

TITLE: CHARACTERIZATION OF THE SRBA TRANSCRIPTION FACTOR IN *PARACOCIDIROIDES* SPP.

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ABSTRACT:

Paracoccidioidomycosis is an endemic systemic mycosis in Latin America, inflicted by the thermomorphogenic fungi *Paracoccidioides* spp. These fungi face low oxygen concentrations in its natural ecological niche as well inside the hosts tissue during infection. When encountering low oxygen levels, metabolic adaptations must occur and are necessary for the fungi survival. SrbA is a transcription factor that induces the expression of genes allowing adaptation in hypoxic environments. Previous researches have shown SrbA involvement on virulence and survival in several fungi, participating in sterol production, heme synthesis and iron uptake. Given this transcription factor importance, our study objective is to characterize SrbA and its importance on metabolic responses to hypoxia, in fungi from the genus *Paracoccidioides*. The alignment of amino acid sequences have shown a high conservation level at the DNA binding domain (basic helix-loop-helix/bHLH) between the species *P. brasiliensis* (strains 03 and 18), and *P. lutzii* (strain 01). This domain is important for protein dimerization and activity, since proteins without the basic domain adjacent the HLH act as negative regulators. Primers for heterologous expression, for gene silencing and for quantitative analysis of the SrbA through RT-qPCR were synthesized. The plasmid pGEX-4T-1::*SrbA* were constructed and expressed in *Escherichia coli* cells. The plasmid construction was confirmed by DNA sequencing. Heterologous proteins expression and polyclonal antibodies production are under progress. In addition, *srbA* knockdown strains are under progress by antisense-RNA (aRNA) strategy. The plasmid pCR35::*PbsrbA*-aRNA was constructed and confirmed by DNA sequencing. In order to verify expression levels of *PbfglA* transcripts in low-oxygen conditions, RT-qPCR assays are under progress.

Keywords: hypoxia, *Paracoccidioides*, SrbA, RT-qPCR, gene silencing

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