Evidence of Heterokaryon compatibility on *Candida albicans* biofilm

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Abstract

**Aims:** *Candida* species, especially *Candida albicans*, are frequently found associated with biomaterials and immunosuppressed patients, and have been described as the most virulent yeasts in human fungi diseases. These yeasts have recently been isolated from periodontal pockets, revealing the penetration of hyphae into the periodontal connective tissue. **Methods:** In this study, 7 periodontal *C. albicans* strains were applied individually in biofilm development on titanium discs and the samples were thereafter analyzed as for the number of colony forming units per milliliter (CFU/mL), dry-weight and scanning electron microscopy (SEM). **Results:** Counting of CFU/mL and determination of dry-weight showed that all samples formed biofilm. SEM analysis showed the development of a polymorphic network in the biofilms and the presence of hyphal anastomosis in the sites where fusion between the hyphae occurred. **Conclusion:** periodontal *C. albicans* strains present heterokaryon compatibility.

**Keywords:** *C. albicans*, biofilm, heterokaryon compatibility.

Introduction

*Candida* yeasts are opportunistic pathogens that cause diseases in hosts that are compromised by underlying local or systemic pathological processes. Candidiasis is the most common fungal infection in humans. *Candida albicans* is an aerobic/anaerobic commensal that can be cultured from the oral cavity of young adult. In the oral cavity, yeasts commonly colonize the tongue, palate, and oral mucosa, and may occur in subgingival plaque of adults with severe periodontitis. It is estimated that around 75% of healthy people care members of the genus *Candida* in skin and mucosa, and 36% of this population have shown evidence of oral candidiasis. In healthy carriers, various local and/or systemic general predisposing factors give *Candida* species the capacity to invade different mucosal tissues, making them opportunistic pathogens. *C. albicans* is the main specie responsible for the majority of oral mycotic infections, although other members of this genus may be involved. Like other pathogens, virulence in *C. albicans* includes host recognition (adhesion, co-aggregation to host cells and biofilm development), several degrading enzymes, hyphal formation and phenotypic switching.

When fungal colonies grow contiguously, the hyphae may fuse (anastomosis) to form cells with nuclei and cytoplasm from both parent colonies (heterokaryon). The biological significance of this phenomena explain the horizontal transfer of genes, acquisition of new biological characteristics, homeostasis and cellular communication, but on other hand, this phenomena works like a major histocompatibility complex that preserves the integrity of the organism, limit the heterokaryon formation (heterokaryon incompatibility) preventing the transfer of infectious cytoplasmic elements, virus transposons and debilitated organelles. These genes are called het (for heterokaryon formation) and have been extensively studied in *Podospora anserine*. One of these genes, called het-s, has two alleles, het-s and het-S. Strains with the same allele can undergo hyphal anastomosis to form heterokaryons, but when het-s and het-S strains grow together, heterokaryon incompatibility is observed. In oral biofilms, microbial communities consist by more than 700 different microbial species. In subgingival biofilms have been described many bacterial complex and yeasts belonging to *Candida* species. Moreover, several studies have reported virulence factors for *C. albicans*, such as like adhesion, proteinase secretion, hyphal formation, phenotype switching, biofilm growth and antifungal resistance. Considering that biofilm is a vital and natural structure for microorganisms to exchange nutrients and genetic information and that periodontal *C. albicans* is a common yeast in periodontal biofilms associated with
periodontal microbial complexes, and given that heterokaryon compatibility is a phenomena that allows for nutrient and genetic material interchange in fungi, the aim of this study was to investigate the heterokaryon formation of periodontal C. albicans strains in biofilms.

Material and Methods

Yeast Preparation and Adherence Assay Procedures

Samples of C. albicans CBS-562, and the periodontal isolates 3 A1, 15 A1, 31 A1, 34 A1, 41 A1, 47 A1 and 57 A1, which were positive to phospholipases and aspartyl proteinases, were obtained from the culture collection of the Microbiology and Immunology Laboratory of Piracicaba Dental School, Brazil. Candida species were reactivated from their original cultures (Sabouraud agar, SDA) at 37°C in 5 mL of Brain Heart Infusion (BHI) supplemented with 0.5% of yeast extract granulated (BHIY) and incubated for 24 h at 37°C in an aerobic atmosphere. The species were harvested at 2,400 xg for 10 min, suspended in 10 mL of NaCl 0.89% buffered with sodium phosphate 0.1M, pH 7 (PBS, phosphate buffer solution), and agitated for 15 s. After this procedure, the cell concentration was measured spectrophotometrically at 520nm ensuring an OD 800 equivalent to 1 x 10^6 CFU/mL.

Titanium discs (Conexão Sistema de Prótese Ltda., São Paulo, SP, Brazil) were placed in 24-well tissue culture plates (Nunclon; Nunc, Roskilde, Denmark) and 80 µL of standard yeast suspension were dropped on the discs. After 180 min of cell adherence, the non-adhered microorganisms were removed by PBS washing. Next, the titanium discs were placed on a new tissue culture plaque and submerged in 2 mL of BHI for incubation at 37°C in aerobic atmosphere. After 48 h, the discs were washed with PBS and sonicated. Candida cells were plated on SDA for counting of CFU/mL. Dry-weight was carried out according to Koo et al. (2003). Briefly, the removed biofilms were subjected to sonication and the homogenized suspension was used to dry weight determination. Three volumes of cold ethanol (-20°C) were added to 5 mL of the cell suspension and the precipitate was collected (10,000 xg for 10 min, 4°C). The supernatant was discarded, and the cell pellet was washed twice with cold ethanol, and then lyophilized and weighed.

Scanning Electron Microscopy

Some samples were fixed in 2.5% glutaraldehyde, 0.15M PBS (v/v) for 1 h at room temperature. Next, the discs were treated with 1% osmium trioxide for 1 h and washed three times with 3 mL of distilled water. The discs were hydrated in an increasing ethanol series (50% to 100%), critical-point dried, sputter-coated with gold and examined under SEM. This test was done in triplicate for each sample. The anastomosis was identified like a many contiguous hyphal growing fused between them, and the fusion, like a hyphal fusion between two cells.

Statistical analyses

All statistical tests were performed by one-way ANOVA and Bonferroni’s correction at 5% significance level using Sigmastat 3.0 Demo system software (SPSS Inc., Chicago, IL, USA) and the graphs were done using Sigmaplot v.8.02 Demo system software (SPSS Inc.).

Results

C. albicans samples showed biofilm development on the titanium discs. The 41 A1, 47 A1 and 57 A1 samples produced a smaller number of CFU/mL (Figure 1) and less biofilm mass (Figure 2), p <0.05.

SEM analysis of Candida biofilms showed a consisted polymorphic cell network, as reported by Al-Fattani and Douglas (2004), with predominance of hyphal morphology (Figure 3). All strains showed the presence of hyphal anastomosis, but 31A1, 57A1 and 15A1 exhibited more anastomoses and fusions between hyphae, proving the heterokaryon compatibility of C. albicans strains (Figure 4).
The heterokaryon compatibility observed in biofilms models supports the evidence that these strains can fuse with others *C. albicans* clones or yeast species to interchange genetic material, nutrients and other elements providing new virulence characteristics. The importance of studies investigating heterokaryon compatibility as a virulent factor of *C. albicans* relies on fact that het-s/het-S strains can interchange many elements around the biofilm network, increasing *C. albicans* resistance to antimicrobial agents and its virulence, changing from harmless commensals yeasts to fatal human pathogens. In periodontal diseases, bacteria are continuously interchanging genetic material in biofilms. Yeasts can capture and integrate this genetic material in their genomes and exchange it with other yeasts by the heterokaryon compatibility phenomenon, creating a pathogenic entity in periodontal pockets.

In conclusion, periodontal *C. albicans* strains present heterokaryon compatibility. Further research is needed to determine the importance of heterokaryon compatibility as a virulent factor of *C. albicans*.

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**References**


