TITLE: Siderophore biosynthesis pathway in the *Paracoccidioides* genus

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ABSTRACT:
Iron is an essential nutrient for all eukaryotes and prokaryotes. However, iron excess or the incorrect storage of this metal within the cell is deleterious due the production of reactive oxygen species. The control of iron homeostasis is of great importance in host-pathogen interaction, as both compete for this essential micronutrient. Since iron is essential for the success of the infection, fungi have developed high affinity uptake mechanisms for this metal to deal with the nutritional immunity in the host. Siderophore production is one of the mechanisms that fungal pathogens use for acquisition of iron. These low molecular weight compounds provide iron to the cell via solubilization of extracellular Fe^{+3}. Proteomic analyzes showed that metabolic status in *Paracoccidioides* is changed under iron deprivation conditions. Furthermore, the expression of siderophore biosynthesis and transport genes are induced in iron limited conditions. The objective of this proposal is the functional study of the siderophore biosynthetic pathway in *Paracoccidioides* by antisense RNA technology, recombinant protein production and their relation to virulence. Initially, genome of three *Paracoccidioides* strains were analyzed in searching for conserved regions in *sidA* and *sit1* genes. These regions were used as the basis for the generation of antisense fragments. Molecular cloning experiments were performed to construct the cassette containing the antisense fragments of interest in pCR35 plasmid. Cloning was confirmed by sequencing and positive samples were then used for cloning in pUR5750 binary vector. In parallel, *sidA*, *sidH* and *sidI* genes were cloned into the pGEX4T3 vector and SidA, SidH and SidI recombinant proteins were obtained by heterologous expression. The expression of *sidA* and *sidH* genes was induced with 1mM IPTG for 4 hours at 37ºC. Confirmation of protein identity was achieved by NanoUPLC-MS^E. The induced proteins were used for polyclonal antibody production in mouse. After that, *Western blotting* was performed with bacterial protein extract after induction. We observed that the antibodies were specific for each protein. Although siderophore production has already been reported in *Paracoccidiodies* genus, functional studies of this pathway are still unavailable. Therefore, RNA silencing technology coupled to antibody availability will allow a deeper investigation of the role of iron during the host-pathogen interaction.

Keywords: iron, heterologous expression, gene silencing
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