Cell-surface Hydrophobicity of *Candida* Species as Determined by the Contact-angle and Hydrocarbon-adherence Methods

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(Received 13 August 1985; revised 12 November 1985)

Cell-surface hydrophobicities of six *Candida* species were studied by two methods: measurement of the contact angle, and partitioning with aqueous-hydrocarbon (n-octane, n-hexadecane and *p*-xylene) mixtures. *C. tropicalis*, *C. glabrata* and *C. krusei* adhered better to the hydrocarbons than did *C. albicans*, *C. stellatoidea* and *C. parapsilosis*. Contact angles for the less adherent species were smaller than those for the more adherent species. Thus the two methods gave results that were similar overall and indicated that *C. tropicalis*, *C. glabrata* and *C. krusei* have greater cell-surface hydrophobicities than *C. albicans*, *C. stellatoidea* and *C. parapsilosis*.

INTRODUCTION

Bacterial adherence to the surface of animal cells is an important step in the infection process (Ofek & Beachey, 1980), and hydrophobic interactions are thought to be involved in the adherence of a variety of bacteria to host tissues (Mörner *et al.*, 1983; Peres *et al.*, 1977). To study microbial attachment to surfaces, a number of methods to evaluate cell-surface hydrophobicity have been developed (Lindahl *et al.*, 1981; Jonsson & Wadström, 1984). Contact-angle methods and partitioning methods with hydrocarbons have been used in many investigations (Miura *et al.*, 1977; Iimura *et al.*, 1980; Rosenberg *et al.*, 1980; Busscher *et al.*, 1984).

Gerson & Scheer (1980) reported that adherence of bacteria to hydrophobic surfaces is affected by the change in interfacial free energy which corresponds to the process of attachment. In a previous report (Minagi *et al.*, 1985), we described a physico-chemical mechanism for adherence of *Candida* to various resin surfaces. In the present study, we have investigated the adherence of six *Candida* species to three hydrocarbons (n-octane, n-hexadecane and *p*-xylene). The cell-surface hydrophobicities of the organisms were also determined, by Young contact-angle measurements, and results obtained by the two methods were compared.

METHODS

Candida strains and growth conditions. The organisms used were *C. albicans* IFO 1385, *C. tropicalis* IFO 1400, *C. stellatoidea* IFO 0692, *C. parapsilosis* IFO 1396, *C. krusei* IFO 1395 and *C. glabrata* IFO 0622. The yeasts, maintained on slopes of Sabouraud glucose agar [1% (w/v) peptone, 0.5% (w/v) yeast extract and 2% (w/v) glucose], were precultured in Sabouraud glucose broth at 37 °C overnight. A 10 ml portion of the preculture was inoculated into 40 ml Sabouraud glucose broth and incubated at 37 °C with shaking to OD₆₆₀ 2.0 (18 mm light path; Hitachi model 110-10 spectrophotometer). Cells were harvested by centrifugation (Kubota model KN-70) at 1500 *g* for 5 min at 20 °C, washed three times with 0.01 M-phosphate-buffered saline (PBS; 0.15 M-NaCl and 0.01 M-phosphate buffer, pH 7.3) and resuspended in PBS.

Contact angle. To measure the Young contact angle of the yeasts, a layer of cells was prepared on a membrane filter (pore size 0.45 µm; Millipore) as previously described (Minagi *et al.*, 1985), and air dried. The Young contact angle of distilled water on the yeast cell layer was measured by the horizontal-projection technique with a contact angle meter (Kyowa type CA-A) at 20 °C. Contact angles were measured at ten points on the yeast cell layer and the mean value was calculated.

Adherence to hydrocarbons. The adherence of the yeasts to hydrocarbons was measured by the method described by Rosenberg *et al.* (1980). To round-bottomed test tubes (10 mm internal diameter) containing washed cells suspended in 3 ml PBS and adjusted to an OD_{660} of approximately 0.5 were added various volumes (0–0.5 ml) of n-octane, n-hexadecane or *p*-xylene (Katayama Chemical Industries Co.). The surface tensions of n-octane, n-hexadecane and *p*-xylene are 21.7, 27.7 and 28.3 mJ m⁻², respectively (Schäfer & Lax, 1956). The mixtures were vigorously agitated for 60 s using a flat-top mixer (model MT-51 Touch Mixer; Yamato Scientific Co.). Immediately after separation of the two phases, the aqueous phase was carefully removed with a Pasteur pipette and transferred to a cuvette, and the OD_{660} was measured. Results were expressed as the percentage of the initial OD_{660} of the aqueous suspension as a function of hydrocarbon volume added.

Cell surface free energy. The surface free energy of a micro-organism (γ^{MV}) is given by equation (1) and some derivatives described by Neumann *et al.* (1974)

$$\cos \theta = \frac{(0.015\gamma^{MV} - 2.00)(\gamma^{MV} \gamma^{LV})^{1/2} + \gamma^{LV}}{\gamma^{LV} [0.015(\gamma^{MV} \gamma^{LV})^{1/2} - 1]} \quad (1)$$

where θ is the Young contact angle of distilled water on the cell layer, and γ^{LV} is the surface tension of distilled water.

RESULTS

Contact angle measurements. Table 1 shows the mean contact angle of distilled water for the six *Candida* species. *C. tropicalis* demonstrated the largest contact angle, and *C. stellatoidea* the smallest.

Adherence to hydrocarbons. The adherence of the six *Candida* species to n-hexadecane is shown in Fig. 1, and their adherence to 50 μ l and 500 μ l of three hydrocarbons is shown in Table 1. These results demonstrate that *C. albicans* and *C. stellatoidea* show low adherence, whereas *C. tropicalis*, *C. glabrata* and *C. krusei* are highly adherent. Although *C. tropicalis* was the most adherent to 50 μ l n-hexadecane, *C. krusei* had the highest affinity to 200–500 μ l n-hexadecane of the test strains.

Fig. 1. Fractional decrease of OD_{660} of suspensions of *Candida* species as a function of hexadecane volume added. PBS suspensions of (a) *C. albicans*, (b) *C. stellatoidea*, (c) *C. parapsilosis*, (d) *C. tropicalis*, (e) *C. glabrata* and (f) *C. krusei* were used. The results are presented as the percentage OD_{660} of the cell suspension after mixing with hexadecane, relative to that of the initial cell suspension.

Table 1. Contact angles, surface free energies and adherence to hydrocarbons of the six *Candida* species

Species	Contact angle* (degrees)	$\gamma^{MV}\dagger$ (mJ m ⁻²)	Percentage of original OD ₆₆₀ ‡					
			n-Octane		n-Hexadecane		p-Xylene	
			50 μ l	500 μ l	50 μ l	500 μ l	50 μ l	500 μ l
<i>C. stellatoidea</i>	45.9 $\pm$ 3.1	55.6	100.0 $\pm$ 1.3	97.5 $\pm$ 1.8	100.0 $\pm$ 2.6	99.4 $\pm$ 2.5	100.0 $\pm$ 5.3	99.9 $\pm$ 2.8
<i>C. albicans</i>	51.1 $\pm$ 1.9	52.6	100.0 $\pm$ 0.1	99.0 $\pm$ 0.2	100.0 $\pm$ 0.1	99.6 $\pm$ 0.4	100.0 $\pm$ 0.1	99.0 $\pm$ 0.4
<i>C. parapsilosis</i>	54.0 $\pm$ 3.0	50.9	91.6 $\pm$ 4.2	58.6 $\pm$ 1.9	95.8 $\pm$ 1.8	88.7 $\pm$ 2.6	96.2 $\pm$ 3.1	49.8 $\pm$ 4.3
<i>C. krusei</i>	94.2 $\pm$ 5.8	26.4	64.7 $\pm$ 5.8	3.6 $\pm$ 0.6	53.4 $\pm$ 5.3	4.2 $\pm$ 0.9	42.0 $\pm$ 6.2	0.0 $\pm$ 0.0
<i>C. tropicalis</i>	118.6 $\pm$ 4.1	11.7	36.2 $\pm$ 4.3	11.0 $\pm$ 2.0	33.8 $\pm$ 4.8	11.2 $\pm$ 2.0	34.7 $\pm$ 7.4	12.2 $\pm$ 1.4
<i>C. glabrata</i>	75.7 $\pm$ 2.7	38.0	58.8 $\pm$ 5.3	14.5 $\pm$ 2.0	56.6 $\pm$ 5.0	11.1 $\pm$ 2.0	22.0 $\pm$ 2.6	2.9 $\pm$ 1.0

* Mean $\pm$ SD ($n = 10$).

† Surface free energy of the micro-organism calculated from the mean contact angle by equation (1) (see Methods).

‡ Adherence to hydrocarbons was measured as described in Methods, and results expressed as mean percentage of original OD₆₆₀ $\pm$ SD ($n = 10$).

Cell-surface free energy. The surface free energies (γ^{MV}) of the yeasts, calculated from the mean contact angles by equation (1), are shown in Table 1. The calculated value of the surface free energy of *C. tropicalis* was the smallest and that of *C. stellatoidea* was the largest of the six species.

DISCUSSION

A number of methods to evaluate cell surface hydrophobicity have been developed; the two-liquid partition method with hydrocarbons has been used in many investigations (Rosenberg *et al.*, 1980; Ofek *et al.*, 1983; Gibbons *et al.*, 1983). In a previous report, we described a physico-chemical mechanism for adherence of *Candida* to various resin surfaces, and showed that the change in interfacial free energy, calculated from the contact angles of the resin surface and the yeast cell layer, correlated well with the adherence of the yeasts (Minagi *et al.*, 1985). In the present study, we examined the relation between the contact-angle method and the partitioning method for evaluating yeast surface hydrophobicity.

The adherence of *Candida* species to n-octane, n-hexadecane and *p*-xylene was examined with the partitioning method. *C. tropicalis*, *C. glabrata* and *C. krusei* adhered well to the hydrocarbons, while *C. albicans*, *C. stellatoidea* and *C. parapsilosis* were less adherent. The contact angles of the less adherent species were smaller than those of the more adherent species. Although the contact angles of the yeasts were measured on dry cell layers in the present study, measurements on moist bacterial layers have been reported (Busscher *et al.*, 1984). In general, contact angles measured on dry layers are somewhat larger than those on wet layers. *C. tropicalis* showed the largest contact angle of the six species, 118.6 degrees (Table 1), a value similar to that reported for *Streptococcus mitis* L2 (111 degrees; van Pelt *et al.*, 1984). Overall similarity of the results obtained from the partitioning method and the contact angle method on dry cell layers was demonstrated in the present study. However, a more accurate method of determining cell-surface hydrophobicity will be necessary for the exact physico-chemical analysis of yeast cell adherence.

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