

TITLE: EVALUATION OF BIOFILM FORMATION ABILITY IN DIFFERENT *PARACOCCIDIOIDES* STRAINS

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ABSTRACT:

The *Paracoccidioides* complex involves different phylogenetic species that cause severe systemic mycosis, the paracoccidioidomycosis (PCM). This fungal disease has a wide spectrum of clinical presentations that affect different countries in Latin America, especially Brazil. Recently, the ability of *Paracoccidioides brasiliensis* to form biofilms has been described. This study aimed to evaluate if strains of different species of the *Paracoccidioides* complex have the capacity to form biofilms. The strains of *P. brasiliensis* (Pb18), *P. lutzii* (Pb01 and 8334) and *P. restrepiensis* (339 and 192) were studied for biofilm formation and their characteristics. Firstly, the strains were reactivated in macrophages (RAW 264.7) and maintained in solid Fava-Netto medium at 37°C. The biofilms were formed in 96-wells microplates, the inoculum adjusted to 10⁶ cells/ml and the plates were incubated for 24 h to 144 h. The biomass was quantified by the crystal violet method, the extracellular matrix by the safranin method, while the metabolic activity of the cells was measured by the tetrazolium salt reduction test (XTT). Additionally, confocal scanning laser microscopy (CLSM) was used to visualize the biofilm structure through the fluorescent markers FITC (fungal cell), Hoescht (nucleus) and SYPRO Ruby (protein content). Data were analyzed using the GraphPad Prism Version 5.0 for Windows software (GraphPad Software, Inc., La Jolla, CA, USA). A value of $p \leq 0.05$ was considered significant. Although the different species of the *Paracoccidioides* complex have been studied on different aspects, it is not known if all can form biofilm. Our results showed that most of the studied strains of different species were able to form biofilms in vitro up to 144 h with few differences. There are studies that demonstrate the difference between *P. brasiliensis* and *P. lutzii* species in relation to virulence, antigen production and susceptibility to antifungal drugs. Interestingly, our results showed that there was a greater amount of extracellular matrix in Pb01 when compared to Pb 18. All *Paracoccidioides* strains used in this study were able to form biofilm in an abiotic surface with some differences related to features of each strain.

Keywords: biofilms, matrix, *Paracoccidioides*.

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