TRANSCRIPTIONAL CHANGES IN CANDIDA ALBICANS GENES AT AN EARLY STAGE OF QUORUM SENSING FOLLOWING TREATMENT WITH FARNESOL AND AT HIGH CELL DENSITY

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Candida albicans is an opportunistic fungal pathogen found in the normal flora of healthy humans. In 2001, a quorum sensing molecule of C. albicans (farnesol: C9, H16, O) was identified. Farnesol is a metabolic product of mevalonate/sterol synthesis in eukaryotes. Generally this molecule functions endogenously. However, in C. albicans, farnesol acts in an autocrine manner and quorum sensing through farnesol regulates virulence and morphogenesis. On the other hand, we devised a minimal medium (N-acetyl-D-glucosamine: GlcNAc medium) in order to understand the mechanism of the yeast-to-hyphal conversion. In this paper, we focused on global transcription profiling of cells in the initial stage in the GlcNAc medium to which farnesol was added or in which the medium was inoculated at a high cell density of C. albicans. Therefore, global transcription profiling at an early stage of quorum sensing by C. albicans was analyzed using genomic microarrays. Twenty-two known genes of a total of 53 responded to both farnesol and high cell density. From in silico analysis and previous published data, nine of those including genes encoding amino acid biosynthesis were controlled by the Gcn4p regulator. Nine others which included genes encoding central carbon metabolism were controlled by negative regulators including Nrg1p, Tup1p, Sun1p, and/or Mig1p.

Other genes not controlled by these regulators included genes related to oxidative stress, glucose metabolism, and agglutination. Expression of genes related to amino acid biosynthesis and central carbon metabolism in this study is similar to a previous report of transcription profiling in C. albicans following its internalization by phagocyte cells and adaptation to host challenges. From these data, we hypothesize that the addition of farnesol or a high cell density inoculum in GlcNAc medium temporarily prolongs starvation conditions for the organism and its adaptation to environmental changes; a consequence of this is the postponement of the morphogenetic switch.

IN-VIVO CANDIDA BIOFILMS IN SCANNING ELECTRON MICROSCOPY

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Candida biofilms are an increasing problem on indwelling devices from patients attended in intensive care units. For better understanding the occurrence and frequency of biofilms removed catheter material was investigated. A total of 172 catheters, which were positive in routine laboratory fungal diagnostics, were collected. C. albicans identification was performed using CAN2 agar plates, other species were identified using Vitek2 and/or API 32C. Pieces of catheters which turned out to be positive for yeast species were fixed in 2% paraformaldehyde solution. For scanning electron microscopy (SEM) the pieces were dried in ascending concentrations of ethanol and finally air dried in a drying chamber. The pieces were cut diagonally into two halves and sputter coated with gold. Investigation of the lumina was performed with a Philips XL30 SEM using the high vacuum mode, which offered the best resolution. The catheters were collected from 105 male (61%) and 67 female (39%) patients, catheters from men were statistically significant (X2: n= 67; P<0.01) more often yeast positive than those of women. The patients were between three weeks and 98 years old. In SEM 97 (56.4%) catheters turned out to be positive for biofilm formation. Fifty-seven (58.8%) of them came from male patients and 40 (41.2%) from female patients, again catheters from male patients were statistically significant (X2: n= 40; P<0.01) more often positive than those of women. Each catheter was examined for occurrence of fungal structures on its surface. A catheter was designated positive for biofilm, if yeast cells, hyphae and matrix material were found. But there were also some positive catheters without hyphae, because of the isolated species alone. C. utilis and C. glabrata are not able to switch into filamentous growth form. As expected, C. albicans (73.8%) was the most common species, followed by C. parapsilosis (9.3%), C. glabrata (8.1%) and C. tropicalis (4.1%). Recapitulating we can say, that more than 50% of all catheters showed a fungal biofilm on its surface. That huge amount of biofilm positive catheters should lead us to a more sensible use of antifungal agents as Candida biofilms are known for their higher resistance to antymycotics caused by e.g. their reduced metabolism. The epidemiology of Candida biofilms on indwelling devices should be a target for further investigations.

CORRELATION BETWEEN E-TEST, DISK DIFFUSION, AND MICRODILUTION METHODS FOR ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES

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Agar-based susceptibility testing methods have been a focus of interest to many researchers and include the classical disk diffusion methods and the E-test method. These tests are very attractive due to their simplicity, reproducibility, and lack of requirements for specialized equipment. The correlation between the microdilution, E-test, and disk diffusion methods was determined for amphotericin B, itraconazole and fluconazole against Candida spp. The minimal inhibitory concentration (MIC) of those antifungal agents was established for a total of 70 Candida spp. isolates identified. The overall concordance (based on the MIC value obtained within two dilutions) between the E-test and microdilution was 90% for amphotericin B, 67.14% for itraconazole and 58.5% for fluconazole. Considering the breakpoint, the agreement between the disk diffusion and microdilution methods was 71% for itraconazole and 67% for fluconazole. The E-test and microdilution MICs shown good concordance and interpretative agreement. The disk diffusion zone diameters are highly reproducible and correlate well with the microdilution method, making agar-based methods a viable alternative to microdilution for susceptibility testing. Although there is little data on agar-based tests for itraconazole and amphotericin B, results for E-test and
disk diffusion methods provide a good correlation with the microdilution method. However, further research must still be carried out to ensure the standardisation of other antifungal agents.

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CANDIDA TROPICALIS CLINICAL ISOLATES: BIOFILM COMPOSITION AND ARCHITECTURE

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The number of infections caused by Candida species has greatly increased in the past ten years. This has been attributed to an increase in the number of AIDS patients, the elderly population and immunocompromised patients. Moreover, the increased use of indwelling medical devices has also been implicated with the rise of Candida infections. Most candidiasis have been attributed to Candida albicans, however, recently, new non-Candida albicans Candida (NCAC) species have been identified as common pathogens, namely Candida tropicalis. Formation of C. tropicalis biofilms has important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses. Thus, the aim of this study was to compare biofilms formed by different clinical isolates of C. tropicalis. A total of 6 C. tropicalis strains isolated from the vagina (n=2), urinary (n=2) and oral tract (n=2) were used. A reference strain, C. tropicalis ATCC 750, was also assayed. Biofilms were formed in 96-well microtiter plates, in Sabouraud dextrose broth at 37°C (agitated at 130 rpm). The ability of biofilm formation was assessed after 48h through total biomass quantification by crystal violet staining and cellular activity by the reduction of a tetrazolium salt (XTT). Moreover, the number of viable C. tropicalis cells in biofilms was determined by Colony Forming Units (CFUs). Matrix material was extracted from biofilms by sonication and their protein and total carbohydrate contents were determined by the Lowry and Dubois methods, respectively. The ultrastructure of the C. tropicalis strains biofilms was observed by Scanning Electron Microscopy (SEM). The results showed that all clinical isolates of C. tropicalis were able to form biofilms, although there were differences on biomass and biofilm activity depending on strains. Furthermore, comparison of biofilm biomass with cell activity did not reveal any correlation. Matrix recovered from C. tropicalis biofilms present an high amount of proteins and small amounts of carbohydrates per gram of biofilm cell dry weight. C. tropicalis biofilms revealed a multilayer structure that consists of a dense network of yeast, hyphae and pseudohyphae. As a general conclusion, it was possible to infer that clinical isolates of C. tropicalis present different behaviors in terms of biofilm formation, structure and chemical composition.

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ZOOONOTIC ASPECT OF CANDIDIASIS IN GHARBIYA GOVERNORATE, EGYPT

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Abstract: In this study the following samples were examined for detection of candida species: -180 vulvovaginal swabs of pregnant and non pregnant women claiming for vulvovaginitis. -100 skin swabs from the diaper area in infants suffering from dermatitis in the diaper area. - 200 chicken comb swabs from chicken farms and chicken homes. - 90 air samples were collected from the chicken farms that have comb candidiasis, human hospitals in which their patients infected with vulvovaginal or diaper candidiasis and patient’s homes. - 90 swabs from equipments and instruments were collected from the same places in which the air samples were collected. All samples located in Gharbia governorate, Egypt.

The result indicates that the infection rate of vulvovaginal candidiasis in the pregnant women was 53.3%, in non pregnant women 23.3% and in the pregnant women administrated the Yoghurt (Probiotic) during the pregnancy was 28.3%. The rate of infection by diaper candidiasis in infants used the cloth diapers was 44% and 14% in infants used superabsorbant disposable diapers. In comb candidiasis the infection rate in chicken administrated antibiotics was 37% and 6% in chicken without antibiotics use. The rate of candida pollution in the air of chicken farms was 23.3% and in the air of human hospitals and patient’s homes were 16.6% and 13.3% respectively. Equipments and instruments contamination in the chicken farms was 23.3%; in the human hospitals and patient’s homes were 26.6% and 10% respectively. The public health importance of candidiasis was discussed as well as suggestive control measures.

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P-113 BINDING TO THE C. ALBICANS CELL WALL IS SALT SENSITIVE AND IS REQUIRED FOR SUBSEQUENT ENERGY-DEPENDENT CYTOSOLIC TRANSLATION

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P-113 is a 12 amino acid active fragment of Histatin 5 (Hst 5) that retains complete candidacidal activity of the parent peptide against Candida albicans. P-113 toxicity is initiated intracellularly, thus cell wall binding and cytosolic translocation are prerequisites for peptide activity. Incubation of cells in 100 nM extracellular salt or with inhibitors of the energy metabolism (sodium azide and carbonyl cyanide m-chlorophenylhydrazone (CCCP)) renders C. albicans cells resistant to the killing action of P-113.

Objective: To identify whether salt, sodium azide and CCCP inhibit binding of P-113 with the yeast cell wall, or if subsequent translocation and/or cytosolic functions are affected. Methods: Antifungal activities of P-113 and its inactive mutant P-113Q10 were tested against C. albicans by using standard microdilution plate assays. P-113 localization within C. albicans was examined using two sequential cellular fractionation steps consisting of β-mercaptoethanol (β-ME) cell wall extraction, followed by cytosolic fractionation. P-113 was quantified in each cellular compartment by Western blotting. Results: P-113 had potent killing activity against C. albicans cells in 10 mM NaPB, but was completely inactivated in the presence of 100 mM NaPB, 10 mM sodium azide or 500 μM CCCP. Similarly, P-113 was translocated to the C. albicans cytosol within 5 min under low salt conditions; while P-113 was absent in cytosolic extracts from cells incubated in the presence of 100 mM NaPB, azide or CCCP. However, in cell wall binding assays, P-113 was detected in cell wall extracts within 5 min in cells suspended in 10 mM NaPB, azide or CCCP, whereas little to no P-113 was found in the cell wall of C. albicans cells incubated in 100 mM NaPB. The translocation incompetent derivative P-113Q2.10 remained bound at saturated levels with the cell wall, showing that peptide association with the wall is a transitional step prior to internalization, and that translocation is dependent upon the primary structure of the substrate peptide.

Conclusions: Initial binding of P-113 with the C. albicans cell wall requires low salt conditions, suggesting that binding is predominantly through electrostatic interactions rather than by binding with specific peptide motifs. Translocation of P-113 to the cytosolic compartment first requires initial cell wall binding and is significantly reduced in cells energy depleted by sodium azide or CCCP. Therefore, intracellular transport of P-113 likely occurs via energy dependent permeases or other energy-dependent processes such as endocytosis which recognize specific peptide sequences.