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# Adherence of clinical isolates of *Saccharomyces cerevisiae* to buccal epithelial cells

A. R. MURPHY & K. A. KAVANAGH
*Medical Mycology Unit, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland*

A number of isolates of *Saccharomyces cerevisiae* have been associated with disease in immunocompromised individuals. Such isolates display a variety of characteristics that enable colonization and persistence in the host. The aim of the work presented here was to establish whether clinical isolates of *S. cerevisiae* were capable of adhering to epithelial tissue. Adherence to host tissue has been shown to be crucial to the virulence of the pathogenic yeast *Candida albicans*, and identification of this ability in *S. cerevisiae* might indicate a role for adherence in tissue colonization by this emerging pathogen. Clinical *S. cerevisiae* isolates were found to be capable of adhering to exfoliated buccal epithelial cells (BECs) but to a lesser degree than *C. albicans*. In contrast to the situation evident with *C. albicans*, the adherence of *S. cerevisiae* isolates to BECs was not influenced by the carbon source in which the yeast was grown. Treatment of *S. cerevisiae* with trypsin or proteinase K resulted in a significant reduction in adherence ability while adherence was unaffected by treatment of cells with mannosidase, thus indicating a possible role for proteins rather than mannoproteins in the adherence of *S. cerevisiae* to BECs.

**Keywords** adherence, emerging pathogen, *Saccharomyces*, virulence factor

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## Introduction

*Saccharomyces cerevisiae* is used extensively in traditional (i.e. brewing, winemaking and baking) and modern (i.e. for the production of recombinant proteins) forms of biotechnology and as a dietary supplement. There have been numerous reports of *S. cerevisiae* inducing disease in both healthy and immunocompromised individuals [1] and the yeast has been found in many diverse body sites including the lungs [2–4], the liver and blood [2], oesophagus, scrotum and urine [4]. *S. cerevisiae* has also been found to be the aetiological agent of vaginitis in a number of instances [5,6]. *S. cerevisiae* has recently been upgraded from 'generally regarded as safe' (GRAS) status to biosafety level one (BSL-1) status [7].

Correspondence: Kevin A. Kavanagh, Medical Mycology Unit, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland. Tel.: +353 1 7083859; fax: +353 1 7083845; e-mail: Kevin.Kavanagh@may.ie

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Clinical isolates of *S. cerevisiae* exhibit several virulence factors common to other pathogenic yeasts. The ability of some *S. cerevisiae* isolates to grow at 42 °C has been shown to be important since this allows survival in the febrile host. Virulent isolates are capable of producing protease, growing as pseudohyphae [8] and of persisting and proliferating in the brains of CD-1 mice [9].

*Candida albicans* is the most frequent cause of fungal infection in humans and displays a variety of factors including adherence to epithelial tissue [10], phenotypic switching [11], production of phospholipase [12,13] and secretory aspartyl proteases (SAPs) [14], and dimorphism [10] which are considered to be important to the success of the pathogenic process. Adherence to epithelial tissue allows the yeast to colonize, penetrate and invade host tissue and is achieved by a combination of specific and non-specific mechanisms [15]. Due to the importance of adherence to the pathogenicity of *C. albicans*, an investigation into the potential of clinical

isolates of *S. cerevisiae* adhering to epithelial tissue was undertaken. Adherence could represent a novel virulence attribute in the development of *S. cerevisiae*-induced disease.

## Materials and methods

### Yeast strains

*S. cerevisiae* CBS 1227 and CBS 1464 were obtained from the Centraalbureau voor Schimmelcultures Yeast Division (Delft, the Netherlands). *S. cerevisiae* yjm 128, yjm 145 and yjm 436 were donated by Dr Karl Clemons (Santa Clara Valley Medical Center, San Diego, USA). All the above strains are clinical isolates whose sources have been described [9]. *C. albicans* ATCC 10231 was obtained from the American Type Culture Collection (Manassas, VA, USA). *S. cerevisiae* JJ1A ($a$ $arg^-$, $thr^-$) was provided by the Department of Genetics, Trinity College Dublin, Ireland. A commercially available Bakers' yeast (BY) product (McDougalls Ltd., Hampshire, UK) was also used.

### Growth conditions

All cultures were grown at 30 °C overnight in 50 ml YEPD broth (1% (w/v) yeast extract (Oxoid, Basingstoke, UK), 2% (w/v) bacteriological peptone (Oxoid) and 2% (w/v) glucose (Sigma Chemical Co., Dorset, UK)) in 100 ml conical flasks in an orbital incubator at 200 rpm. Where used, sucrose (Sigma) (YEPSuc) and galactose (Sigma) (YEPGal) replaced glucose at the same concentration.

### Adherence assay

Buccal epithelial cells (BECs) were collected from healthy human volunteers using sterile tongue depressors by gently rubbing the inside of the buccal cavity. Cells were resuspended in 5 ml of phosphate-buffered saline (PBS; pH 7·4) (GibcoBRL, Paisley, UK). BECs from four to five volunteers were pooled, centrifuged in a Beckman GS-6 centrifuge (760 *g*, 5 min, 18 °C) at 760 *g* for 5 min, washed twice by centrifugation in sterile PBS and resuspended in PBS at a concentration of $2 \times 10^5$ cells $ml^{-1}$. Yeast cultures were grown to stationary phase overnight, harvested by centrifugation at 2100 *g*, washed twice with 5 ml PBS and resuspended in PBS at a density of $1 \times 10^7$ cells $ml^{-1}$. Yeast cell suspension (1 ml) and BEC suspension (1 ml) were pooled (giving a ratio of 50:1 yeast:BEC) and incubated for 2 h at 30 °C and 200 rpm. BECs with adherent yeasts were collected by filtering through a polycarbonate membrane containing 12 $\mu$m pores and then washed gently with 10 ml PBS in order to remove any non-adhering yeast. BECs were collected in 2 ml PBS and 1 ml of this suspension was placed onto each of two glass slides per treatment. These were allowed to air dry overnight, heat-fixed by passing through a bunsen flame and stained for 30 s using 0·5% (w/v) crystal violet solution (Sigma). The number of yeasts adhering to each of 200 BECs per treatment was determined microscopically. All assays were performed in duplicate on at least two independent occasions and results calculated using the SigmaStat (Jandel Corporation, Erkrath, Germany) software programme. A Dunns *t*-test was performed to establish whether a significant difference existed between the treatments and control.

### Enzymatic degradation of cell wall components

Trypsin (Sigma) was dissolved at 1 mg $ml^{-1}$ in sterile PBS; proteinase K (Boehringer Mannheim, Germany) was dissolved in PBS and used at a concentration of 1 $\mu$g $ml^{-1}$; and mannosidase (Sigma) was dissolved in PBS and used at 1 $\mu$g $ml^{-1}$. Stationary phase yeast cells were harvested by centrifugation, washed with 5 ml PBS and resuspended at a density of $1 \times 10^7$ cells $ml^{-1}$ in PBS containing one of the above enzymes. Cells were incubated in trypsin or proteinase K for 2 h at 30 °C and 200 rpm. Cells were harvested by centrifugation, washed with PBS and mixed with exfoliated BECs in a ratio of 50:1. Adherence was measured as described.

In the case of the treatment of cells with mannosidase, controls were resuspended in PBS and incubated at 30 °C and 200 rpm for 3 h prior to the adherence assay. Cells to be treated with enzyme were resuspended in PBS and incubated at 30 °C and 200 rpm. In the 1-h treatment, the enzyme was added for the final hour of the incubation period, while in the 3-h treatment the enzyme was added at the beginning of the incubation period. In each case cells were incubated at 30 °C and 200 rpm for 3 h prior to the adherence assays being performed.

## Results

Adherence to host tissue has been established as a critical factor in the pathogenicity of *C. albicans* [10]. The primary aim of the work presented here was to establish whether clinical isolates of *S. cerevisiae* were also capable of adhering to epithelial tissue. Yeast cells were cultured as described and mixed with exfoliated BECs at a ratio of 50:1 (yeast:BEC). Adherence of yeasts to exfoliated BECs was assayed as described. The adherence of *S. cerevisiae* cells to BECs compared to that of *C. albicans* 10231 can be seen in Figure 1. In the case of all the clinical *S. cerevisiae* isolates, adherence is significantly greater ($P < 0{\cdot}05$) than that of *S. cerevisiae*

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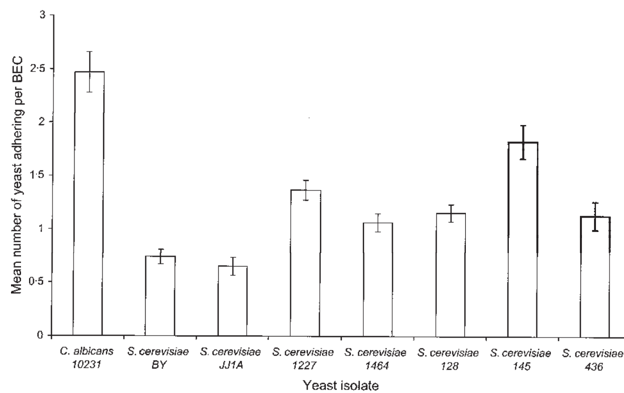


**Fig. 1** Adherence of yeast isolates to exfoliated buccal epithelial cells (BECs). Adherence of yeast isolates was determined by mixing yeasts and BECs (50:1) and incubating for 2 h at 30 °C and 200 rpm. Data are the mean adherence ±SE.

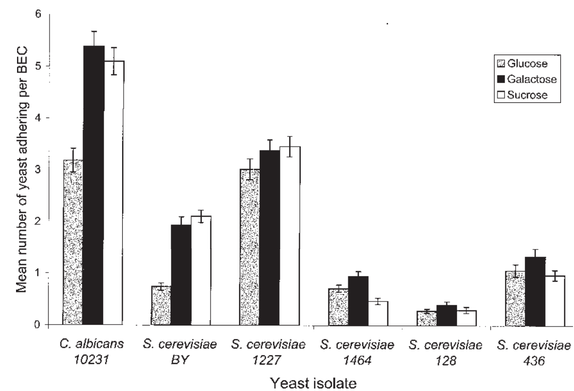


**Fig. 2** Effect of carbon source on the adherence of yeast isolates to buccal epithelial cells (BECs). Yeasts were grown to the stationary phase in YEPD, YEPGal or YEPSuc. Adherence assays were performed as described. Data are the mean adherence ±SE.

JJ1A and Baker's yeast, but less than that of *C. albicans*. The clinical *S. cerevisiae* isolates demonstrate adherence levels of between 43 and 74% that of *C. albicans* with strain 145 exhibiting the highest adherence level.

Previous investigations into factors affecting the adherence of *C. albicans* have shown that the level of adherence to epithelial tissue is influenced by the carbon source in which the yeast is cultured [16,17]. *C. albicans* 10231, a commercially available Bakers' yeast product and four of the clinical *S. cerevisiae* isolates (1227, 1464, 128 and 436) were grown to the stationary phase in YEPD, YEPGal or YEPSuc and adherence assays were performed as described. The results indicate that *C. albicans* cells grown in galactose or sucrose exhibit elevated levels of adherence (Fig. 2). Bakers' yeast manifested low levels of adherence when grown in glucose but greatly increased levels were evident when cells were cultured in galactose or sucrose (Fig. 2). *S. cerevisiae* 1227 exhibited no significant ($P < 0.05$) change in adherence ability regardless of the carbon source, showing levels similar to that of the control *C. albicans* isolate when grown in glucose medium. The other three *S. cerevisiae* isolates (1464, 128 and 436) also displayed no significant change ($P < 0.05$) in adherence ability when cultured in the different growth media.

Elements of the *C. albicans* cell wall, including proteins and mannoproteins, have been implicated in adherence of the yeast to host tissue [18]. A number of cell surface-modifying agents were employed against *S. cerevisiae* in order to establish the relative importance of the major cell wall components in the adherence process. Stationary phase yeast cells were harvested, washed and incubated in PBS containing trypsin (1 mg ml$^{-1}$) at 30 °C and 200 rpm for 2 h. Cells were subsequently washed twice in 5 ml PBS prior to pooling with BECs at a ratio of 50:1 (yeast:BECs). The results (Table 1) indicate that *C. albicans* exhibited a 64% reduction in adherence to BECs when treated with trypsin. Treatment of Bakers' yeast with trypsin reduced the adherence ability by approximately 78%. Three of the clinical *S. cerevisiae* isolates (1227, 1464 and 128) showed reductions in adherence ability of between 47 and 84%

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**Table 1** Effect of trypsin and proteinase K on the adherence ability of yeast isolates to buccal epithelial cells (BECs)

| | Trypsin 1 mg ml$^{-1}$ | | Proteinase K 1 mg ml$^{-1}$ | |
|---|---|---|---|---|
| | Control cells | Treated cells | Control cells | Treated cells |
| *C. albicans* 10231 | 2·47 ± 0·14 | 0·92 ± 0·08 | 1·17 ± 0·08 | 0·64 ± 0·06 |
| *S. cerevisiae* BY | 1·16 ± 0·09 | 0·61 ± 0·07 | 0·99 ± 0·10 | 0·22 ± 0·03 |
| *S. cerevisiae* 1227 | 1·45 ± 0·09 | 0·23 ± 0·03 | 0·58 ± 0·04 | 0·20 ± 0·03 |
| *S. cerevisiae* 1464 | 1·92 ± 0·08 | 0·46 ± 0·04 | 0·49 ± 0·02 | 0·14 ± 0·05 |
| *S. cerevisiae* 128 | 0·96 ± 0·10 | 0·50 ± 0·05 | 0·27 ± 0·03 | 0·18 ± 0·02 |
| *S. cerevisiae* 436 | 1·10 ± 0·10 | 1·08 ± 0·12 | 0·76 ± 0·08 | 0·57 ± 0·07 |

Data are the mean number of yeast adhering to a single BEC cell ± SE.
Stationary phase yeast cells were treated with trypsin or proteinase K for 2 h at 30 °C and 200 rpm. Cells were harvested by centrifugation, washed with PBS and resuspended with BECs at a ratio of 50:1. Adherence was measured as described.

when exposed to trypsin. Only isolate 436 showed no significant reduction in adherence capability after treatment with this enzyme.

Pre-treatment of yeast cells with proteinase K for 2 h at 30 °C produced a 45% reduction in adherence of *C. albicans* to BECs (Table 1). A reduction of 78% in adherence ability was observed when Baker's yeast was treated with proteinase K and all the clinical *S. cerevisiae* isolates treated with this enzyme showed reductions in adherence ranging from 25% to approximately 70%.

Stationary phase yeast cells were treated with mannosidase for 1 or 3 h before being harvested by centrifugation, washed and mixed with BECs. The results of these assays (Table 2) indicate a decrease in *C. albicans* adherence to BECs of approximately 60% after 1 h but no further significant change ($P < 0{\cdot}05$) after 3 h exposure to mannosidase. Treatment of *S. cerevisiae* 1227 with mannosidase for 1 h produced no reduction in adherence although a slight inhibition was evident after three hours exposure. *S. cerevisiae* isolates 1464, 128 and 436 exposed to mannosidase for 3 h showed no reduction in adherence ability but may have displayed a small elevation in adherence ability to BECs. Similarly, the treatment of Bakers' yeast with mannosidase for 3 h may have resulted in a slight increase in adherence ability.

## Discussion

Adherence is crucial to the pathogenicity of *C. albicans* [10] and is mediated by receptor-ligand interactions [19,20] and non-specific factors [21]. The aim of the work presented here was to establish whether isolates of *S. cerevisiae* that had been implicated in cases of disease were capable of adhering to epithelial tissue since this could represent a previously uncharacterized virulence factor.

**Table 2** Effect of mannosidase on the adherence ability of yeast isolates to buccal epithelial cells (BECs)

| | Mannosidase 1 μg ml$^{-1}$ | | |
|---|---|---|---|
| | Control cells | 1-h samples | 3-h samples |
| *C. albicans* 10231 | 2·57 ± 0·12 | 1·03 ± 0·07 | 0·95 ± 0·07 |
| *S. cerevisiae* BY | 1·33 ± 0·10 | 1·10 ± 0·11 | 1·49 ± 0·14 |
| *S. cerevisiae* 1227 | 1·71 ± 0·09 | 1·70 ± 0·08 | 1·02 ± 0·08 |
| *S. cerevisiae* 1464 | 0·66 ± 0·06 | ND | 0·82 ± 0·08 |
| *S. cerevisiae* 128 | 0·75 ± 0·08 | ND | 0·96 ± 0·10 |
| *S. cerevisiae* 436 | 0·68 ± 0·06 | 0·92 ± 0·07 | 0·81 ± 0·07 |

Data are the mean number of yeast cells adhering to a single BEC cell ± SE. Stationary phase yeast cells were treated with mannosidase for 1 or 3 h at 30 °C and 200 rpm. Cells were harvested by centrifugation, washed with PBS and resuspended with BECs at a ratio of 50:1. Adherence was measured as described. ND, not determined.

Clinical *S. cerevisiae* isolates adhered to exfoliated BECs but to a lesser extent than *C. albicans* 10231. *C. albicans* 10231 and Bakers' yeast showed significant increases in adherence ability when cultured in galactose or sucrose while the adherence of clinical *S. cerevisiae* isolates to BECs showed no significant change when cultured in different carbon sources. The nature of the carbon source has been shown previously to have a profound effect on the adherence ability of *C. albicans* to BECs [16] and cultured cells [22] and has been linked to changes in the yeast cell surface, particularly in the production and secretion of mannoprotein, a putative adhesin associated with the fibrillar layer of the cell surface [23]. This work indicates that while the adherence of Bakers' yeast to BECs is influenced by the nature of the carbon source used for growth this effect is not evident in clinical *S. cerevisiae* isolates.

Modification of cell wall proteins using either trypsin or proteinase K (Table 1) resulted in significant reductions in adherence of clinical *S. cerevisiae* isolates to BECs. Only *S. cerevisiae* 436 showed no change in adherence ability when treated with trypsin. Proteinase K treatment reduced the adherence of clinical *S. cerevisiae* isolates from 25 to 65%. Mannoproteins play an important role in the adherence of *C. albicans* to host tissue [18]. The treatment of *C. albicans* with mannosidase caused a substantial decrease (60%) in adherence to BECs. *S. cerevisiae* 1227 exhibited a small decrease in adherence following treatment with mannosidase for 3 h while no significant reduction was observed with isolates 1464, 128 and 436. Previous work has demonstrated that *S. cerevisiae* mannan is insensitive to the action of mannosidase [24]. The results presented here may indicate some degree of heterogeneity in the susceptibility to this enzyme among clinical *S. cerevisiae* isolates. Cell wall proteins seem to play an important role in the adherence ability of clinical *S. cerevisiae* isolates and mannoproteins do not have as important a role as evident in *C. albicans*. Flocculation in brewing strains of *S. cerevisiae* can be reduced substantially by treatment of cells with protease indicating that cell surface associated proteins are important in mediating cell–cell interactions leading to floc formation [25]. Similar proteins may play a role in the adherence of clinical *S. cerevisiae* isolates to BECs.

Proteins which have been identified as potential adhesins in *C. albicans* include the products of the *ALS1* gene [26] and the *ALA1* gene [27]. The *ALS1* gene has extensive homology to the *S. cerevisiae* gene *AGα1* which produces an agglutination protein [26]. The expresion of the AGα1 protein product may be enhanced in clinical *S. cerevisiae* isolates thus mediating adherence. Clinical isolates of *S. cerevisiae* have been

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shown previously to be capable of aggregating and blocking capillaries in experimental models [9]. This suggests that cells must have some ability to adhere to one another and/or to the capillary wall. Several *Candida* species and clinical *S. cerevisiae* isolates have been examined for integrin expression [20] and it was found that clinical isolates of *S. cerevisiae* exhibited low levels of integrin analogue expression but demonstrated some ability to adhere to a cervical epithelial cultured cell line (Hela S3).

Clinical isolates of *S. cerevisiae* show significantly greater levels of adherence to exfoliated BECs when cultured in YEPD compared to a laboratory *S. cerevisiae* isolate (JJ1A) and Baker's yeast ($P < 0{\cdot}05$) but levels of adherence for all clinical isolates were less than that of *C. albicans* 10231. The ability of clinical *S. cerevisiae* isolates to adhere to epithelial cells may represent a novel virulence factor. In particular, adherence may be important in cases where the vagina [5,6] or oropharynx [28] are colonized by this yeast. Adherence to epithelial tissue has previously been shown to be crucial for the successful colonization of these body sites by *C. albicans* [15]. The results presented here suggest that *S. cerevisiae* adherence appears to be mediated by adhesins which are proteinaceous in nature and this may indicate that proteins associated with aggregation or flocculation are involved in facilitating adherence to epithelial tissue. Adherence may assist in the colonization of tissue by *S. cerevisiae in vivo*.

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