

TITLE: THE ROLE OF LEUCINE ZIPPER BASIC DOMAIN TRANSCRIPTION FACTORS (BZIP), DEPENDENT ON SAKA (HOG1), INVOLVED IN THE ADAPTIVE RESPONSE TO OSMOTIC AND CELL WALL STRESS IN *ASPERGILLUS FUMIGATUS*.

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ABSTRACT

Different metabolic pathways are studied to understand the mechanisms of resistance to immune system defenses and virulence of *Aspergillus fumigatus*. The pathways of mitogen-activated protein kinases (MAPK-HOG1) are highlighted in the present study because they are related to the phosphorylation and activation of transcription factors (TFs) leucine zipper basic domain (bZIP) ATF1 family. In this study, deletions were made for the four TFs $\Delta atfA$, $\Delta atfB$, $\Delta atfC$ and $\Delta atfD$. The isolated strains were grown in different stressors such as Congo Red (CR), Calcofluor White (CFW) and caspofungin causing cell wall damage, as well as in the presence of osmotic stress causing sorbitol (1.2M). In addition, the proteins AtfA, AtfB, AtfC and AtfD were fused to green fluorescent protein (GFP) with stress agents. AtfA: GFP, was constitutively present in the nucleus, regardless of the treatments performed. However, the other AtfB-D: GFP proteins migrated to the nucleus after 10 or 30 minutes of treatment with different stressors. It is known that in *A. nidulans*, AtfA is dependent on SakA. To evaluate the physical interaction of SakA with TFs the *A. fumigatus*, co-immunoprecipitation (Co-IP) assays were performed during induction of CR or sorbitol for 10 min. The results show that AtfA, AtfB, AtfC and AtfD interact directly with SakA, showing the importance of TFs during the transcription of genes that may respond to different stresses. In addition to the interaction of TFs with SakA, one of the main regulators of the HOG1 pathway in *A. fumigatus*, the study also studies the possible interaction between AtfA-D TFs. Double deletions were made, and we can observe that there is a possible interaction between the TFs, since the data obtained through the tests performed with the double mutant strains, it was possible to observe that, the double deletion had an additive effect on the phenotype when compared to the wild. In addition, deletions of the *atfA* and *atfB* genes, resulted in loss of virulence and attenuation, respectively, as well as the double deletions also resulted in a decrease in the virulence of *A. fumigatus* in the larval model *Galleria mellonella*.

Keywords: Transcription factors; cell wall stress; osmotic stress; *Aspergillus fumigatus*; MAPK-HOG1.

Development Agencies: FCFRP