Coaggregation of *Streptococcus sanguis* and Other Streptococci with 
*Candida albicans*

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Thirteen strains of viridans group streptococci and two strains of other streptococci were tested for coaggregation with *Candida albicans*. *Streptococcus sanguis* strains generally exhibited low levels of adherence to 28°C-grown exponential-phase yeast cells, but starvation of yeast cells for glucose at 37°C (or at 28°C) increased their coaggregating activity with these streptococci by at least tenfold. This was a property common to four *C. albicans* strains tested, two of which were able to form mycelia (6406 and MEN) and two of which were not (MM2002 and CA2). The expression of the coaggregation adhesin during yeast cell starvation was inhibited by addition of trichodermin or amphotericin B. The strains of *S. sanguis*, *Streptococcus gordonii*, and *Streptococcus oralis* tested for coaggregating activity encompassed a diverse range of physiological and morphological types, yet all exhibited saturable coaggregation with starved *C. albicans* cells. There was no correlation of cell surface hydrophobicity, of either yeast or streptococcal cells, with their abilities to coaggregate. Strains of *Streptococcus anginosus* also coaggregated with starved yeast cells; *Streptococcus salivarius* and *Streptococcus pyogenes* coaggregated to a lesser degree with *C. albicans*, and the coaggregation with *S. pyogenes* was not promoted by yeast cell starvation; *Streptococcus mutans* and *Enterococcus faecalis* did not coaggregate with yeast. The coaggregation reactions of *S. sanguis* and *S. gordonii* with *C. albicans* were inhibited by EDTA and by heat or protease treatment of the yeast cells and were not reversible by the addition of lactose or other simple sugars. These observations extend the range of intergeneric coaggregations that are known to occur between oral microbes and suggest that coaggregations of *C. albicans* with viridans group streptococci may be important for colonization of oral surfaces by the yeast.

More than 300 microbial species coexist in the oral cavity, with *Streptococcus* spp. and *Actinomyces* spp. being the predominant cultivable flora in early plaque (47). Yeasts such as *Candida albicans* are usually a minor component of the oral flora, except in situations where the host is compromised, and then oral candidosis may be evident (41). Increased numbers of *Candida albicans* also occur in denture stomatitis (41), a condition in which the normal bacterial plaque flora appear not to be greatly altered (48). Adhesins present on the surfaces of oral microbial cells are considered necessary for their growth and survival in the mouth (49). Probably best characterized are the adherence properties of oral streptococci and actinomycetes that enable these organisms to attach to the salivary pellicle (7, 9, 11, 34, 40). *C. albicans* adheres to mucosal surfaces, to plastic, and to acrylic, but modes of attachment are less clearly defined than those in oral streptococci (reviewed in references 10, 22, 44).

Oral streptococci, in particular *Streptococcus sanguis*, exhibit coaggregation reactions with a variety of different bacteria (13, 27). These reactions are not random and involve specific coaggregating pairs of organisms (6), thus enabling strains of *S. sanguis* and *Actinomyces viscosus* to be delineated into coaggregation groups (6, 32, 33). The coaggregation reactions that *S. sanguis* displays with *Actinomyces* species and with other oral genera, including *Bacteroides* (30), *Capnocytotapha* (28), and *Fusobacterium* (31, 35), are mediated by complex surface components on the partner cell types and involve lectin-carbohydrate interactions (4, 29, 32, 39, 52). These networks of intergeneric coaggregations account to a greater degree for the diversity of oral species present in plaque (27).

In view of the importance of interbacterial coaggregations in the establishment and maintenance of the oral bacterial flora, it is possible that interactions of bacteria with *C. albicans* may influence colonization of the oral cavity by the yeast. A number of oral and nonoral bacteria can agglutinate with *C. albicans* cells (1), and in this paper we describe and quantify the adherence of cells from a number of streptococcal species to *C. albicans*. The results show that strains of *S. sanguis*, *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus anginosus* coaggregate with *C. albicans* and that this is promoted by subjecting the yeast cells to glucose starvation. Evidence suggests that the coaggregations involve protein-carbohydrate interactions and, in this respect, they are analogous to intergeneric coaggregations of oral bacteria.

**MATERIALS AND METHODS**

**Bacterial and yeast strains.** The following strains of streptococci (with references where appropriate) were used: *S. gordonii* DL1 (Challis), *S. gordonii* ATCC 10558 (NCTC 7865), *S. oralis* ATCC 10557 (NCTC 7864), *S. sanguis* NCTC 7869 (Channon), *S. sanguis* CR311 (genotype 3) (15), *S. sanguis* CN3410 (14), *S. sanguis* PS1Hb (15), *S. sanguis* FW213 (11), *S. anginosus* NCTC 10713, *S. anginosus* ATCC 27335, *S. anginosus* NCTC 10709, *Streptococcus mutans* ATCC 25175 (NCTC 10449, serotype c), *Streptococcus salivarius* HB (51), *Streptococcus pyogenes* M4, and *Enterococcus faecalis* JH2-2. The *S. gordonii* strain Challis and ATCC 10558 were previously classified as *S. sanguis* (25), and *S. anginosus* strains ATCC 27335 and NCTC 10709 were

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previously called *Streptococcus intermedius* and *Streptococcus milleri*, respectively (8).

*C. albicans* MEN was a clinical isolate from an eye infection and strain MM2002 was a spontaneous mutant derived from strain MEN that was unable to form mycelia (3). Strain 6406 was also a clinical isolate (from The Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine). Strain CA2 was derived from strain NCPF 3153 and, like strain MM2002, was unable to form mycelia (37).

**Bacterial growth media.** Streptococci were cultured on BHYN agar (19) at 37°C in a Gas-Pak System (BBL Microbiology Systems, Cockeysville, Md.). Liquid cultures of streptococci were grown in screw-capped bottles or tubes as stationary cultures at 37°C in either BHY medium (19) or in TY medium (5 g of tryptone per liter, 5 g of yeast extract per liter, 4 g of K2HPO4 per liter [pH 7.5]) containing glucose or sucrose (5 g/liter).

**Growth of yeast and germ-tube formation.** *C. albicans* cells were cultured at 30°C on yeast extract-peptone-dextrose agar (46). Liquid cultures were grown with vigorous aeration at 28 or at 37°C in salts-biotin medium, pH 5.3, containing 20 g of glucose per liter (18, 46) (SB-glucose) or at 28°C in yeast nitrogen base (YNB; Difco Laboratories, Detroit, Mich.) medium containing either 20 g of glucose per liter or 180 g of sucrose per liter (38). To induce germ-tube (mycelium) formation, cells were grown at 28°C in SB-glucose to a density of 2 × 10^7 cells per ml (A_600 = 1.0) and were harvested by centrifugation at 2,000 × g for 5 min. The yeast cells were suspended in prewarmed salts-biotin medium without glucose (pH 6.5) and starved with shaking at 37°C for 3 h. Glucose (2 g/liter) was then added, and after a further 2 h of shaking at 37°C, >90% of the cells had formed germ tubes, as assessed by phase-contrast microscopy (18). All yeast cells for adherence assays were collected from cultures by centrifugation at 2,000 × g for 5 min and were washed by three cycles of centrifugation and suspension of the pellet in 0.15 M NaCl. Cells were suspended at a density of approximately 5 × 10^7 cells per ml (A_600 = 25) in 0.15 M NaCl containing 0.02% (wt/vol) NaN3. Fresh cells were used when comparing adherence of yeast cells grown under different conditions. Storage of yeast cells for up to 2 weeks at 4°C did not affect the adherence properties of starved cells, but exponential-phase cells became more adherent on storage.

**Radioactive labeling of cells.** Bacteria were grown with [methy1-3H]thymidine (3.5 or 5 μCi/ml) to late exponential phase and harvested by centrifugation (12,000 × g for 3 min in a microcentrifuge). The cells were washed by two cycles of centrifugation and suspension of the pellet in 0.15 M NaCl; they were then suspended at a density of about 5 × 10^7 cells per ml in 0.15 M NaCl containing 0.02% (wt/vol) NaN3 and stored at 4°C. Before using the bacterial cells in coaggregation assays, they were collected by centrifugation as described above and washed once with coaggregation buffer (1 mM Tris adjusted to pH 8.0 with HCl, 10^-4 M CaCl2, 10^-4 M MgCl2, 6 H2O, 0.15 M NaCl) (6) to remove any leached radioactivity. To measure radioactivity, aqueous samples (0.1 to 0.3 ml) were added to 3 ml of Aquasafe II scintillation cocktail (LKB Instruments, Inc., Rockville, Md.) and radioactivity was counted with a liquid scintillation counter (model LS3801; Beckman Instruments, Inc., Fullerton, Calif.).

**Radioactive assay for adherence.** The adherence assay was essentially that described by Kolenbrander and Andersen (29) for measuring interbacterial coaggregation. Radioactively labeled bacterial cell suspensions were mixed with suspensions of yeast cells in polypropylene tubes (1.5 ml capacity) that had been precoated with a solution of 0.05% (vol/vol) Tween 80 and dried at 55°C. The volumes were adjusted to 0.5 ml with coaggregation buffer, and the suspensions were vortex mixed for 10 s. Tubes containing bacterial suspensions alone (no yeast) were included as controls with every assay. The tubes were placed on a VibraShaker shaker at 900 rpm (sufficient to keep the yeast cells suspended) at 30°C for 20 min, after which time they were centrifuged at 400 × g for 1 min to sediment yeast cells and attached bacteria. The centrifugation speed and time were kept constant, a condition essential for reproducibility of the assay. The 1-min centrifugation time included a period of 35 s during which the rotor head was attaining its set revolutions per minute but did not include the period taken to return to rest (about 20 s). Duplicate portions of the supernatants (usually 0.1 ml) were then counted for radioactivity as described above. The remaining contents of each tube were centrifuged at 12,000 × g for 5 min to sediment all cells, and portions of the supernatants were removed and counted for radioactivity to determine amounts of cell-free radioactivity. These amounts were subtracted from the total counts per minute associated with the bacterial cells in the supernatants after the initial low-speed centrifugation.

A critical assessment of this assay method and the validity of sampling the supernatant are described by Kolenbrander and Andersen (29). By measuring directly the bacterial radioactive counts per minute associated with the yeast cell pellets and adding these amounts to the total supernatant radioactive counts per minute (as described in reference 29), it was shown that 94 to 100% of the assay input radioactivity could be recovered. The centrifugation step was sufficient to sediment >95% of the yeast cells (as measured by agar plate counts of samples taken before and after centrifugation), while at the same time >93% of streptococcal cells of most strains remained unsedimented (measured by radioactive counts per minute of samples taken before and after centrifugation). Cells of some of the streptococcal strains, e.g., *S. sanguis* CR311, under some growth conditions, autoaggregated, and a greater proportion of cells (about 20%) was sedimented by the centrifugation. Autoaggregation was corrected for with the bacteria-only controls run alongside the bacteria and yeast mixtures. None of the strains for which coaggregation data are presented showed >20% autoaggregation of BHYN-grown cells.

The relationships between viable count, A_600, protein concentration, and radioactive counts per minute were determined for the various suspensions of streptococcal cells. For most streptococci growing in short chains, the relationships did not vary more than ±10%; for *S. gordonii* strain DL1 (Challis), a suspension of cells containing 1 × 10^9 CFU/ml had an A_600 of 0.7 and a protein concentration of 0.25 mg/ml as measured by the Folin method (36). Typically, a suspension of 5 × 10^7 streptococcal cells per ml contained 8 × 10^6 to 4 × 10^7 radioactive cpm/ml. Adherence measurements were recorded as radioactive counts per minute bound, but because the specific activities of cell labeling varied with batches, coaggregation was expressed as the percentage of total bacterial radioactive counts per minute bound. All experiments were repeated at least three times with either duplicate or triplicate tubes, and unless otherwise indicated, the errors in measurement were ±6% of the values reported.

**Protease or heat treatment of cells.** Cell suspensions of streptococci (A_600 = 1.0; 1.4 × 10^9 cells per ml) or yeast (A_600 = 5.0; 1.0 × 10^8 cells per ml) in 10 mM Tris hydroxyl-
ride, pH 7.5, containing 0.15 M NaCl were treated with pronase (Type XIV; Sigma Chemical Co., St. Louis, Mo.: final concentration, 100 μg/ml) at 28°C for 30 min or heated as appropriate, and the cells were collected by centrifugation as described above. The pellets were washed twice with suspension in 0.15 M NaCl and centrifugation and were then suspended in coaggregation buffer for adherence assays.

Measurement of surface hydrophobicity. Surface hydrophobicity of bacterial or yeast cells was estimated by the hexadecane partition method of Rosenberg et al. (43), with either 1 × 10^9 streptococcal cells per ml or 1.5 × 10^9 yeast cells per ml suspended in 1 mM Tris hydrochloride, pH 7.0, containing 15 mM NaCl (3 ml) and 0.2 ml of hexadecane. Hydrophobicity was expressed as the percentage of total cells entering the hexadecane phase (20) after vortex mixing the tubes and allowing them to stand for 15 min at room temperature.

Determination of protein. Bacterial or yeast cells were suspended in 0.5 ml of 1 M NaOH and heated at 100°C for 15 min. Solutions were diluted to 1 ml with water, and protein concentration was determined by the Folin method (36), with bovine serum albumin as the standard.

Chemicals. Trichodermin was from Leo Pharmaceutical Products, Ballerup Denmark, and amphotericin B was from Calbiochem-Behring, San Diego, Calif. Radiochemicals were purchased from Amersham International plc, Little Chalfont, England. All other special chemicals and reagents were from Sigma unless otherwise stated.

RESULTS

Adherence of \textit{S. gordonii} and \textit{S. sanguis} to \textit{C. albicans}. Initial experiments were performed to determine suitable conditions for promoting adherence of streptococcal cells to yeast cells. Attempts were made to devise a visual coaggregation assay similar to those used for estimating coaggregating activity of oral bacterial pairs (6, 27), but a visual assay was not suitable because the yeasts rapidly settled out of suspension. Several streptococcal species were tested for their abilities to adhere in the radioactive coaggregation assay, as described in Materials and Methods, to cells of \textit{C. albicans} that were in either the yeast (blastospore) morphology or mycelial (germ-tube) morphology. Of the streptococci tested, \textit{S. sanguis} and \textit{S. gordonii} strains adhered best, and only to \textit{C. albicans} cells that had formed germ tubes. These experiments were extended by using various relative concentrations of yeast and bacterial cells, and it was found that when approximately equal numbers of cells were mixed, >50% of the input streptococcal cells attached to the germ-tube forming cells. Optimum conditions of pH, salt concentration, and time were established for the assay: adherence of \textit{S. gordonii} Challis was found to be maximal between pH 7.0 and 8.0, it was promoted by divalent cations (Ca^{2+} and Mg^{2+}), and adherence was better with NaCl present rather than KCl; adherence was not affected by temperatures in the range of 6 to 30°C, and maximum coaggregation occurred within 20 min of mixing the cell partners.

To test the effects of cell growth conditions on adherence, strains of \textit{S. gordonii} and \textit{S. sanguis} were grown in either TY-glucose or TY-sucrose medium and their adherence to \textit{C. albicans} strain 6406 cells produced under six different culture conditions was measured. Table 1 presents results for coaggregation with \textit{C. albicans} of two streptococcal strains representing distinct physiological and morphological groups. Strain Challis produces cells that have sparse peritrichous fibrils on their surfaces and which synthesize extra-cellular glucan from sucrose (R. J. Buchan and H. F. Jenkinson, Oral Microbiol. Immunol., in press), whereas strain PSH1b cells have lateral tufts of fibrils and do not synthesize extracellular glucan from sucrose (15).

The patterns of adherence of \textit{S. gordonii} Challis and \textit{S. sanguis} PSH1b to the yeast cells produced under the various growth conditions were virtually identical (Table 1) and irrespective of whether the streptococci were grown with glucose or sucrose. Coaggregation of streptococci with exponential-phase yeast cells grown at 28°C was low, better with 37°C-grown yeast cells, and best to yeast cells that had been starved for glucose at 37°C for 3 h or to cells that had, after starvation, been induced to form germ tubes (Table 1). Sucrose- or glucose-grown streptococcal cells coaggregated similarly (Table 1), and the presence of glucan associated with cells of strain Challis grown in TY-sucrose did not make them any more adherent to \textit{C. albicans}. Neither did inclusion of sucrose in the medium used to grow \textit{C. albicans} promote adherence of streptococcal cells to the yeasts (Table 1). It was important when comparing coaggregating abilities of yeast cells grown under different conditions that the same yeast biomass was present in each assay. Because it was impossible to determine individual cell numbers for mycelia by viable plate counts, in the assays where coaggregation of mycelial or nonmycelial cells was compared, a constant amount of yeast cell protein was added as the basis for ensuring valid comparisons (see Table 1).

Several other strains of \textit{S. sanguis} were tested for adherence to \textit{C. albicans} 6406 cells produced under the various conditions shown in Table 1, and in all cases the same overall patterns of coaggregation were observed. However, not all viridans streptococci that had been grown in TY-sucrose could be tested for coaggregation because polysaccharides produced from sucrose promoted their autoaggregation (see Materials and Methods).

Germ-tube formation and coaggregation ability. Glucose starvation is a prerequisite for germ-tube (mycelium) formation by \textit{C. albicans} in the induction system described (18). To determine whether the ability to form mycelia was essential for coaggregation of starved cells with streptococci, strains MEN and MM2002 were compared. Strain MM2002 is a spontaneous mutant of strain MEN and is unable to form germ tubes under standard conditions (3).
Table 2 shows results for coaggregation of S. gordonii ATCC 10558, S. sanguis CN3410, and S. anginosus NCTC 10713, each grown in TY-glucose medium, with C. albicans MEN and MM2002. For the streptococcal strains, their patterns of coaggregation were the same for both the C. albicans strains. Starvation of yeast cells promoted their coaggregation with the streptococci, irrespective of whether the yeast strain was able to form germ tubes (Table 2). All streptococcal strains coaggregated best with starved cells of C. albicans, although strain NCTC 10713 was less adherent than ATCC 10558 and CN3410 (Table 2). The percent coaggregations of S. gordonii Challis to strains MEN and MM2002 (not shown) were similar to its percent coaggregation with strain 6404 (Table 1). In separate experiments, the coaggregations of S. gordonii Challis and of several S. sanguis strains were measured to cells of a fourth C. albicans strain, CA2, which also does not form germ tubes. This strain was similar to, if not better than, the other C. albicans strains in its ability to coaggregate with S. gordonii and S. sanguis.

Cell surface hydrophobicity. There is evidence that the cell surface hydrophobicity of C. albicans, which is strongly influenced by growth conditions (17, 23), is a determinant in adherence of yeast to epithelial cells (16). By using the hexadecane partition assay (43), the cell surface hydrophobicity values for the four strains of C. albicans, 6406, MEN, MM2002, and CA2, were, respectively, 70 ± 5.5%, 52 ± 2.3%, 14 ± 1.8% and 9.1 ± 2.9% for exponential-phase cells grown at 28°C in SB-glucose medium. Starvation of cells at 37°C for 3 h did not alter these values significantly (results not shown). Since starved cells of all four C. albicans strains were more or less similar in their abilities to coaggregate with streptococci, this appeared to rule out yeast cell surface hydrophobicity as the determinant for streptococcal adherence.

Saturability and stability of streptococcus-yeast adherence. To establish optimum cell concentrations for comparison of the adherence properties of different streptococci to C. albicans, labeled streptococcal cells were mixed with increasing numbers of unlabeled C. albicans cells of strain CA2 that had been subjected to the starvation regime. In these experiments, the streptococci were grown in BHY medium because some species did not grow well in TY-glucose medium and cells of some strains, e.g., S. sanguis CR311, formed clumps. Results are presented for two strains of S. sanguis and for one strain each of S. gordonii, S. anginosus, S. mutans, S. pyogenes, and E. faecalis (Fig. 1). The addition of increasing numbers of C. albicans cells to a constant number of streptococcal cells showed for S. sanguis FW213 and CN3410, for S. gordonii Challis, and for S. anginosus ATCC 27335, saturation by the unlabeled yeast cells (Fig. 1). At a streptococcus/yeast cell ratio of unity for each of these strains, the addition of a tenfold excess of homologous unlabeled streptococcal cells did not displace the labeled cells from coaggregates. Adherence of S. pyogenes M4 was almost saturable but at a much lower streptococcus/yeast cell ratio, while S. mutans ATCC 25175 and E. faecalis JH2-2 were virtually nonadherent (Fig. 1) even at a ratio of 1 to 100 (streptococci/yeasts). Bound labeled cells of the latter two organisms were displaced from yeast cell aggregates by the addition of unlabeled homologous cells. At a ratio of about 4 to 1 (streptococci/yeasts), differences in affinity of attachment of the various streptococci were suggested (for example, approximately 80% of CN3410 cells bound, compared with only 30% of FW213 cells). At a ratio of unity, at least 80% of all cells from S. sanguis and S. gordonii strains tested were bound and about 60% of S. pyogenes M4 cells and less than 20% of S. mutans ATCC 25175 or E. faecalis JH2-2 cells were bound.

A compilation of results for coaggregation of 15 streptococcal strains to starved cells of C. albicans 6404 and C. albicans CA2 and to 28°C-grown cells of strain CA2 is given in Table 3. These tests were done at a streptococcus/yeast cell ratio of 2 to 1, this being below saturation for virtually all strains (Fig. 1). All strains of S. sanguis, S. gordonii, and S. oralis and two strains of S. anginosus coaggregated well with starved C. albicans cells but poorly to 28°C-grown exponential-phase yeast cells. S. sanguis CN3410 and Channon were consistently best in coaggregation with starved yeast cells. S. pyogenes strain M4 coaggregated with exponential-phase yeast cells and with starved cells (Table 3) but not as well as S. sanguis or S. gordonii did to starved cells. Differences in surface hydrophobicities of the streptococcal cells did not
correlate with their abilities to attach to the yeast cells. For example, strain Challis was hydrophobic (>80% in the hexadecane partition assay; see also reference 21), but strain PSH1b was not (see also reference 53); nevertheless, they both coaggregated with C. albicans.

**Effect of temperature and metabolic inhibitors on expression of yeast adhesin.** Exponential-phase cells of C. albicans CA2 at 28°C in SB-glucose medium were harvested by centrifugation and were suspended in prewarmed starvation medium, and the culture was then divided into five equal portions. These were incubated at 28 or at 37°C to determine whether temperature was critical for the starvation-induced coaggregation or at 37°C with either amphotericin B (2 μg/ml) or trichodermin (10 μg/ml). Coaggregation of S. gordonii Challis with the yeast cells harvested from the various cultures was measured as before with a Streptococcus/Candida cell ratio of 1.5 to 1. Maximum coaggregation of S. gordonii with C. albicans occurred after yeast cells had been starved for glucose for 3 h; however, even after 30 min of starvation at 37°C, streptococcal attachment was promoted (Table 4). Moreover, starvation did not need to be done at 37°C, and the C. albicans coaggregation adhesin was also induced in cells starved at 28°C (Table 4). The expression of the adhesin by the yeast cells was inhibited by addition of amphotericin B (which alters membrane permeability) and was virtually abolished by addition of trichodermin (Table 4), showing that de novo protein synthesis during starvation was necessary for expression.

**Factors influencing streptococcus–yeast cell coaggregation.** A number of compounds were tested for their ability to influence coaggregation of S. gordonii or S. gordonii with C. albicans. Attachment of S. gordonii Challis to starved CA2 cells was not influenced by pretreatment of the yeast cells with 0.1 M 2-mercaptoethanol nor was coaggregation affected by increasing the Ca²⁺ concentration in the assay to 50 mM. Adherence of S. gordonii was reduced by treatment of yeast cells with bovine serum albumin (200 μg/ml, 20 min, 20°C) before assay but not by treatment under identical conditions with hyaluronidase (EC 3.2.1.17). Coaggregations of S. sanguis and S. gordonii cells with C. albicans cells were >80% inhibited by addition of 10 mM EDTA. Intergeneric coaggregations between pairs of human oral bacteria are often reversible by addition of simple sugars to the reactions (27, 31, 32). The addition of 60 mM D-fucose, D-galactose, D-glucose, lactose, D-mannose, methyl α-D-glucoside, raffinose, L-rhamnose, N-acetyl-D-glucosamine, or N-acetyl-D-galactosamine to the assays did not affect coaggregations of S. sanguis FW213 and CN3410 or S. gordonii Challis with C. albicans.

**Effect of heat and protease treatments on coaggregation.** Heating of starved C. albicans CA2 cells at 80°C for 5 min (or treatment with 100 μg of pronase per ml at 28°C for 30 min) virtually abolished their ability to coaggregate with cells of S. gordonii Challis or S. sanguis FW213 (Table 5). Heat treating starved cells of CA2 also completely abolished their coaggregation with S. pyogenes M4 cells (Table 5). However, heating or pronase treating streptococcal cells had no effect on coaggregation with starved cells of CA2 (Table 5).

**DISCUSSION**

The description of coaggregation between viridans group streptococci and C. albicans extends our knowledge of the
range of possible intergeneric microbial coaggregations that can occur in the human oral cavity (27). The ability of S. sanguis and S. gordonii strains to coaggregate with other oral microbes appears to be a feature of the organisms, since they exhibit the widest range of coaggregating partners of all the oral streptococci (29, 31, 33). Throughout this paper we have used the terms "adherence to" and "coaggregation with" synonymously to describe the interaction of streptococci with yeast cells. It should be noted that while the interactions are considered to be analogous to interbacterial coaggregations, visible clumps are formed in the latter type and these can be scored with the naked eye. However, because noncoaggregated yeast cells rapidly settle out of suspension, and under some conditions (such as germ-tube cells) the yeast cells autoaggregate, coaggregation cannot be scored visually. Maximum streptococcal adherence was observed with most strains at an input ratio between 2 to 1 and 1 to 1, streptococci to yeast cells (Fig. 1), which represents a ratio of biomass of about 1 to 20. Microscopically, individual streptococcal cells or short chains could be seen attached to yeast cells, some acting as bridges between yeast cells. With the streptococcal strains that tended to autoaggregate (mainly strains producing cells with tufts of fibrils), bacterial aggregates were visible among yeast cell aggregates. Coaggregates of streptococci (50) or intergeneric bacterial coaggregates containing streptococci (29), as well as single streptococcal chains, could therefore potentially coaggregate in vivo with C. albicans.

Of the viridans group streptococci tested, all strains of S. sanguis and S. gordonii adhered to starved C. albicans cells and the coaggregation was unrelated to germ-tube formation by the yeast cells. Other streptococcal species showed some coaggregating ability, e.g., S. salivarius, while others were deficient, e.g., S. mutans and E. faecalis (Table 3). Generally the low coaggregation values (<10%), such as for E. faecalis with starved yeast cells and for most of the strains with 28°C-grown exponential-phase yeast cells, may represent nonspecific entrapment of streptococci. Nonspecific entrapment of streptococci was not, however, a factor in the starvation-induced coaggregations where addition of an excess of unlabeled cells failed to displace >10% labeled cells in coaggregates. Strains CN3410 and Shannon were usually better at coaggregation with C. albicans than were other S. sanguis strains (e.g., FW213) (Fig. 1). These differences might be related directly to the streptococcus-yeast cell coaggregation interactions or perhaps due to other factors influencing the coaggregations. For example, S. sanguis Channon was the most hydrophobic streptococcal strain included in this study and there is evidence that hydrophobic force influence coaggregations of streptococci and actinomyces (20, 21). There is no relationship apparent between the cell surface structure morphology of an S. sanguis or S. gordonii strain and its ability to coaggregate with C. albicans. For example S. sanguis CN3410 and PS11b both have tufts of fibrils (15), while S. sanguis Channon and S. gordonii Challis have peripheral fibrils (unpublished observations), and S. sanguis FW213 has filaments (11). Each of these strains coaggregated with starved C. albicans cells. The S. sanguis-C. albicans coaggregations do not show the strain specificities associated with S. sanguis-A. viscosus and S. sanguis-Actinomyces naeslundii coaggregations (6, 32, 33) and are not reversible by addition of lactose. In addition, unrelated isolates of C. albicans were similar in their coaggregation reactions with S. sanguis and S. gordonii, so our results do not enable coaggregation groups to be established as they have been for streptococci-actinomyces coaggregations (6, 33).

It has been proposed that adherence of C. albicans to various surfaces is mediated by specific mannoprotein adhesins (10, 22, 44), possibly associated with a flocculant layer (38), by cell wall adhesins (23), or by components located below the outer wall layer (45). Evidence suggests that both hydrophobic and electrostatic forces influence adherence of C. albicans to mucosal surfaces (17, 26) and that cell surface hydrophobicity plays a role in virulence (16). Starvation of yeast cells at 37°C, while promoting coaggregation, did not change their surface hydrophobicity, indicating that the coaggregation mechanism is not related to cell surface hydrophobicity. This is confirmed by the observation that the four strains of C. albicans used in this work showed varying degrees of hydrophobicity but similar coaggregation properties. Interestingly, the two least hydrophobic strains were those unable to form pseudohyphae.

Two observations suggest that starvation induces expression of new protein(s) at the yeast cell surface: trichodermin (and amphotericin B) inhibited expression of the coaggregation adhesin, and protease or heat treatments of yeast cells inhibited coaggregation with S. gordonii and S. sanguis. The expression of the surface adhesin(s) could involve delivery of new proteins to the surface or the uncovering of underlying protein(s) or both. The coaggregation adhesin was not removed from starved yeast cells by treatment with 2-mercaptoethanol, which extracts components from the outer portion of the cell wall (5), but it was masked by preincubation of yeast cells with bovine albumin, which also acts as a nonspecific blocking agent (22). Some streptococcal adhesion to salivary-gland mucin (12). A detailed analysis of the proteins exposed at the surface of exponential and starved yeast cells is necessary in order to identify the adhesin(s).

Although no simple sugars that were tested were effective in reversing or preventing coaggregation of yeast cells and streptococci, it seems that the mechanism is best explained by proposing a protein-carbohydrate interaction. The specificity of this interaction could be investigated by testing oligosaccharides for competition with coaggregation, similar to the way that streptococcal carbohydrate receptors for A. viscosus-S. sanguis coaggregates have been recently identified (4, 39).

Bagg and Silverwood (1) provided evidence to show that aggregations of various bacteria (including S. sanguis) to C. albicans occurred through the interaction of protein(s) on the bacterial cell surface with carbohydrate components on the yeast cell surface. Clearly, our results do not support this, and it would appear therefore that there is more than one type of complementary interaction between yeast and streptococcal cells. The coaggregations we describe are promoted, in some cases by at least 10-fold, by glucose starvation of the yeast cells. These results emphasize the influence environmental growth conditions can have on C. albicans cell adherence properties (23). Adherence of C. albicans to indigenous attached bacteria is suggested as a means by which yeast can associate with and adhere to uroepithelial cells and gut mucosa (22, 24). The coaggregation reactions described in this paper may be significant in the colonization of mucosal and hard surfaces in the oral cavity by the yeast cells. Once cells of C. albicans become attached to bacteria and to a surface, their proliferation could be enhanced by metabolic products of oral bacteria, e.g., polysaccharides (2, 42). It is also evident that the cell surface properties of C. albicans are modified rapidly in response to environmental changes. A transient expression...
of surface hydrophobicity is proposed to be a mechanism by which C. albicans could become more virulent (16). On the basis of our observations, it is suggested that the surface changes induced by starvation could specifically aid oral colonization by C. albicans. Attachment of slow-growing or non-growing C. albicans cells would be promoted, thus enabling the yeasts to become integral components of mixed microbial cell networks.

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LITERATURE CITED


