Development of a high throughput cytotoxicity assay of Caco-2 cells using a Candida albicans homozygous deletion library

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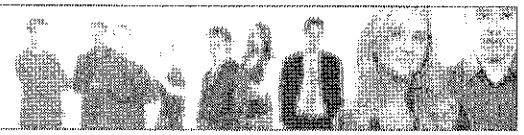

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of bacterial and fungal pathogens co-exist, whereby the direct physical contact between the microorganisms or distant molecular signaling might have an impact on microbial pathogenicity and disease outcome. *Aspergillus fumigatus* is the most important airborne fungal pathogen and until now it is unclear, how this fungus interacts with *Pseudomonas aeruginosa*, the predominant bacterial pathogen in CF-lungs. To get first insights into putative inter-microbial communication processes between *A. fumigatus* and *P. aeruginosa*, co-incubation experiments were performed. Biomass analysis revealed that germination and growth of *A. fumigatus* is inhibited by *P. aeruginosa*. Electron microscopy displayed a direct physical contact between these organisms. In addition, microarray analysis identified differentially expressed fungal genes after co-incubation with *P. aeruginosa*. Genes involved in cell wall synthesis and remodeling, stress response, and secondary metabolism were significantly upregulated in *A. fumigatus* indicating recognition of *P. aeruginosa* and activation of fungal defence mechanisms.

P211
Identification of conidia-associated surface proteins in the human pathogenic fungus *Aspergillus fumigatus*

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The saprophytic fungus *Aspergillus fumigatus* is one of the most important human pathogenic fungi that causes severe invasive lung infections in immunocompromised patients. The asexual reproduction of *A. fumigatus* leads to the formation of conidia which are released into the atmosphere. Based on their small size of 2–3 µm in diameter, they are inhaled by humans and can reach the lung alveoli. Hence, conidia are the fungal entity which have the initial contact with the host's immune system. Besides cell wall polysaccharides the conidial surface proteins are the first molecular structures which are recognised by the host's immune system. To characterise the composition of the *Aspergillus fumigatus* conidial surface proteome, we released surface proteins, especially glycosylphosphatidylinositol-anchored proteins (GPI), by HF-pyridine extraction and subsequent LC-MS/MS analysis. We identified 210 different proteins, of which 50 showed a signal peptide for secretion and 9 proteins a GPI anchor attachment signal. The most abundant surface proteins of conidia of the WT strain ATCC 46645 represented the hydrophobin protein RodA and a so-far uncharacterised protein without assigned function. To elucidate the role of the conidial melanin layer on the composition of the conidial surface proteome we included the melanin-free Af*pksP* mutant in our study, which produces white, melanin-free conidia and which is drastically reduced in virulence. Using spectral counts for peptide quantification we identified 34 proteins, which were not detectable in the HF-pyridine extract of the WT strain. The extract of the mutant strain contained an increased amount of cytoplasmic proteins, e. g. ribosomal proteins, which might indicate a reduction in dormancy. However, in increased contamination of the Af*pksP* extract by cytosolic proteins cannot be ruled out. On the other hand, the lacking melanin layer of the Af*pksP* mutant might influence the composition of surface proteins, because 11 proteins were not present in the HF-pyridine extract of the white sporulating mutant strain. The most promising candidates are currently being investigated by gene deletion studies. In further experiments we aim to search for alterations in cell wall composition and resulting effects *in vitro* and *in vivo*.

P212
Development of a high throughput cytotoxicity assay of Caco-2 cells using a *Candida albicans* homozygous deletion library

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Introduction: The opportunistic yeast *Candida albicans* gains access to the bloodstream mainly through translocation across the intestinal barrier. Using an *in vitro* model of interaction with the enterocytic-like cell line Caco-2, it has been shown that *C. albicans* is capable of adhering to, invading and damaging intestinal cells, probably through a combination of phenotypic properties including adherence, yeast to hyphae transition and secretion of lytic enzymes (Dalle *et al.*, Cell. Microbiol. 12:248, 2010). However the sequence of events together with the genes involved in these processes remain to be clarified.

The aim of our study was to screen a *C. albicans* isogenic collection of homozygous knock-out strains for genes involved in the infectivity of enterocytes using a Caco-2 in *vitro* model

Methods: A high throughput screening tool adapted from our Caco-2 model was used for testing the infectivity of 674 *C. albicans* homozygous deletion strains (equivalent to ~ 11% of the genome, Noble *et al.*, Nat. Genet. 42:590, 2010). Infectivity was measured as the cytotoxic effect of individual mutants upon Caco-2 cell monolayers. For each of the mutants tested, cytotoxicity was expressed as the percentage of variation as compared to the *C. albicans* SC5314 reference strain.

Results: We identified 66 mutants out of 674 with marked increase or decrease in Caco-2 cell cytotoxicity. These mutants were selected as the 10% fraction of the population lying in the tails of the Gaussian distribution, with 5% on each side of the distribution. A gene ontology term analysis of the 66 ORFs showed functional enrichment of genes involved in filamentous growth (p-value of 0.07, false discovery rate of 0%) among the 674 ORFs tested. An additional screen of independent mutants for those 66 genes is currently under investigation to confirm these observations.

Conclusions: Our study demonstrated the potential of our screen to identify genes involved in *C. albicans* ability to cause enterocyte cell damage. Known genes and yet uncharacterized ORFs have been identified for which deletion increases or decreases *C. albicans* virulence *in vitro*. Further studies will be conducted to confirm the involvement of these genes or ORFs in *in vivo* models of disseminated candidiasis originating from the gastrointestinal tract.

P213
Cell wall modifications during maturation and germination of the conidia in the opportunistic fungus *Scedosporium apiospermum*

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Introduction: Recent prevention measures led to an increase in life expectancy of cystic fibrosis (CF) patients; however, this progress remained jeopardized by various microbial infections. *Scedosporium apiospermum* is the second most frequent filamentous fungus found in the respiratory tract of CF patients. Unlike other infectious agents, the pathogenic mechanisms of this fungus are far less studied. We aim

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