INFECTION AND IMMUNITY, May 1995, p. 1827–1834 Vol. 63, No. 5
0019-9567/95/$04.00+0

Adherence of *Candida albicans* to a Cell Surface Polysaccharide Receptor on *Streptococcus gordonii*

ANN R. HOLMES,[1] PRAMOD K. GOPAL,[2] AND HOWARD F. JENKINSON[1]*

Department of Oral Biology and Oral Pathology, University of Otago, Dunedin,[1] and New Zealand Dairy Research Institute, Palmerston North,[2] New Zealand

Received 1 November 1994/Returned for modification 20 December 1994/Accepted 7 February 1995

***Candida albicans* ATCC 10261 and CA2 bound to cells of the oral bacteria *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus sanguis* when these bacteria were immobilized onto microtiter plate wells, but they did not bind to cells of *Streptococcus mutans* or *Streptococcus salivarius*. Cell wall polysaccharide was extracted with alkali from *S. gordonii* NCTC 7869, the streptococcal species to which *C. albicans* bound with highest affinity, and was effective in blocking the coaggregation of *C. albicans* and *S. gordonii* cells in the fluid phase. When fixed to microtiter plate wells, the *S. gordonii* polysaccharide was bound by all strains of *C. albicans* tested. The polysaccharide contained Rha, Glc, GalNAc, GlcNAc, and Gal and was related compositionally to previously characterized cell wall polysaccharides from strains of *S. oralis* and *S. sanguis*. The adherence of yeast cells to the immobilized polysaccharide was not inhibitable by a number of saccharides. Antiserum raised to the *S. gordonii* NCTC 7869 polysaccharide blocked adherence of *C. albicans* ATCC 10261 to the polysaccharide. The results identify a complex cell wall polysaccharide of *S. gordonii* as the coaggregation receptor for *C. albicans*. Adherent interactions of yeast cells with streptococci and other bacteria may be important for colonization of both hard and soft oral surfaces by *C. albicans*.**

Candida albicans colonizes human mucosal surfaces, particularly those within the oral cavity and vagina, and may become hematogenously disseminated in immunocompromised persons. Adherence of *C. albicans* to epithelial cells is an initial event in colonization, and a variety of mechanisms have been proposed. Yeast cells bind to galactoside-containing receptors on epithelial cells (2, 25, 45), express a mannoprotein adhesin that recognizes fucosyl determinants of epithelial cell membrane glycosides (2, 44), and carry surface carbohydrates important for binding to epithelial cells (41) and to macrophages (35). *C. albicans* adheres also to extracellular matrix proteins (1, 5, 26, 37), possibly with the involvement of adhesins that mimic the complement receptors CR2 and CR3 (15). These adhesins may also assist in avoidance by *C. albicans* of host immune defenses (3, 15). Recently it has been shown that *C. albicans* binds to salivary components, including mucins (12, 17), proteoglycan (18), and fractions enriched in proline-rich proteins (4), suggesting that *C. albicans* has multiple mechanisms for adherence in the oral cavity.

The ability of different oral microorganisms to adhere to each other (or coaggregate) is thought to be important for the development of oral microbial plaques (32). Coadherence may be a significant colonization factor for those organisms that are not primary colonizers of salivary pellicle-coated surfaces because it would enable them to adhere to preattached organisms. Coaggregation reactions generally involve protein (lectin)-carbohydrate interactions between partner bacteria (31). The oral streptococci, especially *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus sanguis*, which are primary colonizers of salivary pellicle, elaborate both adhesins and receptors for coaggregation. The best-characterized receptors are the strain-specific polysaccharides produced by *S. oralis* and *S. sanguis*. These polysaccharides have common structural features (30), and some act as receptors for adherence of *Actinomyces naeslundii* mediated by the type 2 fimbrial adhesin (9, 10). A galactoside receptor within the *S. oralis* 34 polysaccharide is recognized by the anaerobic bacterium *Prevotella loescheii* (36).

C. albicans adheres to a variety of bacterial genera (21) and in particular exhibits high-affinity and saturable binding to a range of oral streptococci (23). Previous work has suggested that the binding of *C. albicans* to oral streptococci involves a protease-sensitive component on the yeast cells recognizing a heat- and protease-resistant component of the streptococci (23). Since *S. gordonii* NCTC 7869 was a particularly strong coaggregating partner with *C. albicans*, we have investigated further the molecular basis for this interaction. In this article we show that *C. albicans* binds to a unique cell wall polysaccharide produced by *S. gordonii*.

MATERIALS AND METHODS

Microbial strains and cultivation. The following bacterial strains were used: *S. gordonii* NCTC 7869 (Channon) and DL1 (Challis), *S. oralis* H1 (ATCC 55229) and 34 (from P. E. Kolenbrander, National Institutes of Health, Bethesda, Md.), *S. oralis* ATCC 10557, *S. sanguis* 12 (from B. C. McBride, University of British Columbia, Vancouver, Canada), *S. sanguis* ATCC 10556, *Streptococcus mutans* NCTC 10449, and *Streptococcus salivarius* HB (from P. S. Handley, University of Manchester, Manchester, United Kingdom). Bacteria were cultured in brain heart infusion medium (Difco Laboratories, Detroit, Mich.) containing 0.5% (wt/vol) yeast extract (BHY) or in defined medium (22) containing 0.8% (wt/vol) glucose. Cultures were inoculated from stock cell suspensions stored at −80°C in BHY medium containing 15% glycerol and were grown at 37°C in closed tubes or bottles without shaking. *C. albicans* ATCC 10261, CA2 (from A. Cassone, Instituto Superiore di Sanita, Rome, Italy), and nine independent clinical isolates from Dunedin Public Hospital were grown in salts-biotin medium containing 1% (wt/vol) glucose (20) at 30°C with vigorous aeration. In some experiments, *C. albicans* cells were glucose starved by incubating them in salts-biotin medium (pH 6.5) in the absence of glucose at 37°C for 3 h as previously described (23).

Coaggregation assay. Streptococci were radioactively labeled with [*methyl*-^{3}H]thymidine, and the numbers of cells binding to *C. albicans* were measured in a fluid-phase centrifugation-based assay (23).

Radioactive labeling of yeast cells. Exponential-phase cells of *C. albicans* in salts-biotin-glucose medium were inoculated into fresh, prewarmed medium (20

* Corresponding author. Mailing address: Department of Oral Biology and Oral Pathology, University of Otago, P.O. Box 647, Dunedin, New Zealand. Phone: (64) 3 479 7076 or -7080. Fax: (64) 3 479 0673. Electronic mail address: howard.jenkinson@stonebow.otago.ac.nz.

ml) at an initial optical density at 540 nm (OD_{540}) of 0.2. [^{35}S]methionine (0.62 MBq, 17 μCi, 10^3 Ci/mmol) was added, and the culture was incubated at 30°C until the OD_{540} reached 1.0 (approximately 2×10^7 cells per ml). Cells were harvested by centrifugation (3,000 × *g* for 5 min at 4°C), suspended in TNMC buffer (1 mM Tris-HCl [pH 8.0] containing 0.15 M NaCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$) (8), and centrifuged as before. The cells were washed twice more by suspension and centrifugation and then suspended in TNMC buffer at a concentration of 2.0×10^8 cells per ml (OD_{540} = 10.0). Specific radioactivities of between 20 and 10 cells per cpm were obtained.

Solid-phase coadherence assay. Streptococcal cells in the early stationary phase of growth were harvested by centrifugation (6,000 × *g* for 10 min at 4°C), washed twice by alternate centrifugation and suspension in TNMC buffer, and suspended in TNMC buffer at a cell density of 4×10^8 cells per ml (OD_{600} = 0.4). Portions (50 μl) were dispensed into the wells of 96-well Maxisorp microtiter plates (Nunc, Roskilde, Denmark), which were then centrifuged (800 × *g* for 5 min at 20°C). To each well was added 0.25% (wt/vol) glutaraldehyde in TNMC buffer (0.1 ml), and the plates were centrifuged again and then incubated for 30 min at 20°C. The contents of the wells were aspirated, and the wells were washed twice with 0.1 ml of TNMC buffer containing 0.05% (vol/vol) Tween 20 (TNMC-Tween). The wells were then incubated with TNMC-Tween (0.1 ml) for 16 h at 4°C to block remaining binding sites on the polystyrene. The buffer was aspirated, and portions of radioactively labeled *C. albicans* cells in TNMC buffer (0.1 ml) were added to the wells. Plates were incubated with reciprocal shaking (100 rpm) for 1 h at 20°C. The unattached yeast cells in suspension were removed, and the wells were washed four times with TNMC-Tween (0.1 ml each time). To each well was then added 0.2% (wt/vol) sodium dodecyl sulfate in 0.1 M NaOH (0.1 ml), and the plates were incubated for at least 2 h (up to a maximum of 16 h) at 20°C. This was found to be effective in removing >95% of the bound cells from the wells. The cell suspensions were then transferred to scintillation fluid (Optiphase Hisafe; Wallac Oy, Turku, Finland), and radioactivity was counted with a liquid scintillation counter. Radioactivity measurements were converted to cell numbers on the basis of the specific radioactivities of the cell suspensions.

Scanning electron microscopy. To visualize coaggregates of yeast cells and streptococci in suspension, cells were collected by centrifugation (6,000 × *g* for 5 min) and fixed by incubation in 0.1 M sodium cacodylate buffer (pH 7.4) containing 2.5% (vol/vol) glutaraldehyde for 90 min at 4°C. The cells were collected by centrifugation (12,000 × *g* for 1 min), washed four times in 0.1 M sodium cacodylate buffer, and then postfixed in 1% (wt/vol) osmium tetroxide for 1 h at 20°C. Specimens were dehydrated through a graded ethanol series (30, 50, 70, 95, 100 and 100 [vol/vol]) and were dried in a Polaron critical-point drying apparatus with liquid CO_2. Specimens were sputter coated with gold and examined in a Cambridge stereoscan 360 scanning electron microscope at an accelerating voltage of 20 kV. To visualize adherence of *C. albicans* to immobilized cells of *S. gordonii*, streptococci (5×10^8 cells per ml, 0.5 ml) were first fixed to 13-mm-diameter plastic coverslips (Nunc) contained within wells of multidishes (Nunc) by centrifugation and incubation with glutaraldehyde as described above. The coverslips were blocked with TNMC-Tween, incubated with cells of *C. albicans* ATCC 10261 (5×10^6 cells per ml) for 1 h at 20°C with shaking, and washed twice in TNMC buffer. Specimens were then incubated with 0.1 M sodium cacodylate buffer (pH 7.4) containing 2.5% (vol/vol) glutaraldehyde and were processed for microscopy as described above.

Alkali extraction of streptococcal cell surface polysaccharide. Streptococcal cells were collected from early-stationary-phase cultures (1 liter) by centrifugation at 4,000 × *g* for 10 min at 4°C. Bacteria were washed once with deionized water and then extracted with 0.1 M NaOH at 4°C as previously described (39). A typical extract contained approximately 0.29 mg of carbohydrate per ml, 0.36 mg of protein per ml, and 6 μg of phosphorus per ml. The extract was neutralized, incubated with a mixture of RNase A and DNase I (each at 10 μg/ml) for 30 min at 37°C, and then incubated with pronase (10-μg/ml final concentration) for 30 min at 37°C. The suspension was mixed with an equal volume of phenol (saturated with 10 mM Tris-HCl, pH 7.5) and centrifuged to separate the layers, and the aqueous phase was removed, mixed with an equal volume of chloroform, and centrifuged. The aqueous layer was removed, mixed with 5 volumes of absolute ethanol, and stored for 16 h at −20°C. The polysaccharide precipitate was collected by centrifugation (10,000 × *g* for 20 min at 4°C), suspended in deionized water, and dialyzed against water (5 liters). The contents of the dialysis bag then were stored as portions at −20°C or were freeze-dried. In some experiments polysaccharide extracts were treated with $NaIO_4$ (20 mM) for 1 h at 20°C in the dark. The suspension was then diluted 10-fold with water, and polysaccharides were precipitated with 70% (vol/vol) ethanol for 8 h at −20°C and recovered by centrifugation as described above. Control samples were subjected to the same process but without inclusion of $NaIO_4$.

Autoclave extraction of streptococcal cell surface polysaccharide. Polysaccharide was autoclave extracted from streptococci by a method similar to that described by McIntire et al. (38). Bacteria from an early-stationary-phase culture (1 liter) were collected by centrifugation, washed with buffer I (0.25 M NaH_2PO_4 adjusted to pH 8.0 and containing 25 mM NaCl), and suspended in buffer I (100 ml) containing 0.1% (vol/vol) Triton X-100. The cells were shaken gently for 16 h at 4°C, harvested by centrifugation (4,000 × *g* for 10 min at 4°C), and washed once in buffer II (25 mM Tris-HCl [pH 7.6] containing 0.16 M $CaCl_2$). The cells were suspended in buffer II (10 ml) containing pronase (0.1 mg/ml), incubated for 4 h at 37°C, and then collected by centrifugation and washed in distilled water. Cells were suspended at a density of approximately 5×10^9 cells per ml in water and autoclaved for 45 min at 15 lb/in^2. Finally, the suspension was centrifuged at 10,000 × *g* for 15 min at 4°C, and the supernatant was removed and treated sequentially with RNase, DNase, pronase, phenol, and chloroform as described above for alkali extraction. Polysaccharide was precipitated with 70% (vol/vol) ethanol (final concentration), collected by centrifugation, dialyzed, and stored as described above.

Immobilization of streptococcal polysaccharides on microtiter well surfaces. Polysaccharides were suspended in distilled water, and portions containing up to 50 μg of glucose equivalents (as determined by the phenol-sulfuric acid assay [11] with glucose as a control) were air dried on the bottom inside surfaces of Maxisorp microtiter plate wells. The wells were incubated with 0.25% (wt/vol) glutaraldehyde in TNMC buffer (0.1 ml) at 20°C for 30 min, washed with TNMC-Tween (0.1 ml), and then incubated with TNMC-Tween (0.1 ml) at 4°C for 16 h to block nonspecific binding sites. Amounts of carbohydrate bound were estimated by utilizing an in situ adaptation of the phenol-sulfuric acid assay (11). It was estimated that up to 20 μg of streptococcal carbohydrate could be bound to each well. The adherence of radioactively labeled yeast cells to immobilized polysaccharides in microtiter wells was measured as described for the assay with immobilized streptococcal cells. For sugar inhibition experiments, saccharides were dissolved in TNMC buffer at appropriate concentrations (usually 0.5 M), and the solutions were adjusted to pH 8.0 if necessary and then mixed with *C. albicans* cells just prior to incubation with polysaccharide in plate wells. To determine inhibition of adherence by antibodies, wells were coated with 5 μg of polysaccharide, blocked with TNMC-Tween as described above, and then incubated either with TBS (10 mM Tris-HCl [pH 8.0] containing 0.15 M NaCl) containing 1% (wt/vol) gelatin or with preimmune serum diluted 1:100 in TBS–1% gelatin for 1 h at 37°C. The wells were then washed twice with TNMC-Tween and preincubated with dilutions of preimmune or immune serum in TNMC buffer for 1 h at 37°C. After the wells were washed twice with TNMC-Tween, the adherence of *C. albicans* was determined as described above.

Analysis of polysaccharides. Component monosaccharides of streptococcal cell surface polysaccharides were identified and quantitated after partial or total acid hydrolysis by high-performance anion-exchange chromatography (Dionex Corp., Sunnyvale, Calif.) as previously described (14). Samples of polysaccharide (1 mg) were hydrolyzed by heating with 2 M HCl for 14 h at 100°C under vacuum or with 2 M trifluoroacetic acid for 1 to 3 h at 100°C. Hydrolysates were freeze-dried and reconstituted in water for analysis on the Dionex system (14).

Determination of protein and phosphorus. The protein concentration was measured by the Coomassie blue-binding assay (Bio-Rad Laboratories, Richmond, Calif.) with bovine serum albumin as a standard. Total phosphorus was determined colorimetrically as inorganic phosphate (7) with $Na_2(PO_4)_3$ as a standard.

Antiserum and ELISA. For the generation of antibodies to polysaccharide, four intramuscular injections of alkali-extracted and purified cell wall polysaccharide (0.4 mg) from *S. gordonii* NCTC 7869 in 0.15 M NaCl were made into New Zealand White rabbits at intervals of 5 days. Serum samples were obtained before immunization (preimmune serum) and 5 days following the final injection. To determine antibody reactivities by enzyme-linked immunosorbent assay (ELISA), streptococcal cells or polysaccharides were immobilized onto microtiter plate wells as described above for the coadherence assays, except that TBS replaced TNMC buffer. Antigen-coated wells were incubated with TBS containing 1% (wt/vol) gelatin for 16 h at 4°C to block remaining protein-binding sites on the plastic. The buffer was aspirated, and doubling dilutions of serum in TBS containing 0.1% (wt/vol) gelatin (TBS-gelatin) were added to the wells (0.05 ml per well) and incubated for 1 h at 37°C. The wells were washed three times with TBS-gelatin, and antibody binding was detected with peroxidase-conjugated swine immunoglobulins to rabbit immunoglobulins (Dako Corp., Carpenteria, Calif.) diluted 1:1000 in TBS-gelatin (0.05 ml). Plates were developed with 1,2-phenylenediamine as an enzyme substrate, and OD_{490}s were measured. The activity of the antiserum was measured by the titration endpoint, which was defined as the reciprocal of the highest dilution of antiserum which gave an OD_{490} in ELISA of >0.10 following subtraction of the OD_{490} in ELISA of an equivalent dilution of preimmune serum.

RESULTS

Inhibition of *S. gordonii-C. albicans* coaggregation by streptococcal cell surface polysaccharide. Of a number of oral streptococci examined, *S. gordonii* NCTC 7869 was found to have one of the highest affinities for coaggregation with several strains of *C. albicans* in a fluid-phase assay (23). Scanning electron microscopy of coaggregates of *S. gordonii* NCTC 7869 with *C. albicans* ATCC 10261 indicated that the streptococci attached singly, or occasionally in short chains, to the yeast cells, effectively cross bridging the *C. albicans* cells (Fig. 1A).

Previous work had suggested that the streptococcal receptor

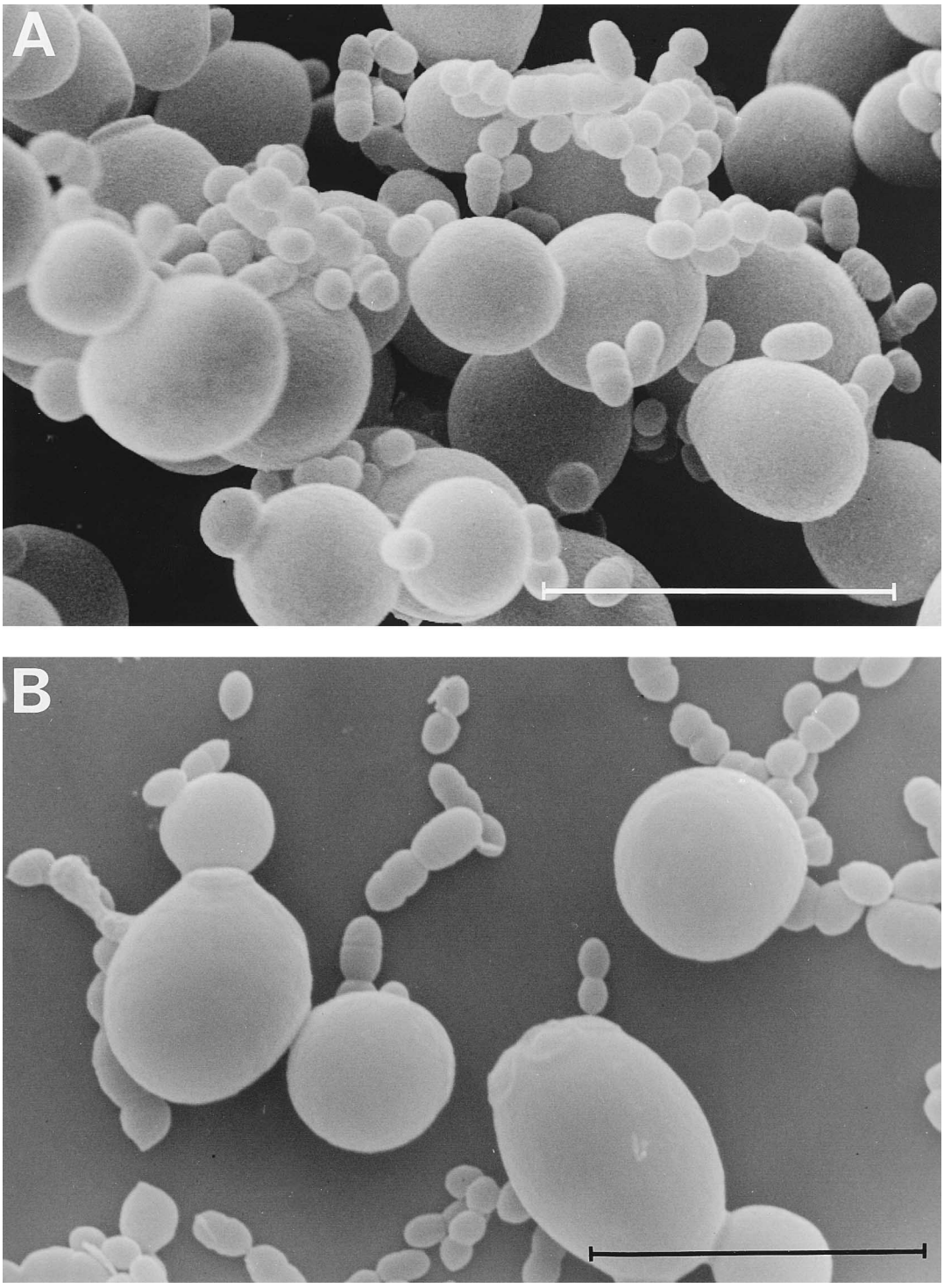

FIG. 1. Scanning electron micrographs of *C. albicans* ATCC 10261 coaggregating with *S. gordonii* NCTC 7869 in fluid phase (A) or adhering to *S. gordonii* NCTC 7869 cells immobilized on plastic (B). Bars, 5 μm.

might be nonproteinaceous (23). To attempt to identify and characterize the receptor on the surface of *S. gordonii* NCTC 7869, bacterial cells were extracted with alkali (0.1 N NaOH). This treatment has been shown previously to remove surface fibrillar material composed of protein and polysaccharides from the cell surface of *S. gordonii* NCTC 7869 (39). The alkali extract (neutralized and dialyzed as described in Materials and Methods) was found to be inhibitory to fluid-phase coaggregation of *C. albicans* ATCC 10261 cells with *S. gordonii* NCTC 7869 cells. Heating (for 30 min at 80°C), pronase treatment (0.1 mg/ml for 30 min at 37°C), and chloroform-isoamyl alcohol (24:1, vol/vol) treatment of the extract had little effect on

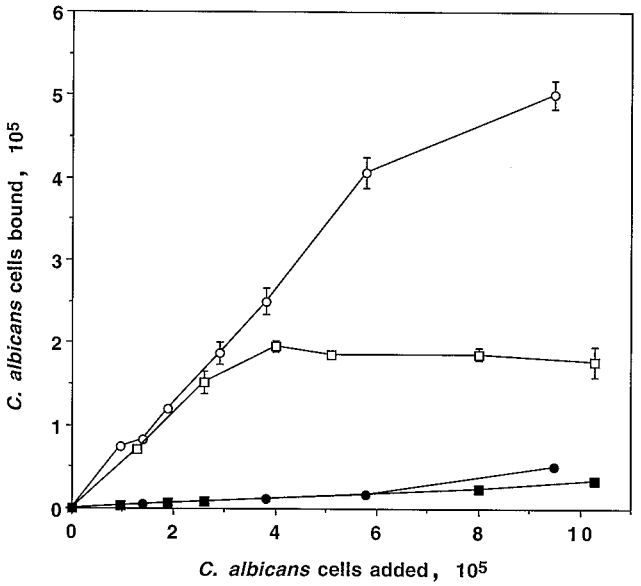

FIG. 2. Adherence of *C. albicans* ATCC 10261 (circles) or CA2 (squares) to *S. gordonii* NCTC 7869 cells immobilized on microtiter plate wells (open symbols) or to blocked wells (closed symbols). Values are the means of quadruplicate samples from a representative experiment repeated twice (error bars are standard errors of the means).

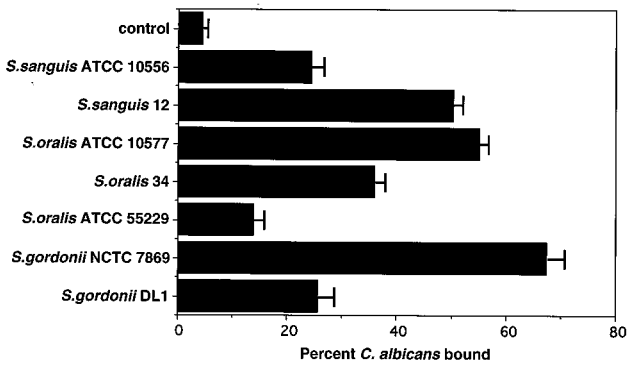

FIG. 3. Adherence of *C. albicans* ATCC 10261 to cells of various oral streptococcal species immobilized on microtiter plate wells. Bacterial cells (2×10^7) were immobilized as described in Materials and Methods, and the numbers of *C. albicans* cells bound are expressed as percentages of the number of input cells (2×10^5 per well). Values are the means of quadruplicate samples from a representative experiment (error bars are standard errors of the means).

its coaggregation-inhibitory activity. However, incubation of the extract with 20 mM periodate abolished its coaggregation-inhibitory activity (results not shown), suggesting that the *C. albicans* receptor was glycosidic.

Adherence of *C. albicans* to immobilized streptococcal cells. To more accurately and conveniently investigate the binding of yeast cells to streptococci, a solid-phase adherence assay was developed. The bottom surfaces of microtiter wells were coated uniformly with cells of *S. gordonii* NCTC 7869 (2×10^7 bacteria per well). The numbers of *C. albicans* ATCC 10261 cells (grown at 30°C) adhering to streptococci were found to be proportional to the numbers of yeast cells added at an input of up to about 6×10^5 cells (Fig. 2). Above this input cell number, the binding curve tailed off but did not appear to reach saturation because of secondary binding interactions between yeast cells. At an input number of 2×10^5 cells, the *C. albicans* cells adhered singly and only to the immobilized streptococcal cells as visualized by scanning electron microscopy (Fig. 1B).

Exponential-phase cells of *C. albicans* CA2 that had been grown at 30°C were shown previously to coaggregate only weakly with streptococci, but their adherence to streptococci was increased 10-fold when the yeast cells were starved for glucose at 37°C (23). Binding of glucose-starved cells of *C. albicans* CA2 to *S. gordonii* NCTC 7869 was found to be of lower affinity than that of exponential-phase cells of strain ATCC 10261 and was saturable (Fig. 2). Adherence of *C. albicans* ATCC 10261 cells to streptococci was also enhanced by glucose starvation of yeast cells at 37°C. However, the percent binding could not be measured accurately because the cells of this strain, unlike those of CA2, clumped following starvation. For both strains, less than 5% of the input yeast cells adhered to blocked wells that did not contain streptococci (Fig. 2). These results established the conditions for a standardized adherence assay with an input of 2×10^5 cells of *C. albicans* per well. By using this assay, exponential-phase cells of nine fresh clinical isolates of *C. albicans* were also tested for adherence to *S. gordonii* NCTC 7869. All strains showed significant degrees of binding, which ranged from 23 to 73% of input cells (data not shown).

Cells of *S. gordonii* NCTC 7869 grown in TY-glucose medium (23) were as effective as BHY-grown cells in supporting adherence of *C. albicans* ATCC 10261. Yeast cell binding to *S. gordonii* cells grown in defined medium containing glucose was reduced by about 40% (results not shown). When streptococcal cells grown in any one of these three media were treated with alkali as described in Materials and Methods, washed, and immobilized at equivalent cell densities on microtiter wells, the binding of *C. albicans* ATCC 10261 cells was reduced by >50% (not shown).

The adherence of exponential-phase cells of *C. albicans* ATCC 10261 or of glucose-starved cells of strain CA2 to *S. gordonii* NCTC 7869 was not inhibited by any of the following saccharides (0.25 M final concentration): *N*-acetyl-D-galactosamine (GalNAc), *N*-acetyl-D-glucosamine (GlcNAc), L-fucose (Fuc), D-galactose (Gal), D-glucose (Glc), lactose, D-mannose (Man), and L-rhamnose (Rha).

Adherence of *C. albicans* ATCC 10261 to various oral streptococci. A number of strains of streptococci within the species *S. gordonii*, *S. oralis*, and *S. sanguis* were found to bind *C. albicans* with high affinities. *S. gordonii* NCTC 7869 cells best supported the adherence of *C. albicans*, while yeast cells bound least well to *S. oralis* ATCC 55229 (Fig. 3). The adherence of *C. albicans* ATCC 10261 to cells of *S. mutans* NCTC 10449 or *S. salivarius* HB was not greater than that of *C. albicans* background binding to blocked wells (Fig. 3). An identical profile of streptococcal cell binding specificity was obtained for glucose-starved cells of *C. albicans* CA2 (not shown), although the overall numbers of cells bound were lower than for strain ATCC 10261 (Fig. 2).

Monosaccharide composition of *S. gordonii* polysaccharide. Acid hydrolysates of purified polysaccharide extracted from *S. gordonii* NCTC 7869 with alkali contained Rha, GalN, GlcN, Glc, and Gal, with Gal being present in a considerably lower molar proportion (Table 1). Acid hydrolysates of autoclave-extracted polysaccharide contained similar molar ratios of monosaccharides (not shown). The same monosaccharides, although in different molar proportions, were identified in hydrolysates of polysaccharides extracted with alkali from *S. oralis* 34 and ATCC 55229 (Table 1). Hydrolysates of the purified coaggregation receptor polysaccharide from *S. oralis* ATCC 55229 (provided by F. J. Cassels, Walter Reed Army Institute

TABLE 1. Monosaccharide composition of cell wall polysaccharides extracted from three oral streptococcal species

Monosaccharide[a]	Mol% monosaccharide in total acid hydrolysate[b] of:			
	Alkali-extracted polysaccharide from:			Purified coaggregation receptor polysaccharide from *S. oralis* ATCC 55229[c]
	S. gordonii NCTC 7869	*S. oralis* 34	*S. oralis* ATCC 55229	
Rha	17	<1	6.9	21
GalN	22	13	19	ND[d]
GlcN	30	12	8.2	ND
Gal	4.1	11	27	57
Glc	25	63	39	22

[a] Monosaccharides are listed in order of their elution from the column. Glycerol was detected in extracts from *S. oralis* ATCC 55229.
[b] Polysaccharides were hydrolyzed with 2 M HCl for 14 h at 100°C, and samples were separated by Dionex column chromatography.
[c] Provided by F. J. Cassels.
[d] ND, not detected.

of Research, Washington, D.C.) did not contain GalN or GlcN, and the presence of Rha, Gal, and Glc, together with glycerol (Table 1), was consistent with the published structure of this polysaccharide (6, 13).

Adherence of *C. albicans* to streptococcal polysaccharides. *C. albicans* cells adhered to polysaccharide that had been extracted from *S. gordonii* NCTC 7869 with alkali and which was bound to microtiter plate wells. The numbers of yeast cells adhering were proportional to the amounts of carbohydrate bound in the wells at up to approximately 0.2 μg (Fig. 4). Attachment followed saturation kinetics, and maximal binding of *C. albicans* ATCC 10261 cells to alkali-extracted polysaccharide (76.5% of input cells) was greater than that to autoclave-extracted polysaccharide (maximal binding, 48.5% of input yeast cells). The addition of soluble alkali-extracted polysaccharide (up to 10 μg/ml) to the microtiter well assay did not block binding of *C. albicans* cells to the immobilized polysaccharide; rather, it led to the promotion of *C. albicans* cell binding, possibly through cross bridging of yeast cells. The inclusion of commercial T10 dextran or of purified *S. gordonii* glucan (16) in the assay mixtures did not affect *C. albicans* cell adherence to immobilized polysaccharide. The adherence of yeast cells to polysaccharides was not inhibited by the addition of 0.25 M Fuc, Gal, Glc, GlcNAc, GalNAc, Man, or Rha to the assay mixtures. Polysaccharides from *S. oralis* 34 and ATCC 55229 also supported adherence of *C. albicans*, but to lesser extents (Fig. 4).

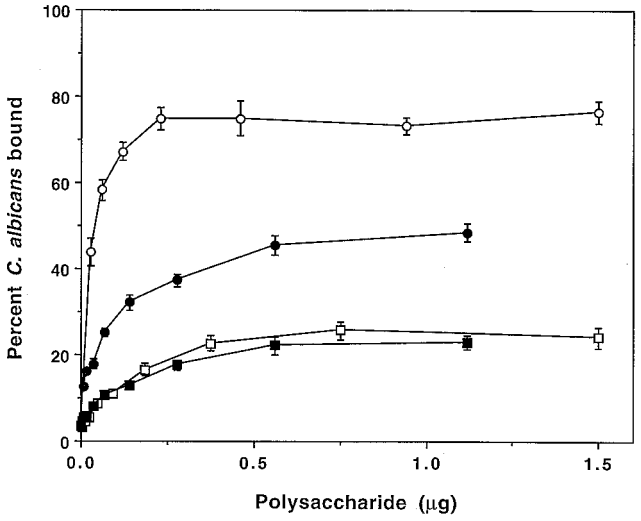

FIG. 4. Adherence of *C. albicans* ATCC 10261 cells to polysaccharides alkali extracted from *S. gordonii* NCTC 7869 (○), *S. oralis* 34 (□), and *S. oralis* ATCC 55229 (■) or autoclave extracted from *S. gordonii* NCTC 7869 (●) and then immobilized onto microtiter plate wells. Numbers of *C. albicans* cells bound are expressed as percentages of the number of input cells (2×10^5 per well). Values represent the means of quadruplicate samples from a representative experiment (error bars are standard errors of the means).

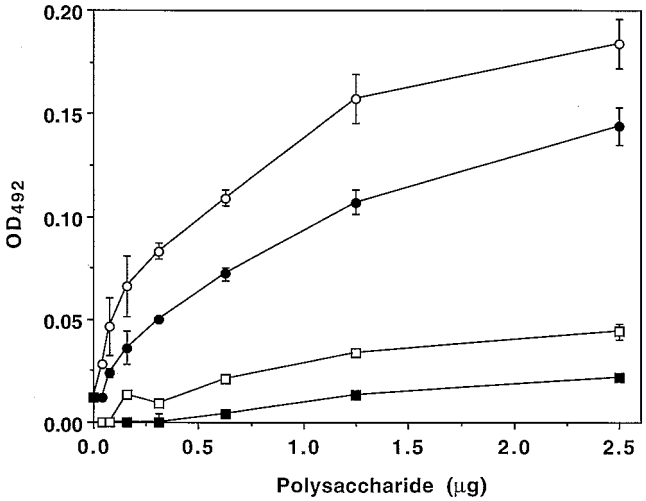

FIG. 5. Reactivity by ELISA of immune serum (diluted 1:100) raised to polysaccharide from *S. gordonii* NCTC 7869 with polysaccharides extracted with alkali from *S. gordonii* NCTC 7869 (○), *S. gordonii* DL1 (●), *S. oralis* 34 (□), and *S. oralis* ATCC 55229 (■) and immobilized on microtiter plate wells. ODs are corrected for values obtained for wells incubated with preimmune serum (diluted 1:100) and are the means of quadruplicate samples from a typical experiment (error bars are standard errors of the means).

Reactivities of antibodies to *S. gordonii* polysaccharide. In whole-cell ELISA, serum from rabbits immunized with *S. gordonii* NCTC 7869 polysaccharide reacted with *S. gordonii* NCTC 7869 and *S. gordonii* DL1 cells with a titer of 800. Antiserum reactivities with cells of *S. oralis* ATCC 55229, 34, and ATCC 10557 were much reduced (titers of <100), and there was no reaction with *S. mutans* NCTC 10449 cells (data not shown). Immune serum bound to immobilized polysaccharide from *S. gordonii* NCTC 7869 and bound with reduced affinity to polysaccharide from *S. gordonii* DL1, showing that these polysaccharides were not antigenically identical (Fig. 5). The immune serum bound better to the *S. oralis* 34 polysaccharide than to the polysaccharide from strain ATCC 55229 (Fig. 5). The antiserum blocked, in a dose-dependent manner, the binding of *C. albicans* ATCC 10261 to immobilized polysaccharide from *S. gordonii* NCTC 7869 (Fig. 6). At a 1:10 dilution of immune serum, binding of yeast cells was inhibited by 50%, while preimmune serum had no effect. The lower level of binding of *C. albicans* to polysaccharide from *S. oralis* 34 was not affected by immune or preimmune serum (Fig. 6), and neither serum affected yeast cell binding to polysaccharide from *S. oralis* ATCC 55229 (not shown).

DISCUSSION

Strains of *S. oralis* and *S. sanguis* produce linear cell wall polysaccharides which have been shown to have several com-

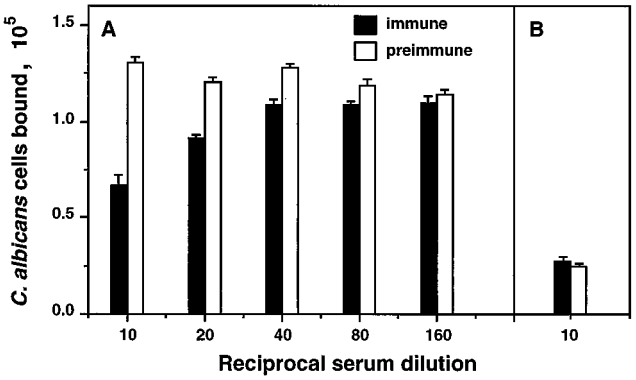

FIG. 6. Effect of dilutions of preimmune or immune serum on the binding of *C. albicans* ATCC 10261 to polysaccharide extracted with alkali from *S. gordonii* NCTC 7869 (A) or *S. oralis* 34 (B) and immobilized on microtiter plate wells (5 μg per well). Values are the means of quadruplicate samples from a representative experiment (error bars are standard errors of the means).

mon structural features. All contain galactopyranose, β-galactofuranose, and phosphodiester linkages, and all are composed of repeat units of six or seven saccharides (30). These polysaccharides act as receptors for a variety of oral bacteria, including strains of *A. naeslundii*, *P. loescheii*, *Veillonella atypica*, and *Capnocytophaga ochracea* (30, 32). The results in this paper demonstrate that *S. gordonii* also produces a cell wall polysaccharide which, in this instance, acts as a receptor for eukaryotic cells of *C. albicans*. Although the structure of the *S. gordonii* polysaccharide has not yet been established, compositional analyses suggest that it might be related to the linear polysaccharides produced by strains of *S. oralis.*

Coaggregation assays of bacterial partners have been usually performed in the fluid phase, in which the extent of coaggregation may be assessed visually (8) or quantitated if one of the partner cell types is radioactively labeled (27). Previously, the development of a coaggregation assay of *C. albicans* with radioactively labeled oral streptococci (23) enabled a distinction between bacteria that were able to coaggregate with yeast cells (e.g., *S. gordonii*, *S. oralis*, and *S. sanguis*) and those that did not appear to coaggregate (e.g., *S. mutans* and *S. salivarius*). The fluid-phase assay allowed us further to reveal that alkali extracts of streptococci were inhibitory to the coaggregation of yeast cells with streptococci. However, we have found assays for coaggregation to be more simple and reproducible if one of the partner cell types can be immobilized (24). To quantitate better the binding of *C. albicans* to streptococci, bacteria were immobilized on microtiter wells in the presence of a low concentration of glutaraldehyde, which, although not essential for bacterial binding, ensured reproducible coverage of the well surfaces. Yeast cells, on the other hand, were found not to remain bound to the wells unless they were fixed with glutaraldehyde, and then their ability to bind streptococci was abolished. The assay as developed to measure *C. albicans* adherence was saturable, although for some strains of *C. albicans*, autoaggregation occurred with higher numbers of yeast cells input. Accordingly, adherence assays were performed at yeast cell input concentrations within the lower end of the linear portion of the binding curve (Fig. 2). Electron microscopic observations confirmed that under these conditions yeast cells did not autoadhere. The oral streptococcal binding profiles of *C. albicans* obtained with the microtiter well plate phase assays paralleled closely those previously obtained with the fluid-phase coaggregation assays (23).

Alkali treatment of oral streptococci appears to be a rapid and efficient method for the isolation of cell wall polysaccharide. Alkali extracts and autoclave extracts from three strains of oral streptococci all contained the same five monosaccharides. The *S. gordonii* NCTC 7869 polysaccharide differed from the other two in that the galactose content was much reduced. The polysaccharide preparations all contained GalNAc and GlcNAc (deacetylated following hydrolysis). These compounds were not present in a purified coaggregation receptor polysaccharide from *S. oralis* ATCC 55229 (6, 13); therefore, carbohydrate polymers in addition to the linear cell wall polysaccharides were present in the alkali extracted material.

The streptococcal polysaccharides, unlike the homopolysaccharides dextran, laminarin, and *S. gordonii* glucan (16), could be bound to microtiter plate wells. This enabled us to demonstrate that the isolated polysaccharide from *S. gordonii* NCTC 7869 was capable of supporting binding of *C. albicans* and therefore that this material was likely to be the coaggregation receptor. This was substantiated by two additional observations: first, the polysaccharide was a potent inhibitor of the fluid-phase coaggregation of *C. albicans* and *S. gordonii* cells, and second, antiserum raised to the *S. gordonii* NCTC 7869 polysaccharide blocked binding of *C. albicans* to the polysaccharide.

The *S. gordonii* NCTC 7869 polysaccharide appears to contain the most preferred ligand for the *C. albicans* coadhesin. Although many interbacterial coaggregations can be inhibited by saccharides, frequently by lactose (31), a number of monosaccharides and lactose were ineffective inhibitors of *C. albicans* binding to *S. gordonii* polysaccharide. This suggests that the receptor recognized by the yeast cell adhesin may be structurally complex and/or conformationally sensitive. Inhibition studies with di- and trisaccharides might reveal further information about the structure of the receptor being recognized by *C. albicans*. The *S. gordonii* polysaccharide did not contain Fuc, which seems to rule out the possibility of a fucoside-mediated adhesive interaction (2, 44). Recently it has been shown that *C. albicans* fimbriae bind to βGalNAc(1-4)βGal receptors that might be present within glycosphingolipids on the surfaces of eukaryotic cells (44). None of the streptococcal linear cell wall polysaccharides characterized to date contains this linkage (30), although the *S. oralis* 34 polysaccharide, to which *C. albicans* bound with lower affinity, contained the linkage βGalNAc(1-3)αGal. This disaccharide is considered to be the binding site for *A. naeslundii* T14V (38, 43). We have recently shown, using the microtiter well plate assay, that the alkali-extracted polysaccharide from *S. oralis* 34 is bound with high affinity by cells of *A. naeslundii* T14V and of *A. naeslundii* ATCC 12104 (19). Conversely, polysaccharides extracted from *S. gordonii* NCTC 7869 and DL1 and from *S. oralis* ATCC 55229 were not bound by actinomycetes (19). Therefore, the sugar binding specificity of the *C. albicans* adhesin is different from that of the *A. naeslundii* type 2 fimbrial lectin.

Bacterial coaggregations may involve one or more adhesin-receptor interactions. Coaggregation of *S. gordonii* PK488 with *A. naeslundii* PK606 is believed to be mediated through a unimodal protein-protein interaction that is not sensitive to inhibition by lactose (28). Conversely, coaggregation of *S. gordonii* DL1 with *A. naeslundii* strains is partly lactose sensitive (28), although not mediated by *A. naeslundii* fimbriae (10), and appears to involve at least three different streptococcal cell surface polypeptides (24, 29, 40). Other bacteria with which *S. gordonii* cells coaggregate include *Fusobacterium nucleatum* (32), *Porphyromonas gingivalis* (34), *Propionibacterium acnes* (32), *S. mutans* (33), and *S. oralis* (32). Our data show that *S.*

gordonii can also provide a receptor for binding of *C. albicans*. Therefore, *S. gordonii* strains are multimediators of intra- and intergeneric coaggregations in the oral cavity. The evidence strongly suggests that the major interaction of *C. albicans* with *S. gordonii* involves recognition by the yeast of the streptococcal polysaccharide receptor. However, the possibility that other yeast-bacterial cell adhesive interactions might be occurring cannot be ruled out. Future studies will involve identifying the *C. albicans* coadhesin, determining its receptor specificity, and establishing the structure of the ligand within the *S. gordonii* polysaccharide.

All strains of *C. albicans* that we have tested were able to bind to *S. gordonii* NCTC 7869, indicating that this might represent a common adherence mechanism among *C. albicans* strains. These adhesin-receptor interactions have implications for colonization of the oral cavity by yeasts. Binding of *C. albicans* to streptococcal polysaccharides could be important for the retention of *C. albicans* in dental plaque (42). Furthermore, if *C. albicans* and *S. gordonii* cells compete for binding to epithelial cell surface receptors, then their ability to coaggregate would allow both organisms to colonize a mucosal surface on which host cell adhesive sites were preferentially occupied by one of the coaggregating partner types.

ACKNOWLEDGMENTS

We are grateful to D. Kant and J. E. Talbot, who assisted with assays at various stages of the work. We thank S. C. Johnstone for electron microscopy and F. J. Cassels for generously providing purified polysaccharide.

This research was supported by the Health Research Council of New Zealand. D. Kant and J. E. Talbot were in receipt of Summer Studentships from the New Zealand Dental Research Foundation Trust Board and Health Research Council of New Zealand, respectively.

REFERENCES

1. **Bouchara, J.-P., G. Tronchin, V. Annaix, R. Robert, and J.-M. Senet.** 1990. Laminin receptors on *Candida albicans* germ tubes. Infect. Immun. **58:**48–54.
2. **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.
3. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. **55:**1 –20.
4. **Cannon, R. D., A. K. Nand, and H. F. Jenkinson.** 1995. Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite. Microbiology **141:**213–219.
5. **Casanova, M., J. L. Lopez-Ribot, C. Monteagudo, A. Llombart-Bosch, R. Sentandreu, and J. P. Martinez.** 1992. Identification of a 58-kilodalton cell surface fibrinogen-binding mannoprotein from *Candida albicans*. Infect. Immun. **60:**4221–4229.
6. **Cassels, F. J., and J. London.** 1989. Isolation of a coaggregation-inhibiting cell wall polysaccharide from *Streptococcus sanguis* H1. J. Bacteriol. **171:** 4019–4025.
7. **Chen, P. S., Jr., T. Y. Toribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28:**1756–1758.
8. **Cisar, J. O., P. E. Kolenbrander, and F. C. McIntire.** 1979. Specificity of coaggregation reactions between human oral streptococci and strains of *Actinomyces viscosus* or *Actinomyces naeslundii*. Infect. Immun. **24:**742–752.
9. **Cisar, J. O., A. L. Sandberg, and W. B. Clark.** 1989. Molecular aspects of adherence of *Actinomyces viscosus* and *Actinomyces naeslundii* to oral surfaces. J. Dent. Res. **68**(Spec. Iss.)**:**1558–1559.
10. **Cisar, J. O., A. E. Vatter, W. B. Clark, S. H. Curl, S. Hurst-Calderone, and A. L. Sandberg.** 1988. Mutants of *Actinomyces viscosus* T14V lacking type 1, type 2, or both types of fimbriae. Infect. Immun. **56:**2984–2989.
11. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–354.
12. **Edgerton, M., F. A. Scannapieco, M. S. Reddy, and M. J. Levine.** 1993. Human submandibular-sublingual saliva promotes adhesion of *Candida albicans* to polymethylmethacrylate. Infect. Immun. **61:**2644–2652.
13. **Glushka, J., F. J. Cassels, R. W. Carlson, and H. van Halbeek.** 1992. Complete structure of the adhesin receptor polysaccharide of *Streptococcus oralis* ATCC 55229 (*S. sanguis* H1). Biochemistry **31:**10741–10746.
14. **Gopal, P. K., and V. L. Crow.** 1993. Characterization of loosely associated material from the cell surface of *Lactococcus lactis* subsp. *cremoris* E8 and its phage-resistant variant strain 398. Appl. Environ. Microbiol. **59:**3177–3182.
15. **Gustafson, K. S., G. M. Vercelloti, C. M. Bendel, and M. K. Hostetter.** 1991. Molecular mimicry in *Candida albicans*. Role of an integrin analogue in adhesion of yeast to human endothelium. J. Clin. Invest. **87:**1896–1902.
16. **Haisman, R. J., and H. F. Jenkinson.** 1991. Mutants of *Streptococcus gordonii* Challis over-producing glucosyltransferase. J. Gen. Microbiol. **137:**483–489.
17. **Hoffman, M. P., and C. G. Haidaris.** 1993. Analysis of *Candida albicans* adhesion to salivary mucin. Infect. Immun. **61:**1940–1949.
18. **Hoffman, M. P., and C. G. Haidaris.** 1994. Identification and characterization of a *Candida albicans*-binding proteoglycan secreted from rat submandibular salivary glands. Infect. Immun. **62:**828–836.
19. **Holmes, A. R., and H. F. Jenkinson.** 1994. Unpublished data.
20. **Holmes, A. R., and M. G. Shepherd.** 1988. Nutritional factors determine germ tube formation in *Candida albicans*. J. Med. Vet. Mycol. **26:**127–131.
21. **Hsu, L. Y., G. E. Minah, D. E. Peterson, J. R. Wingard, W. G. Merz, V. Altomonte, and C. A. Tylenda.** 1990. Coaggregation of oral *Candida* isolates with bacteria from bone marrow transplant recipients. J. Clin. Microbiol. **28:**2621–2626.
22. **Jenkinson, H. F.** 1986. Cell-surface proteins of *Streptococcus sanguis* associated with cell hydrophobicity and coaggregation properties. J. Gen. Microbiol. **132:**1575–1589.
23. **Jenkinson, H. F., H. C. Lala, and M. G. Shepherd.** 1990. Coaggregation of *Streptococcus sanguis* and other streptococci with *Candida albicans*. Infect. Immun. **58:**1429–1436.
24. **Jenkinson, H. F., S. D. Terry, R. McNab, and G. W. Tannock.** 1993. Inactivation of the gene encoding surface protein SspA in *Streptococcus gordonii* DL1 affects cell interactions with human salivary agglutinin and oral actinomyces. Infect. Immun. **61:**3199–3208.
25. **Jimenez-Lucho, V., V. Ginsburg, and H. C. Krivan.** 1990. *Cryptococcus neoformans*, *Candida albicans*, and other fungi bind specifically to the glycosphingolipid lactosylceramide (Galβ1-4Glcβ1-1Cer), a possible adhesion receptor for yeasts. Infect. Immun. **58:**2085–2090.
26. **Klotz, S. A., R. C. Hein, R. L. Smith, and J. B. Rouse.** 1994. The fibronectin adhesin of *Candida albicans*. Infect. Immun. **62:**4679–4681.
27. **Kolenbrander, P. E., and R. N. Andersen.** 1986. Multigeneric coaggregations among oral bacteria: a network of independent cell-to-cell interactions. J. Bacteriol. **168:**851–859.
28. **Kolenbrander, P. E., and R. N. Andersen.** 1990. Characterization of *Streptococcus gordonii* (*S. sanguis*) PK488 adhesin-mediated coaggregation with *Actinomyces naeslundii* PK606. Infect. Immun. **58:**3064–3072.
29. **Kolenbrander, P. E., R. N. Andersen, and N. Ganeshkumar.** 1994. Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene, *scaA*, and ATP-binding cassette. Infect. Immun. **62:**4469–4480.
30. **Kolenbrander, P. E., N. Ganeshkumar, F. J. Cassels, and C. V. Hughes.** 1993. Coaggregation: specific adherence among human oral plaque bacteria. FASEB J. **7:**406–413.
31. **Kolenbrander, P. E., and J. London.** 1992. Ecological significance of coaggregation among bacteria. Adv. Microb. Ecol. **12:**183–217.
32. **Kolenbrander, P. E., and J. London.** 1993. Adhere today, here tomorrow: oral bacterial adherence. J. Bacteriol. **175:**3247–3252.
33. **Lamont, R. J., D. R. Demuth, C. A. Davis, D. Malamud, and B. Rosan.** 1991. Salivary-agglutinin-mediated adherence of *Streptococcus mutans* to early plaque bacteria. Infect. Immun. **59:**3446–3450.
34. **Lamont, R. J., S. Gil, D. R. Demuth, D. Malamud, and B. Rosan.** 1994. Molecules of *Streptococcus gordonii* that bind to *Porphyromonas gingivalis*. Microbiology **140:**867–872.
35. **Li, R. K., and J. E. Cutler.** 1993. Chemical definition of an epitope/adhesin molecule on *Candida albicans*. J. Biol. Chem. **268:**18293–18299.
36. **London, J., and J. Allen.** 1990. Purification and characterization of a *Bacteroides loescheii* adhesin that interacts with procaryotic and eucaryotic cells. J. Bacteriol. **172:**2527–2534.
37. **Lopez-Ribot, J.-L., and W. L. Chaffin.** 1994. Binding of the extracellular matrix component entactin to *Candida albicans*. Infect. Immun. **62:**4564–4571.
38. **McIntire, F. C., L. K. Crosby, A. E. Vatter, J. O. Cisar, M. R. McNeil, C. A. Bush, S. S. Tjoa, and P. V. Fennessey.** 1988. A polysaccharide from *Streptococcus sanguis* 34 that inhibits coaggregation of *S. sanguis* 34 with *Actinomyces viscosus* T14V. J. Bacteriol. **170:**2229–2235.
39. **McNab, R., and H. F. Jenkinson.** 1992. Aggregation-deficient mutants of *Streptococcus gordonii* Channon altered in production of cell-surface polysaccharide and proteins. Microb. Ecol. Health Dis. **5:**277–289.
40. **McNab, R., H. F. Jenkinson, D. M. Loach, and G. W. Tannock.** 1994. Cell-surface-associated polypeptides CshA and CshB of high molecular mass are colonization determinants in the oral bacterium *Streptococcus gordonii*. Mol. Microbiol. **14:**743–754.
41. **Miyakawa, Y., T. Kuribayashi, K. Kagaya, and M. Suzuki.** 1992. Role of specific determinants in mannan of *Candida albicans* serotype A in adherence to buccal epithelial cells. Infect. Immun. **60:**2493–2499.

42. **Slots, J., T. E. Rams, and M. A. Listgarten.** 1988. Yeasts, enteric rods and pseudomonads in the subgingival flora of severe adult periodontitis. Oral Microbiol. Immunol. **3:**47–52.
43. **Strömberg, N., and T. Borén.** 1992. *Actinomyces* tissue specificity may depend on differences in receptor specificity for GalNAcβ-containing glycoconjugates. Infect. Immun. **60:**3268–3277.
44. **Tosh, F. D., and L. J. Douglas.** 1992. Characterization of a fucoside-binding adhesin of *Candida albicans*. Infect. Immun. **60:**4734–4739.
45. **Yu, L., K. K. Lee, H. B. Sheth, P. Lane-Bell, G. Srivastava, O. Hindsgaul, W. Paranchych, R. S. Hodges, and R. T. Irvin.** 1994. Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells. Infect. Immun. **62:**2843–2848.