Virulence factors of non-Candida albicans Candida species

Silva S, Henriques M, Oliveira R and Azeredo J
Department of Biological Engineering
soniasilva@deb.uminho.pt

KEYWORDS
Candida species, candidosis, virulent factors.

ABSTRACT
Candidosis caused by non-Candida albicans Candida (NCAC) species have been increased in recent years, namely due Candida glabrata, Candida tropicalis and Candida parapsilosis. Furthermore, candidosis is facilitated by a number of virulence factors, such as adherence to medical devices and/or host cells, biofilm formation and secretion of enzymes. Thus, the main goal of this study was to characterize clinical isolates of C. glabrata, C. tropicalis and C. parapsilosis recovered from different body sites (oral cavity and urinary and vaginal tracts) in terms of the most important virulence factors (adhesion, biofilm formation ability, tissue colonization and invasion, and expression of secreted aspartly proteinases).

INTRODUCTION
Infections caused by Candida species (candidosis) have greatly increased over recent years, mainly due to the escalation of the AIDS epidemic, population ageing, increasing number of immunocompromised patients and the more widespread use of indwelling medical devices (Fanello et al. 2001; Samaranayake et al. 2002; Kojic et al. 2004). Candida albicans is the main cause of candidosis, however, non-Candida albicans Candida (NCAC) species such as Candida glabrata, Candida tropicalis and Candida parapsilosis are now frequently identified as potential human pathogens (Hajjeh et al. 2004; Bonassoli et al. 2005; Bassetti et al. 2006; Weinberger et al. 2005; Nucci et al. 2007). This increased incidence of NCAC in human infection can be attributed to improved identification methods but might also be a reflection of the high level of resistance often exhibited by these species to certain antifungal agents. Candida species pathogenicity is facilitated by a number of virulence factors, most importantly adherence to medical devices and/or host cells, biofilm formation, and secretion of enzymes, such as proteases. Despite intensive research to identify pathogenic factors in fungi, particularly in C. albicans, relatively little is known about the virulence determinants of NCAC species. Thus, this work described the most important virulence factors (adhesion, biofilm formation ability, tissue colonization and invasion, and expression of hydrolytic enzymes) of clinical isolates of C. glabrata, C. tropicalis and C. parapsilosis recovered from different body sites.

BIOFILMS NON-CANDIDA ALBICANS CANDIDA SPECIES: QUANTIFICATION, STRUCTURE AND MATRIX COMPOSITION
The study of NCAC biofilms, in terms of formation, structure, matrix composition, and metabolic activity, was the first goal of this research. Total biomass quantification, showed that all NCAC strains were able to form biofilms, although this was less extensive for C. glabrata compared to C. parapsilosis and C. tropicalis. Scanning electron microscopy (SEM) revealed structural differences for biofilms with respect to cell morphology and spatial arrangement. Furthermore, C. parapsilosis matrices had large amounts of carbohydrates and low protein. Conversely, matrices extracted from C. tropicalis biofilms had low amounts of carbohydrates and protein. Interestingly, the composition of C. glabrata biofilm matrices' was high in both protein and carbohydrate contents. It was also evident that there were intrinsic differences in terms of metabolic activity amongst biofilms of NCAC species, and no correlation was found concerning colony forming units (CFUs) and biofilm metabolic activities determined by XTT reduction.

SILICONE COLONIZATION BY NON-CANDIDA ALBICANS CANDIDA SPECIES IN PRESENCE OF URINE
Another objective of the work described was to study the adhesion and biofilm formation ability of several clinical urinary isolates on to silicone in the presence of artificial urine (AU) and the role of Candida surface properties (hydrophobicity and zeta potential) in these events. Silicone colonization by NCAC species in
the presence of AU showed that, all urinary isolates were able to adhere to silicone, but in a species and strain dependent manner. However, these differences in adhesion could not be correlated with cell surface properties (hydrophobicity and zeta potential). Moreover, despite the high number of cultivable cells biofilms were not observed and confocal scanning laser microscopy (CLSM) showed an absence of extracellular polymeric material for all strains.

THE ROLE OF SECRETED ASPARTLY PROTEINASES IN CANDIDA PARAPILOSIS AND CANDIDA TROPICALIS INVASION AND DAMAGE OF ORAL MUCOSA

The pathogenesis of C. parapsilosis and C. tropicalis was investigated using a commercially available reconstituted human oral epithelium (RHOE), in conjunction with CLSM observation and lactate dehydrogenase (LDH) determination. Furthermore, the role of secreted aspartly proteinases (Saps) on invasion and damage of RHOE was evaluated by real-time polymerase chain reaction (PCR). It was possible to observe that all C. tropicalis and C. parapsilosis strains were able to colonize the tissue, however, this was in a species- and strain-dependent manner. Candida parapsilosis revealed low invasiveness after 12 h of infection and extensive damage was evident after 24 h when assessed using histological examination and LDH determination. Moreover, C. tropicalis was found to be highly invasive after 12 h of infection, with extensive tissue damage occurring also after 24h. Real time-PCR of SAP genes showed that expression was strain dependent for both species. Furthermore, the results suggested that the proteinases were not involved in invasion of RHOE by C. tropicalis and C. parapsilosis, but indicated a role for these enzymes in tissue damage caused by C. parapsilosis.

CANDIDA GLABRATA AND CANDIDA ALBICANS CO-INFECTION OF AN IN VITRO ORAL EPITHELIUM

Finally, single and mixed infection of RHOE by C. glabrata or and C. albicans were examined, using CLSM and peptide nucleic probes by in situ hybridization (PNA FISH). The ability of C. glabrata and C. albicans strains to colonize and invade human oral epithelium was examined after 12 h of infection. Results showed that all strains colonized the RHOE surface, although the extent of colonization was highly strain dependent. In general, after 12 h of infection, only clusters of C. glabrata yeast were detected at the surface of the keratinocyte layers and on the contrary, C. albicans showed a greater ability to invade the RHOE with hyphal elements completely penetrating all the epithelial layers. Interestingly, the results obtained enhanced invasion and increased tissue damage caused by mixed C. glabrata and C. albicans infections has important clinical significance and highlights the need to identify Candida species involved in oral candidosis.

CONCLUDING REMARKS

In summary, this work underlines both species and strain differences in terms of virulence factors associated with C. glabrata, C. parapsilosis and C. tropicalis. Furthermore, there was clear evidence demonstrating the importance of the use of new techniques including CLSM and molecular analysis tools enabling the elucidation of the mechanisms of virulence. By increasing our knowledge on Candida pathogenesis, potential therapeutic targets may well be identified that can be used as adjuvant for new therapies.

REFERENCES


