Extracellular Polymer of *Candida albicans*: Isolation, Analysis and Role in Adhesion

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Extracellular polymeric material (EP) was isolated from culture supernatants of *Candida albicans* grown on carbon sources (50 mM-glucose, 500 mM-sucrose or 500 mM-galactose) known to promote yeast adhesion to different extents. Galactose-grown yeasts, which are the most adherent, produced more EP than sucrose-grown organisms, particularly after incubation for 5 d, while glucose-grown yeasts (the least adherent) gave the lowest yield. EP produced on all three carbon sources was of similar composition and contained carbohydrate (65 to 82%; mannose with some glucose), protein (7%), phosphorus (0.5%) and glucosamine (1.5%). Serological studies indicated that these EP preparations were immunologically identical but that galactose-grown yeasts had more antigenic determinants than sucrose-grown organisms while glucose-grown yeasts had the fewest determinants. Antigenic differences were apparent between EP preparations of some strains of *C. albicans*. Pretreatment of acrylic strips with EP to form a polymeric coating promoted yeast adhesion to the acrylic surface, but similar pretreatment of buccal epithelial cells with EP inhibited subsequent yeast adhesion. These results indicate that EP originates from the cell surface of *C. albicans* and that it contains the surface component(s), probably mannoprotein in nature, responsible for yeast adhesion.

INTRODUCTION

Oral thrush and denture stomatitis are two of the commonest forms of superficial candidosis (Odds, 1979). Adhesion of *Candida albicans* to buccal epithelial cells or to the acrylic denture can be regarded as the first stage in the pathogenesis of these diseases. Using *C. albicans* strains isolated from active infections, we have previously shown that adhesion to either surface *in vitro* is substantially increased after growth of the yeast in defined media containing high concentrations of certain sugars, notably galactose and sucrose, as the carbon source (McCourtie & Douglas, 1981; Douglas *et al.*, 1981; McCourtie & Douglas, 1984). Organisms grown in medium containing 500 mM-galactose, for example, are up to eleven times more adherent than control yeasts grown in medium with a relatively low concentration (50 mM) of glucose. Enhanced adhesion appears to result from the production of an additional fibrillar layer on the yeast cell surface, a change which also confers increased resistance to spheroplast formation with Zymolyase-5000 and increased virulence of the yeast for mice (McCourtie & Douglas, 1984). Thus, organisms harvested from medium containing 500 mM-galactose are more adherent, more resistant to spheroplast formation and more virulent than are 50 mM-glucose-grown yeasts, in which the fibrillar component is largely absent.

A fibrillar-floccular layer on the surface of *C. albicans* growing *in vivo* has been demonstrated by electron microscopy of scrapings taken from the tongue and buccal mucosa of patients with oral candidosis (Montes & Wiborn, 1968; Mohamed, 1975; Marrie & Costerton, 1981). In such preparations the fibrils could be seen to mediate yeast attachment to epithelial cells. *In vitro*,

Abbreviation: EP, extracellular polymeric material.

synthesis of the fibrillar layer in response to high concentrations of sugar takes place during the stationary phase of growth (Douglas & McCourtie, 1981) and electron microscopy of stationary-phase organisms suggests that fibrils are released from the yeast surface relatively readily (McCourtie & Douglas, 1981). The present paper describes the isolation and analysis of extracellular polymeric material (EP) from culture supernatants of *C. albicans* and provides evidence that this material originates from the yeast surface where it plays a role in adhesion.

METHODS

Organisms. Four strains of *C. albicans* were used in this study. *Candida albicans* MRL 3153 was from the Mycological Reference Laboratory at the London School of Hygiene and Tropical Medicine, London, UK. The remaining three strains were isolated in Glasgow and have recently been deposited with the National Collection of Yeast Cultures (NCYC), Food Research Institute, Norwich, UK. *Candida albicans* BP 3968 (NCYC 1466) was obtained from a cutaneous candidosis lesion; strains GDH 2346 (NCYC 1467) and GDH 2023 (NCYC 1468) were isolated at Glasgow Dental Hospital from patients with denture stomatitis. The organisms were maintained on slopes of Sabouraud dextrose agar (Difco) and subcultured monthly. Every two 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, 500 mM-sucrose or 500 mM-galactose as described previously (McCourtie & Douglas, 1981). 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.

Isolation of EP. EP was isolated from culture supernatants by precipitation with acetone. Batches of medium (500 ml in 2 litre Erlenmeyer flasks) were inoculated with overnight yeast cultures (50 ml) and incubated at 37 °C for 24 h or 5 d in an orbital shaker operating at 150 r.p.m. Yeasts were removed by centrifugation and culture supernatant (500 ml) was added to 1.5 vols cold (4 °C) acetone containing approximately 1 g sodium acetate to aid precipitation. After 2 h at 4 °C, the resultant precipitate was collected by centrifugation at 17000 g for 30 min. The pellet was redissolved in a small volume (10 to 15 ml) of distilled water and any insoluble material was removed by centrifugation. Water-soluble polymer was recovered by acetone precipitation (3 vols; 2 h at 4 °C), washed with ether and evaporated to dryness *in vacuo*. The dried material was weighed and the yield was calculated as a percentage of the combined dry weight of yeast cells and EP.

Crude EP preparations, dissolved in a minimum volume of distilled water, were dialysed at 4 °C for 24 h against two changes (9 litres each) of distilled water. EP in the retentate was precipitated with acetone, dried and weighed. All samples, dialysed and undialysed, were stored at -20 °C. Qualitative or quantitative analyses, adherence assays and immunological studies were done only with dialysed material. Prior to analysis for carbohydrate, in some cases, EP preparations were treated with detergent. EP was dissolved in 0.1% Triton X-100 and the solution was stirred continuously at room temperature for 5 h. Detergent-treated EP was recovered by precipitation with acetone (2 vols; 2 h at 4 °C), washed with ether and evaporated to dryness *in vacuo*.

Analysis of EP. Qualitative analysis of EP preparations was done by paper chromatography of acid hydrolysates; results were confirmed by gas-liquid chromatography (kindly performed by Dr John G. Beeley). EP (2 mg) was hydrolysed in 2 M-HCl (1 ml) at 105 °C for 3 h in sealed ampoules. Hydrolysates were evaporated to dryness *in vacuo*, redissolved in a few drops of distilled water and subjected to descending chromatography on Whatman no. 1 paper in butan-1-ol/pyridine/water (6:4:3, by vol.; Jeanes *et al.*, 1951). Hydrolysis products and standard sugars were visualized with alkaline silver nitrate (Trevelyan *et al.*, 1950).

For quantitative analysis, protein was determined by the Lowry method and phosphorus by the method of Chen *et al.* (1956). Total carbohydrate was estimated according to the procedure of Dubois *et al.* (1956), using mannose as a standard. Glucose content was determined with a Boehringer GOD-Perid test combination after hydrolysis of samples in 0.5 M-HCl at 100 °C for 5 h. Hexosamine was estimated by the method of Blumerkrantz & Asboe-Hansen (1976) using glucosamine as a standard; before analysis, samples were hydrolysed in 1 M-HCl at 105 °C for 6 h.

Adhesion assays. Yeast adhesion to transparent acrylic strips or to exfoliated human buccal epithelial cells was determined by light microscopy as described previously (McCourtie & Douglas, 1984).

Preparation of antisera. Dialysed EP (20 mg) was dissolved in saline (0.5 ml) and mixed thoroughly with 0.5 ml Bayol F (Esso; kindly provided by Dr D. E. S. Stewart-Tull) containing 250 µg freeze-dried *Mycobacterium tuberculosis* and 0.2 ml Arlcel A (Honeywell-Atlas, Carshalton, Surrey, UK). Groups of five HaM/ICR mice, aged 6 weeks, were injected intraperitoneally with 0.1 ml of this mixture of EP in complete Freund's adjuvant. Control mice were inoculated with saline. After 14 d, slide agglutination tests for antibody production proved positive and the mice were bled by cardiac puncture. Sera were collected by centrifugation at 1200 g and samples from each batch of five mice were pooled.

Antiserum was also prepared in guinea pigs (Dunkin-Hartley) by intramuscular injection of 0.2 ml EP in

adjuvant into the inner aspect of the hind limbs. Production of antibodies was monitored after 19 d and the guinea pigs were bled by cardiac puncture after 21 d.

Agglutinin absorption. Portions (1.5 ml) of 24 h yeast cultures were centrifuged in a Beckman microfuge B at 8740 g for 1 min. The organisms were washed in saline and resuspended in 0.5 ml antiserum. These mixtures were incubated at 37 °C for 30 min and then centrifuged to remove the yeast cells. The procedure was repeated (five times in all), using the same sera, until no residual antibodies to the absorbing cells could be detected in slide agglutination tests.

Agglutination assays. The tests were set up in microtitre trays (Sterilin) using doubling dilutions (0.05 ml) of antisera in saline to which were added equal volumes (0.05 ml) of *C. albicans* suspended in saline (10^7 yeast cells ml^{-1}). Control sera were treated in the same way. An additional control consisted of yeast cell suspension (0.05 ml) and saline (0.05 ml). Complete mixing was achieved by sideways agitation and the trays were incubated at 37 °C for 1 h. The endpoint of titration was taken as the highest dilution of antiserum which gave visible agglutination of the yeast suspensions. The trays were incubated at 4 °C overnight to check for any increase in titres. All agglutination tests were done in duplicate and the mean titre calculated. Normal sera gave zero titres.

RESULTS

Yields of EP

EP was isolated from culture supernatants of *C. albicans* GDH 2346 by precipitation with acetone. More EP was recovered from supernatants of cultures grown in 500 mM-sucrose or galactose than from those of cultures grown in 50 mM-glucose (Table 1). This was particularly evident after an incubation period of 5 d when there was a significant increase in the amount of material isolated, especially from galactose-grown cultures; the yield of EP for galactose-grown yeasts was 17.1%, compared with 10.5% for sucrose-grown yeasts and 4.4% for glucose-grown organisms. Similar results were obtained with two other strains of *C. albicans*; strain BP 3968 gave EP yields of 20.7%, 15.2% and 8.6% from 5 d cultures of galactose-, sucrose- and glucose-grown yeasts respectively, while the corresponding figures for strain MRL 3153 were 19.3%, 13.0% and 4.3%.

With all three strains, EP prepared from culture supernatants of glucose- or sucrose-grown yeasts was crystalline and grey. Material obtained from culture supernatants of 500 mM-galactose-grown organisms, however, was grey-green. The green colour was particularly noticeable with EP from strain GDH 2346 and was presumably due to the presence of a green pigment known to be produced by *C. albicans* under certain conditions (Ashford, 1917; Jones & Peck, 1940).

Analysis of EP

Paper chromatography of acid-hydrolysed EP from *C. albicans* GDH 2346 revealed mannose, glucose and glucosamine as the only identifiable sugars. An unidentified component ($R_{\text{GlcN}} = 0.78$) was also visualized with alkaline silver nitrate. These findings were confirmed by gas-liquid chromatography, which also indicated that mannose was the major sugar present.

Table 1. *Yield of EP obtained from culture supernatants of C. albicans GDH 2346*

C. albicans GDH 2346 was grown for 1 d (24 h) or 5 d in yeast nitrogen base medium containing glucose (50 mM), sucrose (500 mM) or galactose (500 mM) as the carbon source. EP was isolated from culture supernatants by precipitation with acetone as described in Methods. Results represent means $\pm$ SEM for 3–7 independent experiments. Yield is expressed as a percentage of the combined dry weight of yeast cells and EP. Probability values (*P*) compare yields with those from corresponding cultures grown in medium containing 50 mM-glucose.

Carbon source	Incubation period (d)	Dry wt of yeasts (g)	Dry wt of EP (mg)	Percentage yield of EP	<i>P</i>
Glucose	1	1.67 $\pm$ 0.19	75 $\pm$ 12	4.3 $\pm$ 0.5	
Sucrose	1	2.69 $\pm$ 0.59	137 $\pm$ 31	4.8 $\pm$ 0.2	NS
Galactose	1	2.35 $\pm$ 0.44	137 $\pm$ 20	5.7 $\pm$ 0.3	<0.05
Glucose	5	1.94 $\pm$ 0.19	92 $\pm$ 20	4.4 $\pm$ 0.8	
Sucrose	5	1.87 $\pm$ 0.07	221 $\pm$ 41	10.5 $\pm$ 1.8	<0.025
Galactose	5	2.72 $\pm$ 0.46	576 $\pm$ 115	17.1 $\pm$ 1.5	<0.001

NS, Not significant.

Table 2. *Analysis of EP obtained from culture supernatants of C. albicans GDH 2346*

C. albicans GDH 2346 was grown for 24 h or 5 d in yeast nitrogen base medium containing glucose (50 mM), sucrose (500 mM) or galactose (500 mM) as the carbon source. EP was isolated from culture supernatants and analysed as described in Methods. Results represent means $\pm$ SD of six independent determinations where assays were done in duplicate.

Carbon source	Incubation period (d)	Percentage composition				
		Carbohydrate	Carbohydrate after Triton treatment*	Glucose	Protein	Phosphorus
Glucose	1	68.6 $\pm$ 2.3	64.5 $\pm$ 1.2	6.8 $\pm$ 0.4	7.6 $\pm$ 0.3	0.49 $\pm$ 0.01
Sucrose	1	74.7 $\pm$ 1.9	73.6 $\pm$ 1.7	14.5 $\pm$ 0.5	7.2 $\pm$ 0.3	0.50 $\pm$ 0.03
Galactose	1	94.2 $\pm$ 5.2	81.9 $\pm$ 1.0	14.8 $\pm$ 0.5	6.8 $\pm$ 0.3	0.46 $\pm$ 0.02
Glucose	5	68.2 $\pm$ 2.2	70.0 $\pm$ 1.6	4.9 $\pm$ 0.2	6.7 $\pm$ 0.3	0.47 $\pm$ 0.01
Sucrose	5	78.0 $\pm$ 1.0	76.1 $\pm$ 1.3	16.1 $\pm$ 0.5	6.5 $\pm$ 1.3	0.40 $\pm$ 0.02
Galactose	5	100.9 $\pm$ 6.4	78.8 $\pm$ 2.6	9.8 $\pm$ 0.3	6.9 $\pm$ 0.2	0.41 $\pm$ 0.02

* Prior to analysis, EP was treated with Triton X-100 as described in Methods.

Table 3. Agglutination titres of serum raised against EP obtained from culture supernatants of *C. albicans* GDH 2346

C. albicans GDH 2346 grown in yeast nitrogen base medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose was assayed with sera raised in (A) guinea pigs or (B) mice. Results shown are mean values for duplicate titres.

Carbon source for yeasts used in assay		Reciprocal titre of antiserum raised against EP produced by yeasts grown on:		
		Glucose (50 mM)	Sucrose (500 mM)	Galactose (500 mM)
A.	Glucose	32	24	32
	Sucrose	128	128	192
	Galactose	512	512	768
B.	Glucose	12	8	8
	Sucrose	192	768	3072
	Galactose	128	4096	8192

The results of quantitative analyses are shown in Table 2. Initially, values obtained for the carbohydrate content of EP produced by galactose-grown yeasts were very high and, on occasion, in excess of 100%. This appeared to be due to interference in the assay by the green pigment present in these preparations, since treatment with 0.1% Triton X-100 for 5 h at room temperature decreased the carbohydrate content to values more comparable with those obtained for the other EP preparations analysed. Prolonged extraction with Triton X-100 had no further effect. The carbohydrate content of EP produced by glucose- or sucrose-grown yeasts was completely unaffected by detergent treatment.

The overall composition of different EP preparations was quite similar, irrespective of the carbon source or incubation period (Table 2). They comprised 65 to 82% carbohydrate (mainly mannose), with smaller amounts of protein (7%), phosphorus (0.5%) and glucosamine (1.5%). EP produced by glucose-grown yeasts had less carbohydrate and a correspondingly lower glucose content than the other EP preparations.

Immunological studies

Possible structural differences between EP preparations were investigated serologically. Antiserum was raised in both mice and guinea pigs against EP produced by *C. albicans* GDH 2346 in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose. The sera were then tested in agglutination experiments with suspensions of yeast cells of the same strain grown on each of the three carbon sources. Increasing titres were obtained in going from glucose-grown yeasts to sucrose-grown yeasts and finally to galactose-grown organisms (Table 3). This was true of all three types of serum raised in either animal species, although mouse sera gave higher titres. These results suggest immunological identity between the different EP preparations. They also indicate that there are more antigenic determinants on the surface of galactose-grown yeasts than on sucrose-grown yeasts and that the latter in turn possess more determinants than organisms grown on 50 mM-glucose.

Immunological identity of the EP preparations was confirmed by means of agglutinin-absorption experiments. Antiserum raised against EP from galactose-grown yeasts was absorbed with organisms grown in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose. Yeasts were removed by centrifugation and the procedure was repeated with the supernatant until no reaction with absorbing cells could be detected in slide agglutination tests. An analogous protocol was employed with the two other sera, and the nine resulting absorbed antisera were then used in agglutination assays with *C. albicans* GDH 2346 grown in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose. No appreciable agglutination of these organisms by any of the absorbed sera could be detected.

Two further strains of *C. albicans* were used in cross-agglutination tests (Table 4). Incubation of suspensions of *C. albicans* MRL 3153 with antisera raised against EP from strain GDH 2346

Table 4. *Cross-agglutination titres of serum raised against EP obtained from culture supernatants of C. albicans GDH 2346*

Sera raised in guinea pigs were assayed with (A) *C. albicans* MRL 3153 and (B) *C. albicans* GDH 2023 grown in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose. Results shown are mean values for duplicate titres.

Carbon source for yeasts used in assay		Reciprocal titre of antiserum raised against EP of <i>C. albicans</i> GDH 2346 grown on:		
		Glucose (50 mM)	Sucrose (500 mM)	Galactose (500 mM)
A.	Glucose	24	96	128
	Sucrose	128	256	512
	Galactose	64	192	384
B.	Glucose	12	8	12
	Sucrose	8	8	8
	Galactose	8	6	12

Table 5. *Effect of pretreatment of acrylic with EP on the subsequent adhesion of C. albicans GDH 2346*

Prior to adhesion assays, acrylic strips were incubated for 4 h at room temperature in solutions (5 mg ml⁻¹) of EP produced by *C. albicans* GDH 2346 in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose. Control strips were preincubated for 4 h in PBS. All strips were then withdrawn, shaken to remove excess liquid and used in adhesion assays with *C. albicans* GDH 2346 grown in medium containing 50 mM-glucose or 500 mM-sucrose.

Carbon source for EP production	Carbon source for yeasts used in assay	Mean no. ($\pm$ SEM) of adherent yeasts per mm ² of acrylic treated with:		Relative adherence*	P†
		PBS	EP		
Glucose	Glucose	104 $\pm$ 3	108 $\pm$ 3	1.0	NS
Sucrose	Glucose	104 $\pm$ 6	198 $\pm$ 16	1.9	<0.001
Galactose	Glucose	106 $\pm$ 8	242 $\pm$ 13	2.3	<0.001
Sucrose	Sucrose	320 $\pm$ 5	339 $\pm$ 4	1.1	<0.025
Galactose	Sucrose	354 $\pm$ 6	450 $\pm$ 8	1.3	<0.001

* Adherence to EP-coated acrylic compared with that to PBS-treated acrylic.

† Probability values comparing adherence to EP-coated acrylic with that to PBS-treated acrylic. NS, Not significant.

produced a pattern of titres similar to that obtained with the homologous yeast cells, indicating a similarity between the EP produced by these two strains. Incubation of the same sera with suspensions of *C. albicans* GDH 2023, on the other hand, gave much lower titres, suggesting marked differences in EP antigenicity between these strains.

Effect of EP on yeast adhesion in vitro

Increased yields of EP from galactose-grown or sucrose-grown organisms after incubation for 5 d (Table 1) correlated with enhanced ability of the yeasts to adhere to acrylic *in vitro*. For example, the adhesion of *C. albicans* GDH 2346 incubated in medium containing 500 mM-sucrose for 5 d was double that of yeasts incubated similarly for only 24 h (data not shown). Moreover, pretreatment of acrylic strips with EP to form a polymeric coating promoted adhesion in most cases (Table 5). EP obtained from glucose-grown yeasts had no effect on the adhesion of these organisms to acrylic but EP isolated from culture supernatants of sucrose-grown cells increased the adherence of sucrose-grown and, to a greater extent, glucose-grown organisms. The biggest increase was observed when glucose-grown yeasts were incubated with acrylic which had been pretreated with EP obtained from galactose-grown organisms: attachment was more than doubled. Thus, precoating acrylic with EP was most effective at promoting adhesion when EP derived from the most adherent yeast type was used in assays with organisms of the least adherent type.

Table 6. *Effect of pretreatment of buccal epithelial cells with EP on the subsequent adhesion of C. albicans GDH 2346 and GDH 2023*

Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37 °C in PBS or in a solution (5 mg ml⁻¹) of EP produced by *C. albicans* GDH 2346 or GDH 2023 in medium containing 500 mM-galactose. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays with the indicated strain of *C. albicans* grown in medium containing 50 mM-glucose, 500 mM-sucrose or 500 mM-galactose.

Strain	Carbon source in growth medium	Mean no. ($\pm$ SEM) of adherent yeasts per 100 epithelial cells pretreated with:		Relative adherence*	<i>P</i> †
		PBS	EP		
<i>(a) EP from strain GDH 2346</i>					
GDH 2346	Glucose	114 $\pm$ 5	104 $\pm$ 6	0.9	NS
GDH 2346	Sucrose	387 $\pm$ 17	292 $\pm$ 18	0.7	<0.01
GDH 2346	Galactose	847 $\pm$ 47	451 $\pm$ 27	0.5	<0.001
<i>(b) EP from strain GDH 2023</i>					
GDH 2023	Galactose	871 $\pm$ 34	419 $\pm$ 38	0.5	<0.001
GDH 2346	Galactose	692 $\pm$ 23	695 $\pm$ 33	1.0	NS

* Adherence to EP-treated epithelial cells compared with that to PBS-treated cells.

† Probability values comparing adherence to EP-treated epithelial cells with that to PBS-treated cells. NS, Not significant.

A different result was obtained when buccal epithelial cells were preincubated with EP obtained from galactose-grown *C. albicans* GDH 2346 (Table 6*a*). Such treatment decreased the adhesion of yeast cells grown in medium containing 500 mM-sucrose or galactose by 30% and 50% respectively. The adhesion of 50 mM-glucose-grown yeasts, however, was unaffected. After incubation with EP for 30 min at 37 °C, epithelial cells tended to aggregate. To prevent this affecting adhesion assays, the epithelial cells were recovered by centrifugation (1200 g for 5 min) and resuspended in PBS; control cells incubated with PBS were treated similarly.

The adhesion of *C. albicans* GDH 2346 and GDH 2023 to buccal cells pretreated with EP obtained from galactose-grown *C. albicans* GDH 2023 was also investigated (Table 6*b*). Adhesion of galactose-grown yeasts of the homologous strain was halved while that of galactose-grown *C. albicans* GDH 2346 was unaffected. These results are consistent with those of the cross-agglutination experiments described above, which indicated differences in the nature of the EP produced by these two strains.

DISCUSSION

Previous work has shown that growth of *C. albicans* in medium containing high concentrations of certain sugars can promote the synthesis of a fibrillar layer on the yeast surface (McCourtie & Douglas, 1981). The fibrils, which have also been described as fungal fimbriae (Gardiner *et al.*, 1982), enhance yeast attachment to epithelial and acrylic surfaces *in vitro* (McCourtie & Douglas, 1984). The results reported in this paper indicate that fibrils are released into the surrounding environment upon prolonged incubation of the yeast at 37 °C and that polymeric material isolated from culture supernatants contains the fibrillar adhesin.

Yeasts grown in medium with a high concentration of galactose or sucrose are more adherent than those grown in a relatively low concentration of glucose (McCourtie & Douglas, 1984). It was not unexpected, therefore, that more EP could be recovered from culture supernatants of galactose- or sucrose-grown yeasts than from those of glucose-grown organisms. The strains of *C. albicans* used in this study grew at similar rates on all three carbon sources irrespective of initial concentration (50 mM or 500 mM). Thus, growth limitation was not due to depletion of the carbon source but must, rather, have resulted from exhaustion of some other constituent of the growth medium. Production of the fibrillar layer by *C. albicans* may therefore be analogous to exopolysaccharide synthesis by bacteria where maximum yields are often obtained in medium

containing excess carbohydrate in which growth is limited by the availability of nitrogen, phosphorus or sulphur (Sutherland, 1977).

Quantitative analysis of EP indicated that it has a high mannose content and is probably largely mannoprotein in nature. This conclusion is consistent with our earlier studies with the antibiotic tunicamycin which, in yeasts, is a specific inhibitor of mannoprotein synthesis. Addition of tunicamycin to 500 mM-galactose-grown cultures at the onset of the stationary phase of growth decreased yeast adhesion and increased yeast sensitivity to spheroplast formation, presumably by inhibiting synthesis of the fibrillar surface layer (Douglas & McCourtie, 1983). Removal of surface mannoprotein from *C. albicans* by warm water washes has been reported by Šikl *et al.* (1964). The same authors (Masler *et al.*, 1966) have also described the isolation from yeast culture supernatants of polysaccharide-protein complexes similar in composition to EP prepared in this study. The extracellular material was obtained from cultures grown in medium containing 2% (w/v) glucose for one week; it was immunologically active and could be separated electrophoretically into two or three distinct components, depending on the strain. Preliminary fractionation of our EP preparations also indicates the presence of more than one component (unpublished results).

Quantitative analysis failed to reveal significant differences in the chemical composition of EP produced by strain GDH 2346 on different carbon sources. Moreover, serological studies indicated that the EP preparations from this strain were immunologically identical, but that 500 mM-galactose-grown yeasts had more antigenic determinants, and thus presumably a more extensive fibrillar layer than 500 mM-sucrose-grown organisms, while 50 mM-glucose-grown yeasts had the fewest determinants/fibrils. The physiological basis of this phenotypic variation in yeast surface composition remains to be established. Cross-agglutination tests indicated a similarity of surface structure in *C. albicans* GDH 2346 and MRL 3153. However, antigenic differences were apparent between strains GDH 2346 and GDH 2023 and preliminary analyses also indicate differences in the chemical composition of EP preparations from these two strains (unpublished data). *C. albicans* MRL 3153 is known to correspond to serotype A of Hasenclever & Mitchell (1961). Although the serotypes of the Glasgow isolates used in this study have not been determined, our present results suggest that strains GDH 2346 and GDH 2023 may represent serotypes A and B, respectively.

The adhesion of micro-organisms to mucosal surfaces is believed to involve specific interactions between microbial adhesins and complementary host-cell receptors. Adhesion to epithelial cells *in vitro* can be blocked by adding an excess of isolated adhesin or receptor (or their analogues) to assay mixtures where either substance will function as a competitive inhibitor (Ofek & Beachey, 1980). In the present investigation, inhibition was observed when epithelial cells were pretreated with EP, indicating that this extracellular material contained the yeast adhesin. These results, together with our analytical data (Table 2), also provide further evidence for the mannoprotein nature of the adhesin (Sandin *et al.*, 1982; Douglas & McCourtie, 1983; Lee & King, 1983). However, the finding that EP from strain GDH 2023 does not inhibit adhesion of strain GDH 2346 (Table 6) suggests that the adhesins of these two strains may be different.

In aquatic environments, adsorption of macromolecules on solid surfaces results in a modified surface charge which may promote or inhibit microbial attachment (Wardell *et al.*, 1983). For example, Corpe (1970) reported that marine bacteria adhered in greater numbers to glass slides precoated with bacterial exopolysaccharide than to uncoated slides. Adhesion of *C. albicans* to acrylic was similarly enhanced when acrylic strips were pretreated with EP (Table 5). The effect was particularly marked with EP preparations from galactose-grown yeasts which presumably contained the highest proportion of yeast adhesin. If non-specific polymeric bridging of this type also occurred in assays with EP-treated buccal cells, it would tend to counteract the specific inhibition due to blocked epithelial receptors. This may explain the apparent absence of inhibition with glucose-grown yeasts in such assays.

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