

Influence of *P. brasiliensis* 14-3-3 protein in biofilm formation by clinical and silenced strains.

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*Paracoccidioides* spp, thermodynamically pathogenic fungi, are the etiological agents of paracoccidioidomycosis (PCM). The disease is endemic in Latin America, with a high incidence in Brazil. The pathogen has several mechanisms that allow its survival in the host cell environment, using several factors of virulence. This general interaction is mediated by binding events between adhesins on the fungal surface and receptors in the extracellular matrix of the host or on the cell surface. The objective of this study was to evaluate the participation of the 14-3-3 protein belonging to a family of highly conserved regulatory molecules that are expressed in all eukaryotic cells and are involved in several cellular functions, virulence and consequently in the formation of biofilms of *Paracoccidioides brasiliensis*. MATERIAL AND METHODS A *P. brasiliensis* strain called Pb18 14-3-3a-RNA, silenced, generated from genetic transformation and a clinical strain of *P. brasiliensis* Pb18 was used. Through the XTT method, a differential metabolic activity was determined between the isolates and also the biomass and a comparative extracellular matrix were also quantified. Finally, the differential expression of some genes involved in the virulence of *P. brasiliensis* was analyzed, comparing the expression related to planktonic and biofilm forms. RESULTS It was able to correlate a value of 14-3-3 in the biofilm formation of *P. brasiliensis* and how the lack of its expression affects the expression of other genes involved in the virulence of *P. brasiliensis*. The results suggest that the decrease of 14-3-3 protein may influence the formation of *P. brasiliensis* biofilm in addition to altering a generic expression of other adhesions involved in the pathogenesis. Our results show that a metabolic activity in the biofilm formation of the silenced strain was higher, indicating that possibly the fungus has a compensatory mechanism. CONCLUSION The results suggest that the decrease of 14-3-3 protein has an influence on the formation of the *P. brasiliensis* biofilm, since the metabolic activity of the silenced strain was higher, whereas an amount of extracellular matrix was very low, when compared to the clinical strain. In addition, a gene expression of other adhesins involved in the pathogenesis was, for the most part, decreased, except for enolase and aspartyl protease genes. A feature of this protein in virulence and biofilm formation is also important for novel markers in cell-host interaction, since a comparative expression among isolates will identify important targets for this interaction.

Keywords: Biofilm, *Paracoccidioides brasiliensis*, virulence.