotaxis are pointed to be important for biofilm formation in other species. Our results also evidenced the toxin-antitoxin system VapBC, which contributes with RNase activity in the late biofilm. Our work is novel in describing the regulatory mechanisms of *Leptospira* biofilm formation and will shed light on the intricate regulatory pathways of this phenotype.

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