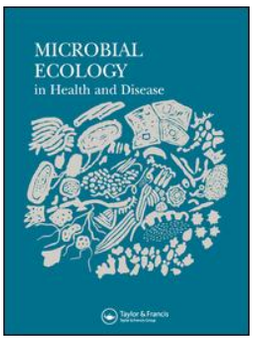

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ORIGINAL ARTICLE

The influence of dietary carbohydrates on *in vitro* adherence of four *Candida* species to human buccal epithelial cells

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Abstract
The adherence of four *Candida* species to human buccal epithelial cells (BECs) following treatment with the most commonly consumed dietary carbohydrates was investigated *in vitro*. Adhesion of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis* was significantly promoted by incubation in minimal medium containing a high concentration (500 mM) of fructose, galactose, glucose, maltose, sorbitol or sucrose ($p < 0.001$). *C. albicans* grown in galactose elicited maximal increase in adhesion followed by glucose and sucrose. Maltose and fructose also promoted adherence of *Candida* spp. but to a lesser extent than galactose and glucose, while no statistical difference in adherence was observed when *Candida* spp. were grown in the presence of lactose and trehalose. Xylitol significantly reduced adherence of *Candida* spp. to BECs. No statistically significant difference in the adherence capabilities of different growth phases of *C. albicans* was noted, and the effect of galactose and glucose persisted irrespective of the phase of growth used. The dietary carbohydrates, therefore, might represent a risk factor for oral candidosis. The limitation of their consumption by substituting xylitol could be of value in the control of oral *Candida* colonization and infection.

Key words: Candida, *adhesion, buccal epithelial cells, carbohydrates*

Introduction

Oral candidosis is a common opportunistic infection in cancer patients and currently ranks as the most common human fungal disease. *Candida albicans*, the major human pathogen of the genus *Candida*, colonizes human mucosal surfaces, particularly those within the oral cavity and vagina, and may become haematogenously disseminated in immunocompromised persons (1,2). Adherence of *Candida* spp. to host surfaces is thought to be a crucial step in the pathogenic process and a prerequisite for colonization of these surfaces (2). This adhesion occurs by the interaction between yeast and epithelial cell receptors, and a variety of mechanisms have been proposed (2,3). Yeast cells bind to galactoside-containing receptors on epithelial cells (4), express a mannoprotein adhesin that recognizes fucosyl determinants of epithelial cell membrane glycosides (5), and carry surface carbohydrates important for binding to epithelial cells (6). *C. albicans* adheres also to extracellular matrix proteins (7,8), possibly with the involvement of adhesins that mimic the complement receptors CR2 and CR3 (9). Moreover, it has been shown that *C. albicans* binds to salivary components, including mucins (10), proteoglycan (11), and proline-rich protein (12), suggesting that *C. albicans* has multiple mechanisms for adherence in the oral cavity. It is well known that *C. albicans* adhesion to mucosal and artificial surfaces in the mouth is enhanced by several factors such as germ-tube production, phospholipase, protease, other extracellular enzymatic activities, carbohydrates, pH and temperature (2,3).

Dietary sugars, such as glucose and sucrose commonly consumed, may be of importance in the pathogenesis of *C. albicans* in the oral cavity, given the effect of such sugars on the adherence of the yeast *in vitro* (13). *C. albicans* cells grown in defined medium supplemented with a high concentration (500 mM) of dietary carbohydrates exhibited increased adherence to acrylic surfaces (14,15). Samaranayake et al. (14) showed that yeasts incubated in sucrose and glucose had a better adhesion to acrylic strips than controls grown in sugar-free

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media. Growth in the presence of lactose and xylitol showed no significant difference when compared with the control yeasts (15). Pre-incubation of *C. albicans* in the presence of a range of sucrose concentrations (50–500 mM) gave a significant positive correlation between the number of yeasts adherent to acrylic surfaces or epithelial cells and the sucrose concentration (14,15). Similarly, Douglas and co-workers (16–18) showed that adherence of *C. albicans* to acrylic surfaces, as well as epithelial cells, was enhanced significantly after growth of the yeast to the stationary phase in media containing high concentrations of various sugars as the carbon source. Galactose was the most effective sugar tested and fructose the least (17). Electron microscopy revealed that incubation in galactose or sucrose results in a greater production of the outer fibrillar-floccular layer of the cell wall (17,18). This layer, composed primarily of mannoproteins, has been recognized as being responsible for the increased adherence to acrylics (19,20). *Candida* adherence to epithelial surfaces appeared to be similarly promoted, but relatively few investigations compared the adhesion of *Candida* cells after growth in various carbohydrates (14–18). The effects of a number of carbohydrates, such as fructose, lactose, maltose and xylitol, are also poorly understood, and there are no data, to the best of our knowledge, on the adhesion of sorbitol- and trehalose-grown yeasts. Furthermore, most of the reports currently available deal with *C. albicans*, thus there is little information on the epithelial adhesion of non-*albicans* species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*, which have emerged as significant pathogens in immunocompromised patients (1,2). Based on the above, it was the aim of this study to investigate the effects of the most commonly consumed dietary carbohydrates on the *in vitro* adhesion of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis* to buccal epithelial cells.

Materials and methods

Organisms

Four species of *Candida* were used in this study: *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. One strain each of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* was isolated from cancer patients with oral candidosis. Methods of isolation, identification and antifungal susceptibility testing have been described elsewhere (21). Four strains from the American Type Culture Collection (*C. albicans* ATCC 36082, *C. tropicalis* ATCC 750, *C. glabrata* ATCC 22553 and *C. parapsilosis* ATCC 22019) were also used. All organisms were stored in Sabouraud dextrose agar (SDA) medium (Difco Laboratories, Detroit, MI, USA), kept at 4°C and subcultured routinely.

Growth conditions

Overnight cultures of various *Candida* spp. were grown at 37°C in yeast nitrogen base medium (Difco) supplemented with 2.5% glucose. Flasks containing 50 ml of minimal media (ammonium sulphate 1 g/l and monopotassium sulphate 1 g/l, adjusted to pH 6.0) and supplemented with 50 mM glucose, or 500 mM fructose, galactose, glucose, lactose, maltose, sorbitol, sucrose, trehalose and xylitol, were inoculated with 1 ml of the overnight culture and some were grown for 12 h (exponential phase), while others were grown for 24 h (stationary phase) in a shaking water bath (160 rpm) at 37°C. Yeast cells were harvested by centrifugation at 1200 *g* for 10 min and the pellet was washed twice, with 10 ml of Hanks's balanced salts solution (HBSS), pH 7.0. A final yeast suspension of 1×10^7 cells/ml was prepared by appropriate dilution in HBSS followed by haemocytometer counting. These cells were used in the adherence assay.

Preparation of buccal epithelial cells for adherence assays

Buccal epithelial cells (BECs) were collected during the early morning from six healthy adult fasting male volunteers by gentle rubbing of the mucosal surface of the cheeks with a sterile tongue depressor. BECs were washed twice with HBSS and collected by centrifugation (500 *g*, 10 min). This step was intended to wash away saliva and other contaminating oral secretions. These cells were then used to study the adhesion of *Candida* spp. to BECs following exposure of yeast to dietary carbohydrates. A final suspension of 2×10^5 BECs/ml was prepared by appropriate dilution in HBSS after haemocytometer counting. Only freshly prepared samples of BECs were used in adherence assays.

Adherence assay

The candidal adhesion assay was performed as described previously (21). Briefly, a mixture of equal volumes of BECs (2×10^5 BECs/ml) and yeast cells (1×10^7 yeast/ml), treated as described above, was incubated at 37°C for 2 h in a shaking bath at 180 rpm. Cells were filtered through a 20 μm pore size filter (Retsch, Idar-Oberstein, Haan, Germany) to remove non-adherent yeast cells. The epithelial cells on the filter were washed twice with 5 ml portions of HBSS and finally suspended in 5 ml of the same buffer. A drop of this suspension was mounted on a glass slide, air-dried, heat-fixed and stained with

crystal violet for 1 min and adherence assayed microscopically at a magnification of $\times 400$. The number of yeast cells adhering to every BEC was counted for 100 BECs taken at random and only uniform, unfolded epithelial cells were included. Each *Candida* species was assayed twice for adhesion to BECs on two separate occasions.

Cells of *C. albicans* ATCC 36082 as well as BECs suspended in HBSS were pretreated separately with galactose and glucose (500 mM). The mixtures were incubated for 30 min at 37°C on an orbital shaker. The cells were harvested, washed twice with HBSS, resuspended in this buffer, then standardized following haemocytometer counting and their adherence was assayed as described above.

Statistical analysis

To evaluate the differences in the adherence value among *Candida* species, the Student's *t* test was used to determine significant differences using statistical analysis software (STATISTICA for Windows, version 5.0, StatSoft Inc., Oklahoma City, OK, USA). Data are expressed as the mean $\pm$SE, and a p value of ≤ 0.05 was considered to be statistically significant.

Results

The effects of carbohydrates on adherence of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis* to BECs are shown in Table I. Results from adhesion assays are expressed as means ($\pm$standard error) of two independent experiments and on two different occasions with duplicate determinations. The adhesion of *Candida* spp. grown in carbohydrates at 500 mM (test organisms) was also compared with that of organisms grown in relatively low concentrations of glucose (50 mM; control organisms) and expressed as relative adherence.

Fructose, galactose, glucose, maltose, sorbitol and sucrose significantly enhanced adhesion of *Candida* spp. to BECs to different extents according to the carbohydrate used ($p < 0.05$ to 0.001). Galactose was the most effective carbohydrate of those tested, and galactose-grown yeasts elicited more than two-fold enhancement in adhesion (relative adherence = 2.59 for *C. albicans*) (Table I). A similar pattern of enhancement was observed for *C. tropicalis*, *C. glabrata* and *C. parapsilosis* (Table I). Lactose and trehalose also increased *C. albicans* adhesion, but the difference in adherence values between test and control organisms did not reach statistical significance ($p > 0.05$). On the other hand, *C. albicans* grown in 500 mM xylitol demonstrated a significant reduction in adherence ($p < 0.001$, relative adherence = 0.80) (Table I).

When comparing the mean number of adherent yeasts after growth in 500 mM galactose, *C. albicans* demonstrated the greatest degree of adhesion in comparison with *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. Thus, the pattern of increased adherence was *C. albicans* > *C. glabrata* > *C. tropicalis* > *C. parapsilosis*. However, lactose and trehalose were observed to be the least favourable, while galactose, glucose and sucrose at 500 mM were the most favourable for adherence among the sugars tested.

No significant difference was observed between the adherence of control exponential and stationary phase grown cells of *C. albicans* to BECs ($p > 0.05$). Growing of *C. albicans* in the presence of 500 mM galactose and glucose for 12 h (exponential phase) or for 24 h (stationary phase) enhanced the adherence of this yeast to BEC ($p < 0.05$) (Figure 1). Inclusion of galactose or glucose in the assay medium produced a reduction in the observed enhancement of adherence of *C. albicans* grown in the presence of these sugars (Figure 1). Furthermore, no significant difference was observed between the adherence to BECs of exponential grown cells of *C. albicans* in the presence of 500 mM glucose and inclusion of 500 mM glucose in the assay medium ($p > 0.05$).

The effects of pretreatment of yeast and/or buccal cells for 30 min with 500 mM galactose or glucose before assay are presented in Table II. It is apparent that the adherence was substantially enhanced in both cases as compared with the control. However, the relative adherence of 1.77 and 1.35 was obtained when both types of cells, i.e. yeast cells and BECs, were pretreated at the same time with galactose and glucose, respectively.

Discussion

We have investigated the effects of a number of dietary carbohydrates on the *in vitro* adherence of four different *Candida* spp. to BECs. The most commonly consumed dietary carbohydrates, i.e. fructose, galactose, glucose, lactose, maltose and sucrose, together with sorbitol, trehalose and xylitol, were selected for the adhesion assays. Dietary carbohydrates, such as glucose and sucrose, may be of importance in the pathogenesis of *C. albicans* in the oral cavity, given the effect of such sugars on the adherence of the yeast *in vitro*. Results from adhesion assays indicated that incubation in fructose, galactose, glucose, maltose and sucrose significantly promotes adherence of various *Candida* spp. to BECs. In contrast, yeast cells grown in 500 mM of lactose and trehalose show no significant effect on the adherence to BECs, which is consistent with

Table I. The effect of growth of various *Candida* spp. in saccharide-containing media on adherence to buccal epithelial cells (BECs).

	Candida spp.											
	C. albicans ATCC 36082			*C. tropicalis* ATCC 750			*C. glabrata* ATCC 22553			*C. parapsilosis* ATCC 22019		
Saccharide[a]	No. of yeast cells adhering to 100 BECs (mean ± SE)	Relative adherence[b]	*p* value[c]	No. of yeast cells adhering to 100 BECs (mean ± SE)	Relative adherence	*p* value	No. of yeast cells adhering to 100 BECs (mean ± SE)	Relative adherence	*p* value	No. of yeast cells adhering to 100 BECs (mean ± SE)	Relative adherence	*p* value
Glucose (50 mM)	514 ± 24	1	–	425 ± 23	1	–	486 ± 24	1	–	286 ± 24	1	–
Fructose	664 ± 29	1.29	<0.05	530 ± 21	1.25	<0.05	546 ± 26	1.12	<0.05	366 ± 21	1.28	<0.05
Galactose	1329 ± 38	2.59	<0.001	849 ± 31	2.00	<0.001	912 ± 31	1.88	<0.001	449 ± 22	1.57	<0.001
Glucose	1128 ± 34	2.19	<0.001	686 ± 26	1.61	<0.001	896 ± 26	1.84	<0.001	428 ± 21	1.50	<0.001
Lactose	540 ± 21	1.05	NS	488 ± 21	1.15	NS	532 ± 24	1.09	NS	312 ± 19	1.09	NS
Maltose	945 ± 31	1.84	<0.001	642 ± 29	1.51	<0.001	584 ± 21	1.20	<0.05	406 ± 19	1.42	<0.001
Sorbitol	600 ± 26	1.17	<0.05	512 ± 22	1.20	<0.05	539 ± 22	1.11	<0.05	348 ± 17	1.22	<0.05
Sucrose	1014 ± 39	1.97	<0.001	724 ± 29	1.70	<0.001	862 ± 29	1.77	<0.001	426 ± 24	1.49	<0.001
Trehalose	530 ± 14	1.03	NS	466 ± 19	1.10	NS	516 ± 23	1.06	NS	309 ± 14	1.08	NS
Xylitol	413 ± 19	0.80	<0.001	332 ± 14	0.78	<0.001	417 ± 22	0.86	<0.001	202 ± 18	0.71	<0.001

Values represent the means of independent experiments, each run with duplicate determination. SE, standard error. [a]Saccharides were present at 500 mM, except where indicated. [b]Adherence is expressed relative to that of 50 mM glucose-grown yeasts. [c]Differences in adhesion between yeasts grown in different saccharides at 500 mM and in 50 mM glucose were tested by Student's *t* test. NS, not significant.

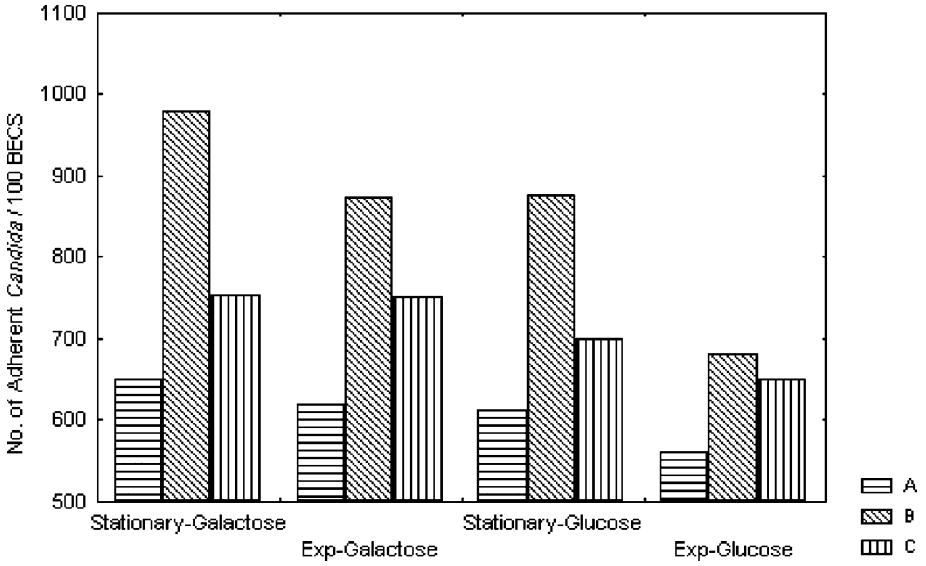

Figure 1. Effect of galactose and glucose on the adherence of *C. albicans* ATCC 36082 to buccal epithelial cells (BECs). (A) Yeasts grown for 24 h (stationary phase) or 12 h (exponential phase) without sugars (control). (B) Yeasts grown for 24 h (stationary phase) or 12 h (exponential phase) in the presence of sugars (500 mM). (C) Sugars (500 mM final concentration) included in the assay medium.

published data on the relationship between intra-oral concentration of these carbohydrates, candidal carriage and candidosis (2,13). McCourtie and Douglas (17) showed that the adherence of yeast to acrylic surfaces *in vitro* was increased after growth of the yeast in a medium containing high levels of different sugars, particularly galactose. Samaranayake and MacFarlane (15) showed that various strains of *C. albicans* pre-incubated in a medium containing glucose, sucrose, galactose, xylitol or maltose, exhibited enhanced adherence to HeLa epithelial monolayers and to BECs. The most effective sugar in this respect was maltose and the least effective was glucose (15). In these studies it was noted that adhesion was directly proportional to the sugar concentration. McCourtie and Douglas (17) found that the *C. albicans* grown on 500 mM galactose adhered to acrylic surfaces at a maximal linear rate throughout an incubation period of 1 h, whereas non-linear adhesion rates were observed with cells grown on 500 mM sucrose, 50 mM glucose or 50 mM galactose (17). However, these studies concentrated mainly on a single strain of *C. albicans* isolated from a patient with denture stomatitis, and the disparity could also be due to such an origin. Our results indicated that the adherence-promoting effect of sucrose was observed for *C. tropicalis*, *C. glabrata* and *C. parapsilosis*; maximal adhesion to BECs occurred after incubation in galactose and glucose as shown in Table I. As far as we know, there is no information available on the modulating effect of sucrose or glucose on epithelial adhesion of *C. tropicalis* and *C. parapsilosis*. However, it is conceivable that the enhanced adhesion is due to the production of a fibrillar-floccular layer, as reported for *C. albicans* (19). Glucose can also promote acid production and lower pH, with consequent activation of acid proteinases and extracellular phospholipases, factors involved in yeast adhesion (22,23).

Yeasts incubated in media containing lactose showed no significant difference in adherence to

Table II. Effect of pretreatment of yeast cells and/or BECs with galactose and glucose on the adherence of *C. albicans* ATCC 36082 to BECs.

Pretreatment		Galactose (500 mM)		Glucose (500 mM)	
Yeast cells	Epithelial cells	No. of yeast adhering to 100 BECs (mean ± SE)	Relative adherence[a]	No. of yeast adhering to 100 BECs (mean ± SE)	Relative adherence[a]
–	– (control)	434 ± 21	1	434 ± 21	1
+	–	571 ± 27	1.32*	530 ± 25	1.22*
–	+	560 ± 24	1.29*	500 ± 23	1.15*
+	+	770 ± 31	1.77*	586 ± 28	1.35*

[a]Compared with control.
*Significant difference from control ($p < 0.05$).

BECs when compared with control yeasts (14). Moreover, Samaranayake and MacFarlane (15) found that the xylitol-grown *C. albicans* cells are up to two times more adherent to epithelial surfaces than control-grown cells. Our results indicated that *Candida* spp. incubated in 500 mM xylitol exhibited a significant inhibition in adhesion. Xylitol is incorporated in toothpaste and mouth rinse formulations, as well as in chewing-gum, for its anticariogenic effect. This alditol is not metabolized by oral bacteria and lower levels of *Streptococcus mutans* are found in plaque and saliva of subjects consuming such sugar substitute (24). Xylitol does not increase the *in vitro* growth of *C. albicans*, being metabolized poorly, if at all (25). The dietary intake of xylitol was reported to reduce oral candidal carriage (26), and inhibit colonization and invasion of the gastrointestinal tract in a neutropenic mouse model (27). Furthermore, giant cell production by *C. albicans* cultured in xylitol was also described (28), and it has been explained by the inability of yeast cells to catabolize or excrete the xylitol products that accumulate in the cytoplasm and induce an increased osmotic strength and cell swelling. Such conditions could account for reduced adherence through a poor production of an additional fibrillar-floccular layer on the yeast cell surface (18,19).

No statistical difference in the adherence ability of different growth phases of *C. albicans* was observed in this study. Although stationary phase yeast showed greater adherence than exponential phase cells (650 ± 22 compared to 612 ± 26 yeast adhering to 100 BECs, respectively), this was not high enough to be statistically significant ($p > 0.5$). These findings agree with those reported by Ghannoum and Abu-Elteen (29). King et al. (30) reported that stationary phase yeasts attached to vaginal epithelial cells in greater numbers than exponential phase organisms, although prolonged incubation of the culture (for more than 24 h) did not significantly enhance adhesion. In contrast, Segal et al. (31) reported a higher rate of adhesion with exponential phase yeasts. The reason for this difference is not immediately evident, although it may be attributable to the different growth media used by the three different groups. In addition, King et al. (30) and Segal et al. (31) used vaginal epithelial cells, while BECs were used in the present study and in the study by Ghannoum and Abu-Elteen (29). The effect of galactose and glucose on adherence of *C. albicans* as reported above persisted, irrespective of the growth phase.

Our findings on the adherence-promoting effect caused by the pretreatment of either the yeast cells, the epithelial cells, or both, by 500 mM galactose or glucose generally support the mechanism by which carbohydrates enhance adherence, which appears to be by the production of an additional fibrillar-floccular layer that mediates *Candida* adhesion. Tokunaga et al. (32) showed that the adhesion of *Candida* cells to BECs corresponded with increased density of the fibrillar structure at the outermost *Candida* cell wall. Another study associated adherence with the condensation or disposal of this layer, facilitating contact between epithelial cells and the deeper layer of the fungal wall (18,19,33).

In conclusion, our results indicate that epithelial adhesion of *Candida* spp. is affected to different extents by dietary carbohydrates. However, sorbitol was observed as the least favourable and galactose as the most favourable sugar for adherence among those tested. Maltose and fructose promoted adherence to a lesser extent than galactose, glucose and sucrose. Significant inhibition was observed after incubation in xylitol. Lactose and trehalose did not appear to significantly affect adhesion. These findings imply that the frequent consumption of dietary carbohydrate such as galactose, sucrose, glucose, maltose or fructose, especially in association with poor oral hygiene, might represent a risk factor for oral candidal colonization and infection. Interestingly, a high local glucose concentration leads to an increased incidence of *Candida* paronychia in sugar cane workers (34) and rinsing with sucrose leads to *Candida*-induced stomatitis in humans (35). A propagating role of a carbohydrate-rich diet in relapsing *Candida* vulvovaginitis was proposed in a study involving 240 women (36). A limited intake of these carbohydrates by substituting xylitol could be of value in the management of oral candidosis in denture wearers, diabetics and patients undergoing topical steroid therapy or prolonged antibiotics, as well as in the control of oral *Candida* colonization in patients at high risk of developing candidosis.

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