**TITLE:** UNMASKING THE NEW HAEM-PROTEIN RELATED TO $O_2$-LIMITATION RESPONSE IN *PARACOCCIDIOIDES* SPECIES.

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## ABSTRACT

Fungi of the genus *Paracoccidioides* face $O_2$-limitation in its natural ecological niche, as well in host infection. To survive in this environment, metabolic and respiratory adaptations are necessary, and key proteins play an important role in this response. Fungoglobin (FglA) contains a haem-group, and was revealed in *Aspergillus fumigatus* as $O_2$ sensor being involved in cell growth during hypoxia stress. The protein role in *Paracoccidioides* spp. is unknown; thus, we aimed to characterize the homologous to FglA in *Paracoccidioides* and check its importance in $O_2$-limited adaptation. *In silico* analysis of *fglA* ortholog of *Paracoccidioides*, reveals the presence of the *fglA* in *P. brasiliensis* (*Pb*03 and *Pb*18), and although non annotated in *P. lutzii* (*Pb*01) genome, the gene was obtained by PCR amplification and DNA sequencing. In addition, the levels of *PbfglA* transcripts in low-oxygen was assayed. Yeast cells of the *Pb*01 and *Pb*18 were incubated *in vitro* upon normoxia (~21% $O_2$) and hypoxia (1% $O_2$, 5% $CO_2$, 94% $N_2$) in a Multi-Gas Incubator. After RNA extraction and cDNA production, the relative expression level of the transcript encoding *PbfglA* was determined by RT-qPCR. As result, the fungus increases the levels of *PbfglA* after 1h upon hypoxia in comparison to normoxia, in both strains. Thus, *Paracoccidioides* spp. presents a similar transcriptional response when compared to *A. fumigatus*. To perform the protein functional characterization, assays of heterologous expression and knockdown mutant construct are under progress. The plasmid pGEX-4T-1::*PbfglA* was obtained and the recombinant FglA (rFglA) was confirmed by mass spectrometry. Antisense-RNA (aRNA) strategy mediated *Agrobacterium tumefaciens* transformation, is used to obtain knockdown mutants; the plasmid pCR35::*PbfglA*-aRNA was constructed and cloning was confirmed by DNA sequencing. Pull-down assays with the rFglA, as well as sub-cloning in a parenteral vector pUR5750 for the knockdown mutant experiments, are in progress. In conclusion, it is evident that the FglA is involved in the *Paracoccidioides* response during hypoxia, although its role needs to be better elucidated.

**Keywords:** fungoglobin, hypoxia, stress response, gene silencing

**Development agency:** INCT-IPH, CNPq, CAPES and FAPEG.