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Mini Review

# Host–pathogen interactions and virulence-associated genes during *Candida albicans* oral infections

Ronny Martin[a,b,d], Betty Wächtler[b], Martin Schaller[c], Duncan Wilson[b], Bernhard Hube[b,d,*]

[a] *Center for Innovation Competence Septomics, Research Group Fungal Septomics at the Leibniz Institute for Natural Products Research and Infection Biology–Hans Knoell Institute (HKI), Jena, Germany*
[b] *Dept. of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Products Research and Infection Biology–Hans Knoell Institute (HKI), Jena, Germany*
[c] *Dept. of Dermatology, Eberhard Karls University, Tübingen, Germany*
[d] *Friedrich Schiller University, Jena, Germany*

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ABSTRACT

Oral infections with *Candida albicans* are very common diseases in even only mildly immunocompromised patients. By using genome-wide microarrays, in vitro infection models and samples from patients with pseudomembranous candidiasis, several genes have been identified which encode known and unknown fungal factors associated with oral infection. The expression of selected genes has been investigated via qRT-PCR in both in vitro models and in vivo samples from patients. Several lines of evidence suggest that fungal morphology plays a key role in adhesion to and invasion into oral epithelial cells and mutants lacking regulators of hyphal formation are attenuated in their ability to invade and damage epithelial cells. Adhesion is mediated by hyphal-associated factors such as Hwp1 and the Als adhesin family. Hyphal formation facilitates epithelial invasion via two routes: active penetration and induced endocytosis. While induced endocytosis is predominantly mediated by the adhesin and invasin Als3, active penetration seems to be supported by hydrolase activity and mechanical pressure. Expression profiles reflect the morphological switch and an adaptive response to neutral pH, non-glucose carbon sources, and nitrosative stress.



## Introduction

Although *Candida albicans* is widely recognised as one of the leading causes of hospital-acquired bloodstream infections and sepsis (Martin et al., 2003; Perlroth et al., 2007), it is also a frequent cause of superficial infections of the oral and vaginal mucosa. Unlike systemic infections, which can have attributable mortality rates of approximately 40%, superficial infections are not life-threatening (LaFleur et al., 2006; Pfaller and Diekema, 2007). However, in contrast to systemic infections, cases of vaginal and oral candidiasis are very common and can occur in otherwise healthy or in only mildly immunosuppressed individuals (Sobel, 1988). In this review, we will focus on oral candidiasis and the host–pathogen interactions which take place before and during this disease.

*C. albicans* and to a lesser extent other *Candida* spp. are present in the oral cavities of 15–75% of the population (reviewed by ten Cate et al. (2009)). This fungus, which is normally a harmless commensal, is also associated with root caries (Zaremba et al., 2006), is part of oral biofilms on teeth (Zijnge et al., 2010), and has been observed associated with infected gingival crevices (Shen et al., 2002). During the most common type of oral candidiasis, oropharyngeal candidiasis (OPC), *C. albicans* invades the oral mucosa and persists within the epithelium causing superficial lesions (Fidel, 2006; Schaller et al., 1999). HIV-positive individuals frequently suffer from oral candidiasis, with an incidence as high as 90% before the advent of highly active anti-retro viral therapy (Challacombe and Naglik, 2006; Ruhnke and Maschmeyer, 2002). An interesting and still not well-understood fact is the observation that HIV patients including those with oral candidiasis very rarely develop systemic infections with *C. albicans*, suggesting that although oral candidiasis is an important disease manifestation in its own right, it does not necessarily precede systemic infections.

## Environmental challenges for *C. albicans* in the oral cavity

The mucosal epithelium of the oral cavity is one of the first body sites confronted with potentially pathogenic microorganisms.

* Corresponding author at: Dept. of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Products Research and Infection Biology–Hans Knoell Institute (HKI), Beutenbergstraße 11a, D-07745 Jena, Germany. Tel.: +49 0 3641 532 1401; fax: +49 0 3641 532 0810.
*E-mail address:* bernhard.hube@hki-jena.de (B. Hube).

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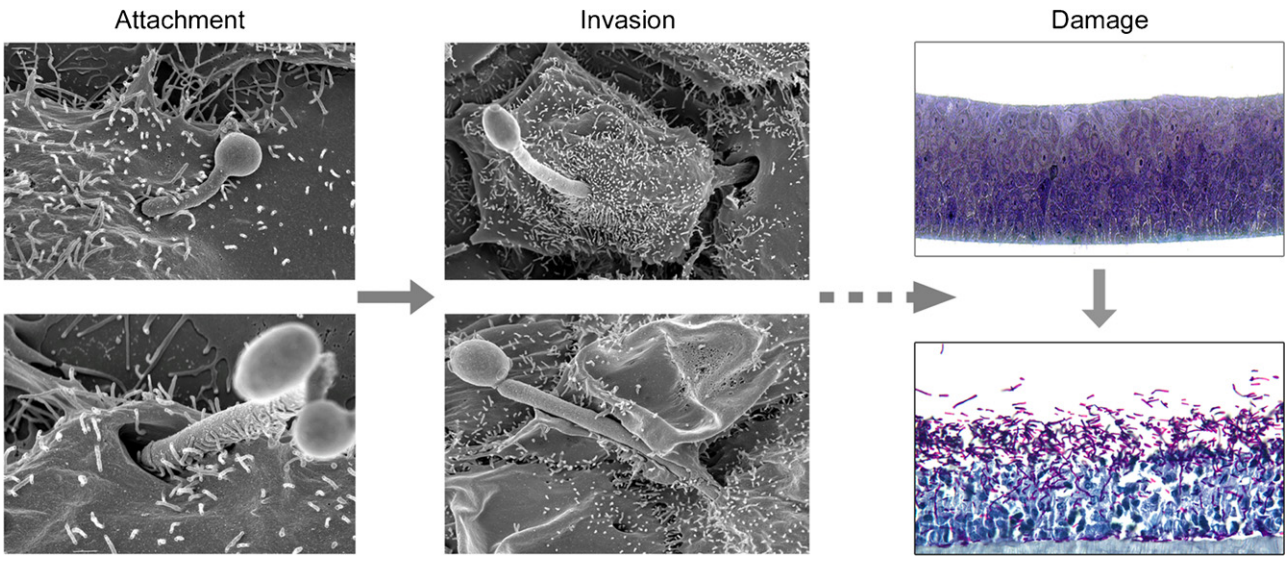


**Fig. 1.** Summary of the interactions of *C. albicans* with oral epithelial cells. Attachment of *C. albicans* is characterised by a close interaction of surface components and triggers hyphal formation by the fungus. Invasion of these hyphae into epithelial cells can then occur by either active penetration (upper picture) or induced endocytosis, characterized by membrane ruffling (lower picture). Damage occurs at later time points, characterised by gross tissue destruction.

*C. albicans* often colonises this niche, along with other members of the microbial flora (which consists mainly of bacterial species) in healthy individuals without causing disease. Within the oral cavity, *C. albicans* and other *Candida* spp. reside in mixed biofilms where bacterial-fungal interactions influence the overall survival and proliferation of the respective species (for review, see Mukherjee et al., 2005). Colonisation of mucosal epithelium with non-pathogenic microbes is thought to provide a barrier effect by protecting the host from infection by exogenous pathogens as well as by inhibiting the commensal-to-pathogenic transition of endogenous microbes, such as *C. albicans*. Despite the relevance of asymptomatic carriage to the development of oral candidiasis, very little is known about the commensal lifestyle of *C. albicans*. In the oral cavity, *C. albicans* interacts with various bacteria, and these relationships can influence pathogenesis. Some of these interactions benefit the host: For example, it was shown that oral administration of *Lactobacillus acidophilus* enhanced clearance of *C. albicans* from the oral cavity of mice (Elahi et al., 2005). On the other hand, *C. albicans* interactions with other bacteria, such as *Streptococcus* sp. or *Pseudomonas aeruginosa* within mixed biofilms can contribute to increased antimicrobial resistance of both fungus and bacteria (Hogan and Kolter, 2002; Pierce, 2005; Wargo and Hogan, 2007). For more detailed information about *C. albicans* biofilm formation in the oral cavity, readers are directed to recently published reviews (Blankenship and Mitchell, 2006; ten Cate et al., 2009). In addition to the direct interaction with bacteria in the oral cavity, the fungus is confronted with unstable physical and chemical conditions, such as changing pH values. How the fungus deals with these environmental challenges is not fully understood. Previous studies have shown that *C. albicans* reacts to changing pH by altering its morphology (reviewed by Biswas et al., 2007), gene expression (as has been shown for *PHR1* and *PHR2*) (Mühlschlegel and Fonzi, 1997), and Rim101-dependent cell wall alterations – all of which potentially contribute to pathogenic interactions with human oral epithelial cells (Nobile et al., 2008b). Another environmental challenge for the fungus is the acquisition of iron. In oral epithelial cells, iron is stored within ferritin, which is normally not accessible to microbes. However, *C. albicans* is able to gain iron from ferritin in a mechanism mediated by the multifunctional cell surface protein Als3 (Almeida et al., 2008).

## Models for experimental oral infections

In addition to interaction with commensal bacteria, *C. albicans*–oral epithelial interactions are the key events which are important to elucidate in order to understand how the fungus can cause oral infections. Mouse models to study oral infections have been described (reviewed by Naglik et al. (2008a)), however, their relevance to understanding oral candidiasis in humans is limited, due to physiological differences between human and murine oral tissues. Moreover, strains traditionally used for infection experiments, SC5314 and its derivatives, are poor colonisers of oral epithelial surfaces of mice in vivo (Rahman et al., 2007; Taylor et al., 2000). This problem was addressed by Schaller and coworkers with the establishment of the reconstituted human oral epithelium (RHE) as a model for oral candidiasis (Schaller et al., 1998, 2006). These bioengineered tissues mimic several aspects of the human epithelium: For example, RHE represents a stratified three-dimensional structure of epithelial tissue. Alternatively, confluent monolayers of epithelial cells can be used to investigate several aspects of *C. albicans*–epithelial interactions. Commonly used cell lines for monolayer infection experiments include TR146 and FaDu (Dalle et al., 2009; Park et al., 2009). Additionally, investigations using biopsy samples from patients suffering from oral candidiasis have been performed, providing valuable insight into the processes which actually occur during infections (Green et al., 2006; Naglik et al., 2005, 2006, 2008b; Schaller et al., 1998; Zakikhany et al., 2007).

## Stages of oral infections with *Candida albicans*: attachment

Initial contact of *C. albicans* to host tissues, whether during commensal or pathogenic phases, requires adhesion of the fungus to host cells. In fact, the physical contact of *C. albicans* yeasts to epithelial cells is sufficient to trigger rapid hyphal formation and the expression of genes which mediate adhesion (Fig. 1, and Wächtler et al., 2011; Zakikhany et al., 2007), such as adhesins, including members of the agglutinin-like sequence (Als) family and hyphal wall protein 1 (Hwp1). Genes encoding these adhesins were shown to be expressed in samples from patients suffering from oral candidiasis (Green et al., 2006; Naglik et al., 2006; Zakikhany et al., 2007). Adhesion via Hwp1 is due to an astonishing example