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FUNCTIONAL ANALYSIS OF bZIP TRANSCRIPTION FACTORS AtfA AND AtfB IN *ASPERGILLUS NIDULANS*

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The eukaryotic bZIP type transcription factors AtfA and AtfB, regulating secondary metabolism, sexual development and stress responses, play critical roles in the organismal response to the environment. To examine the physiological functions of these bZIPs we constructed and phenotypically studied deletion and overexpression mutants of *atfA* and *atfB* in all combination (*ΔatfA*, *ΔatfB*, *ΔatfAΔatfB*, *ΔatfAatfBOE*, *ΔatfBatfAOE*, *atfAOE*, *atfBOE* and *atfAOEatfBOE*) in *Aspergillus nidulans*. We studied the stress sensitivity of the mutants with stress agar plate assays, in the presence of oxidative (2.0 mM diamide, 0.8 mM tert-butyl hydroperoxide or 0.08 mM menadione sodium bisulfite), hyperosmotic (2.0 M sorbitol or 1.5 M NaCl), heavy metal (300 μM cadmium chloride) and cell wall stress (54 μM CongoRed) generating agents. *ΔatfAatfBOE*, *ΔatfAΔatfB*, *atfAOEatfBOE* mutants showed increased sensitivity to the oxidative stress inducing agent diamide. Only one mutant, *ΔatfA* was sensitive to MSB, while the overexpression of *atfB* compensated this sensitivity in *ΔatfAatfBOE* mutant. *atfAOE*, *atfBOE*, *atfAOEatfBOE* showed increased tolerance to tBOOH meanwhile *ΔatfA* as well as *ΔatfAΔatfB* were sensitive to tBOOH. *atfAOEatfBOE* mutant showed increased tolerance to NaCl. The growth of *ΔatfB* mutant significantly reduced in the presence of NaCl, however this mutant was the most tolerant to sorbitol. After heavy metal stress treatment the growth of *ΔatfAatfBOE* mutant was slightly reduced but in *atfBOE*, *atfAOEatfBOE* mutants showed sensitivity to $CdCl_2$. The cell wall stress inducing CongoRed affected only the *ΔatfA* mutant, moderate tolerance was observed. Quantitative determination of the sterigmatocystin production was carried out by HPLC analysis from the point-inoculated surface cultures incubated for 5 days at 37°C. The production of this mycotoxin was reduced in *ΔAtfAatfBOE* and *ΔatfBatfAOE* and *AtfAOEatfBOE* mutants. The deletion of *atfA* led to the loss of sterigmatocystin production while *ΔatfAΔatfB* was able to synthesize this compound. We also determined the size of conidiospores. Based on light and scanning electron microscopy images, *atfBOE* mutant can be characterized by larger spore size compared to that of the control strain. We also tested the viability of the conidiospores under 50°C thermal stress for 10 min. *ΔatfAatfBOE* and *ΔatfBatfAOE* showed increased viability, meanwhile conidia of the *ΔatfB* showed reduced viability compared to the control strain. Conidiospore production was also quantified in all mutants. In *ΔatfA*, *ΔatfAΔatfB*, *atfBOE* mutants reduced conidiospore formation was observed, while in *ΔatfBatfAOE* the number of asexual spores increased compared to the control. We are also planning bimolecular fluorescence complementation experiments (BiFC) for the confirmation of the possible AtfA-AtfB heterodimer formation in vivo.

ETIOLOGIC AND PATHOGENIC ROLES OF PERSISTENT VIRAL INFECTIONS

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The course of a persistent infection is classified as either latent or chronic or slow infection. During the persistent infectious course, the host suffers limited but either prolonged or repeating exposures from the microorganism in concern. As a result, a disease can or cannot develop and even if a disease manifested, typically a long term infectious course with accumulating injuries will eventually lead to the pathologic condition. This is to discuss the etiologic and pathogenic roles in three different virus-disease associations, namely, human papillomaviruses in cervical carcinogenesis, herpes viruses in periapical periodontitis, and measles virus in otosclerosis. The methodological approach applied in the projects was based on molecular Koch postulates involving cirteria for disease associations, consistency, time factor, reversibility and plausibility with a final goal to build evidences for the roles of infections in the investigated diseases.

IMPACT OF N-ACYL-HOMOSERINE LACTONE, QUORUM SENSING MOLECULE, ON *CANDIDA AURIS* ISOLATES

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Candida albicans-Pseudomonas aeruginosa mixed infections and/or colonization are commonly found in various clinical cases. This mixed species cooperation is well studied; however, the number of data focusing on *P. aeruginosa*–non-albicans species (as *C. auris*) are limited. *P. aeruginosa* significantly affects the morphological and virulence-related properties of *C. albicans*. This effect was mediated primarily by N-3-oxo-C12 homoserine lactone (3OC12HSL), a molecule studied for its role in cell-cell signalling. In this study, we examined the effect of 3OC12HSL on *C. auris* growth and invasion ability by epithelial transmigration assay with Caco-2 human epithelium model. In growth-related experiments, *C. auris* pre-cultures were grown in 5 mL yeast peptone dextrose (YPD) medium at 37ºC for 18 hours, diluted to an optical density of 0.1 (OD640) with YPD then grown further at 37ºC and at 2.3 Hz shaking frequency. Following a 4-hour incubation period, some cultures were supplemented with 3OC12HSL (200 μM and 400 μM), and microbial growth was followed by measuring changes in optical density. In invasion-related experiments, the effect of 200 and 400 μM 3OC12HSL were tested using Transwell cell culture insert. To obtain monolayers, 6×10^4 Caco-2 cells of the same passage number were seeded into Transwell cell culture inserts with 8 μm pore size, 1×10^5 pores per cm^2 density and 0.33 cm^2 area, polycarbonate membrane, and placed in 24 well plates. In all cases, the volume of the apical compartment was set to 200 μL and the basolateral was set to 1250 μL. Before the infection, *C. auris* strains grown overnight at 30 °C in YPD were washed with PBS and resuspended in the cell culture medium in 1×10^6 cells mL^{-1} concentration and were put into the apical compartment and incubated at 37°C in a humidified atmosphere of 5% CO_2. The medium in the apical and basolateral compartments were changed daily without disturbing the developing yeast layer. At 12, 24, 48 and 72 hours quantitative culturing was performed to determine the number of migrated *Candida* cells. Growth was significantly inhibited within 2 hours after the addition of 3OC12HSL as assessed by observed absorbance values (1.17±0.02, 1.05±0.007 and 0.97±0.01 for untreated control 200 μM-exposed and 400 μM-exposed cells, respectively), at OD640) ($p<0.05$) and quantitative culturing. The 3OC12HSL significantly enhanced the invasion of *C. auris* cells in concentration dependent manner compared to untreated control and *C. albicans* SC5314 reference strain ($p<0.01$-0.05). Our results help to understand the cell-cell relationship between *C. auris* and *P. aeruginosa*.

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DEVELOPMENT OF PCR FOR THE SPECIFIC DETECTION OF *BOTRYTIS CINEREA*

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Botrytis cinerea is considered as one of the most important plant pathogenic fungi due to its broad host specificity and high degree of damage causing ability. Detecting the presence of the fungus before the appearance of symptoms is therefore very important, but very challenging. Most of the PCR-based diagnostic methods developed so far target different regions of the rRNA gene cluster, but results have also been achieved with primers designed for other genes. The aim of this study was to develop a PCR method based on gene sequences different from the previous ones, which enables the specific detection of *B. cinerea*. Based on data from data banks and publications, we selected three genes for development: *BMP1* and *BOS5*, which encode kinases (MAPK, MAPKK) that play an important role in intracellular signaling processes and through this in pathogenesis, and the *BcLCC2* gene, which encodes a laccase and whose role in pathogenicity can also be assumed. We designed primers for the three selected *B. cinerea* genes (*BMP1*, *BOS5* and *BcLCC2*). Since greater diversity can be expected in the case of introns, we designed the primers for the conservative regions of the exon sequences. During the planning, we took into account that the length of the sequence to be amplified should not exceed two hundred base pairs. The primers were characterized based on secondary structure and expected specificity. We designed and tested a total of 40 primer pairs for the three genes, and then selected those with the appropriate parameters. For *BMP1* one (Bmp1), for *BOS5* two (Bos-X1, Bos-X2), while for *BcLCC2* three (Lcc-X1-1, Lcc-X1-2, Lcc-X4) sets seemed suitable for further experiments. As a first step, we performed and, if necessary, optimized the PCR reactions by adding *B. cinerea* genomic DNA to the reaction mixture, and determined the appropriate annealing temperatures in terms of specificity. With the exception of Bos-X2, a specific product was formed in all cases, but in some reactions, despite the optimization, non-specific extra products were also generated. Among the reactions performed, the Bmp1, Bos-X1 and Lcc-X1-2 were deemed suitable for further experiments, in which we also performed the reactions with the involvement of other fungal species. Based on the results we found that the Lcc-X1-2 primer pair produces an amplicon only in the case of *B. cinerea* (the specific product is about 150 bp in size), thus the primer pair designed for the *BcLCC2* laccase gene is specific for *B. cinerea* species, and so we plan to continue our real-time PCR tests with these oligonucleotides.