

SUB-ÁREA: Genética

Cyclic di-GMP signaling during *Leptospira biflexa* biofilm development

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Leptospire form biofilms in the renal tubules of *Rattus norvegicus* and integrate environmental biofilms in rural areas. This phenotype occurs in response to environmental cues mediated by signaling systems. Cyclic di-GMP (c-di-GMP) is the main second messenger involved in bacterial biofilm formation. However, signaling processes in *Leptospira* and c-di-GMP roles in leptospiral biofilm are poorly known. Thus, we aimed to identify metabolic and effector components of c-di-GMP signaling in the model saprobic species *Leptospira biflexa*. First, we constructed a manually curated database containing 10,955 c-di-GMP-related proteins in bacteria. Then, we performed similarity searches on *L. biflexa* serovar Patoc strain Patoc 1 (Paris) proteome (PRJNA20133, assembly GCA_000017685.1 ASM1768v1) using BLASTp. To predict the functionality of enzymatic (GGDEF, EAL and HD-GYP) and binding (PilZ and MshEN) c-di-GMP domains, we performed domain analyses using InterProScan and PFAM, followed by multiple sequence alignments analyses using Clustal Omega and Jalview. In this step, we used as references well-characterized proteins from other bacteria and *Leptospira interrogans* proteins with confirmed enzymatic activity. Finally, we used the public transcriptome dataset of *L. biflexa* biofilms (PRJNA288909) to analyze the expression profiles of c-di-GMP genes during biofilm development (False Discovery Rate <math><1e-2</math> and L. biflexa. We also identified nine effector proteins and predicted four proteins harboring PilZ domains as functional. All effector genes were transcribed in biofilms, including an YajQ protein-encoding gene. Pnp, a c-di-GMP effector with an unknown binding structure, was more expressed in biofilms at the mature state. Based on these findings, we conclude that *Leptospira biflexa* presents the genetic components that are necessary to a c-di-GMP signaling circuit. Furthermore, the expression of c-di-GMP-related genes suggests that c-di-GMP modulates biofilm formation in *L. biflexa*.

Keywords: leptospirosis, signal transduction, second messenger, diguanylate cyclases, phosphodiesterases, effector proteins,

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