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Interactions of *Candida albicans* with bacteria and salivary molecules in oral biofilms

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The yeast *Candida albicans* coaggregates with a variety of streptococcal species, an interaction that may promote oral colonization by yeast cells. *C. albicans* and *Candida tropicalis* are the yeasts most frequently isolated from the human oral cavity and our data demonstrate that both these species bind to *Streptococcus gordonii* NCTC 7869 while two other *Candida* species (*Candida krusei* and *Candida kefyr*) do not. Adherence of *C. albicans* was greatest when the yeast had been grown at 30° C to mid-exponential growth phase. For 21 strains of *C. albicans* there was a positive correlation between the ability to adhere to *S. gordonii* and adherence to experimental salivary pellicle. Whole saliva either stimulated or slightly inhibited adherence of *C. albicans* to *S. gordonii* depending on the streptococcal growth conditions. The results suggest that the major salivary adhesins and coaggregation adhesins of *C. albicans* are co-expressed.

Keywords: *Candida albicans*; *Streptococcus gordonii*; oral biofilm; adherence

Introduction

Candida albicans is a human commensal yeast that colonizes oral, intestinal and vaginal mucosae. *Candida* species can be isolated from the oral cavities of between 18 and 40% of healthy adults, and *C. albicans*, followed by *Candida tropicalis*, is the most commonly isolated species [18]. In individuals carrying *Candida*, colonization can be persistent [4,22] and epidemiological evidence indicates that the source of infection for candidosis is often endogenous [19]. Adherence of yeast cells to host surfaces is a primary event in colonization. Survival of the organism depends on a complex interaction of factors such as the avidity of yeast cell binding, their growth rate and the effectiveness of host defences. In the oral cavity, *C. albicans* adheres to buccal and lingual epithelial cells [1], to saliva-coated surfaces [6,8] and to oral bacteria [14]. The build-up of plaque starts by the adsorption of salivary proteins to a surface to form the acquired pellicle. Bacteria that have high affinity for salivary pellicle (strains of *Streptococcus* and *Actinomyces*) are the primary colonizers, and pave the way for the adherence and accumulation of other oral microorganisms. This oral plaque biofilm represents a protected reservoir for organisms such as *Candida* species which are often not present in high numbers but which can be part of the dental plaque consortium [1,11].

Some specific *C. albicans* adherence mechanisms of relevance to oral colonization have been identified. These include *Candida* adhesins that recognize fucose or *N*-acetyl-D-glucosamine residues on human epithelial membrane glycosides [7,21], fucosyl determinants on human buccal epithelial cells [3], or human extracellular matrix proteins [16]. *C. albicans* also expresses surface adhesins that mimic the complement receptors CR2 and CR3 [5] and these adhesins may contribute to avoidance of immune protection mechanisms [9]. In this paper we show that *Candida* cells adhere to both oral bacteria and to salivary proteins, and that these interactions may be interdependent and influenced by environmental growth conditions.

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Materials and methods

Microbial strains and cultivation

S. gordonii NCTC 7869 (Channon) was cultured in Brain Heart Infusion medium (Difco Laboratories, Detroit, MI, USA) containing yeast extract (Difco), (BHY) or in Tryptone-yeast extract (Difco) medium containing 0.8% (w/v) glucose (TY-glucose) [14] at 37° C in closed tubes or bottles without shaking. Cultures were grown from standard inocula comprised of cell suspensions in BHY medium containing 15% (v/v) glycerol and stored at −80° C. Yeast strains (Table 1) were grown in glucose-salts-biotin (GSB) medium [12] containing per litre: 1 g $(NH_4)_2SO_4$, 2 g KH_2PO_4, (pH 4.5), 0.05 g $MgSO_4(7{\cdot}H_2O)$, 0.05 g $CaCl_2(2\ H_2O)$, 0.02 mg biotin, 10 g glucose.

Radioactive labelling of yeasts

Yeast cells were grown in GSB medium at 30° C or at 37° C with shaking for 16 h, and used to inoculate fresh, pre-warmed GSB (20 ml) at an initial concentration of 4×10^6 cells per ml. [^{35}S] Methionine (0.62 MBq, 17μCi, 1×10^3 Ci mmol^{-1}) was added and the cultures were incubated at 30° C or 37° C until the culture density was approximately 2×10^7 cells per ml. The cells were harvested by centrifugation (3000 *g*, 5 min, 4° C), washed twice in either TNMC buffer (1 mM Tris-HCl, pH 8.0 containing 0.15 M NaCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$) or KCl buffer (2 mM KH_2PO_4/K_2HPO_4, pH 6.5, containing 5 mM KCl and 1 mM $CaCl_2$) and suspended in the required buffer at a cell concentration of 2.0×10^8 cells per ml. The specific activity of the cells was calculated by measuring the radioactivity of a cell suspension and by using a stan-

Table 1 Yeast strains used

Yeast	Strain[a]	Source
C. albicans	10261	ATCC, 12301 Parklawn Drive, Rockville, MD 20852-1776
	S1(HMHc2)[a]; S2(ko-2c); S3(hp10bt); S4(hp36bt); S5(wo-1c); S6(RIH017); S7(hp31an); S8(hp41bt); S9(hp6ch); S10(HMHc11); S11(hp2bt); S12(jan-2c) S13(HMHc7); S14(HMHc4); S15(pra-1c); S16(crr-1c); S17(RIH010); S18(hp33bt); S19(hp42bt); S20(h01-c)	J Schmid, Massey University, Palmerston North, New Zealand
C. tropicalis	MY 820738 MY 820567	ESR Health, NZ Communicable Disease Centre, Porirua, New Zealand
C. parapsilosis	90.493	
C. krusei	89.102 90.147	
C. kefyr	78.256	

[a]Original strain designation given in brackets

dard curve relating the OD_{540} of the suspension to cell concentration. Specific activities of between 10 and 20 cells per cpm were obtained. Radioactively-labelled yeast cells were stored on ice for up to 48 h before use in adherence assays. In some experiments, radioactively-labelled *C. albicans* cells were glucose-starved by incubating them at 37° C or 30° C for 3 h in salts-biotin medium (pH 4.5) in the absence of glucose.

Saliva-coated hydroxylapatite bead adherence assay

This assay was performed as described by Cannon *et al* [6]. Saliva (unstimulated) was collected on ice from five donors, clarified, and a 50% (v/v) suspension in KCl buffer was used to coat hydroxylapatite beads (12 mg, BDH Ltd, Poole, UK). Adherence of radioactively-labelled yeast cells was determined by measuring the radioactivity associated with the beads following end-over-end mixing of beads at 22° C for 90 min with 2.5×10^6 *C. albicans* cells [6].

Assay of Candida *adherence to* S. gordonii *NCTC 7869*

Early stationary-phase streptococcal cells were harvested by centrifugation (6000 *g*, 10 min, 4° C), washed twice in TNMC buffer, and were suspended in TNMC buffer at a cell density of 4×10^8 cells per ml. Portions (50 μl) were dispensed into the wells of microtitre plates (Maxisorp, Nunc, Roskilde, Denmark) which were then centrifuged (800 *g*, 5 min, 20° C). Glutaraldehyde (0.25% w/v) in TNMC buffer (0.1 ml) was added to each well of the plate and the plates were recentrifuged and incubated at 20° C for 30 min. The contents of the wells were aspirated and the wells were washed twice with TNMC buffer containing 0.05% (v/v) Tween 20 (TNMC-Tween; 0.1 ml) and subsequently incubated with TNMC-Tween (0.1 ml) at 4° C for 16 h to block non-specific binding sites. The TNMC-Tween was aspirated and radioactively-labelled yeast cells (100 μl, final concentration 2×10^5 cells per well) in TNMC buffer were added to the wells. Plates were incubated with reciprocal shaking at 20° C for 2 h. The unattached yeast cells in suspension were discarded and the wells were washed four times with TNMC-Tween (0.1 ml). To detach bound yeast cells, 0.2% (w/v) sodium dodecyl sulphate (SDS) in 0.1 M NaOH (0.1 ml) was added to each well and the plates were incubated for at least 2 h (up to a maximum of 16 h) at 20° C. The cell suspensions were then transferred to scintillation fluid (Optiphase Hisafe, Wallac Oy, Turku, Finland) and the radioactivity was counted. Each assay was performed at least twice, using quadruplicate samples in each assay.

The effect of saliva on adherence of *C. albicans* to immobilised *S. gordonii* was determined by including whole saliva (from a single donor, blood group O, nonsecretor of Lewis antigen) diluted 1 : 5 with TNMC buffer (50 μl) in the microtitre plate assay. Alternatively, yeast or bacteria were incubated with diluted saliva at 20° C for 1 h before washing with TNMC buffer and proceeding with the assay. Yeast inputs were adjusted to a final input concentration of 2×10^5 cells per microtitre well, and adherence was determined as described above.

Results

Adherence of Candida *species to cells of* S. gordonii *NCTC 7869*

Approximately 50% of input cells of *C. albicans* ATCC 10261 bound to immobilised *S. gordonii* cells that had been cultured in BHY medium (Figure 1). *C. albicans* ATCC 10261 is a well characterized laboratory strain, which showed moderately high binding relative to 20 other strains (Table 1). Between 30 and 38% of input cells of two strains of *C. tropicalis* were bound (Figure 1). In all assays of radiolabelled *Candida* cell attachment to immobilised *S. gordonii* cells, controls were included to determine the attachment of yeasts to uncoated microtitre wells, blocked as described for the streptococcal-coated wells. Adherence to control wells did not exceed 5% of input cells. Attachment of *C. albicans* and *C. tropicalis* to immobilised BHY-grown *S. gordonii* cells was significantly higher than background binding to control wells (Student's *t*-test, $P < 0.005$). Binding of *C. parapsilosis* was also signifi-

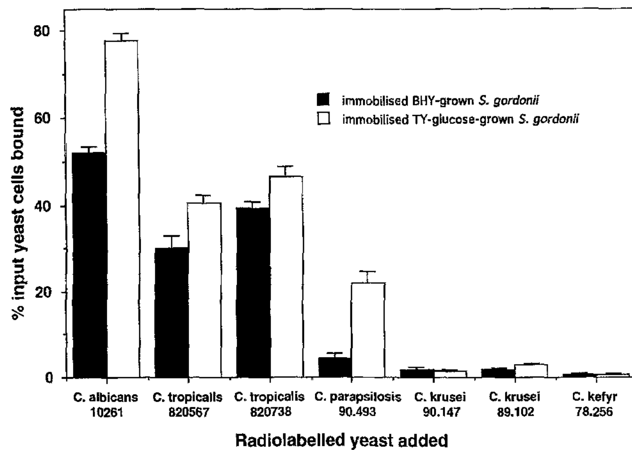

Figure 1 Relative adherence of cells of five *Candida* species to immobilised cells of *S. gordonii* NCTC 7869 that had been grown in either BHY or TY-glucose medium. Results are the mean values (±SE) from two experiments carried out in quadruplicate ($n = 8$). Adherence values are expressed as the percentage of input radiolabelled cells (2×10^5 cells per well) bound. Adherence of yeast cells to wells not coated with streptococci was <5% of input cells

cantly ($P < 0.05$) higher than background binding, whereas attachment of *C. krusei* and *C. kefyr* was not. Since complex growth media such as BHY contain blood group substances that may interfere with adhesin-receptor interactions, we also compared the ability of the same *Candida* strains to adhere to *S. gordonii* grown in TY-glucose medium. Interestingly, adherence of yeast cells to TY-glucose grown streptococci was greater in all instances, and in particular was greater for adhesion of *C. albicans* or *C. parapsilosis* cells (Figure 1).

Effect of yeast cell growth conditions on adherence of C. albicans *to* S. gordonii

Binding of *C. albicans* ATCC 10261 to immobilized *S. gordonii* cells was affected by the yeast culture conditions (Figure 2). Optimal binding was obtained using *C. albicans* cells from exponential phase cultures grown at 30° C. Yeast cells that were glucose-deprived (at pH 4.5) were less adherent than exponential phase cells. These results demonstrate that the expression of the *C. albicans* coadhesin was affected by a variety of culture conditions including temperature and nutritional status. In subsequent experiments, yeast cell adherence to *S. gordonii* NCTC 7869 was assessed utilizing exponential-phase *C. albicans* cells grown at 30° C.

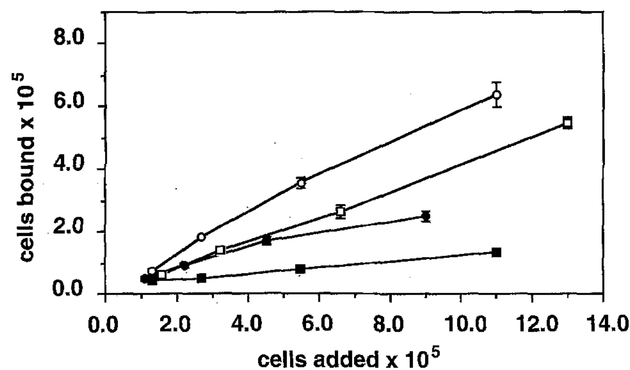

Figure 2 Effect of yeast culture conditions on the ability of *C. albicans* ATCC 10261 cells to adhere to cells of *S. gordonii* NCTC 7869 that had been grown in BHY medium. Adherence values are expressed as the mean number of yeast cells (± SE; $n = 4$) bound to quadruplicate microtitre wells coated with streptococci. (○) Exponential growth phase yeast cells grown at 30° C; (●) glucose-deprived yeast cells grown at 30° C (see Materials and methods); (□) exponential growth phase yeast cells grown at 37° C; (■) glucose-deprived yeast cells grown at 37° C. Results are from a single experiment

Comparison of adherence of various C. albicans *isolates to saliva-coated hydroxylapatite beads and adherence to cells of* S. gordonii *NCTC 7869*

A number of clinical isolates of *C. albicans* were examined for their ability to bind to immobilised cells of *S. gordonii* (grown in TY-glucose) and also to saliva-coated hydroxylapatite beads (Figure 3). Adherence data are expressed as percentage adherence relative to that of strain ATCC 10261. All strains tested adhered both to *S. gordonii* and to experimental salivary pellicle, and only four isolates (out of 20) bound better to *S. gordonii* than the laboratory strain (Figure 3). Likewise, only four isolates bound better to saliva-coated hydroxylapatite beads than did ATCC 10261, two of these were strains that also bound better to *S. gordonii* (Figure 3). A positive correlation ($r = 0.681$, $P = 0.001$) existed between the ability to adhere to *S. gordonii* and to saliva-coated hydroxylapatite beads.

Only three strains of *C. albicans* (S2, S4 and S14) showed binding to immobilised TY-glucose-grown *S. gordonii* cells of <20% (relative to binding of *C. albicans* ATCC 10261). This degree of attachment was less than that shown by *C. parapsilosis* 90.493 (Figure 1). These results show that binding to *S. gordonii* was a common phenotypic property of *C. albicans*. Selected strains were tested also for binding to BHY-grown *S. gordonii*. Even though the latter cells bound *C. albicans* to a lesser extent than TY-glucose-grown cells, the binding relative to ATCC 10261 was equivalent. For example, strain S14, which was one of the weakest binding strains to TY-grown streptococci (Figure 3), also showed only 20.2 ± 0.5% of the binding of strain ATCC 10261 to BHY-grown cells of *S. gordonii*.

Effect of salivary molecules on adherence of C. albicans *ATCC 10261 to* S. gordonii *NCTC 7869*

Since the oral cavity is bathed in salivary secretions, we determined the effects of salivary molecules on the ability of yeast cells to bind to streptococci. When the coadherence assay was performed in the presence of 20% whole saliva there was a slight (17%) inhibition of yeast binding to TY-glucose grown streptococci. In contrast, yeast-binding to streptococci was greatly stimulated by saliva if the streptococcal cells had been cultured in BHY medium (Table 2). To determine whether these effects were due to salivary molecules binding to yeast or to *S. gordonii* cells, yeast cells or immobilised streptococci were pretreated with whole saliva prior to assay for coadherence. Pretreatment of the streptococci with saliva stimulated adherence of yeast to BHY-grown cells but did not affect adherence to TY-glucose grown cells (Table 2). Conversely, treatment of yeast with saliva partially blocked binding to BHY-grown *S. gor-*

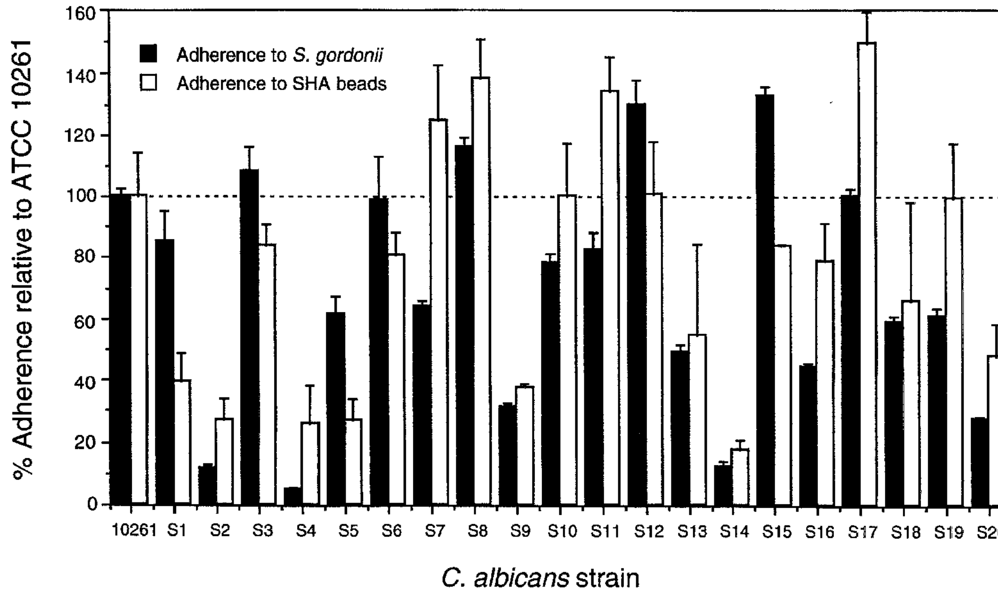

Figure 3 Adherence of 21 strains of *C. albicans* to immobilised cells of *S. gordonii* or to saliva-coated hydroxylapatite beads. Adherence values for the streptococcal assays are expressed as the mean percent *C. albicans* cells bound to quadruplicate wells (±SE; $n = 4$) relative to binding of strain ATCC 10261 (mean number of ATCC 10261 cells bound per microtitre well = 1.60×10^5). Adherence values for the saliva-coated hydroxylapatite bead assays are expressed as the percent *C. albicans* cells bound to 12 mg beads relative to binding of strain ATCC 10261 (mean number of ATCC 10261 cells bound to 12 mg beads = 9.13×10^5); where the values given are the means of triplicate samples from duplicate experiments ($n = 6$)

Table 2 Effect of saliva on coadherence of *C. albicans* ATCC 10261 with immobilised *S. gordonii* NCTC 7869

Assay conditions	Percent input[a] *C. albicans* bound ± SE	
	TY-glucose-grown *S. gordonii*	BHY-grown *S. gordonii*
Standard (no added saliva)	40.0 ± 2.9	32.4 ± 2.5
Saliva added with yeast[b]	33.3 ± 2.7*	55.9 ± 2.4**
Streptococci pretreated with saliva[c]	39.5 ± 0.7	51.1 ± 1.3**
Yeast pretreated with saliva[d]	39.6 ± 2.5	20.1 ± 1.5**

Results are from a single experiment, and the values given are the means of quadruplicates ($n = 4$). *Values significantly different from standard values (Student's *t*-test, $P < 0.05$); **significantly different from standard values ($P < 0.005$)

[a]The number of input yeast cells was 2×10^5 per microtitre well

[b]Clarified whole saliva was mixed with radioactively-labelled *C. albicans* immediately before addition to the microtitre assay

[c]Microtitre wells containing immobilised *S. gordonii* were incubated with clarified whole saliva before being washed twice with TNMC-Tween and used in the coadherence assay

[d]Radioactively-labelled *C. albicans* cells were incubated with clarified whole saliva before being washed twice with TNMC-Tween and used in the coadherence assay

donii, yet did not affect adherence to TY-glucose grown cells.

Discussion

Microbial cells, microbial macromolecules and host glycoproteins are the major components of oral biofilms. In this work we used assays to measure the adherence of *Candida* cells to experimental salivary pellicle, and to streptococcal cells, to investigate the nature of interactions that might occur within oral biofilms. Adherence to the oral bacterium *S. gordonii*, found in dental plaque and on mucosal surfaces, was shown for three *Candida* species that are frequently isolated from the oral cavity. *C. albicans* ATCC 10261 adhered to a greater degree to streptococci than did *C. tropicalis* and *C. parapsilosis* strains, while *C. krusei* and *C. kefyr* strains did not adhere significantly to streptococci. Interestingly, *C. albicans* and *C. tropicalis* are the more pathogenic species in comparative studies using animal models, and also are the species isolated more frequently from the human mouth [18]. These trends support the notion that adherent interactions of yeasts with sub-

strates in the oral cavity are associated with colonization and pathogenesis.

Environmental conditions during growth can affect yeast adherence particularly to buccal epithelial cells [15] but also to experimental salivary pellicles [6]. It was shown previously that starvation of yeast cells for glucose (at pH 6.5) stimulated ability to adhere to streptococci [14]. However, for *C. albicans* ATCC 10261 starvation at pH 6.5 also promoted autoaggregation. Therefore, yeast cell adherence to *S. gordonii* NCTC 7869 was routinely assessed using exponential-phase yeast cells grown at 30° C since they consistently bound in higher numbers to streptococcal cells (Figure 2). As growth conditions affected the adherence of yeast to streptococci, care was taken to ensure that culture conditions were standardized for all comparative experiments. Under these conditions we demonstrated a correlation between the ability of cells to adhere to streptococci and their ability to adhere to salivary pellicle. Increased growth temperature (from 30° C to 37° C) or glucose starvation at pH 4.5, 37° C, resulted in decreased binding to both experimental salivary pellicle [6] and to streptococci. These data suggest that expression of the adhesins on the yeast cell surface for streptococci and pellicle is co-regulated, a hypothesis supported by the observed correlation between the ability to adhere to *S. gordonii* and to saliva-coated hydroxylapatite beads in the present study (Figure 3).

Two alternative explanations for the results were considered. Either the coaggregation and salivary adhesins of *C. albicans* were different proteins recognising different ligands, or they were similar molecules recognising the same ligands present in saliva and on the streptococcal surface. The latter was an attractive possibility since the streptococcal cell receptors could be present in the fluid phase of whole saliva. However we know from previous work [6] that *C. albicans* cells bind to parotid saliva equally as well as to whole saliva, and parotid saliva does not contain bacterial cells or products. To try to resolve the question of whether or not the salivary and coaggregation adhesins were one and the same, the effects of saliva on coaggregation were assessed. For TY-glucose grown streptococci, pretreatment of yeast cells with saliva did not block adherence to streptococci (Table 2). Thus it seems likely that the salivary and coaggregation adhesins are different molecules. The effects of saliva on adherence of *C. albicans* to BHY-grown streptococci are less easy to interpret. It seems that streptococci, grown under these conditions, adsorb different salivary molecules that are also able to provide receptors for *C. albicans* adhesins, resulting in promotion of yeast attachment (Table 2). A similar phenomenon has been shown for oral bacterial coadherence. Adsorption of salivary molecules by *Streptococcus mutans* promotes adherence of streptococcal cells to *Actinomyces naeslundii*, *Streptococcus sanguis* and *Streptococcus mitis* [17]. In other instances salivary proteins block coadherence, such as that between the gram-negative organism *Porphyromonas gingivalis* and *S. gordonii* [20]. Saliva had only a weakly inhibitory effect on adherence of *C. albicans* to BHY-grown streptococci when preadsorbed to the yeast cells (Table 2).

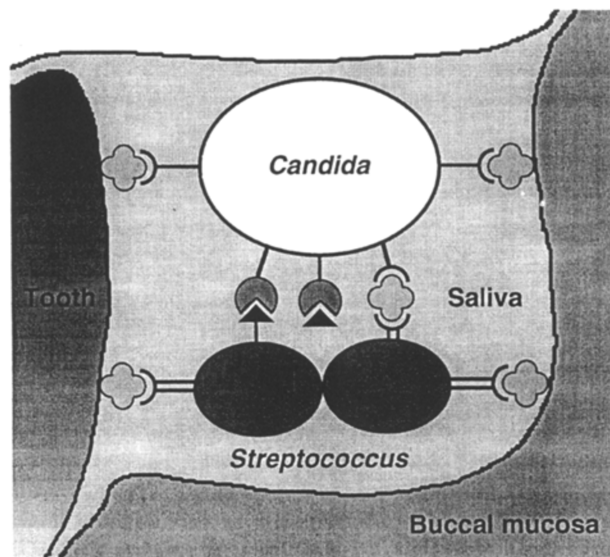

Figure 4 Schematic diagram showing possible interactions between *C. albicans*, oral streptococci, and saliva in oral biofilms. *C. albicans* adheres to salivary glycoproteins associated with the salivary pellicle and adsorbed to epithelial cells (♢). Streptococci also bind to salivary pellicle glycoproteins. Coadherence between streptococcal receptors (▶) and *C. albicans* adhesins (◎) is influenced by salivary molecules. Streptococci may bind salivary proteins (♢) that act to promote yeast–streptococcal adhesion. Alternatively, depending on the surface properties of the streptococci (influenced by growth conditions), salivary molecules may partially inhibit coadherence

Possible interactions of streptococci, yeast and salivary molecules are postulated in Figure 4. Oral streptococci possess surface adhesins for salivary receptors [10,13], and C. albicans also binds to salivary components including mucins [8] and fractions enriched in proline-rich proteins [6]. The host epithelial enzyme transglutaminase can covalently cross-link acidic proline-rich proteins to epithelial cell surface proteins [2] thus providing more receptors for *C. albicans* adherence to buccal surfaces. Some of these interactions are shown in Figure 4. In the simplified oral biofilm composed of *Streptococcus*, *Candida* and salivary molecules, the coaggregation reactions of yeast and streptococci may be enhanced by adsorption of salivary molecules by the cells to provide additional adhesin-receptor interactions. Clearly both salivary molecules and streptococci can contribute to the ability of *C. albicans* to colonize successfully the human oral cavity. Our current efforts are concentrated on characterizing the adhesins mediating binding to salivary glycoproteins and to oral streptococci.

Acknowledgements

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References

1 Arendorf TM and DM Walker. 1980. The prevalence and intra-oral distribution of *Candida albicans* in man. Arch Oral Biol 25: 1–10.
2 Bradway SD, EJ Bergey, FA Scannapieco, N Rama Subbu, S Zawacki and MJ Levine. 1992. Formation of salivary-mucosal pellicle: the role of transglutaminase. Biochem J 284: 557–564.

3 Brassart D, A Woltz, M Golliard and J-R Neeser. 1991. *In vitro* inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fucα1->2Galβ-bearing complex carbohydrates. Infect Immun 59: 1605–1613.
4 Burford-Mason AP, JM Willoughby and JC Weber. 1993. Association between gastrointestinal tract carriage of *Candida*, blood group O, and non-secretion of blood group antigens in patients with peptic ulcer. Dig Dis Sci 38: 1453–1458.
5 Calderone RA, L Linehan, E Wadsworth and AL Sandberg. 1988. Identification of C3d receptors on *Candida albicans*. Infect Immun 56: 252–258.
6 Cannon RD, AK Nand and HF Jenkinson. 1995. Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite. Microbiology 141: 213–219.
7 Critchley IA and LJ Douglas. 1987. Role of glycosides as epithelial cell receptors for *Candida albicans*. J Gen Microbiol 133: 637–643.
8 Edgerton M, FA Scannapieco, MS Reddy and MJ Levine. 1993. Human submandibular-sublingual saliva promotes adhesion of *Candida albicans* to polymethylmethacrylate. Infect Immun 61: 2644–2652.
9 Fukayama M, E Wadsworth and R Calderone. 1992. Expression of the C3d-binding protein (CR2) from *Candida albicans* during experimental candidiasis as measured by lymphoblastogenesis. Infect Immun 60: 8–12.
10 Gibbons RJ, DI Hay and DH Schlesinger. 1991. Delineation of a segment of adsorbed salivary acidic proline-rich proteins which promotes adhesion of *Streptococcus gordonii* to apatitic surfaces. Infect Immun 59: 2948–2954.
11 Grevstad HJ and KN Leknes. 1993. Ultrastructure of plaque associated with polytetrafluoroethylene (PTFE) membranes used for guided tissue regeneration. J Clin Periodontol 20: 193–198.
12 Holmes AR, RD Cannon and MG Shepherd. 1991. Effect of calcium ion uptake on *Candida albicans* morphology. FEMS Microbiol Lett 77: 187–194.
13 Jenkinson HF. 1994. Cell surface protein receptors in oral streptococci. FEMS Microbiol Lett 121: 133–140.
14 Jenkinson HF, HC Lala and MG Shepherd. 1990. Coaggregation of *Streptococcus sanguis* and other Streptococci with *Candida albicans*. Infect Immun 58: 1429–1436.
15 Kennedy MJ and RL Sandin. 1988. Influence of growth conditions on *Candida albicans* adhesion, hydrophobicity and cell wall ultrastructure. J Med Vet Mycol 26: 79–92.
16 Klotz SA, MJ Rutten, RL Smith, SR Babcock and MD Cunningham. 1993. Adherence of *Candida albicans* to immobilized extracellular matrix proteins is mediated by calcium-dependent surface glycoproteins. Microb Pathogen 14: 133–147.
17 Lamont RJ and B Rosan. 1990. Adherence of mutans streptococci to other oral bacteria. Infect Immun 58: 1738–1743.
18 Odds FC. 1988. Candida and Candidosis. Baillière Tindall, London.
19 Powderly WG, K Robinson and EJ Keath. 1993. Molecular epidemiology of recurrent oral candidiasis in human immunodeficiency virus-positive patients: evidence for two patterns of recurrence. J Infect Dis 168: 463–466.
20 Stinson MW, GG Haraszthy, XL Zhang and MJ Levine. 1992. Inhibition of *Porphyromonas gingivalis* adhesion to *Streptococcus gordonii* by human submandibular-sublingual saliva. Infect Immun 60: 2598–2604.
21 Tosh FD and LJ Douglas. 1992. Characterization of a fucoside-binding adhesin of *Candida albicans*. Infect Immun 60: 4734–4739.
22 Williamson JJ. 1972. A study of extent of variation in daily counts of *Candida albicans* in saliva. Aust Dent J 17: 106–109.