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Germ Tube Formation Changes Surface Hydrophobicity of *Candida* Cells

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ABSTRACT

Hydrophobic interaction is generally considered to play an important role in the adherence of microorganisms to eukaryotic cells and also to certain inert surfaces. Using a microbe adhesion assay to hydrocarbons (n-hexadecane), 68 strains of *Candida albicans* and 30 non-*albicans* strains were studied. Influence of source of isolate, age of the culture, and percentage of germ tube formation on adhesion were studied. *C. albicans* blastoconidia were found to be hydrophilic; conversely, blastoconidia of non-*albicans* strains were slightly more hydrophobic. Germ tube formation was associated with a significant rise in cell surface hydrophobicity. Infect. Dis. Obstet. Gynecol. 7:222–226, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS

Candida albicans; Candida non-*albicans;* cell surface hydrophobicity; adhesion; cell adhesion

The importance of germ tube formation for the invasive capability of *Candida albicans* has been stressed.[1] It is commonly considered that the blastoconidia represent the morphological cell form associated with asymptomatic colonization of mucosal surfaces. Conversely, germ tube/hypha formation is assumed to represent apotentially tissue-invasive form. Most species produce germ tubes in vivo, but in vitro *C. albicans* is the only species capable of doing such following incubation for 4 hours at 37°C.[2] Adherence to liquid hydrocarbons, especiallyn-hexadecane, has been described as an indirect method to assess microbial cell surface hydrophobicity.[3] Hydrophobic interaction is generally thought to play an important role in the adherence of pathogenic microorganisms to host cells[4] by facilitating contact between the parasite and host cell.

Using a modified assay for microbial adhesion to hydrocarbons,[5] the cell surface hydrophobicity (CSH) of *C. albicans* and non-*albicans* strains presenting and not presenting germ tubes was studied. The possible influence of source of isolate, age of culture, and percentage of formed germ tubes were also evaluated.

MATERIAL AND METHODS

Strains

A total of 98 *Candida* strains were used, including 68 strains of *C. albicans* and 30 non-*albicans* strains, i.e., five strains of *Candida glabrata*, eight of *Candida krusei*, five of *Candidatropicalis*, three of *Can-*

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dida guillermondii, five of *Candida parapsilosis*, two of *Candida kefyr*, and two of *Candida rugosa*. With the exception of ATCC 10231 type strain of *C. albicans*, all the other strains were clinical isolates, i.e., from either the vagina, rectum, blood, or respiratory tract. The strains were identified by API 32C (Biomérieux).

Germ Tube Formation

Briefly, the germ tube test was performed as follows: 100 µL of a yeast cell suspension containing 10^5 cells/mL of phosphate-buffered saline (PBS) were added to 500 µL of RPMI 1640 medium (Sigma Chemical, St. Louis, MO). The percentage of germ-tube-forming cells was evaluated after 4 hours of incubation at37°C. Formation of germ tubes by the non-*albicans* strains was achieved by extended incubation (up to 18 hours) in RPMI 1640 medium (Sigma) at 37°C. In each test run, a sample of 200 yeast cells were studied using a Burke's chamber (×400). The percentage of cells that had formed germ tubes was determined. Only suspensions with a percentage of germ-tube-forming cells >90% and in which the tubes were at least twice as long as the diameter of blastoconidia were considered. For the non-*albicans* strains, suspensions with different percentages of germ tubes were included in the test result, with sizes twice as long as the diameter of the blastoconidia.

Inoculum Preparation

Fifty mL of Sabouraud dextrose broth (Difco Laboratories, Detroit, MI) was inoculated with yeast cells and incubated overnight at 37°C during shaking. Cells grown under similar conditions were also studied for germ tube formation, using the method described above. We also evaluated the influence on adherence of blastoconidia by variations of the incubation time, i.e., 24, 72, and 120 hours.

Adhesion Assay

Hydrophobicity of resting blastoconidia and of cells after germ tube formation was evaluated according to Van der Mei.[6] Briefly, yeast cells were harvested, washed twice in 10 mmol/L phosphate buffer, pH 7.0. A yeast suspension was prepared in the same buffer, to hold an optical density (A0) of 0.4–0.6 (at 600 nm). To 3 mL of this yeast suspension, 150 µL of hexadecane was added in acid-washed spectrophotometer glass tubes. After 10 minutes of incubation at 30°C, the tubes were vortexed twice for 30 seconds. After allowing phase separation for 10 minutes, the optical density of the lower aqueous-phase (A1) was measured and compared with that obtained prior to the mixing procedure (A0). The percentage of cells in the hexadecane layer (adhered cells) was used to estimate the hydrophobicity, using the following formula:

$$\text{percent cell adhesion} = [1 = (A1/A0)] \times 100.$$

All tests were run in duplicate. The results shown represent the mean of two consecutive experiments. No significant statistical interexperimental variations were observed.

Statistics

For statistical analysis of data the Wilcoxon signed rank test[7] was used; data were compared at a significance level of 0.05.

RESULTS

Increasing incubation periods did not result in any significant variation of CSH both for *C. albicans* and the non-*albicans* strains (Fig. 1), even after incubation up to 5 days. No difference was found between the sampling sites from which the *C. albicans* strains had been collected (Fig. 2).

Blastoconidia of the non-*albicans* strains were slightly more hydrophobic than those of *C. albicans* ($P = 0.003$) (Fig. 3). No differences were found among the non-*albicans* strains, with the exception of *C. parapsilosis*, which showed significantly higher CSH values, and a strain of *C. guillermondii*, which repeatedly showed hydrophobicity values around 70%. Figure 4 shows the results from 28 randomly chosen strains of *C. albicans*, indicating that germ tube formation by *C. albicans* is associated with a notable increase of CSH, in most cases higher than 40%, which represents a change from hydrophilic to hydrophobic surface properties. Similar conclusions can be extracted from Figure 5, which shows successive increases in hydrophobicity with the increasing rate of germ tube formation by the non-*albicans* strains, although the changes are not as obvious as with *C. albicans*. The difficulty of the non-*albicans* strains in producing a high percentage

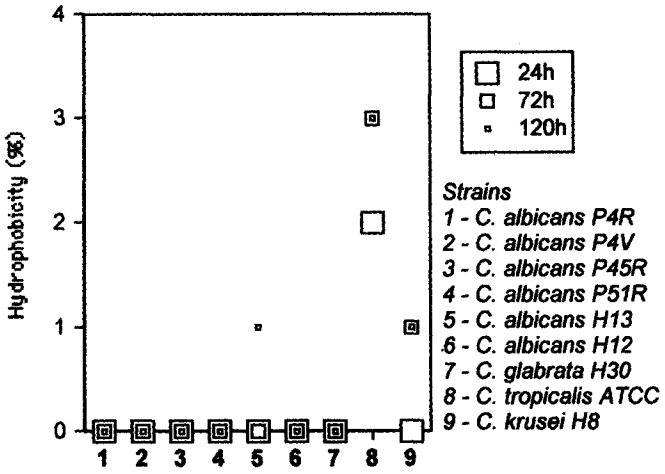

Fig. 1. Cell surface hydrophobicity of *Candida* strains after incubation for 24, 72, and 120 hours.

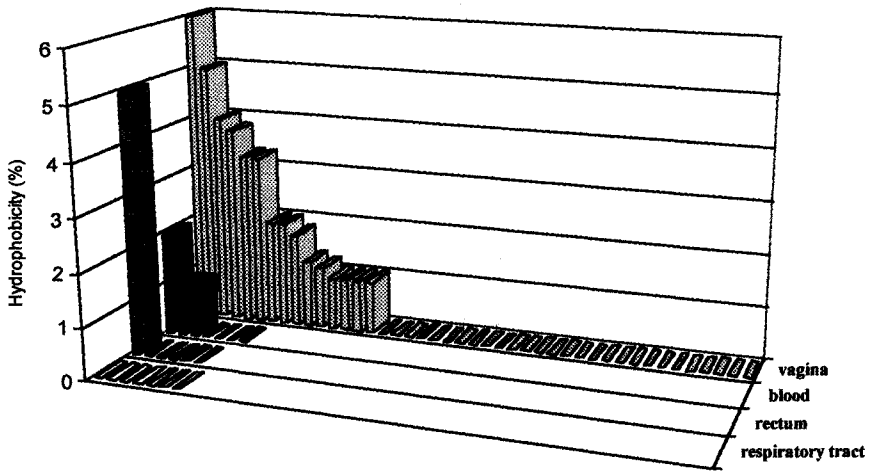

Fig. 2. Cell surface hydrophobicity of *Candida albicans* strains isolated from blood, respiratory tract, rectum, and vagina.

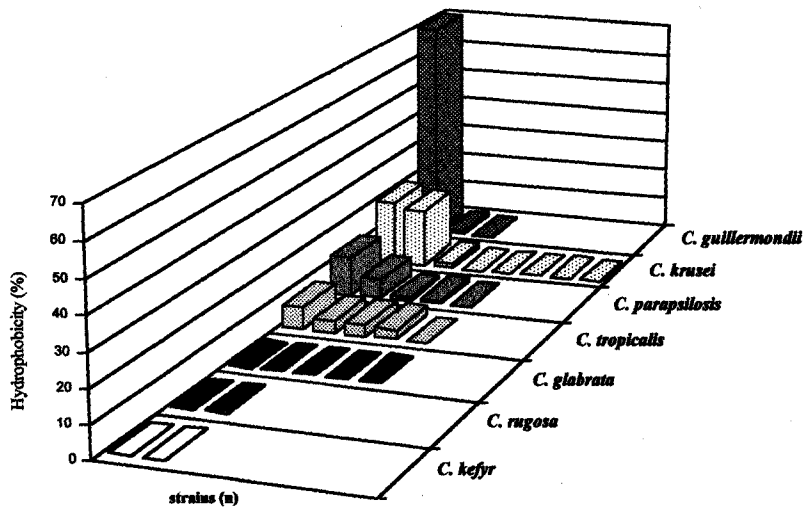

Fig. 3. Cell surface hydrophobicity of the non-*albicans* strains.

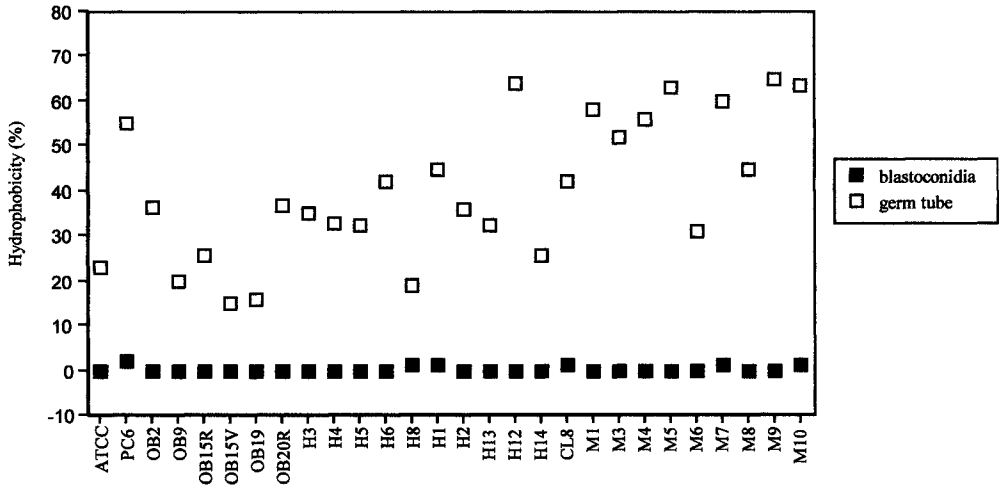

Fig. 4. Cell surface hydrophobicity of *Candida albicans* (28 strains) before and after germ tube formation.

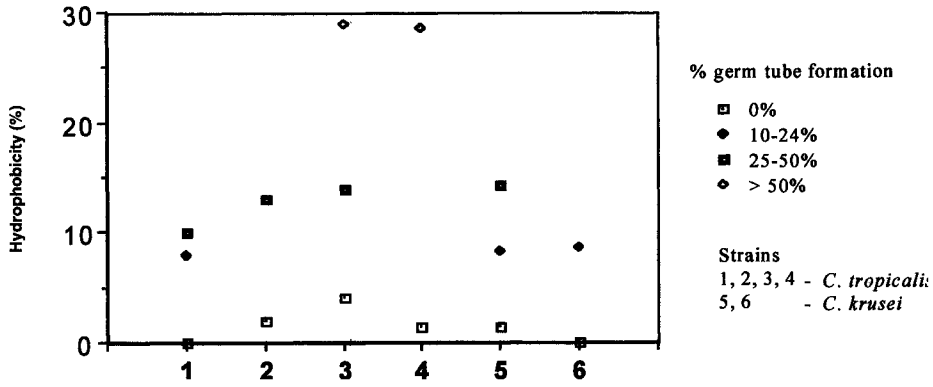

Fig. 5. Cell surface hydrophobicity of non-*albicans* strains before and after germ tube formation.

of germ tubes of proper size may have influence in the test result with those strains.

DISCUSSION

Microbial adherence is an essential initial step in the infectious process. Along with the presence of microbial adhesins and receptors in host cells, microbial surface hydrophobicity has been described as a major factor influencing adhesion of microorganisms to biological surfaces, but also to inert surfaces, including plastic ones.[8]

The development of candidosis involves the attachment of yeast and hyphae to host cell surfaces, possibly through multiple adhesion mechanisms. Cell surface hydrophobicity plays an important role in mediating the adhesion of yeasts to epithelial cells,[9, 10]as well as to splenic, kidney, and lymph node cells.[11]Increase in CSH also enhances the virulence of *C. albicans* in an animal model.[12] Knowing that CSH may change according to incubation temperature of the culture,[10] we conducted our assays using yeast cells (blastoconidia, cells that had formed germ tubes) grown at 37°C, in that way to mimic the in vivo situation.

Germ tube formation was a strong promoter of CSH both in the *albicans* and non-*albicans* strains. Higher rates of longer pseudomycelial filaments were invariably associated with higher hydrophobicity. In contrast, blastoconidia were found to be essentially non-hydrophobic, independently of the source of isolate or incubation time of culture.

Hydrophobic interactions may be of importance in promoting tissue invasion by filamentous yeast cells. Germ tubes of *C. albicans* are able to adhere to fibronectin, fibrinogen, and complement via cell surface receptors.[13] Recently, it has been shown that CSH favors attachment of *C. albicans* to extracellular matrix components (ECM), namely fi-

brinogen, fibronectin, and vitronectin, substances intimately associated with host cell surfaces.[14] Enhanced ability of hydrophobic cells to bind to ECM proteins appears to be responsible for a diffuse binding pattern of hydrophobic cells to splenic tissue.

The increase of CSH inherent to germ tube formation could represent an attachment mechanism not only to epithelial cell surfaces but also to inert surfaces, e.g., to endotracheal plastic tubes and catheters. This may perpetuate local colonization. In the case of *Candida* fungemia, the exposure of hydrophobic sites could influence the initial distribution of yeast cells within the body and potentially determine whether successful colonization and dissemination will occur.

Experiments have suggested that increase in CSH produces impairment of phagocytosis, increasing resistance to blood clearance[15] and thereby the virulence of *Candida* cells. Additionally, we showed that the CSH of blastoconidia of non-*albicans* strains was slightly higher than that of *C. albicans,* a fact that may explain a higher rate of fungal colonization and disseminated candidal infections in patients who receive transplants or other patients admitted to intensive care units, as they are frequent carriers of plastics or similar foreign bodies.

The strains recovered from mucosal surfaces were mainly hydrophilic, suggesting the relative unimportance of CSH in vivo in the colonization of those epithelia; germ tube formation was associated with a notable rise of CSH, suggesting a pathogenic role for this morphological presentation of the yeast.

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