

TITLE: C-DI-GMP SIGNALING DURING BIOFILM DEVELOPMENT IN *Leptospira biflexa*

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ABSTRACT:

Leptospirosis is a zoonosis that affects more than 1,000.000 people annually. *Leptospira* form biofilms, which may contribute to environmental survival and host colonization. Biofilm formation in Bacteria is commonly mediated by *Quorum sensing* (QS) and two-component systems (TCS). C-di-GMP is an important second messenger involved in biofilm formation in Bacteria. C-di-GMP has a role in leptospiral virulence, but its participation in biofilm development is poorly known. We aimed to identify c-di-GMP-related genes in *Leptospira biflexa* genome and their expression profile during biofilm formation. We constructed a database with orthologs involved in c-di-GMP signaling during biofilm formation in Bacteria. We performed BLASTP using *L. biflexa* serovar Patoc str. Patoc 1 (Paris) genome (e-value $\leq 10^{-5}$, identity $\geq 55\%$). We performed domain analysis using InterProScan and analysis of transcriptional profiles using the transcriptome dataset of *L. biflexa* (BIOPROJECT PRJNA288909). To determine if the c-di-GMP-binding genes are potentially functional, we searched for conserved domains (GGDEF, EAL, HD-GYP, PilZ, MshEN). Since some genes found were histidine kinases (HK) or response regulators (RR), we searched for complementary HKs or RRs, using MaGe. We found 42 c-di-GMP-related genes. From them, 14 had differential expression, including a TetR encoding gene, which was up regulated in late biofilms (120 h). Eight genes presented shifts in their regulation status between mature (48 h) and late (120 h) biofilms. They encode: chemotaxis methyltransferase CheR, stress response kinase A, GGDEF/response regulator protein, two GGDEF proteins, sigma factor FliA, two-component response regulator and polyribonucleotide nucleotidyltransferase Pnp. Among the c-di-GMP-binding proteins, five diguanylate cyclases, three phosphodiesterases and one PilZ protein were considered functional. TetR proteins are involved in QS, which in *Vibrio cholerae*, regulates biofilm formation inhibiting c-di-GMP signaling. The eight genes with regulation profile shift indicate that those genes are involved in phenotypic and physiological aspects of 48 h and 120 h biofilms of *L. biflexa*. Our results suggest that an integration of two different signaling circuits, QS and c-di-GMP signaling, may occur. We conclude that c-di-GMP signaling occurs during biofilm development in *L. biflexa*. Our study indicates potential targets for future functional studies.

Keywords: biofilm, c-di-GMP signaling, leptospirosis, bioinformatic analysis

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