TITLE: In *silico* PREDICTION OF REGULATORS OF GENETIC EXPRESSION IN *Leptospira* GENOMES IN THE CONTEXT OF BIOFILM FORMATION

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ABSTRACT:

Pathogenic *Leptospira* cause leptospirosis, a disease of public health impact. *Leptospira* form biofilms, which may improve the survival of microorganisms. The regulatory mechanisms of this phenotype are poorly known. We aimed to identify gene expression regulators involved in *Leptospira* biofilm formation. We manually searched in the literature for genes coding for regulators with proven biological role in bacterial biofilms. Amino acid sequences were obtained and compared with *Leptospira* genomes using Protein BLAST (NCBI database). We used Pfam and InterPro to identify regulatory domains. We checked for regulatory gene expression levels using previous data from *Leptospira biflexa* transcriptome of biofilm (BIOF) *versus* planktonic cells (PLANK) at 48 h (mature biofilm) and 120 h (late biofilm). We found 41 regulatory genes in prokaryotic biofilms. From those, 17 were *Leptospira* orthologs, comprising master and global regulators, sigma factors, transcription factors, post-transcriptional and quorum sensing regulators. Five genes presented transcriptional differences: BolA, CsrA, Pnp, ClpP and FliA. BolA, CsrA, Pnp and ClpP were widely conserved in all 22 *Leptospira* species. FliA was present in 18 *Leptospira* species. FliA and PnP were upregulated in BIOF when compared to PLANK in the mature biofilm, whereas BolA was upregulated in BIOF in relation to PLANK in late biofilm. FliA is a sigma factor for flagellar synthesis and chemotaxis. PnP is a negative regulator of poli-n-acetylglucosamine production, a component of matrix biofilms. The global regulator BolA negatively regulates motility and c-di-GMP synthesis. Our results suggest that FliA, PnP and BolA are regulators of biofilm formation in *Leptospira*. They also suggest that biofilm formation is regulated by c-di-GMP levels. CsrA and ClpP were downregulated in BIOF in relation to PLANK in late biofilm. The global carbon storage regulator CsrA is a post-transcriptional regulator important when nutrients are scarce. ClpP is involved in the biosynthesis of massetolides, which are required for biofilm formation. Those results indicate that during late biofilm stage, bacterial cells may tolerate nutrient scarcity more than planktonic cells. It also suggests that massetolides are not required in this phase. Although additional studies are necessary to build regulatory networks for biofilm formation in *Leptospira*, our study will pave the way for an initial comprehension of these regulatory mechanisms.

Keywords: transcription factor; transcriptional regulator; biofilm; regulation; leptospirosis

Development Agency: CNPq.