TITLE: GROWTH SHIFT OF *Leptospira biflexa* DURING BIOFILM FORMATION

AUTHORS: PAULA RISTOW; PRISCYLA RIBEIRO; ELDER MÜLLER; TÂNIA SILVA.

INSTITUTION: UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR, BA (INSTITUTO DE BIOLOGIA, CAMPUS ONDINA, LABORATÓRIO DE BACTERIOLOGIA E SAÚDE, RUA BARÃO DE JEREMOABO, 668, 1º ANDAR, CEP 40170-115, SALVADOR - BA, BRASIL). *E-mail*: paularistow3@gmail.com

ABSTRACT:

Leptospirosis is a worldwide important zoonosis caused by pathogenic *Leptospira*. Those bacteria form environmental mixed biofilms, which may increase survival in hostile conditions. Bacteria in biofilm present metabolic changes to adapt to a sessile social environment. However, there is very poor information about leptospiral biology or metabolism when forming biofilms. We used the saprophyte *Leptospira biflexa* as a model to study the growth metabolism during biofilm formation. We cultivated *L. biflexa* biofilms (BIOF) in glass tubes in static condition and planktonic cells (PLANK) in plastic tubes under agitation, both at 29º C. We counted and measured leptospires every 12 hours, during five days. We analyzed bacterial growth in the following conditions: biofilm with no exchange of culture medium (BIOFnMex); biofilm with medium exchange (BIOFMex); and planktonic with no medium exchange (PLANK). We analyzed the expression of *L. biflexa* growth genes during biofilm formation by accessing the available transcriptome data (BioProject accession number PRJNA288909). Both biofilm and planktonic cells presented classic growth curves. The average generation time (GT) for BIOFnMex (5.6 h) and PLANK (6.43) was not statistically different. Despite of the GT similarity, cell population density in BIOFnMex was lower (maximum ~10^8 cells/mL) when compared with PLANK (maximum ~10^9 cells/mL). Likewise, the average growth was significatively lower in BIOFnMex (5.0E+7 cell/mL), compared to PLANK (4.8E+8). Additionally, we observed a shorter exponential phase for BIOFnMex (12 to 48 h) in comparison to PLANK (12 to 60 h). Those results show that leptospiral biofilm presents a growth diminution, demonstrated by both cell density and duration of exponential phase. During lag and exponential phases we did not observe statistical differences in cell size for BIOF and PLANK. When we exchanged the culture medium in BIOFMex, cell density and size were equal to BIOFnMex, suggesting that growth differences are not related to nutrient availability. Genes related to bacterial growth (*ftsA2*) and DNA replication (*parA*, *parB*, *gidA*, *gidB*, *dnaK*) were downregulated in leptospiral BIOF when compared to PLANK. Collectively, our results suggest that leptospires present metabolic shifts when forming biofilms, with growth diminution traits and downregulation of growth genes, indicating a quiescent behavior.

Keywords: Growth curve, metabolism, quiescence.

Development Agency: CNPq and FAPESB.