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Surface-Active Properties of *Candida albicans*

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Cell surface hydrophobicity may be an important factor contributing to the virulence of *Candida* yeast cells. Surface hydrophobic and surface polar groups would be required for a yeast cell to act as a surface-active agent. In this report, the surface activities of whole yeast cells were measured. Yeast cells added at 10^8/ml reduced the surface tension (γ_s) of saline by 20% as determined by the du Nouy method. A 1% suspension of yeast cell wall fragments reduced γ_s of saline by 36%. Whole yeast cells caused a reduction in interfacial tension (γ_I) between hexadecane and saline. The reduction of γ_I was proportional to the surface hydrophobicity of the yeasts. Yeast cells grown in glucose as the sole carbon source (thus possessing a relatively more hydrophilic cell surface) reduced γ_I by 30%, whereas yeast cells grown in hexadecane (thus possessing a more hydrophobic cell surface) reduced γ_I by 41%. The reduction of γ_I was reversed upon the addition of a strong surfactant. It was also demonstrated that yeast cells blended with nonionic surfactants during growth in a glucose broth in order to change their cell surface hydrophobicity adhered to solid surfaces in direct proportion to their cell surface hydrophobicity. Thus, the surface-active properties of *Candida* yeast cells may significantly contribute to the accumulation of yeast cells at various biological interfaces such as liquid-solid, liquid-liquid, and liquid-air, leading to their eventual adhesion to solid or tissue surfaces.

Candida albicans and related yeast cell species are the most frequently isolated opportunistic fungal pathogens in humans (10). These fungi not only infect tissue but frequently cause infection at sites where biomaterials such as those found in peripheral venous catheters (25), central catheters (6), contact lenses (3), and bladder catheters are exposed (8). These materials are bathed in biological fluids which provide novel interfaces which may serve as sites of ingress for microorganisms into the host. Interfaces such as liquid with solid, liquid with liquid, and liquid with air are thus important in determining the adherence of yeasts to biomaterials and tissue. In previous work, we showed that hydrophobic and electrostatic forces largely determined the adhesion of *Candida* yeasts to plastic surfaces (16). More recently, others have shown that the hydrophobicity of *C. albicans* directly correlated with the ability of each isolate to adhere to buccal epithelial cells (19) and with the virulence of *C. albicans* in mice (1). In this report, surface properties of yeast cells which favor the concentration of yeast cells at interfacial boundaries are described. The concentration of yeast cells at interfacial boundaries may enhance yeast cell adherence to tissue and biomaterials.

MATERIALS AND METHODS

Yeasts. *C. albicans* was a clinical isolate maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) and transferred monthly. For experimental purposes, a loopful of yeast cells was added to 50 ml of broth and agitated for 20 to 120 h at 26°C. The broth was yeast nitrogen base (YNB) (Difco) plus D-glucose (23.3 g/liter), YNB plus hexadecane (10 g/liter), or Sabouraud dextrose broth (Difco). In experiments investigating the adhesion of yeasts to liquid-solid interfaces, *C. albicans* was grown in Sabouraud dextrose broth with 2% solutions of nonionic surfactants (L61, P105, and F127; BASF Wyandotte Corp., Parsippany, N.J.). Following incubation, yeast cells were washed three times by centrifugation with 0.85% saline and suspended in saline to desired concentrations according to hemacytometer counts.

Adsorption of yeasts to interfaces. (i) Determination of percent change in absorbance. Yeast cells were suspended in saline at 5×10^6 yeasts per ml, and the A_{540} was obtained in a spectrophotometer. This suspension (2 ml) was placed in a glass test tube (12 by 75 mm), covered by 0.5 ml of hexadecane, vortexed for 3 min, and allowed to settle for 10 min. After the suspension settled, the absorbance value was determined from a portion of the saline layer, and the percent change in absorbance was calculated by dividing the absorbance obtained after vortexing by the absorbance of the original sample and multiplying by 100 (16).

(ii) Determination of γ_I. Saline (20 ml) containing the desired concentration of washed yeast cells was added to 50-ml beakers. Hexadecane (6 ml) was layered onto the saline, the liquids were mixed by a stirring bar for 3 min, and the interfacial tension (γ_I) was measured by the du Nouy method with a tensiometer (Fisher Scientific Co., Pittsburgh, Pa.).

(iii) Determination of γ_s. Saline (20 ml) containing yeast cells or yeast cell wall fragments at desired concentrations was placed in a 50-ml beaker, and the surface tension (γ_s) was determined by the du Nouy method. Cell wall fragments were prepared by fracturing yeast cells with glass beads (7, 17) and were separated by differential centrifugation.

(iv) Determination of adhesion of yeast cells to a liquid-solid interface. Planchets (3 by 1 in. [~8 by 2.5 cm]) of polyvinyl chloride (PVC) and polymethyl methacrylate (Rohm & Haas Co., Philadelphia, Pa.) were washed with detergent, rinsed with deionized water, and submerged in a saline suspension of *C. albicans* at 5×10^7 yeast cells per ml. The yeast cells had been blended with nonionic surfactants during growth in Sabouraud dextrose broth. After being incubated for 24 h at 26°C, the plastic strips were removed and washed by immersion in saline, fixed in Bouin fluid, and stained with crystal violet. Adherent yeast cells were counted by using light microscopy and expressed as yeast cells per square millimeter of polymer surface.

Detection of surfactant activity in yeast filtrates. *C. albicans* yeast cells were grown in YNB plus hexadecane. Samples

TABLE 1. Reduction in γ_s upon the addition of whole *C. albicans* yeast cells or cell wall fragments[a]

Additive	γ_s (dynes/cm ± SD)
Control (no yeasts)	73 ± 1
Whole yeasts (10^8/ml)	58 ± 2
Cell wall fragments (concn)	
0.01%	60 ± 2
0.1%	56 ± 2
1%	45 ± 3

[a] γ_s was determined by the du Nouy method. Each experiment used a minimum of three samples and was repeated at least three times.

TABLE 2. Ability of yeast cells to collect at a liquid-liquid interface[a]

Carbon source	θ (mean degrees ± SD)	%Δ Abs (± SD)[b]	γ_I (dynes/cm ± SD)[c]	CSH[d]
Glucose	37.5 ± 1.3	44 ± 1	31 ± 1	68 ± 11
Hexadecane	54.0 ± 0.0	88 ± 3	26 ± 1	66 ± 14

[a] Each experiment used a minimum of three samples and was repeated at least three times.
[b] %Δ Abs, Change in absorbance of saline phase containing yeast cells.
[c] γ_I for saline alone was 44 ± 1 dynes/cm. To detect a change in γ_I, 2.5 × 10^7 yeast cells per ml were used.
[d] CSH, Cell surface hydrophobicity, i.e., percentage of yeast cells with three polystyrene beads per cell.

were refrigerated overnight at 4°C, causing the hexadecane to freeze, and were then filtered through a 0.45-μm-pore-size filter. The effect of the filtrate on γ_I was measured by the technique described above.

Determining cell surface hydrophobicity. (i) θ method. Yeast cells grown in hexadecane or the surfactants displayed a range of hydrophobicity of the cell surface, as determined by the contact angle (θ) method (16, 26). In this method, yeasts were washed and pelleted by centrifugation and then layered onto agar-covered slides. The slides were allowed to dry for 3 h in a moist chamber at 26°C. A 1-μl portion of alpha-bromonaphthalene was used as the sensing liquid because it provides a sensitive and reproducible θ (2, 16). θ was measured by a telescope equipped with a goniometer (Gaertner Scientific Corp., Chicago, Ill.). Systematic studies showed that the addition of less than 2% surfactant did not cause a measurable difference in θ of the yeast cell surface, whereas more than 2% surfactant caused clumping of yeasts.

(ii) Microsphere method. The method of Hazen and Hazen was used (11). Briefly, approximately 10^9 blue polystyrene microspheres (diameter, <1.0 μm) were mixed with 2 × 10^6 yeast cells in equal volumes of sodium phosphate buffer and vortexed for 30 s. Yeast cells were then examined under a microscope, and the relative cell surface hydrophobicity was determined by counting the numbers of beads per yeast cell. A CFU with three or more beads was considered hydrophobic, whereas a CFU with less than three beads was considered hydrophilic (11).

RESULTS

C. albicans yeast cells are hydrophobic enough to be excluded from bulk physiologic saline and to accumulate at the air-saline interface. The movement of yeast cells to the interface was detected by measuring a decline in γ_s of the saline (Table 1). γ_s declined from 73 dynes/cm (1 dyne = 10^{-5} N) (saline only) to 62 dynes/cm upon the addition of 10^5 yeast cells per ml and fell to 58 dynes/cm after the addition of 10^8 yeast cells per ml, a 20% reduction of γ_s. A dilution (0.01% suspension) of cell wall fractions reduced γ_s to 60 dynes/cm, whereas a 1% suspension caused a decline in γ_s to 45 dynes/cm, or a total reduction in γ_s of 36% (Table 1).

The concentration of yeast cells at a liquid-liquid interface was investigated by measuring the adsorption of yeast cells to a saline-hexadecane interface. *C. albicans* was grown in glucose or hexadecane as the sole carbon source in order to change the surface hydrophobicity as measured by θ (17). θ was greater for yeast cells grown in hexadecane (Table 2), but cell surface hydrophobicity as determined by the microsphere method of Hazen and Hazen (11) indicated no difference in the two yeast cell populations. The movement of yeast cells to the saline-hexadecane interface was detected by measuring the percent change in absorbance (Table 2). It is evident that the greater the value of θ, the greater the percent change in absorbance and hence the greater the movement of the yeast cells to the liquid-liquid interface (Table 2). However, movement of yeast cells to the interface was negligible (the percent change in absorbance was 5%) when either 1% bovine serum albumin or 0.1% polyol (a surfactant) was added to the saline. Change in γ_I between hexadecane and saline was also determined upon the addition of yeast cells grown in either glucose or hexadecane (Table 2). γ_I between saline and hexadecane was 44 dynes/cm and decreased to 31 dynes/cm when yeasts grown in glucose were suspended in the saline. When *C. albicans* was grown in hexadecane, however, γ_I decreased to 26 dynes/cm. This represented a reduction in γ_I of 41%.

The adsorption of yeast cells to a liquid-solid interface was then investigated. Figure 1 shows the results of experiments which tested the abilities of yeast cells with various surface hydrophobicities (as determined by θ) to adhere to PVC. Yeast cell surfaces were made more hydrophobic by growing them in 2% solutions of nonionic surfactants, and the cell

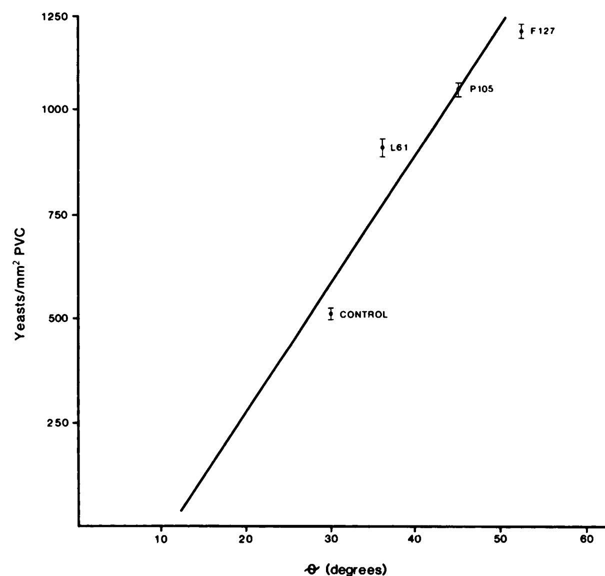

FIG. 1. Relationship between θ and the number of *C. albicans* yeast cells adhering to PVC. Yeast cells were grown in Sabouraud dextrose broth (control) with 2% solutions of the nonionic surfactants L61, P105, and F127. Each point represents the mean of 18 to 25 replicates. The line is a best-fit straight line (r = 0.9345). Bars represent 2 standard errors of the mean.

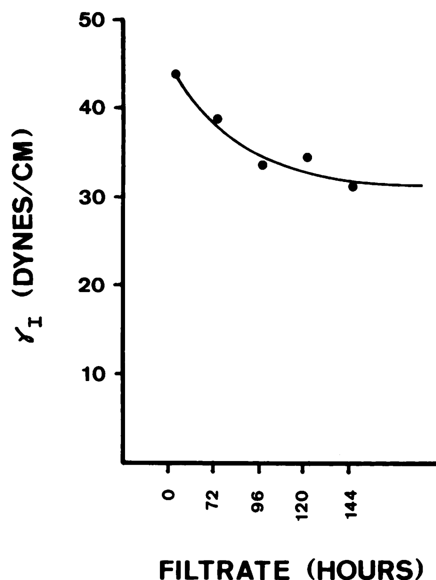

FIG. 2. Surface-active nature of ultrafiltrates of *C. albicans* growth. Yeast cells were grown in YNB plus hexadecane for various periods of time. γ_I between the ultrafiltrate and hexadecane was determined by the du Nouy method.

surface hydrophobicity was determined by the θ method. The more hydrophobic the yeast cell surface was (i.e., the greater the value of θ), the more numerous were the yeasts adhering to PVC (Fig. 1). Similar results were obtained using polymethyl methacrylate as the target substratum (data not shown).

It was also noted that during the growth of *C. albicans* in YNB plus hexadecane or glucose, γ_s of the broth declined over time. Ultrafiltrates of a YNB-hexadecane broth caused a 32% decline in γ_I of hexadecane and saline. γ_I decreased from 44 dynes/cm without filtrate to 30 dynes/cm with the 144-h filtrate, which appeared to be a point of saturation since there was no further reduction in γ_I (Fig. 2). This indicated the production of an extracellular surface-active agent by *C. albicans*.

DISCUSSION

The tendency of some microorganisms to accumulate at interfaces was noted many years ago. Mudd and Mudd (21) demonstrated the stability of non-acid-fast bacteria at aqueous-oil interfaces, whereas ZoBell (27) demonstrated that some microorganisms concentrated at liquid-solid interfaces and adsorbed to the solid matter rather than remaining free in seawater. Recent work suggests that concentration of microorganisms at the liquid-solid interface may occur in some cases because nutrients concentrate at this interface (20), an observation supported by the fact that the metabolic activity of adherent cells is greater than that of suspended cells (18). The accumulation of most microorganisms at these interfaces is passive. Some microorganisms, however, may use their powers of motility to influence their approach and adsorption to the interface (13). Because *Candida* spp. do not possess means of motility, the forces of diffusion and turbulence dictate their dispersion in a liquid.

The accumulation of substances at liquid-liquid and liquid-solid interfaces in aqueous solutions is obviously enhanced by the hydrophobic surface moieties of the suspended particle or microorganism. Because of the strong attraction of water molecules for one another and their resistance to dissolving nonpolar molecules, these nonpolar solutes are excluded from bulk water (24). Whole *Candida* yeast cells are sufficiently hydrophobic to be excluded from bulk water. They concentrate at liquid-air and liquid-liquid interfaces and, in so doing, reduce γ_s and γ_I. The more hydrophobic the yeast cell surface is, the greater is the concentration of yeast cells at an interface; e.g., yeast cells grown in hexadecane caused a greater reduction in γ_I than did yeast cells grown in glucose. In addition, yeast cells grown in hexadecane caused a greater percent change in absorbance than did yeast cells grown in glucose (Table 2). Cell surface hydrophobicity may be the sum total of the numerous lipids present in the cell wall of *C. albicans*, whereas polysaccharides alone and the fatty acids complexed to polysaccharides (12) may provide the polar groups necessary for surface activity. Although fatty acids may function as the moieties required for surface activity, they apparently do not directly mediate adherence of yeast cells to solid surfaces such as buccal epithelial cells (9). It is also interesting to note the discrepancy between cell surface hydrophobicity measured by the microsphere assay and that measured by the θ method. It may be that these two methods are measuring different hydrophobic components. Whereas alpha-bromonaphthalene, the sensing liquid used in this study, primarily measures dispersion forces, it is conceivable that the microspheres would be subject to polar forces as well. Furthermore, it is possible that the θ method with a sensing liquid measures hydrophobic forces on the surface of cells as well as forces removed from the surface, whereas the microsphere method reflects only the superficial surface hydrophobic forces.

Cell surface hydrophobicity directly correlates with the adhesion of yeast cells to PVC and polymethyl methacrylate planchets on which cells interact with a liquid-solid interface. In prior work, we have shown that electrostatic and hydrophobic forces largely influence the adhesion of yeast cells to various plastic surfaces (16) and contact lenses (4), a feature that is true of *Pseudomonas aeruginosa* as well (15). In those experiments, the surfaces to which yeast cells adhered varied over a wide range of hydrophobicity. In the present experiments, the target surfaces were identical, but yeast cells with various degrees of cell surface hydrophobicity were used. Yeast cell surface hydrophobicity correlated directly with the adhesion of the yeast cells to the substratum; therefore, it appears that hydrophobic interactions exert a measurable effect upon the adsorption of yeasts to the liquid-solid interface (Fig. 1) as well as to liquid-liquid and liquid-air interfaces.

Having once accumulated at an oil-water interface, yeast cells tend to remain at that interface, possibly because of their ability to stabilize oil in water emulsions. This characteristic of *C. albicans* yeast is particularly evident when the percent change in absorbance is measured. *C. albicans* yeast cells are round to oval and approximately 3 to 5 μm in diameter. The ability of small particles to stabilize emulsions is well known. Barium sulfate particles, for example, ranging in size from 0.5 to 10 μm, form good emulsions (23). The fact that cell wall fragments further reduced γ_I below that of whole yeast cells is consistent with this concept. Transmission electron microscopy demonstrated the presence of some cell membrane fragments adhering to the cell wall preparations, and this may have made the cell wall fragments more surface active (data not shown).

The related yeast *Candida* (or *Yarrowia*) *lipolytica* has shown a decrease in γ_s of filtrates of the growth medium (22).

This decrease in γ_s is due to the production of bioemulsifiers, some of which are surface active (5). Ultrafiltrates of the growth medium of *C. albicans* decrease γ_l of hexadecane and saline and therefore reflect the production of a surface-active agent(s). A bioemulsifier with weak surface activity has been isolated from the aqueous phase of *C. albicans* filtrates (14), but chloroform-extractable substances (lipids) were ignored in that study. How extracellular surfactants produced by *C. albicans* affect the adherence process of yeast cells is unknown, but the bioemulsifier with weak surface activity clearly enhanced yeast cell adherence to living cells (14).

It has been shown in this study that yeasts are excluded from a bulk aqueous environment by physicochemical factors and therefore act as surface-active agents. This exclusion from the aqueous phase causes the microorganisms to accumulate at interfaces where other interactions, such as ionic or weak hydrophobic forces, may play roles in the stabilization of yeasts at the interface and, eventually, the adhesion of yeast cells to a solid surface.

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