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EFFECT OF *CANDIDA TROPICALIS* IN PLANKTONIC AND BIOFILM FORM ON URINARY EPITHELIAL CELLS

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KEYWORDS
Candida tropicalis; epithelial cells; biofilm; adhesion.

INTRODUCTION

Urinary tract infections (UTI) are usually the most common type of hospital acquired infection in developed countries. Although medical devices are indispensable in the management of critically ill patients, about 20% of fungal UTI are associated to the use of urinary catheters. *Candida* species are the most frequently isolated fungi, corresponding to approximately 80% of fungal associated nosocomial infections and are the second most common species responsible for patients' mortality. *Candida tropicalis* has been reported to be one of the *Candida* species which is most likely to cause bloodstream and urinary tract infections in hospitals being responsible for a high rate of patients' mortality. Adhesion to host surfaces (epithelial cells and medical devices), as well as biofilm formation, are considered the first step to initiate *Candida* infection. Hence, the colonization of indwelling devices like urinary catheters by *C. tropicalis* poses a critical problem. Therefore, more knowledge has to be acquired in order to understand and prevent the formation of these biofilm infections.

Objective

The aim of this study was to investigate the influence of *C. tropicalis* growth form (planktonic or biofilm) in its adhesion to TCC-SUP cells (human urinary bladder).

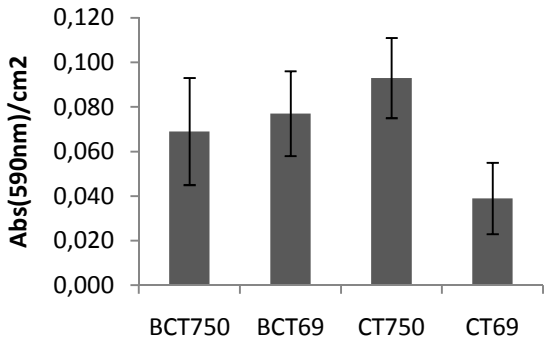
MATERIALS AND METHODS

This study was conducted with one isolate of *C. tropicalis* obtained from a patient with candiduria admitted to the intensive care unit at the University Hospital in Maringá, Paraná, Brazil and *C. tropicalis* ATCC 750 was also used, as a control. Adhesion assays were performed incubating one silicone cupon with pre-formed *C. tropicalis* 24h biofilm or 1 ml of *C. tropicalis* cell suspension (1.0 x 10^7 cells/mL), at 37ºC, on a confluent layer of epithelial cells. The extent of adhesion was evaluated after 2 h of incubation using an adaptation of the crystal violet staining method. Moreover, cell viability was also assessed, after contact with yeasts, either by trypan blue staining and using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) viability assay. Samples were also observed under scanning electron microscopy (SEM).

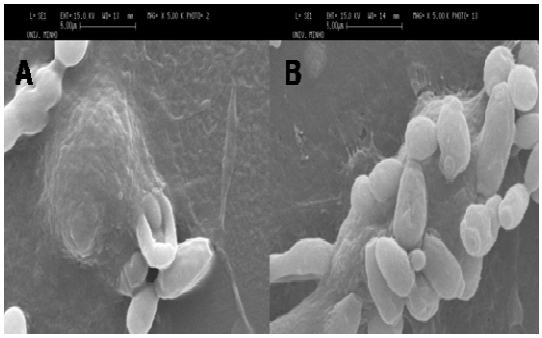
RESULTS

From the results obtained it was possible to verify that, in general, Candida cells adhered to epithelium (Fig 1). Furthermore, the clinical isolate biofilm cells adhered in higher extent than planktonic cells. Nevertheless, comparing both strains, it can be highlighted that the reference strain grown planktonically adhered significantly more ($p<0.05$) to epithelial cells than *C. tropicalis* from candiduria, which was confirmed through ultra structure analysis by SEM (Fig. 1). *C. tropicalis* in biofilm form caused higher epithelial cells death than their planktonic counterparts (Fig. 2). Moreover, epithelial cells showed less metabolic activity when in contact with biofilms.

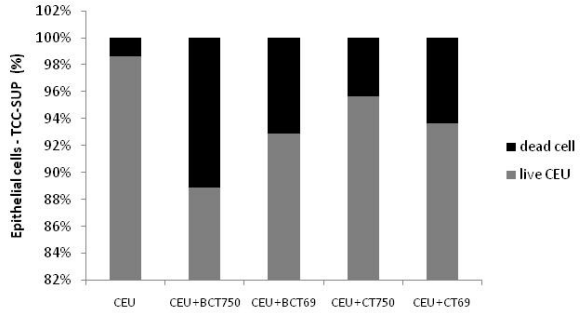
(I)



(II)



Figures 1: (I) *C. tropicalis* extent of adhesion to TCC-SUP cells after 2 hours of incubation measured by crystal violet staining expressed as absorbance/cm² (Abs/cm²). BCT750 - *C. tropicalis* ATCC 750 biofilm; BCT69 - *C. tropicalis* clinical isolate biofilm; CT750 - *C. tropicalis* ATCC 750 plantonic; CT69 - *C. tropicalis* clinical isolate plantonic. (II) SEM of yeasts adhered to TCC-SUP:. A) *C. tropicalis* from candiduria and B) *C. tropicalis* ATCC 750.



Figures 2: Death of the cells of the epithelial cell (TCC-SUP). The cell number was quantified by Trypan blue after 2h of incubation to TCC-SUP cells. CEU - epithelial cell TCC-SUP; CS - silicone coupon; BCT750 - *C. tropicalis* ATCC 750 biofilm; BCT69 - *C. tropicalis* from candiduria biofilm; CT750 - *C. tropicalis* ATCC 750; CT69 - *C. tropicalis* from candiduria.

CONCLUSIONS

Thus, it is possible to conclude that *C. tropicalis* were able to cause more epithelial cell death when in biofilm form. This highlights the importance of biofilm formation, associated to the use of urinary catheters, on *C. tropicalis* virulence.

AUTOBIOGRAPHIES

MELYSSA NEGRI graduated in Biology Science at University Pontifícia Católica of Paraná, Brazil, in 2002. She obtained her master degree in Microbiology on pathogenic yeast, in 2006 at the University of São Paulo, Brazil. She is working on Professor Rosário Oliveira's group, performing her PhD on analysis of gene expression of virulence factors: correlation with biofilms and adhesion of Candida tropicalis to epithelial cells. Her e-mail is melyssanegri@deb.uminho.pt and her web page is http://www.ceb.uminho.pt/pessoas/pid.aspx?id=200.

MARIANA HENRIQUES graduated in Biological Engineering in 1998 at University of Minho. She then post-graduated in Industrial Engineering in 2000 and obtained her PhD degree in Chemical and Biological Engineering in 2005 at the University of Minho. She is currently an invited assistant at the Department of Biological Engineering at University of Minho, and her main research interests are *Candida* species virulence factors, monoclonal antibodies production processes, and characterization of biomaterials. Her e-mail address is mcrh@deb.uminho.pt and her web page can be found at http://www.deb.uminho.pt/pessoas/docente.aspx?id=28.

ROSÁRIO OLIVEIRA graduated in Chemical Engineering at University of Luanda, in 1975. She obtained her PhD degree in Engineering Science in 1991 at the University of Minho. She is currently a full professor at the Department of Biological Engineering of the University of Minho. She is the head of the master degree in Environmental Management and head of the Biofilm research group. Her main research interests are in microbiology and environmental sciences, and animal cell culture technology. Her e-mail is roliveira@deb.uminho.pt and her web page is http://www.deb.uminho.pt/pessoas/en/docente.aspx?id=36.