Study of the hypothetical protein codifying gene Afu6g07200 of *Aspergillus fumigatus*: First granulin activity in fungi?

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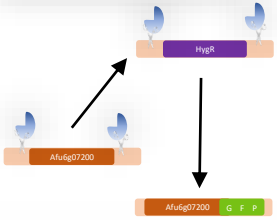
INTRODUCTION

Germination is the first adaptive process that the fungus experiences to colonize the environment or infect animals. *Aspergillus fumigatus* germination process involves a first step known as swollen. This study focuses on the transcriptomic analysis of the genes differentially expressed between swelling conidia and early hyphae output to find new molecular targets for diagnosis or treatment. Between the most overexpressed genes, we selected the Afu6g07200 gene that codifies a hypothetical protein with a domain that has 95.4% homology in 3D structure to human Granulin A. In humans, this secreted protein is implicated in development, inflammation, cell proliferation, and protein homeostasis. In addition, it plays an important role in neuronal development as it is involved in the polarization of neurons, but its mode of action has not been elucidated yet.

OBJECTIVE

Study the implication of Afu6g07200 gene in *A. fumigatus* biology and the similarities with human granulin

Mutation strategy



Gene edition. The *A. fumigatus* reference strain *ΔakuBku80* was used as genetic background wild type strain (WT) to generate mutants using the CRISPR-Cas9 gene-editing technique. A null mutant (*Δ72*), its complemented, and GFP-fused mutant strains were generated and validated by PCR and sequencing.

CONCLUSIONS

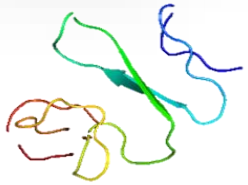
The results obtained in this work indicate that the gene Afu6g07200 has an important role in hyphal polarization, morphogenesis and cell wall building. This could have similar functions as human granulin although more studies are needed to confirm this hypothesis.

RESULTS

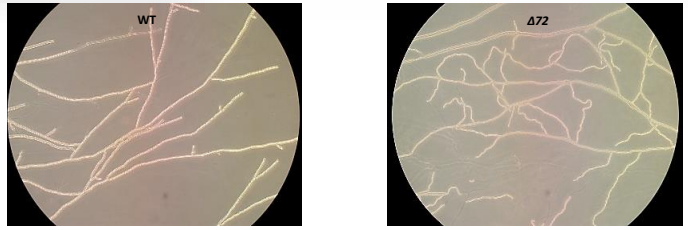
Protein structure and homology

Protein structure and domains. The N-terminal of the protein consists of a signal peptide that would target the protein to the secretory pathway and the transmembrane region would localize the protein in the outer membrane and/or organelles. There is a granulin-like domain characterized by twelve conserved cysteines in the external part of the protein.

3D homology. The protein sequence was analyzed with Phyre 2 software. The confidence between the 7 human granulins and zebrafish granulin and 93 Amino acids of Afu6g07200 gene codifying protein reached 95.4%. i.e. there is a 95.4% probability that these domains will fold the same. This could indicate that they also share function since it has been shown that the protein functionality depends on its hairpin structure conferred by the conserved cysteines.

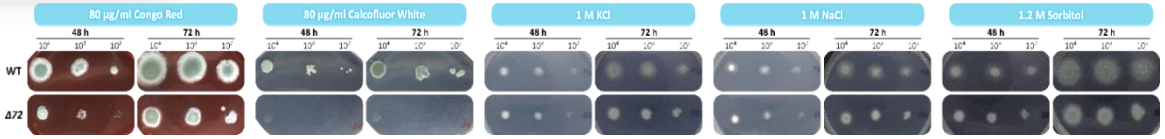


Microscopic visualization



The visualization of microscopic growth (x400) was done with the same plates as radial growth. The deletion strain displayed a curved phenotype. In the WT strain, the branch angle was 45° while mutant presented angles from 45° to over 90°. This phenotype is characteristic of mutants affected in the microtubule system such as the microtubule polymerase AlpA, the kinesin KipA and the cell-end markers TeaA and TeaR.

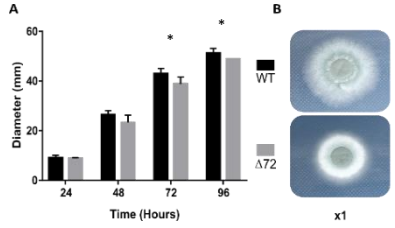
Stress characterization



Stress resistance analysis. The characterization of the mutant strain was performed seeding a 5 µl drop that contained 10^4, 10^3 or 10^2 conidia per plate. On the one hand, 80 µg/ml of Congo red or Calcofluor White were used as cell wall stressors. On the other hand, osmotic resistance to 1 M of KCl or NaCl and 1.2 M of Sorbitol was determined. As we can see in the above images mutant strain has remarkably more sensitivity than WT to all stresses assayed indicating the importance of the gene in cell wall integrity and osmotic balance.

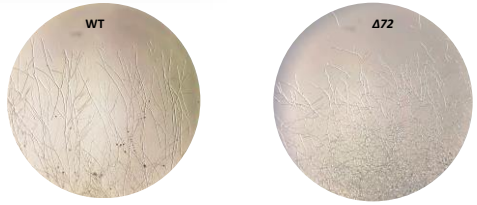
Radial growth

Radial growth. The analysis was performed using 10^5 conidia per plate of Glucose Minimal Medium (GMM) agar. Colony diameter was measured in two directions and all assays were performed at least in triplicate. **A)** Graphic representation of the colony diameter at different time points. **B)** Colony growth at 96 hours.



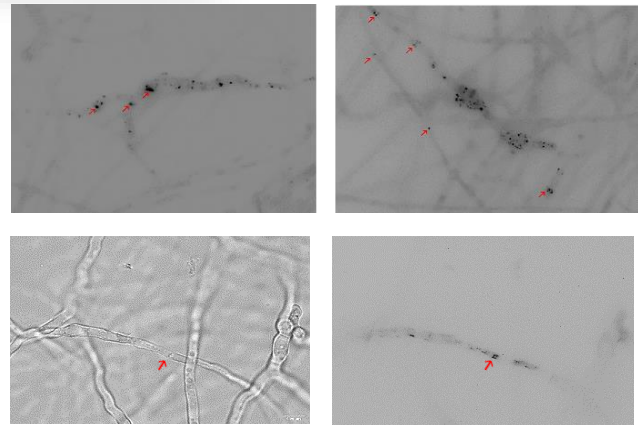
We had significant differences in the diameter of the colonies after 72 hours of growth. The deletion strain had a smaller conidiation surface, higher hyphal density and undefined edge. *p<0,05

Colony appearance



The same plates used for radial growth were observed with x40 magnification. The strain *Δ72* was found to branch more and at anomalous angles causing many apices to grow back to the center of the colony.

Protein localization



The GFP-fused mutant strain was incubated in RPMI for 16 hours and visualized with Eclipse Ni fluorescence microscopy. Images were processed with ImageJ, converted to grayscale and color inverted. **A)** The fluorescence was observed near the outer cell membrane, concentrating especially near the septa (Red arrow) or moving within the cytoplasm. **B)** In some cases a double ring near the septa was observed. This co-localized with septins double rings characteristic of new-formed septa.