Scanning electron microscopy as a tool for the analysis of colony architecture produced by phenotypic switching of a human pathogenic yeast *Candida tropicalis*

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**Abstract.** *Candida tropicalis* has been identified as one of the most prevalent pathogenic yeast species of the *Candida-*non-*albicans* group. Phenotypic switching is a biological phenomenon related to the occurrence of spontaneous emergence of colonies with different morphologies that provides variability within colonizing populations in order to adapt to different environments. Currently, studies of the microstructure of switching variant colonies are not subject of extensive research. SEM analysis was used to verify the architecture of whole *Candida* colonies. The strain 49/07 exhibited a hemispherical shape character, while the strain 335/07 showed a volcano shape with mycelated-edge colony. The ring switch variant is characterized by a highly wrinkled centre and an irregular periphery. The rough phenotype exhibited a three-dimensional architecture and was characterized by the presence of deep central and peripheral depressions areas. The ultrastructural analysis also allowed the observation of the arrangement of individual cells within the colonies. The whole smooth colony consisted entirely of yeast cells. Differently, aerial filaments were found all around the colony periphery of the volcano shape colony. For this colony type the mycelated-edge consisted mainly of hyphae, although yeast cells are also seen. The ring and rough colonies phenotypes comprised mainly yeast cells with the presence of extracellular material connecting neighbouring cells. This study has shown that SEM can be used effectively to examine the microarchitecture of colonies morphotypes of the yeast *C. tropicalis* and further our understanding of switching event in this pathogen.

1. Introduction

*Candida tropicalis* is an opportunistic yeast pathogen that causes superficial and systemic mycoses [1-3]. In Latin America, *C. tropicalis* accounts for the majority of non-*albicans* Candida species associated with candidemia episodes [4].

Among several virulence factors that contribute to pathogenesis of *Candida* it has been suggested that phenotypic switching provides variability within colonizing populations in order to adapt to challenges at different environments, including various anatomical sites in the human body [5].

Phenotypic switching represents an epigenetic state that occurs in a small fraction of the population, is random and reversible. For fungi, phenotypic switching is defined as the spontaneous emergence of colonies with altered colony morphology at rates higher than the somatic mutations rates that enables the microorganism to undergo rapid microevolution [6]. For *Candida albicans*, a relationship between colony shape of switching variants and the constituent cells (blastospores, true and pseudo-hyphae) has been demonstrated by scanning electron microscopy analysis [7]. According
to these authors the relationship between switched variant colonies and microstructure may help elucidate the relationship between pathogenicity in vivo and colonial morphology in vitro [7].

In contrast to the species *C. albicans*, the study of switching in *C. tropicalis* has not been the subject of extensive research. Our work focuses on using scanning electron microscopy for the analysis of switched variant colonies of clinical isolates of *C. tropicalis*. An understanding of this virulence determinant would provide insight into *C. tropicalis* pathogenic mechanisms.

2. Experimental techniques

2.1 Fungal strains

*C. tropicalis* strains 49/07 and 335/07 included in this study were recovered from tracheal secretion and belong to the *Candida* culture collection of the Fungal Genetics Laboratory, The University of Londrina-Brazil. The switched variants ring and rough were obtained as previously described [8].

2.2. Scanning electron microscopy of intact colonies

To verify the architecture of *C. tropicalis* colonies morphotypes, whole yeast colonies were removed from YPD (1% yeast extract, 2% peptone, 2% dextrose) agar plates using a scalpel blade. Colonies were fixed for 18 h at 4°C in 3% glutaraldehyde (Electron Microscopy Sciences) in 0.1 M phosphate buffer, pH 7.2. They were then immersed in liquid nitrogen for 30 sec and freeze-dried for 90 min at 2x10⁻³ MPa (Juan LP3). Then, colonies were coated with gold (BALTEC SDC 050 Sputter Coater) and viewed in a FEI Quanta 200 Scanning Electron Microscope at 30kV.

3. Results and Discussion

3.1. Microarchitecture of whole Candida colony

Despite the pioneer study on colony variants from *C. tropicalis* clinical strains [9] little information is available concerning the microstructure of individual colonies. Thus, we employed SEM to verify the architecture of *C. tropicalis* morphotypes. The preparation of colonies by a freeze-drying technique allowed their architecture preservation (Fig. 1) with maintenance of the phenotypes observed at lower magnitude (data not shown). The strain 49/07 exhibited a hemispherical shape character (Fig. 1A), while the strain 335/07 showed a volcano shape with mycelated-edge colony (Fig. 1B). The ring switch variant is characterized by a highly wrinkled centre and an irregular periphery (Fig. 1C). The rough phenotype exhibited more complex architecture and was characterized by the presence of deep central and peripheral depressions areas (Fig. 1D). França et al. [8] were the first to describe the architecture of whole *Candida* colonies at ultrastructural level. Here, we extend these observations of both non-variant colonies and switch variants produced by phenotypic switching of *C. tropicalis*. 
Figure 1 Scanning electron micrographs showing the architecture of *C. tropicalis* colonies morphotypes following 96 h incubation on YPD agar. (A) smooth, (B) volcano shape with myceliated-edge, (C) ring and (D) rough. Scalebar = 1mm.

3.2. Ultrastructural analysis of morphotypes

The ultrastructural analysis allowed the observation of the arrangement of individual cells within the colonies. After 4 days of colony development, the whole smooth colony consisted entirely of yeast cells (not shown). Aerial filaments were found all around the colony periphery of the volcano shape colony (Fig. 2A). Although the central area of this colony morphotype also comprises blastoconidia, the myceliated-edge consisted mainly of hyphae (Fig. 2B), although yeast cells are also seen.

Figure 2 Electron micrographs of the *C. tropicalis* volcano shape with myceliated-edge morphotype following 96 h incubation on YPD agar. Scalebar = 200µm (A); 50µm (B). (B) Insert shows detail of myceliated edge that is composed of hyphae and blastoconidia at higher magnification (5000x)

The ring and rough colonies phenotypes also comprised mainly yeast cells as observed at the deep peripheral depressions areas (Fig. 3). Most interesting is the presence of extracellular material exclusively at these areas (Fig. 3 A, B), where many of the cells are almost hidden by this material. It was observed as fibrils, with enlarged structures, connecting neighbouring cells. In a recent study we report the presence of extracellular material, resembling a biofilm-like colony, throughout the
development of C. tropicalis switch colonies, suggesting that its presence is correlated with the complex architecture of colonies in C. tropicalis [8].

Figure 3 Electron micrographs of the C. tropicalis rough morphotype following 96 h incubation on YPD agar. Extracellular material (arrows) is seen forming a biofilm-like colony. Scalebar = 200µm (A); 50µm (B).

4. Conclusions

This study has shown that scanning electron microscopy can be used effectively to examine the microarchitecture of colonies morphotypes of the yeast pathogen C. tropicalis. Thus, SEM seems to be a useful tool to analyse the relationship between switched variant colonies and microstructure. The information derived from this study will further our understanding of the switching event in C. tropicalis.

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6. References

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