Journal of Medical Microbiology (2011), **60**, 1270–1275 DOI 10.1099/jmm.0.031195-0

An *in vitro* evaluation of *Candida tropicalis* infectivity using human cell monolayers

Melyssa Negri, Cláudia Botelho, Sónia Silva, Luís Miguel Reis Henriques Lopes, Mariana Henriques, Joana Azeredo and Rosário Oliveira

Correspondence
Mariana Henriques
mcrh@deb.uminho.pt

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The aim of this study was to investigate the interaction of *Candida tropicalis* with three different human cell lines: TCC-SUP (epithelial cells from urinary bladder), HeLa (epithelial cells from cervical carcinoma) and Caco-2 (epithelial cells from colorectal adenocarcinoma). In particular we sought to assess the degree of cell damage and activity reduction induced by *C. tropicalis* adhesion and the role of secreted aspartyl proteinase (*SAP*) gene expression in this process. Two *C. tropicalis* strains were used: the reference strain ATCC 750 and a clinical isolate from urine (U69). The ability of *C. tropicalis* to adhere to a confluent layer of human cells was determined using an adaptation of the crystal violet staining method; cell damage and cell activity inhibition induced by the adhesion of *C. tropicalis* were assessed by measuring lactate dehydrogenase and tetrazolium salt (MTS) reduction, respectively. *C. tropicalis SAP* gene expression was determined by real-time PCR. Both *C. tropicalis* strains were able to adhere to the different human cells, although in a strain- and cell-line-dependent manner. Concerning the cellular response to *C. tropicalis*, the highest inhibition of cell activity was obtained for Caco-2, followed by TCC-SUP and HeLa cells. The highest percentage of cell damage (around 14 %) was observed for TCC-SUP cells in contact with the U69 isolate and for Caco-2 in contact with the reference strain. Real-time PCR analysis revealed a wide range of expression profiles of *SAP* genes for both *C. tropicalis* strains in contact with the different types of epithelial cells. *SAPT3* was the gene expressed at the highest level for both *C. tropicalis* strains in contact with the three human epithelial cell lines. The results highlight that the response of human cells to *C. tropicalis* adhesion, as well as production of SAPs, is dependent on both the strain and the epithelial cell line.

Received 10 February 2011
Accepted 9 May 2011

INTRODUCTION

Adhesion to host surfaces, such as human epithelial cells, and secretion of hydrolytic enzymes are considered important factors for *Candida tropicalis* virulence (Negri *et al.*, 2010b; Okawa *et al.*, 2008; Silva *et al.*, 2011; Zaugg *et al.*, 2001). The capacity of *C. tropicalis* to adhere to and infect host cells becomes a serious problem when patients are in intensive care units, mainly because this yeast is associated with higher dissemination potential and mortality, particularly in oncological patients (Chakrabarti *et al.*, 2009; Kothavade *et al.*, 2010; Nucci & Colombo, 2007). In order to clarify the infectivity of *Candida* species, several *in vitro* studies have been performed using human cell monolayers from urinary, vaginal and intestinal epithelia (Bendel & Hostetter, 1993; Negri *et al.*, 2010b; Sohn *et al.*, 2006).

In addition, hydrolytic enzymes such as secreted aspartyl proteinases (Saps) are known to facilitate invasion and colonization of tissues by disrupting the host mucosal membranes and by degrading important immunological and structural defence proteins (Rüchel *et al.*, 1992; Zaugg *et al.*, 2001). *C. tropicalis* possesses at least four genes encoding Saps; these are designated *SAPT1*, *SAPT2*, *SAPT3* and *SAPT4* (Zaugg *et al.*, 2001).

Since *C. tropicalis* colonizes specific body sites such as the gastrointestinal and urinary tract and is associated with severe and invasive candidosis (Pfaller, 1992), it is important to understand the mechanisms of interaction between these epithelia and *C. tropicalis*. The aim of this work was to study the effect of *C. tropicalis* colonization of different human epithelial cells and the levels of expression of *SAP* genes by *C. tropicalis* when interacting with different human cells.

Abbreviations: CV, crystal violet; LDH, lactate dehydrogenase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

METHODS

Yeasts and growth conditions. Two strains of *C. tropicalis* were used in this study, a reference strain from the American Type Culture Collection (ATCC 750) and an isolate (U69) obtained from a urine

sample of an 84-year-old female patient with candiduria admitted to the intensive care of an oncological unit and belonging to the archive collection of the University Hospital in Maringá, Paraná, Brazil. For each experiment, strains were inoculated in Sabouraud dextrose broth (SDB; Merck) and incubated for 18 h at 37 °C with agitation at 120 r.p.m. After incubation, cells were harvested by centrifugation at 8000 ***g*** for 5 min at 4 °C and washed twice with phosphate buffered saline (PBS: NaCl 8 g l^{-1}, KCl 0.2 g l^{-1}, Na_2HPO_4 1.44 g l^{-1}, KH_2PO_4 0.2 g l^{-1}; pH 7.5).

Human epithelial cell lines. To assess the adhesion ability of *C. tropicalis* to human epithelial cells, the following cell lines were used as models: (i) TCC-SUP cells, derived from human urinary bladder epithelial cells (DSMZ – German Collection of Microorganisms and Cell Cultures); (ii) HeLa cells, derived from a human cervical carcinoma, donated by 'Instituto Gulbenkian de Ciência', Lisbon, Portugal; (iii) Caco-2 cells, derived from a human colorectal adenocarcinoma cell line (ATCC HTB-37), kindly donated by Carla Nunes, Department of Biochemistry, Faculdade de Farmácia da Universidade de Coimbra, Coimbra, Portugal. Cells were cultured at 37 °C under 5 % CO_2 in Dulbecco's modified Eagle's medium (D-MEM; Gibco) containing 10 % fetal bovine serum (Gibco) and 1 % penicillin/streptomycin (P/S; Gibco). After achieving 80 % confluence, cells were detached using 25 % trypsin-EDTA (Gibco) solution; the cell concentration was adjusted to 1×10^6 cells ml^{-1} with fresh D-MEM without P/S and 1 ml of the suspension was added to the wells of a 24-well plate and incubated at 37 °C under 5 % CO_2 for 24 h. Prior to the adhesion assays, the wells were washed twice with PBS.

Adhesion assay

The yeast cells were suspended in D-MEM, without phenol red, to a final concentration of 1×10^7 yeast ml^{-1} using a Neubauer chamber (Boeco). Then, 1 ml of this suspension was added to each well of a 24-well plate covered with a confluent layer of a human epithelial cell line. After 2 h incubation at 37 °C under 5 % CO_2, each well was washed once with PBS to remove unattached yeasts.

***C. tropicalis* quantification.** The adhered yeasts were quantified using the crystal violet (CV) staining method, according to Negri *et al.* (2010a). The mean absorbance of yeasts was expressed as the absorbance per area of each well and standardized by the number of adhered yeasts per area of each well using a *C. tropicalis* standard curve (Negri *et al.*, 2010a, b). All the procedures were repeated in triplicate in at least three separate assays.

Determination of percentage inhibition of cell activity. After the washing step with PBS, the remaining adhered yeasts were killed by incubating the well plates for 2 h and adding a 1 % amphotericin B (AB) solution (Sigma, USA, 250 μg ml^{-1}) in D-MEM without phenol red at 37 °C and 5 % CO_2. The AB solution was then discarded and the epithelial cells' activity was determined using the CellTiter 96 assay (Promega), based on the reduction of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] with 1 % AB in D-MEM without phenol red. MTS is bioreduced by human epithelial cells into a formazan product that is soluble in tissue-culture medium. After 2 h incubation at 37 °C in the dark, the absorbance of the formazan was measured at 490 nm. A control was performed by measuring the cellular activity of human cells grown in the same conditions but in the absence of yeast cells. The effect of *C. tropicalis* on human epithelial cells was expressed as the percentage inhibition of cell activity standardized by the number of adhered yeast cells, with the MTS control corresponding to 100 % activity of human cells, according to the following equation: percentage inhibition $= 100 \times [(MTS_{Control} - MTS_{With\ \textit{C. tropicalis}})/MTS_{Control}]$/No. of adhered yeasts.

All the procedures were repeated in triplicate in at least three separate assays.

Assay for epithelial cell damage. The release of lactate dehydrogenase (LDH) by epithelial cells into the culture medium was used as a measure of cell damage. The LDH concentration in the medium was measured after 2 h of adhesion using the CytoTox-ONE kit (Promega), following the manufacturer's instructions. Two controls for LDH activity were prepared: (i) epithelial cells grown in the absence of *C. tropicalis* and (ii) yeast cells as sole culture. The LDH concentrations of both controls were subtracted from the LDH released by epithelial cells infected with yeasts. The effect of *C. tropicalis* on epithelial cells was expressed as the percentage of LDH released per number of adhered yeast cells, taking as 100 % the concentration of LDH released by a completely killed epithelial cell monolayer (using the killing buffer provided with the kit), according to the following equation: percentage cell damage $= 100 \times \{LDH_{Assay} - [(LDH_{Control\ (i)} + LDH_{Control\ (ii)})]/LDH_{Killed\ epithelial\ cells}\}$/No. of adhered yeast cells.

All experiments were performed in triplicate.

Analysis of *SAP* gene expression

RNA extraction. Prior to RNA extraction, the adhered yeasts were removed with 500 μl lysis buffer (Invitrogen) and transferred to screw-cap tubes (Bioplastics). Then glass beads (0.5 mm diameter, approximately 500 μl) were added and the tubes were homogenized twice for 30 s, using a Mini-BeadBeater-8 (Stratech Scientific). After disruption of the yeast cells, the PureLink RNA Mini kit (Invitrogen) was used for total RNA extraction according to the manufacturer's recommended protocol. To avoid potential DNA contamination the samples were treated with RNase-free DNase I (Invitrogen).

Primers. The primers used for real-time PCR (RT-PCR) were described by Silva *et al.* (2011) and their sequences are listed in Table 1.

Synthesis of cDNA. To synthesize the complementary DNA (cDNA) the iScript cDNA Synthesis kit (Bio-Rad) was used according to the manufacturer's instructions. For each sample 10 μl extracted RNA was used.

Real-time PCR. Real-time PCR (CF X96 Real-Time PCR System; Bio-Rad) was used to determine the relative levels of *SAPT1–4* mRNA transcripts, with actin 1 (*ACT1*) as a reference housekeeping gene. Each reaction mixture consisted of a working concentration of SsoFast EvaGreen Supermix (Bio-Rad), 300 nM forward and reverse primer, and 1 μl cDNA, in a final reaction volume of 20 μl. Negative controls (water) were included in each run. The relative quantification of *SAPT1–4* gene expression was performed by the ΔC_t method. Each reaction was performed in triplicate and mean values of relative expression were determined for each *SAP* gene.

Statistical analysis. The results obtained were analysed using the SPSS 18 (Statistical Package for the Social Sciences) program. One-way ANOVA with the Bonferroni test was used to compare the number of yeasts adhering to epithelial cells, and cell activity and damage. All tests were performed with a confidence level of 95 %. All the experiments were performed in triplicate and in three independent assays.

RESULTS

The cellular activity and the degree of cell damage as a result of the interaction of *C. tropicalis* with the different human epithelial cell lines (TCC-SUP, HeLa and Caco-2) are presented in Table 2; the results for *SAPT1–4* gene expression are presented in Table 3. In general, the

Table 1. Primers used for real-time-PCR analysis of *SAP* and control (*ACT1*) gene expression

Sequence (5′→3′)	Primer	Target	PCR product size (bp)
GGAAGATCTGATGTGCCAACTACATTGA	Forward	*SAPT1*	1005
CGTGCGGCCGCTCTACAAAGCCGAGATGTCT	Reverse		
TTCTTCTAGTGGTACCTGGGTCAAAG	Forward	*SAPT2*	762
CATAGATCTCTAAACAATAGTGACATTAGA	Reverse		
ACTTGGATTTCCAGCGAAGA	Forward	*SAPT3*	165
AGCCCTTCCAATGCCTAAAT	Reverse		
GTACTCGAGCTCCTACAACTTCACCTCCT	Forward	*SAPT4*	1130
CATGGATCCCTATGTAAGTGGAAGTATGTT	Reverse		
GACCGAAGCTCCAATGAATC	Forward	*ACT1*	181
AATTGGGACAACGTGGGTAA	Reverse		

C. tropicalis strains were able to adhere to the different epithelial cells and to cause a certain degree of cell damage and activity reduction. Moreover *SAP* genes were also expressed during epithelium colonization. However, these parameters were dependent on the yeast strain and on the epithelial cell line.

Curiously, strain U69, which is a clinical isolate from urine, adhered in significantly higher numbers (2.45×10^6 yeasts cm^{-2}) to intestinal cells (Caco-2) than to urinary cells (TCC-SUP) (Fig. 1). In addition, this isolate induced a greater reduction in the cellular activity of intestinal cells than of urinary cells and showed the highest levels of *SAPT1–3* gene expression (0.04, 0.03 and 6.52, respectively) when interacting with these cells. However, when in contact with urinary cells, strain U69 induced a greater percentage of cell damage (14.24 %) and a higher expression of *SAPT4* (0.11) than when in contact with the other cell lines. The interaction of this urinary isolate with cervical cells (HeLa) resulted in low levels of both inhibition of cellular activity (4.81 %) and cell damage (1.56 %), and this strain expressed only *SAPT3*.

The reference strain (ATCC 750) adhered to TCC-SUP cells (2.30×10^6 yeasts cm^{-2}), HeLa cells (1.56×10^6 yeasts cm^{-2}) and Caco-2 cells (1.48×10^6 yeasts cm^{-2}) to a similar extent ($P>0.05$). Concerning the cellular response caused by this strain, the highest inhibition of cellular activity occurred in Caco-2 cells (50.27 %); the degree of cell damage (13.79 %) and expression of *SAPT3* and *4* genes (9.71 and 0.49, respectively) were also highest for ATCC 750 when interacting with this line. HeLa cells showed less cellular response to the reference strain but higher expression of *SAPT1* and *2* (0.06 and 0.05, respectively).

It is important to highlight that both *C. tropicalis* strains affected HeLa cells to a lower extent than the other cell lines, causing low inhibition of cell activity and cell damage. *SAPT3* was the gene that exhibited the highest level of expression.

DISCUSSION

C. tropicalis is an opportunistic human pathogen, which colonizes several anatomical sites, including skin and the gastrointestinal and genito-urinary tracts (Biasoli *et al.*, 2002; Oksuz *et al.*, 2007). Colonization by *C. tropicalis*, especially from specific body sites such as the gastrointestinal and urinary tracts, is associated with a high risk of development of infection (Pfaller, 1992). Several virulence factors seem to be responsible for *C. tropicalis* infections, which have high potential for dissemination, invasion and lethality (Krcmery & Barnes, 2002; Okawa *et al.*, 2008); these include the ability to adhere to human cells and to secrete enzymes such as proteases (Galán-Ladero *et al.*, 2010; Negri *et al.*, 2010b; Zaugg *et al.*, 2001).

Table 2. Activity inhibition and damage of human cells evaluated by MTS and LDH, respectively, after *C. tropicalis* adhesion to three human cell lines

The *P*-value obtained from the comparison between the two strains is also presented. All values are means ± SD.

Cell line	Percentage inhibition of cell activity (± SD)			Percentage cell damage (± SD)		
	U69 isolate	Reference strain	*P*-value	U69 isolate	Reference strain	*P*-value
TCC-SUP	17.96 (±3.90)	6.05 (±1.32)	0.00*	14.24 (±3.73)†	11.07 (±2.17)	0.35
HeLa	4.81 (±0.91)†	5.12 (±0.99)	1.00	1.56 (±0.54)†	2.39 (±0.59)†	1.00
Caco-2	31.53 (±3.91)†	50.27 (±2.60)†	0.00*	6.16 (±0.99)	13.79 (±0.50)†	0.00*

*Statistically significant difference comparing between strains, but with the same cell lines ($P<0.05$).
†Statistically significant difference comparing among cell lines, but with the same strain ($P<0.05$).

Table 3. Secreted aspartyl proteinase (*SAPT1–4*) gene expression associated with adhesion of *C. tropicalis* to three human cell lines

Cell line	Relative expression of SAP genes*							
	U69 isolate				Reference strain			
	SAPT1	*SAPT2*	*SAPT3*	*SAPT4*	*SAPT1*	*SAPT2*	*SAPT3*	*SAPT4*
TCC-SUP	0.02 ± 0.01	0.01 ± 0.00	5.01 ± 0.63	0.11 ± 0.05	0.04 ± 0.00	0.01 ± 0.00	0.77 ± 0.85	0.07 ± 0.03
HeLa	ND	ND	0.09 ± 0.00	ND	0.06 ± 0.04	0.05 ± 0.00	5.91 ± 0.37	0.08 ± 0.01
Caco-2	0.04 ± 0.00	0.03 ± 0.01	6.52 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.02 ± 0.00	9.71 ± 0.01	0.49 ± 0.00

ND, No gene expression detected.
*Mean (±SD) arbitrary mRNA transcript levels in quantitative real-time PCR based upon triplicate measurements, presented as a percentage relative to the respective *ACT1* transcript level.

Several studies have been performed to clarify the behaviour of *Candida* species in the adhesion process, colonization and infection using human cell lines as a study model for *Candida* pathogenesis *ex vivo* (Bendel, 2003; Negri *et al.*, 2010b; Pacheco *et al.*, 2007; Saegusa *et al.*, 2007; Sohn *et al.*, 2006). However, compared to *Candida albicans*, only a few investigations have been performed to assess the virulence of *C. tropicalis*, particularly in the presence of different human cell lines. Thus, the major goal of the present study was to investigate, *ex vivo*, the potential of *C. tropicalis* to colonize and damage urinary, vaginal and intestinal epithelium. Therefore, three different cell lines were used: one from human bladder (TCC-SUP), one from human cervical carcinoma (HeLa), and one from human colorectal adenocarcinoma (Caco-2). These cell lines are very often used to study, *in vitro*, mechanisms of interaction between *Candida* and the gastrointestinal and genito-urinary tracts (Bendel & Hostetter, 1993; Negri *et al.*, 2010a, b; Sohn *et al.*, 2006).

In the present study, and corroborating other authors, *C. tropicalis* yeasts were able to adhere to and to damage all three epithelial cell lines used (Bendel & Hostetter, 1993; Negri *et al.*, 2010a; Saegusa *et al.*, 2007). Furthermore *C. tropicalis* showed a range of expression profiles of *SAP* genes (Silva *et al.*, 2011; Zaugg *et al.*, 2001), although in a strain- and cell-line-dependent manner (Negri *et al.*, 2010b; Okawa *et al.*, 2008). In previous studies, *C. tropicalis* strains have shown different behaviours in their ability to invade human tissue, with dramatic histopathological tissue alteration (Jayatilake *et al.*, 2006; Okawa *et al.*, 2008; Saegusa *et al.*, 2007; Silva *et al.*, 2011).

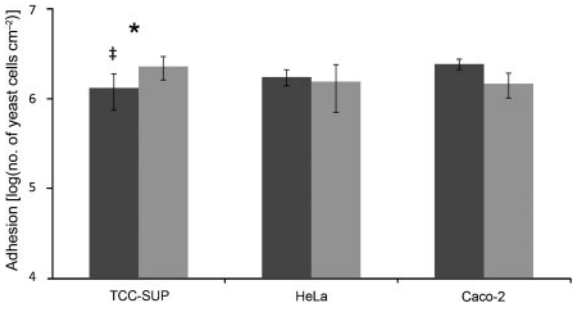

Fig. 1. *C. tropicalis* adhesion to the three cell lines (TCC-SUP, HeLa and Caco-2), analysed by the CV staining method. Dark grey bars, U69; light grey bars, reference strain (ATCC 750). *Isolates with statistically significant difference in extent of adhesion ($P<0.05$). ‡*C. tropicalis* adhesion to cell line statistically different from other cell lines ($P<0.05$). Error bars represent SD.

Comparing our results with other studies (Bendel, 2003; Negri *et al.*, 2010b; Silva *et al.*, 2011), shows that *Candida* species do not adhere to the same extent to the different types of mucosal cells, and also that there is not a strong correlation between the ability to adhere to a particular type of epithelial cells and the tissue site from which the yeast was isolated. It is important to highlight that the two strains used in this study, U69 and the reference strain, are from different clinical specimens and showed different infectivities. Strain U69 is a clinical isolate from a urine sample of a female patient from an oncology unit; the reference strain, ATCC 750, is a clinical isolate from a patient with bronchomycosis. Interestingly, strain U69 adhered to a higher extent to Caco-2 cells than to the other human cells and the reference strain adhered in similar extents to the different cell lines; curiously, it adhered in higher number to the urinary cell line TCC-SUP than did strain U69 (Fig. 1). Sohn *et al.* (2006) reported that the interaction of *C. albicans* with Caco-2 cells is distinct from the interaction with an epidermoid vulvo-vaginal cell line (A-431) and these authors suggested that this fact can be related to epithelial cells' morphology and molecular events occurring during adhesion. Furthermore, in a previous study with the same strains (Negri *et al.*, 2010b), it was confirmed that *C. tropicalis* isolate U69 adhered to the TCC-SUP cell line in lower numbers than the reference strain.

The cellular response to *C. tropicalis* adhesion was also studied, and interestingly both cell activity and integrity were affected. Although there is no direct correlation between inhibition of cell activity and cell damage, it was noticed (Table 2) that when cell inhibition was significantly high, cell damage was also more severe. To the authors'

knowledge previous studies have only evaluated cell damage (by LDH measurement) or cell activity inhibition but never both approaches. Pacheco *et al.* (2007) showed that *Candida famata* caused a low inhibition of cell activity after 2 h of contact, in contrast to *C. tropicalis* strains adhered to Caco-2 cells, which showed high inhibition of cell activity. Opportunistic pathogenic yeasts, such as *C. tropicalis*, are able to promote cellular immune response and progressive cell damage during infection (Okawa *et al.*, 2008; Saegusa *et al.*, 2007; Silva *et al.*, 2011). Moreover, Silva *et al.* (2011) demonstrated that *C. tropicalis* strains were able to cause significant tissue damage, measured by LDH release, after long periods of yeast contact with cells (12–24 h). Unfortunately, there are few studies regarding the effect of *Candida* species on the activity of human cells. Therefore, the present study is a step forward for the understanding of *C. tropicalis* pathogenesis.

Among the three cell lines, the lowest damage was observed in HeLa cells, highlighting that cell damage is dependent on the cell line. According to Sohn *et al.* (2006) *C. albicans* adhesion to epidermoid vulvo-vaginal cells seemed to occur more slowly than adhesion to Caco-2 cells and hyphal penetration into monolayers of the cell lines started after 4 h, leading to damage of the cellular substrate and marking the beginning of the tissue invasion phase. Injury of host cells by *Candida* has been described as a complex mechanism of interaction between yeasts and host cells (Filler & Sheppard, 2006; Silva *et al.*, 2011; Sohn *et al.*, 2006). However, this is mainly related to the time of infection, morphogenesis of *Candida*, morphology of host cells, and production of hydrolytic enzymes.

C. tropicalis adhesion to the epithelial cells also affected *SAP* gene expression. During the process of adhesion and invasion of host tissues, *Candida* species are known to secrete hydrolytic enzymes that damage the host cells' membrane integrity, leading to dysfunction or disruption of host structures (Zaugg *et al.*, 2001). Furthermore, the expression of *SAP* genes by *C. tropicalis* has also been demonstrated during penetration of tissues, and evading macrophages after phagocytosis of yeast cells (Monod *et al.*, 2002; Togni *et al.*, 1991; Zaugg *et al.*, 2001).

In this study, Caco-2 cells showed the highest inhibition of activity when in contact with each of the tested strains (Table 2), and strain U69 and the reference strain expressed the highest levels of *SAPT1–3* and *SAPT3–4*, respectively, when interacting with these cells (Table 3). However, strain U69 in contact with TCC-SUP cells promoted a greater percentage of cell damage and a higher expression of *SAPT4* and the same happened with the reference strain when in contact with Caco-2 cells. Studies with *C. albicans* indicate that each *SAP* gene may be related to the yeast form and to a specific function (Monod *et al.*, 2002; Naglik *et al.*, 2003; Silva *et al.*, 2011; Yang, 2003; Zaugg *et al.*, 2001). Recent data for *C. albicans* indicated that *SAP1–SAP3* genes are expressed by yeast cells only and contribute to the adhesion to human cells and tissue damage, whereas *C. albicans SAP4* expression is confined to hyphae, and has been further implicated in systemic infection and in the evasion of phagocytosis (Yang, 2003).

Among the *SAP* genes, *SAPT3* showed the highest level of expression for both strains (Table 3). Silva *et al.* (2011) studied the expression profiles of *SAP* genes for seven *C. tropicalis* strains in contact with reconstituted human oral epithelium and also determined the expression of the four genes, but *SAPT2* and *SAPT4* transcripts were detected to a similar extent to *SAPT3*. This difference in results may be related to the epithelium type and also to the different *C. tropicalis* strains studied. In the present case, the *SAP* gene expression was strain and human cell line dependent. It is important to highlight that only a few studies (Silva *et al.*, 2011; Zaugg *et al.*, 2001) have reported *C. tropicalis SAPT* gene expression during adhesion to human cells and there is also limited knowledge about the role of these enzymes in *C. tropicalis* adhesion and tissue damage.

In summary, this study shows that *C. tropicalis* is able to adhere to different human cells, influencing their response in a manner dependent on cell type and yeast strain. Our results also stress the importance of using more than one methodology to assess cell injury caused by *Candida* species. Cell damage and activity caused by *C. tropicalis* seem to be related to the expression of different *SAP* genes. As a preliminary study, this work only explored a short period of contact between yeasts and epithelial cells; more studies, including longer contact times, would be of interest, as would the inclusion of different clinical isolates. Greater knowledge of the response of human cells to *Candida* stimuli could help in the development of new therapeutic strategies.

ACKNOWLEDGEMENTS

The authors acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for supporting M. N.'s work through the grant BEX 4642/06-6 and FCT, Portugal, for supporting C. B. (SFRH/BPD/20987/2004) and S. S. (SFRH/BD/28341/2006). The authors would like to acknowledge Ana Rita Costa for the revision of the written English.

REFERENCES

Bendel, C. M. (2003). Colonization and epithelial adhesion in the pathogenesis of neonatal candidiasis. *Semin Perinatol* **27**, 357–364.

Bendel, C. M. & Hostetter, M. K. (1993). Distinct mechanisms of epithelial adhesion for *Candida albicans* and *Candida tropicalis*. Identification of the participating ligands and development of inhibitory peptides. *J Clin Invest* **92**, 1840–1849.

Biasoli, M. S., Tosello, M. E. & Magaró, H. M. (2002). Adherence of *Candida* strains isolated from the human gastrointestinal tract. *Mycoses* **45**, 465–469.

Chakrabarti, A., Chatterjee, S. S., Rao, K. L. N., Zameer, M. M., Shivaprakash, M. R., Singhi, S., Singh, R. & Varma, S. C. (2009). Recent experience with fungaemia: change in species distribution and azole resistance. *Scand J Infect Dis* **41**, 275–284.

Filler, S. G. & Sheppard, D. C. (2006). Fungal invasion of normally non-phagocytic host cells. *PLoS Pathog* **2**, e129.

Galán-Ladero, M. A., Blanco, M. T., Sacristán, B., Fernández-Calderón, M. C., Pérez-Giraldo, C. & Gómez-García, A. C. (2010). Enzymatic activities of *Candida tropicalis* isolated from hospitalized patients. *Med Mycol* **48**, 207–210.

Jayatilake, J. A., Samaranayake, Y. H., Cheung, L. K. & Samaranayake, L. P. (2006). Quantitative evaluation of tissue invasion by wild type, hyphal and *SAP* mutants of *Candida albicans*, and non-*albicans Candida* species in reconstituted human oral epithelium. *J Oral Pathol Med* **35**, 484–491.

Kothavade, R. J., Kura, M. M., Valand, A. G. & Panthaki, M. H. (2010). *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol* **59**, 873–880.

Krcmery, V. & Barnes, A. J. (2002). Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J Hosp Infect* **50**, 243–260.

Monod, M., Capoccia, S., Léchenne, B., Zaugg, C., Holdom, M. & Jousson, O. (2002). Secreted proteases from pathogenic fungi. *Int J Med Microbiol* **292**, 405–419.

Naglik, J. R., Challacombe, S. J. & Hube, B. (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* **67**, 400–428.

Negri, M., Gonçalves, V., Silva, S., Henriques, M., Azeredo, J. & Oliveira, R. (2010a). Crystal violet staining to quantify *Candida* adhesion to epithelial cells. *Br J Microbiol* **67**, 120–125.

Negri, M., Martins, M., Henriques, M., Svidzinski, T. I., Azeredo, J. & Oliveira, R. (2010b). Examination of potential virulence factors of *Candida tropicalis* clinical isolates from hospitalized patients. *Mycopathologia* **169**, 175–182.

Nucci, M. & Colombo, A. L. (2007). Candidemia due to *Candida tropicalis*: clinical, epidemiologic, and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. *Diagn Microbiol Infect Dis* **58**, 77–82.

Okawa, Y., Miyauchi, M. & Kobayashi, H. (2008). Comparison of pathogenicity of various *Candida tropicalis* strains. *Biol Pharm Bull* **31**, 1507–1510.

Oksuz, S., Sahin, I., Yildirim, M., Gulcan, A., Yavuz, T., Kaya, D. & Koc, A. N. (2007). Phospholipase and proteinase activities in different *Candida* species isolated from anatomically distinct sites of healthy adults. *Jpn J Infect Dis* **60**, 280–283.

Pacheco, M., Pisa, D., García-Gómez, P., Carrasco, L. & Juarranz, Á. (2007). Attachment and entry of *Candida famata* in monocytes and epithelial cells. *Microsc Res Tech* **70**, 975–986.

Pfaller, M. A. (1992). Laboratory aids in the diagnosis of invasive candidiasis. *Mycopathologia* **120**, 65–72.

Rüchel, R., de Bernardis, F., Ray, T. L., Sullivan, P. A. & Cole, G. T. (1992). *Candida* acid proteinases. *J Med Vet Mycol* **30** (Suppl. 1), 123–132.

Saegusa, S., Totsuka, M., Kaminogawa, S. & Hosoi, T. (2007). Cytokine responses of intestinal epithelial-like Caco-2 cells to non-pathogenic and opportunistic pathogenic yeasts in the presence of butyric acid. *Biosci Biotechnol Biochem* **71**, 2428–2434.

Silva, S., Hooper, S. J., Henriques, M., Oliveira, R., Azeredo, J. & Williams, D. W. (2011). The role of secreted aspartyl proteinases in *Candida tropicalis* invasion and damage of oral mucosa. *Clin Microbiol Infect* **17**, 264–272.

Sohn, K., Senyürek, I., Fertey, J., Königsdorfer, A., Joffroy, C., Hauser, N., Zelt, G., Brunner, H. & Rupp, S. (2006). An *in vitro* assay to study the transcriptional response during adherence of *Candida albicans* to different human epithelia. *FEMS Yeast Res* **6**, 1085–1093.

Togni, G., Sanglard, D., Falchetto, R. & Monod, M. (1991). Isolation and nucleotide sequence of the extracellular acid protease gene (*ACP*) from the yeast *Candida tropicalis*. *FEBS Lett* **286**, 181–185.

Yang, Y. L. (2003). Virulence factors of *Candida* species. *J Microbiol Immunol Infect* **36**, 223–228.

Zaugg, C., Borg-Von Zepelin, M., Reichard, U., Sanglard, D. & Monod, M. (2001). Secreted aspartic proteinase family of *Candida tropicalis*. *Infect Immun* **69**, 405–412.