TITLE: *LEPTOSPIRA BIFLEXA* BIOFILM: GROWTH CHARACTERIZATION

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ABSTRACT:

Biofilm is the most common lifestyle for microorganisms in nature. In this sessile way of living, bacteria gain protection against noxious agents and hostile environments. Leptospires, the causative agent of leptospirosis, form biofilms *in vitro* and in colonized kidneys of rat reservoirs. Leptospires are spirochetes with fastidious growth. The *in vitro* generation time (GT) of planktonic cells is typically four hours for *Leptospira biflexa* (non-pathogenic) and eight hours for *Leptospira interrogans* (pathogenic). Although the GT is for long known for planktonic leptospires, in biofilms both the growth characteristics and the GT have never been studied. The goal of this work was to characterize the growth of *L. biflexa* in biofilm and identify the GT in this phenotype. For that, we cultivated leptospiral biofilms in glass tubes in static condition, and planktonic cells in plastic tubes under agitation, both at 29º C. We used Petroff-Hausser chamber to count bacteria during the following 30 days. Experiments were done in triplicate and data was further analysed. Three phenotypes were analysed: BIOF (biofilm) - leptospiral cells adhered to the glass tube wall; PLANK (planktonic) - leptospiral cells collected from the plastic tubes; SN (supernatant) - free cells collected from the culture media of the glass tubes (i.e., cells that were not adhered to glass). We observed classic growth curves for PLANK, with the phases *lag* with nearly 12 hours, exponential reaching 10E9 within 144 hours and GT equal to sex hours, and decline. SN cells had a similar growth curve pattern when compared to PLANK, with similar GT. However, it only reached 10E9 cells with 360 hours. Moreover, within 12 hours of cultivation, we observed a decrease in number of SN, which could be a result of planktonic cells adhering to the glass support, forming biofilm first layer. Growth curve of BIOF presented a similar shape to PLANK and SN. Nevertheless, it presented ten times less in number of organisms when compared to the other phenotypes. Furthermore, BIOF growth was faster during the first hours, reaching a maximum of 2,0E7 cells within 96 hours and GT equal to five hours, after what growth started to decrease. This result may be interpreted as a decrease in the expression of growth genes as HepA and Fis during *in vitro* biofilm formation of *L. biflexa*. We believe this work will contribute with the understanding of leptospiral biology and its growth under different phenotypes, as biofilms.

Keywords: Growth curve, *Leptospira biflexa*, biofilm

Development Agency: FAPESB