CRISPR-Cas9 mutation and characterization of the most overexpressed transcription factor of swollen state of Aspergillus fumigatus

INTRODUCTION

Aspergillus fumigatus is the main pathogenic filamentous fungus in the world. Its small conidia are excellently dispersed in the environment and they can reach even the human respiratory tract. The germination is the first adaptive process that the fungus must undergo to colonize the environment or infect animals. At these points conidia must transform the minimum metabolism of the dormant state to an active growth of the hyphae in order to survive all external stresses. The transcription factors are in charge of directing the complex metabolism that this implies. The Afu3g08050 gene was the most overexpressed transcription factor in swollen state and it was selected to carry out a disruption mutant for phenotypical characterization.

RESULTS

Disruption mutant PCR validation

The characterization of the mutant strain and its response to cell membrane and cell wall stress compounds, were made using the same number of conidia per plate, in Glucose Minimal Medium (GMM) agar plates supplemented with 0.5% SDS or 0.0125% of Calcofluor White (CFW) or 80 µg/ml of Congo Red (CR) respectively. As we can see in the images the Afu3g08050 mutant is very sensitive to cell wall stress assays. The transcription factor maybe is implicated in cell wall and membrane integrity.

Radial growth

The pH sensitivity analysis was performed using the same number of conidia per plate of Glucose Minimal Medium (GMM) agar at its standard pH (6.5), acid pH (5) and alkaline conditions (8). Colony diameter was measured in two directions and all assays were performed at least in triplicate. As we can see in the images the mutant strain grew worse in all conditions and most notably in alkaline. Condensation was also altered in the disruption mutant due to the conidia formation occurred later in the mutant than in WT.

Osmotic stress characterization

The osmotic characterization of the mutant strain was performed seeding a 5 µl drop that contained 104, 103 or 102 per plate, but in this case the GMM agar used was GMM 1M in NaCl, GMM 1 M in KCl and GMM 1.2 M in Sorbitol. As we can see in the above images mutant strain is more sensitive to osmotic shocks than WT indicating the importance of the gene in the osmotic balance.

Social networks

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CONCLUSIONS

CRISPR/Cas9 is an accurate technique that allowed us to generate the ∆Afu3g08050 strain using the A. fumigatus genetic background Af-293 with a great efficiency.

The transcription factor Afu3g08050 could be important to normal growth in different pHs and when nitrate is the unique nitrogen source. As well for hyphal density, conidiation, osmotic balance and cell wall and membrane integrity.