INFECTION AND IMMUNITY, July 1984, p. 6–12
0019-9567/84/070006-07$02.00/0
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Vol. 45, No. 1

Relationship Between Cell Surface Composition, Adherence, and Virulence of *Candida albicans*

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Received 28 December 1983/Accepted 21 March 1984

A comparison was made of the adherence to acrylic and to human buccal epithelial cells of seven strains of *Candida albicans* isolated from active infections (I strains) and two strains obtained from asymptomatic carriers (C strains). After growth in defined medium containing a relatively low concentration (50 mM) of glucose as the carbon source, the adherence of I and C strains to either surface was similar and all strains were sensitive to spheroplast formation with Zymolyase 5000. Growth in medium containing a high concentration (500 mM) of sucrose or galactose enhanced the adherence of I strains up to 5- and 11-fold, respectively, and there were corresponding increases in resistance to spheroplast formation. Sucrose- or galactose-grown C strains showed only small increases in adherence and remained relatively sensitive to spheroplast formation. When inoculated intravenously into mice, I strains grown in 500 mM sucrose were up to five times more virulent than organisms grown in 50 mM glucose, while I strains grown in 500 mM galactose showed a 5- to 24-fold increase in virulence. Fifty percent lethal doses obtained for C strains were similar after growth on all three carbon sources. We conclude that I strains are able to modify their surface composition in response to high concentrations of certain sugars in the growth environment. Such modification can enhance both their ability to adhere to surfaces and their virulence. C strains lack this capability, or possess it to a lower degree, and may therefore have a lower pathogenic potential.

In contrast to the enormous research effort devoted to the study of bacterial adherence, particularly during the last decade (1), relatively few investigations have focused on yeast adhesion. Recently, however, increasing recognition of the importance of adherence in the pathogenesis of many microbial infections has stimulated considerable interest in the adhesion of the opportunistic yeast pathogen, *Candida albicans*, to mucosal and other surfaces. Superficial candidosis now ranks among the most common of all infectious diseases, with oral and vaginal infections as its most prevalent forms (8). For this reason, human buccal and vaginal epithelial cells have been frequently used in studies of *C. albicans* adherence in vitro (9, 10, 12, 25, 27; for a review, see L. J. Douglas, *in* A. H. Rose and J. S. Harrison, ed., *The Yeasts*, 2nd ed., vol. 1, in press).

In a previous study relating to the role of *C. albicans* in denture stomatitis, we measured adherence of the yeast to acrylic surfaces (5, 14). Adhesion to acrylic in vitro was enhanced to different extents after growth of the yeast in defined medium containing a high concentration of galactose, sucrose, maltose, glucose, or fructose as the carbon source. Galactose was the most effective carbon source of those tested; organisms grown in 500 mM galactose showed more than 10-fold-greater adherence than control yeasts grown in medium with a relatively low concentration (50 mM) of glucose. Subsequent work indicated that adherence of *C. albicans* to human buccal epithelial cells was similarly promoted by growth in medium with a high sugar content (4).

Enhanced adherence appeared to be due to the production of an additional fibrillar surface layer which also conferred increased resistance to spheroplast formation with Zymolyase 5000 (14). Synthesis of the fibrillar layer in response to high concentrations of sugar occurred during the stationary phase of growth, since mid-exponential-phase yeasts harvested from such media were poorly adherent and were sensitive to Zymolyase 5000 (5). The antibiotic tunicamycin, when added to cultures at the onset of the stationary phase, inhibited this synthesis, suggesting that the fibrillar component consisted largely, if not entirely, of mannoprotein (6).

These earlier studies concentrated mainly on a single strain of *C. albicans* (GDH 2346) which had been isolated from a patient with denture stomatitis. In the present investigation, we have extended our survey to encompass nine *C. albicans* strains, including some isolated from active infections and others obtained from asymptomatic carriers. For all of these strains, an attempt was made to correlate cell surface composition with adherence to acylic and to buccal epithelial cells after growth of the yeasts in defined medium containing glucose, sucrose, or galactose as the carbon source. The virulence for mice of each strain after growth under these conditions was also determined.

MATERIALS AND METHODS

Organisms. Nine strains of *C. albicans* were used. *C. albicans* MRL 3153 was from the Mycological Reference Laboratory at the London School of Hygiene and Tropical Medicine, London, England. The remaining eight strains were all isolated in Glasgow and have recently been deposited with the National Collection of Yeast Cultures (NCYC), Food Research Institute, Norwich, England. *C. albicans* BP 3968 (NCYC 1466) was obtained from a cutaneous candidosis lesion; strains GDH 2346 (NCYC 1467), GDH 2036 (NCYC 1469), and GDH 2023 (NCYC 1468) were isolated at Glasgow Dental Hospital from patients with denture stomatitis; strains GRI 681 (NCYC 1472) and GRI 682 (NCYC 1473) were obtained from routine cervical smears taken from asymptomatic women at Glasgow Royal Infirmary; and strains GRI 2773 (NCYC 1470) and GRI 272 (NCYC 1471) were isolated from patients with vaginitis. The organisms

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were maintained on slopes of Sabouraud dextrose agar (Difco Laboratories) and subcultured monthly. Every 2 months the cultures were replaced by new ones freshly grown from freeze-dried stocks.

Growth conditions. The yeasts were grown in yeast nitrogen base medium (Difco) containing 50 mM glucose or 500 mM glucose, sucrose, galactose, maltose, or fructose, as described previously (14). The organisms grew at similar rates and exclusively in the budding yeast phase in all of these media. They were harvested after 24 h (stationary growth phase) and washed twice in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).

Adherence to acrylic. Yeast suspensions (1.25×10^7 to 2.5×10^7 cells ml^{-1} in PBS) were incubated with transparent acrylic strips, and the numbers of adherent organisms were determined by microscopy, as reported previously (14). All adherence values quoted were obtained by counting 30 fields on each of at least 12 acrylic strips.

Adherence to buccal epithelial cells. Standardized suspensions of human buccal epithelial cells (2.5×10^6 cells ml^{-1} in PBS [0.1 ml]) and yeasts (2.5×10^9 organisms ml^{-1} in PBS [0.1 ml]) were mixed and incubated at 37°C, with gentle shaking, for 45 min. Epithelial cells were then collected by filtration, washed, and stained as described previously (4). The numbers of adherent yeasts on each of 100 epithelial cells were counted on every filter. Duplicate filters were prepared for each assay. All adherence values quoted represent mean figures derived from four independent assays. Epithelial cells were obtained from the buccal mucosa of a single donor (4) and were always collected at the same time of day to minimize variability.

Sensitivity to Zymolyase 5000. The rate of spheroplast formation during treatment of yeasts with Zymolyase 5000 (Miles Laboratories, Slough, England) was monitored as described previously (14). Cells were suspended in 50 mM Tris-hydrochloride buffer (pH 7.2) containing 10 mM $MgCl_2$ and 1 M sorbitol. Zymolyase 5000 (3 mg ml^{-1}) was added, and the suspensions were incubated at 30°C with gentle shaking. At intervals, samples (0.1 ml) were removed from the mixtures and diluted 30-fold in water; after 10 min their absorbance at 600 nm was measured.

Determination of LD_{50}. Five groups of 10 HaM/ICR mice, aged 8 to 10 weeks, were inoculated via the lateral tail vein with yeast suspensions (0.1 ml in saline) containing 10^7, 10^6, 10^5, 10^4, or 10^3 CFU. Deaths were recorded three times daily over 7 days, and 50% lethal dose (LD_{50}) values were calculated by the Kärber method (3).

Statistical analyses. The Student *t* test was used to evaluate differences in yeast adherence. A P value of <0.05 was considered significant. Differences in virulence were assessed after calculating the 95% confidence limits for LD_{50} values.

RESULTS

Adherence to acrylic and to buccal epithelial cells. Of the nine strains of *C. albicans* used in this study, seven (I strains) were originally isolated from active infections, while two (C strains) were obtained from asymptomatic carriers. For each strain, the adherence of organisms grown in medium with a relatively low concentration (50 mM; 0.9%, wt/vol) of glucose as the carbon source was compared with that of yeasts harvested from medium containing a high concentration (500 mM) of sucrose or galactose. All of the I strains adhered in greater numbers to acrylic and to epithelial cells after growth in medium containing sucrose or galactose, although the extent to which adherence was enhanced varied from strain to strain (Fig. 1). Sucrose-grown yeasts were up to five times more adherent than organisms of the same strain grown in 50 mM glucose, while galactose-grown yeasts showed a 5- to 11-fold increase in adherence. Of the seven I strains tested, *C. albicans* GDH 2346 gave the greatest increase in adherence after growth in galactose-containing medium, and strain GDH 2023 gave the smallest increase; absolute adherence values, on the other hand, were highest with strain GDH 2023 after growth on all three carbon sources (Fig. 1).

The two C strains (GRI 681 and GRI 682) adhered to acrylic and to epithelial cells in numbers comparable to those of I strains after growth in 50 mM glucose medium. However, growth in medium with either 500 mM sucrose or 500 mM galactose enhanced the adherence of these strains relatively little (Fig. 1).

Sensitivity to spheroplast formation. Differences in yeast cell surface composition were investigated by measuring the sensitivity of the *C. albicans* strains to spheroplast formation with Zymolyase 5000. In the assay procedure used, samples were removed from buffered mixtures containing yeasts and enzyme and were diluted in water; the rate of decrease in absorbance of successive samples corresponded to the rate of spheroplast formation (14). Figure 2 shows the results obtained with two representative I strains (GDH 2036 and GRI 2773). In each case, yeasts grown in medium with 500 mM galactose or sucrose were more resistant to Zymolyase than were yeasts grown in 50 mM glucose. Moreover, the rate of spheroplast formation correlated well with the relative adherence of the yeasts to acrylic and to buccal epithelial cells (Fig. 1). Galactose-grown yeasts were most resistant to Zymolyase and most adherent to either surface. Similar results were obtained for the other seven I strains. By contrast, the two C strains (GRI 681 and GRI 682) remained relatively sensitive to spheroplast formation after growth in medium containing 500 mM sucrose or galactose (Fig. 3); the same growth conditions also affected the adherence of these strains relatively little (Fig. 1).

Virulence for mice. To investigate whether the changes in yeast cell surface composition which result from growth on certain carbon sources at high concentration affect the virulence of *C. albicans*, 7-day LD_{50} tests in mice were done with all nine strains. Table 1 compares results obtained using an I strain (GDH 2346) and a C strain (GRI 681) after growth of each organism on a variety of carbon sources. The virulence of strain GDH 2346 varied quite markedly according to the carbon source present in the growth medium, whereas that of strain GRI 681 was only slightly affected. With strain GDH 2346, galactose-grown organisms were the most virulent and gave an LD_{50} some 16-fold lower than that obtained with yeasts grown in 50 mM glucose. Strain GRI 681, on the other hand, was most virulent after growth in medium containing 500 mM maltose, with an LD_{50} approximately one-half that of organisms grown in 50 mM glucose. The highest LD_{50} values for both strains were recorded with yeasts grown in 500 mM fructose (Table 1).

LD_{50} values for all nine strains of *C. albicans* after growth in medium containing 50 mM glucose, 500 mM sucrose, or 500 mM galactose are compared in Fig. 4. Although there was some overlap in 95% confidence limits, galactose-grown organisms were consistently more virulent than sucrose-grown yeasts of the same strain, and the sucrose-grown yeasts, in turn, were more virulent than glucose-grown cells. With I stains, sucrose-grown yeasts were up to five times more virulent than organisms grown in 50 mM glucose, while

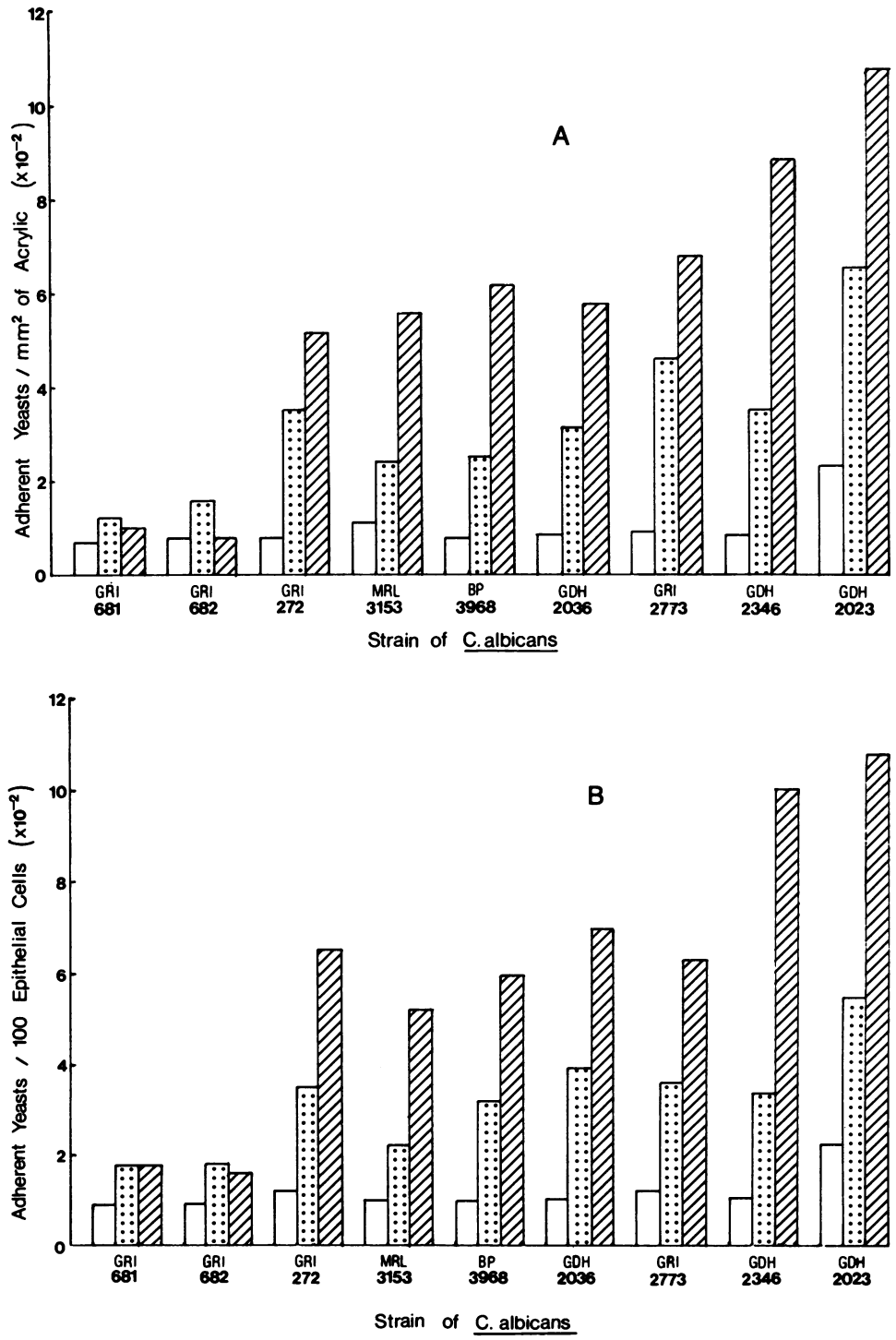

FIG. 1. Adherence of nine strains of *C. albicans* to acrylic (A) and to buccal epithelial cells (B) after growth in medium containing 50 mM glucose (□), 500 mM sucrose (▦), or 500 mM galactose (▨). Standard errors range from 1.3 to 7.8% of mean values in (A) and from 2.3 to 9.3% of mean values in (B).

galactose-grown yeasts showed a 5- to 24-fold increase in virulence. By contrast, differences in virulence for the C strains (GRI 681 and GRI 682) were very small (Table 1 and Fig. 4). LD_{50} values recorded for *C. albicans* GDH 2023 were considerably higher than those obtained for any other strain; however, the relative virulence of this organism after growth on galactose, sucrose, or glucose was similar to that of other I strains (Fig. 4).

DISCUSSION

The results presented here indicate that strains of *C. albicans* can be broadly divided into two categories. The

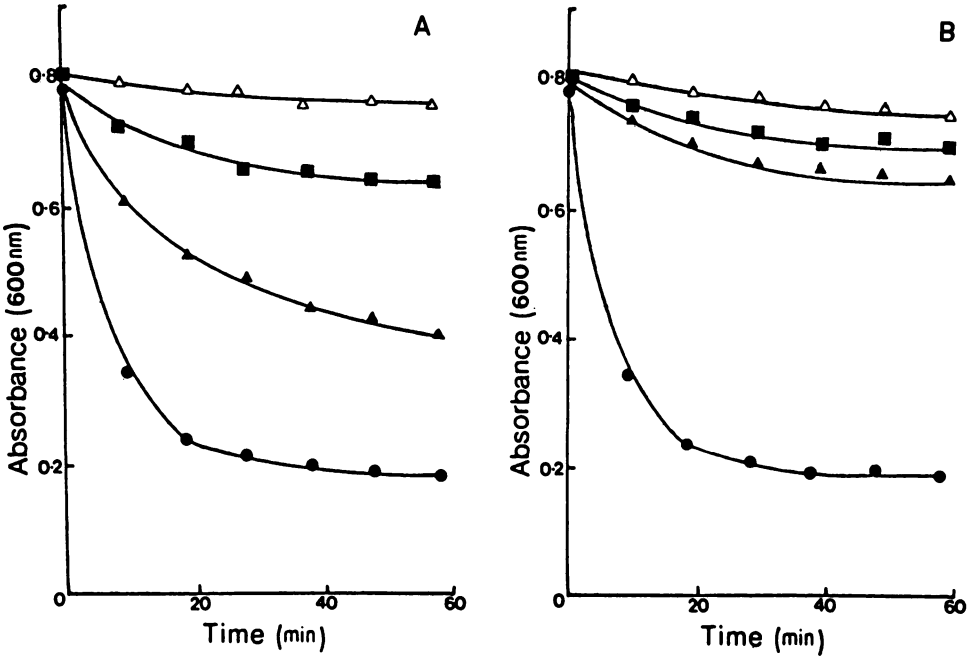

FIG. 2. Sensitivity to Zymolyase 5000 of stationary-phase *C. albicans* GDH 2036 (A) and *C. albicans* GRI 2773 (B), both grown in yeast nitrogen base medium containing 500 mM galactose (■), 500 mM sucrose (▲), or 50 mM glucose (●). Results for control yeasts grown in medium with 500 mM galactose and incubated without Zymolyase are also shown (△).

first category comprises strains which have the ability to modify their cell surface composition in response to high concentrations of certain sugars, notably galactose, in the growth medium. Such a modification promotes adherence of the yeast to acrylic and epithelial cell surfaces and enhances its virulence for mice. Strains in the second category either lack this capability completely or possess it to a much lower degree. Thus, growth of these strains in medium with a high galactose content has relatively little effect on either adherence or virulence. Of the nine *C. albicans* strains used in this

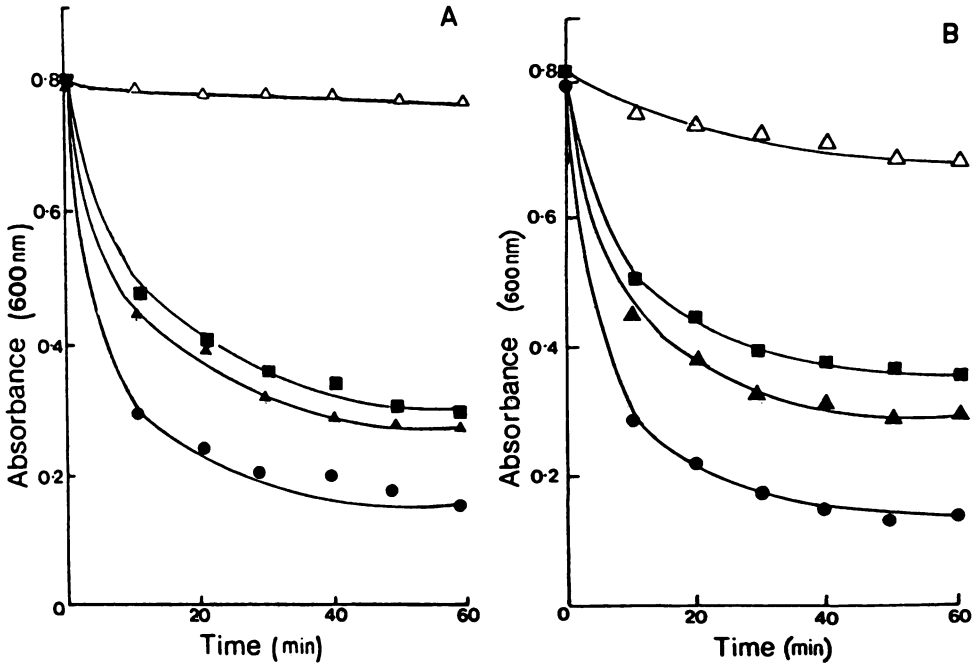

FIG. 3. Sensitivity to Zymolyase 5000 of stationary-phase *C. albicans* GRI 681 (A) and *C. albicans* GRI 682 (B), both grown in yeast nitrogen base medium containing 500 mM galactose (■), 500 mM sucrose (▲), or 50 mM glucose (●). Results for control yeasts grown in medium with 500 mM galactose and incubated without Zymolyase are also shown (△).

TABLE 1. Virulence for mice of *C. albicans* GDH 2346 and GRI 681 after growth in defined medium with different carbon sources

Strain	Carbon source[a]	LD_{50} (10^5 viable units)	Relative virulence[b]
GDH 2346	Glucose (50 mM)	5.1	1.0
	Fructose	8.2	0.6
	Glucose	2.2	2.3
	Maltose	1.6	3.2
	Sucrose	0.96	5.3
	Galactose	0.31	16.5
GRI 681	Glucose (50mM)	7.9	1.0
	Fructose	14.9	0.5
	Glucose	6.9	1.1
	Maltose	4.1	1.9
	Sucrose	7.1	1.1
	Galactose	6.8	1.2

[a] Carbon sources were present at 500 mM, except where indicated.

[b] Virulence relative to that of yeasts of the same strain grown in medium containing 50 mM glucose.

study, seven fell into the first category and two into the second. Each of the seven strains in the first group had been isolated originally from an active infection, while both strains in the second group were obtained from asymptomatic carriers. Clearly, nine is too small a number of strains to judge whether this apparent correlation is genuine, although presumably the ability to modify cell surface composition would be advantageous to the organism in vivo and could be an important virulence factor. In this connection, it is interesting that Poulain et al. (17) have recently provided evidence for antigenic differences between *C. albicans* strains freshly isolated from patients with candidosis and those isolated from healthy subjects. The same workers have also demonstrated phenotypic variability in the antigenic structure of *C. albicans* in vitro and in vivo (18).

Our earlier ultrastructural studies indicated that the modification in surface composition which renders I strains resistant to treatment with Zymolyase involves the production of an outer fibrillar layer (14). The fibrils may represent appendages analogous to bacterial fimbriae, whose importance in adhesion is widely recognized (1). Ultrastructural and serological evidence for the existence of fimbriae in a variety of yeast species has been produced recently (7); two of the *C. albicans* I strains used here (GDH 2346 and MRL 3153) gave positive results in this survey. Strain differences in sensitivity to Zymolyase could therefore reflect different degrees of fimbriation. Similarly, differences between galactose- and glucose-grown yeasts of the same strain could reflect phenotypic variation in the extent of fimbriation. Such variation has been demonstrated with certain bacteria; it is known, for example, that the synthesis of fimbriae by *Salmonella typhimurium* is dependent on cyclic AMP and subject to catabolite repression by many carbohydrates (24).

The physiological basis for phenotypic modification of surface composition in I strains remains to be established. However, it is already clear that the surface changes induced in these organisms by high concentrations of different sugars could be important in vivo. Sucrose, glucose, and maltose are common dietary sugars (16), while galactose may be produced in the mouth as a result of lactose degradation by oral bacteria (2). The observation that all of these sugars can promote adherence of I strains to acrylic or to buccal epithelial cells (4, 14; Fig. 1) could partly account for the clinical finding that a carbohydrate-rich diet often contributes to the development and persistence of oral candidosis (22, 26).

Phenotypic variation in yeast surface composition could

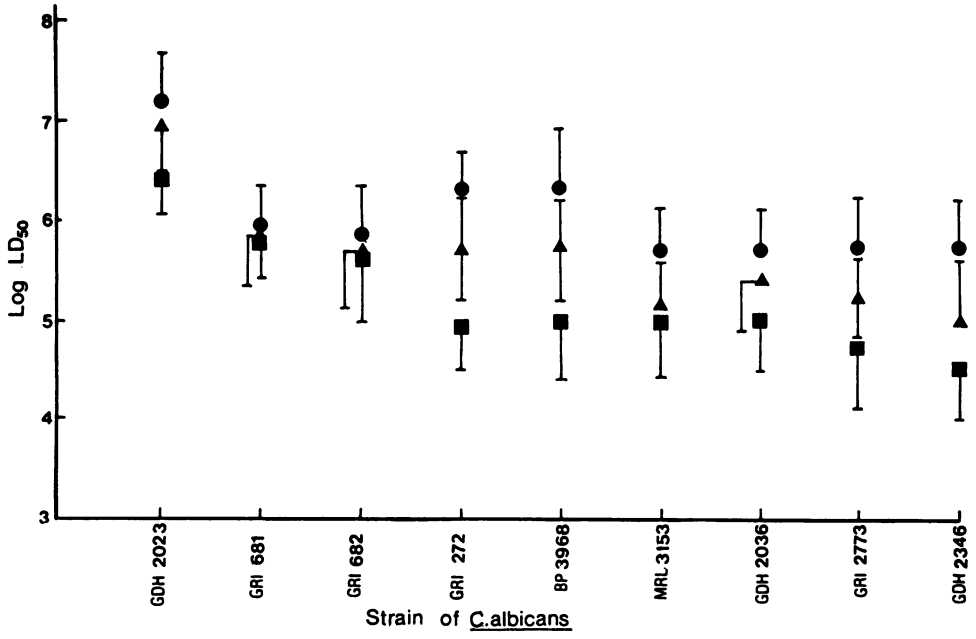

FIG. 4. Virulence for mice of nine strains of *C. albicans* after growth to stationary phase in medium containing 50 mM glucose (●), 500 mM sucrose (▲), or 500 mM galactose (■). Bars represent 95% confidence limits.

also play a significant role in the pathogenesis of vaginal candidosis. Diabetic and pregnant women, who are especially susceptible to this infection, have high levels of vaginal glycogen which can be converted to glucose by enzymes present in the tissue or produced by the normal flora (23). We did not investigate the adherence of I strains to vaginal epithelial cells in the present study. However, earlier experiments with *C. albicans* GDH 2346 showed that adherence to acrylic (14) or to buccal epithelial cells (4) more than doubled when the concentration of glucose in the growth medium was increased from 50 to 500 mM.

The mechanism by which growth in medium containing a high concentration of sucrose or galactose enhances the virulence of I strains has yet to be determined. One obvious possibility would be that the fibrillar surface layer synthesized by the yeasts under these conditions affords protection against phagocytosis. Another pathogenic yeast, *Cryptococcus neoformans*, is known to resist phagocytosis by producing a polysaccharide capsule which inhibits the attachment of yeast to phagocyte (11). Fimbriae can also be antiphagocytic; fimbriated gonococci, for example, are more resistant to phagocytosis than are nonfimbriated variants (19). An alternative explanation for the enhanced virulence of sucrose- or galactose-grown I strains could be increased resistance to intracellular killing by phagocytes. In a recent study (20), strains of *C. albicans* known to differ in their virulence for mice were found to be equally susceptible to phagocytosis. However, the more virulent strains showed a greater propensity for intracellular survival. Myeloperoxidase-mediated killing of *C. albicans* requires binding of the enzyme to target yeasts and is antagonized by mannan solubilized from the yeast cell wall (28). Since the fibrillar layer synthesized by I strains is released from the yeast surface relatively readily (14) and appears to consist largely of mannoprotein (6), it too might inhibit candidacidal activity by interfering with the binding of myeloperoxidase to the cell wall proper.

One of the I strains used in this study, *C. albicans* GDH 2023, was markedly less virulent for mice than any other organism tested, including both C strains. Paradoxically, this organism was the most adherent I strain. Growth in 500 mM galactose or sucrose medium enhanced its adhesiveness and its virulence to an extent similar to that observed with other I strains, presumably by promoting synthesis of the fibrillar surface layer. It is possible that the diminished virulence of this organism is due to the presence, under all growth conditions, of an additional, unique surface component which somehow increases the affinity of the yeast for both epithelial and phagocytic cells. On the other hand, strain GDH 2023 may be deficient in some other virulence factor. The determinants of virulence in *C. albicans* are poorly understood (15) but are thought to include the ability to form hyphae (21) and to produce hydrolytic enzymes such as proteinases (13), as well as the ability to adhere to mucosal surfaces. Investigation of these other properties in strain GDH 2023 might provide further insight into their relative importance in the native virulence of *C. albicans*.

ACKNOWLEDGMENTS

We thank D. E. S. Stewart-Tull and H. Shannon for expert assistance with the animal experiments.

LITERATURE CITED

1. **Beachey, E. H.** 1980. Bacterial adherence. Receptors and recognition, series B, vol. 6. Chapman and Hall, London.
2. **Chauncey, H. H., F. Lionetti, R. A. Winer, and V. F. Lisanti.** 1954. Enzymes of human saliva. 1. The determination, distribution, and origin of whole saliva enzymes. J. Dent. Res. **33**:321–334.
3. **Cruickshank, R.** 1969. Medical microbiology, 11th ed., p. 883. E. & S. Livingstone Ltd., Edinburgh.
4. **Douglas, L. J., J. G. Houston, and J. McCourtie.** 1981. Adherence of *Candida albicans* to human buccal epithelial cells after growth on different carbon sources. FEMS Microbiol. Lett. **12**:241–243.
5. **Douglas, L. J., and J. McCourtie.** 1981. Adherence of *Candida albicans* to denture acrylic as affected by changes in cell-surface composition, p. 375-380. *In* G. G. Stewart and I. Russell (ed.), Current developments in yeast research. Pergamon Press, Toronto.
6. **Douglas, L. J., and J. McCourtie.** 1983. Effect of tunicamycin treatment on the adherence of *Candida albicans* to human buccal epithelial cells. FEMS Microbiol. Lett. **16**:199–202.
7. **Gardiner, R., C. Podgorski, and A. W. Day.** 1982. Serological studies on the fimbriae of yeasts and yeastlike species. Bot. Gaz. **143**:534–541.
8. **Hurley, R.** 1980. The pathogenic *Candida* species and diseases caused by candidas in man. Soc. Appl. Bacteriol. Symp. Ser. **9**:231–248.
9. **Kimura, L. H., and N. N. Pearsall.** 1978. Adherence of *Candida albicans* to human buccal epithelial cells. Infect. Immun. **21**:64–68.
10. **King, R. D., J. C. Lee, and A. L. Morris.** 1980. Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. Infect. Immun. **27**:667–674.
11. **Kozel, T. R., and R. P. Mastroianni.** 1976. Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis. Infect. Immun. **14**:62–67.
12. **Lee, J. C., and R. D. King.** 1983. Characterization of *Candida albicans* adherence to human vaginal epithelial cells in vitro. Infect. Immun. **41**:1024–1030.
13. **MacDonald, F., and F. C. Odds.** 1983. Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-deficient mutant. J. Gen. Microbiol. **129**:431–438.
14. **McCourtie, J., and L. J. Douglas.** 1981. Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. Infect. Immun. **32**:1234–1241.
15. **Odds, F. C.** 1979. Candida and candidosis. Leicester University Press, Leicester.
16. **Page, L., and B. Friend.** 1974. Level of use of sugars in the United States, p. 94–107. *In* H. L. Sipple and K. W. McNutt (ed.), Sugars in nutrition. Academic Press, Inc., New York.
17. **Poulain, D., G. Tronchin, B. Lefebvre, and M. O. Husson.** 1982. Antigenic variability between *Candida albicans* blastospores isolated from healthy subjects and patients with *Candida* infection. Sabouraudia **20**:173–177.
18. **Poulain, D., G. Tronchin, A. Vernes, R. Popeye, and J. Biguet.** 1983. Antigenic variations of *Candida albicans in vivo* and *in vitro*—relationships between P antigens and serotypes. Sabouraudia **21**:99–112.
19. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
20. **Richardson, M. D., and H. Smith.** 1981. Resistance of virulent and attenuated strains of *Candida albicans* to intracellular killing by human and mouse phagocytes. J. Infect. Dis. **144**:557–564.
21. **Richardson, M. D., and H. Smith.** 1981. Production of germ tubes by virulent and attenuated strains of *Candida albicans*. J. Infect. Dis. **144**:565–569.
22. **Ritchie, G. M., A. M. Fletcher, D. M. G. Main, and A. S. Prophet.** 1969. The etiology, exfoliative cytology, and treatment of denture stomatitis. J. Prosthet. Dent. **22**:185–200.
23. **Rogosa, M., and M. E. Sharpe.** 1960. Species differentiation of human vaginal lactobacilli. J. Gen. Microbiol. **23**:197–201.
24. **Saier, M. H., Jr., M. R. Schmidt, and M. Leibowitz.** 1978. Cyclic AMP-dependent synthesis of fimbriae in *Salmonella typhimurium*: effects of *cya* and *pts* mutations. J. Bacteriol. **134**:356–358.
25. **Sandin, R. L., A. L. Rogers, R. J. Patterson, and E. S. Beneke.** 1982. Evidence for mannose-mediated adherence of *Candida*

albicans to human buccal cells in vitro. Infect. Immun. **35**:79–85.

26. **Shuttleworth, C. W., and F. J. Gibbs.** 1960. The aetiological significance of *Candida albicans* in chronic angular cheilitis and its treatment with nystatin. Br. Dent. J. **108**:354–356.
27. **Sobel, J. D., P. G. Myers, D. Kaye, and M. E. Levison.** 1981. Adherence of *Candida albicans* to human vaginal and buccal epithelial cells. J. Infect. Dis. **143**:76–82.
28. **Wright, C. D., J. U. Bowie, G. R. Gray, and R. D. Nelson.** 1983. Candidacidal activity of myeloperoxidase: mechanisms of inhibitory influence of soluble cell wall mannan. Infect. Immun. **42**:76–80.