Semana de Engenharia 2010
Guimarães, 11 a 15 de Outubro

Virulence factors of non-*Candida albicans Candida* species

Silva S, Henriques M, Oliveira R and Azeredo J
Department of Biological Enginnering
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 asparty 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 PARAPSILOSIS* 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 colonisation 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. Interestyling, 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.

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