

**Title: ANALYSIS OF COOPERATIVE BEHAVIOUR IN BIOFILMS OF *LEPTOSPIRA* SPP. BY *IN SILICO* APPROACH**

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**Abstract:**

Leptospirosis is an endemic zoonosis in developing countries. It has medical and veterinary importance and is caused by *Leptospira* spirochetes. We demonstrated that *Leptospira* form biofilms *in vivo* and *in vitro*. Biofilms are agglomerates of microorganisms adhered to a surface involved by extracellular matrix. They promote bacterial protection against environmental factors and host immune response. Biofilm formation involves cooperative behaviors, including biological altruism. Bacterial altruism is characterized by fitness reduction in favor of a population. Bacterial programmed cell death (PCD) and autolysis may be altruistic processes and result in DNA release, contributing to biofilm architecture. In addition, the synthesis of toxins may be related to altruistic processes. The objective of this study was to identify biofilm cooperative and altruistic genotypes in *Leptospira*. We have searched for genes related to cooperation and altruism (autolysins, proteases, cell division genes, toxins, and others) in prokaryotic biofilms, using the databases: PubMed NCBI; Magnifying Genomes, Kegg Orthology and UniProt. To search for orthologs, gene sequences were compared by Blast with 191 genomes of *Leptospira* spp.. A total of 25 genes were found. From those, 17 exhibited orthologs in the genomes of pathogenic, intermediate and saprophytic *Leptospira* spp.. Genes were grouped by function: ten genes coding cell division proteins (DnaA, DNA polimerase III subunit, dnaX1, dnaX2, two ParB, GidA, GidB, Rec F, GyrB1); three coding for cell growth control proteins (HepA, Fis, pyrrole-quinolone quinone); two orthologs coding for MazE/F (VagC and hypothetical protein), which regulate PCD under stress; an ortholog coding for BfmR, involved in cell protection from phage mediated lysis; and RpoS Sigma factor, which is induced in response to multiple stress conditions. We have found that orthologs involved in bacterial biofilm formation were also identified in *Leptospira* genomes, indicating that those genes may contribute to the phenotype. Among the orthologs, it called our attention that the majority was related to cell division. Cells indeed can diminish their growth rate benefiting the development of the population in the biofilm. Understanding altruistic mechanisms in biofilms may help to unravel molecular mechanisms of this important phenotype. Additionally, this study will contribute to the understanding of pathogenicity and biology of *Leptospira*.

**Keywords:** leptospirosis, biofilm, cooperative behavior, altruistic genes.

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