Identification of novel transcription factors involved in *Aspergillus fumigatus* biofilm formation

Shuai Liu [1]*, Yazan Abu Yousef [2,3,#]*, Donald C. Sheppard [2,3], Shizhu Zhang [1], **François Le Mauff [2,3]**

[1] Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Microbiology, College of Life Sciences, Nanjing Normal University, Nanjing, China
[2] Departments of Medicine, Microbiology and Immunology, McGill University, Montréal, Canada
[3] Infectious Diseases in Global Health Program, Research Institute of the McGill University Health Centre, Montréal, Canada
* Contributed equally to the work, # Current address: McMaster University Medical School, Hamilton, Ontario, Canada

Background

Adherence to cells is a key step in fungal pathogenesis. In *Aspergillus fumigatus*, hyphal adherence to host cells is mediated by the exopolysaccharide galactosaminogalactan (GAG). While several studies have identified genes whose product is required for GAG biosynthesis, little is known about the genetic regulation of biofilm formation.

Methodology

A library of 400 *A. fumigatus* transcription factor knock-outs was screened for their capacity to form adherent biofilms using the crystal violet assay. Selected transcription factor mutants were re-constructed to confirm the role of each candidate gene in adherence regulation. Mutants were tested for potential growth defects by visual observation and XTT metabolic activity. GAG synthesis was quantified by ELISA and immunofluorescence microscopy. Transcriptomic analysis was performed by RNA-seq.

Results

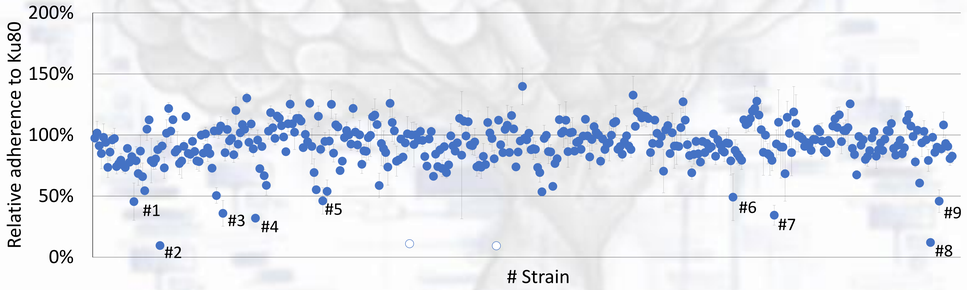

Figure 1. 9 transcription factor knock-outs display a defect in adherence. Adherence of each strains was evaluated after 21h of growth and compared to the adherence of their parent Ku80. 11 strains passed the selection threshold for defect arbitrarely fixed at 50% of Ku80 adherence. 2 strains were excluded from the study (white filled dot) due their auxotrophy.

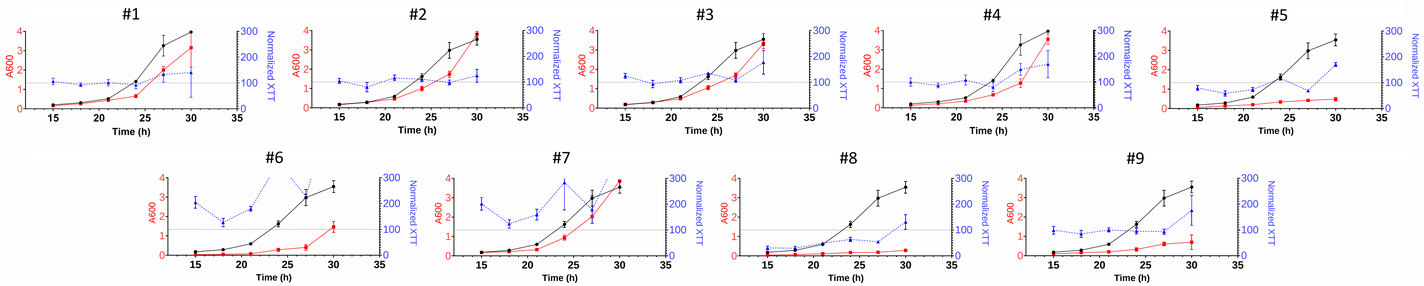

Figure 2. Only strain #9 conserves an adherence defect across a 30-hour time course and does not display a growth defect. 3 strains (#5, 6 and 8) display a growth defect, while 5 strains recover a comparable adherence to Ku80 at the later time points (#1, 2, 3, 4, and 7). Adhesion is referred on the left Y-axis and represented by the solid lines (Ku80 in black, mutant in red). Metabolic activity is referred on the right Y-axis and represented by the dotted blue line as a relative metabolic activity to Ku80.

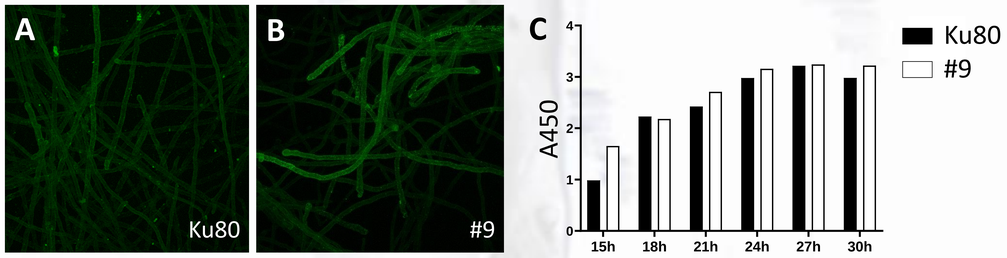

Figure 3. Adhesion defect of strain #9 is not due to a defect in cell-associated GAG or GAG secretion. Cell associated GAG was observed by confocal microscopy and revealed that (A) Ku80 and (B) strain #9 hyphae are both covered with GAG. (C) ELISA quantification of secreted GAG showed that Ku80 and strain #9 are producing similar amount of GAG at every time point analyzed.

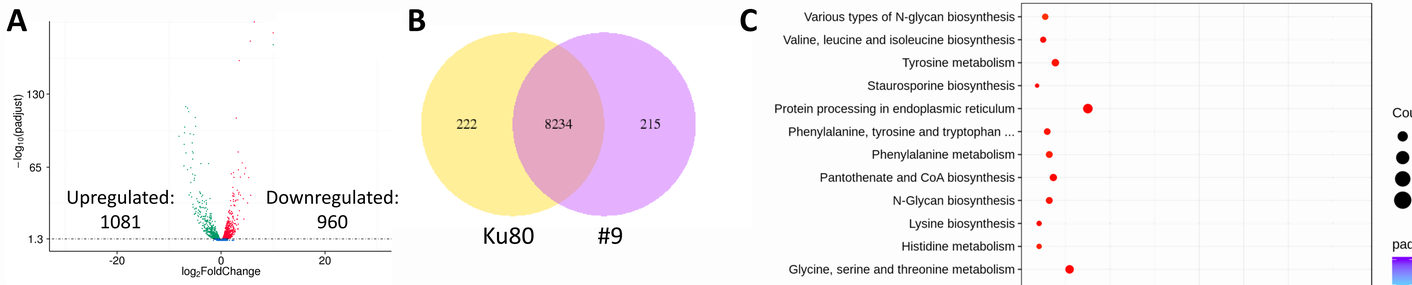

Figure 4. RNA-seq analysis of strain #9 revealed that more than 2000 genes are dysregulated in strain #9 with more than 20% of them being uniquely expressed in either Ku80 or strain #9. (A) Volcano plot of differentially expressed genes in strain #9. (B) Ven diagram of uniquely expressed genes in Ku80 and strain #9. (C) KEGG metabolic pathways most affected in strain #9.

Conclusion

This work demonstrates that *Aspergillus fumigatus* adhesion and biofilm formation is not entirely mediated by GAG. Other factors are at play and their identification is on going.