TITLE: FUNCTIONAL CHARACTERIZATION OF *arfB*, AN ADP-RIBOSYLATION FACTOR DOMAIN - ENCODING PROTEIN IN THE PATHOGENIC FILAMENTOUS FUNGI *Aspergillus fumigatus*

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ABSTRACT:

Aspergillus fumigatus is an opportunistic pathogenic fungus of the respiratory system that affects immunocompromised individuals. The inhalation of asexual conidia causes aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis. ADP-Rybosilation factor (ARF) are GTP-binding proteins which in turn belong to the Ras superfamily in eukaryotes. ARF proteins are assisted by other proteins in order to switch between binding to GTP and GDP. ARF-GTPase activating proteins (GAPs) force ARF to hydrolyze bound GTP to GDP, and Guanine nucleotide exchange factors force ARF to adopt a new GTP molecule in place of a bound GDP. With important functions, ARFs recruit coated proteins that promote vesicle screening, recruitment and activation of enzymes that will act to alter the lipid composition of the membrane. Three myristoilated members of the ARF family have been identified, *arfA, arfA* and *arlA* n *A. fumigatus. gcsA* was additionally identified as an ARF-GAP encoding protein. In this study we performed the functional characterization of *arfB* and its relationship with *gcsA*. In this regard, a deletion was constructed with the fusion of *arfB* 5' Untranslated Region (UTR) fragment, auxotrophic gene *pyrG* and *arfB* 3'UTR fragment. After, the deletion cassette (*arfB 5'UTR::pyrG::arfB 3'UTR*) was transformed into *A. fumigatus* protoplasts and the deleted strains were tested regarding morphology (macro and micro) and drugs sensitivity. The macromorphological aspects of Δ*arfB* and Δ*gcsA* are similar to the parental strain. The micromorphological analysis demonstrated greater production of conidia by Δ*arfB* strain. The double mutant strain has shown depleted development of the conidiophores (specialized hypha upon which conidia develop). Δ*gcsA*, Δ*arfB* and the double mutant strain Δ*gcsA* Δ*arfB* are sensitive to congo red (100 mg/ml), miriocin (20 µg/ml) and 5-flocitosine (1,5 ng/ml). These drugs affect, respectively, cell wall, sphingolipids synthesis and DNA synthesis. The expression of *arfB* and *gcsA* are up regulated at the presence of myriocin (30 µg/ml). Thus, our results indicate that the ARF is required for cell wall, sphingolipids and DNA integrity which can lead to depletion on *A. fumigatus* asexual development. Greater changes in phenotypes due de absence of *arfB* and *gcsA* are not observed possibly due to the overlapping activity of the other ARFs, as *arfA* and *arlA*.

Keywords: *Aspergillus fumigatus*, gene deletion, ADP- ribosylation factors

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