New Assay for Measuring Cell Surface Hydrophobicities of *Candida dubliniensis* and *Candida albicans*

M. A. JABRA-RIZK,1* W. A. FALKLER, JR.,2 W. G. MERZ,3 AND T. F. MEILLER1

Department of Oral Medicine1 and Department of OCBS,2 Dental School, University of Maryland, and Department of Pathology, The Johns Hopkins University,3 Baltimore, Maryland

Received 20 October 2000/Returned for modification 15 December 2000/Accepted 27 February 2001

Hydrophobic interactions, based on cell surface hydrophobicity (CSH), are among the many and varied mechanisms of adherence deployed by the pathogenic yeast *Candida albicans*. Recently it was shown that, unlike *C. albicans*, *C. dubliniensis* is a species that exhibits an outer fibrillar layer consistent with constant CSH. Previously, *C. dubliniensis* grown at 25 or 37°C was shown to coaggregate with the oral anaerobic bacterium *Fusobacterium nucleatum*. *C. albicans*, however, demonstrated similar coaggregation only when hydrophobic or grown at 25°C. This observation implied that coaggregation of *Candida* cells with *F. nucleatum* is associated with a hydrophobic yeast cell surface. To test this hypothesis, 42 *C. albicans* and 40 *C. dubliniensis* clinical isolates, including a *C. albicans* hydrophobic variant, were grown at 25 and 37°C and tested with the established hydrophobicity microsphere assay, which determines CSH levels based on the number of microspheres attached to the yeast cells. The coaggregation assay was performed in parallel experiments. All *C. dubliniensis* isolates grown at either temperature, hydrophobic 25°C-grown *C. albicans* isolates, and the *C. albicans* hydrophobic variant, unlike the 37°C-hydrophobic *C. albicans* isolates, exhibited hydrophobic CSH levels with the microsphere assay and simultaneously showed maximum, 4+, coaggregation with *F. nucleatum*. The parallel results obtained for *C. dubliniensis* using both assays support the use of the CoAg assay both as a rapid assay to determine CSH and to differentiate between *C. dubliniensis* and *C. albicans*.

As with many other infectious processes, the adherence of the yeast *Candida albicans* to host tissues is an important first step in its successful colonization (3, 5, 15–17). The mechanisms of adherence deployed by *C. albicans*, however, are varied and extremely complex (18). Yeast cell surface hydrophobicity (CSH) has been shown to be involved in the adherence process of the yeast by providing hydrophobic interactions (2, 9). CSH is characterized by the presence of hydrophobic proteins embedded in the yeast cell wall matrix beneath an outer fibrillar layer and can be accurately assessed using a hydrophobicity microsphere assay (HMA) (9). Exposure of these hydrophobic proteins results in CSH; therefore, CSH is subject to cell surface variations such as a shortened or rearranged fibrillar layer (9, 10, 13). Variation in growth temperature is one of the factors that is known to affect CSH (9, 10). In fact, *C. albicans* cells are hydrophobic when grown at 25°C and hydrophilic at 37°C (9, 10). In addition to the increased adherence ability that hydrophobic yeast cells possess, virulence and phagocytosis studies showed cells grown at 25°C to be more virulent than those grown at 37°C (1, 6, 11). Recent investigations involving the characterization of *C. dubliniensis* (19, 20) have shown that this new species possesses an outer fibrillar layer which does not vary with growth temperature and which is consistent in length and arrangement with a hydrophobic yeast cell status (13). This property may allow *C. dubliniensis* to, unlike *C. albicans*, exhibit constant CSH (13). Follow-up studies on the CSH of *C. dubliniensis* relative to adherence have shown that *C. dubliniensis* has greater adherence to pooled human buccal epithelial cells in vitro than do typical *C. albicans* strains (in press).

In characterization studies of *C. dubliniensis*, coaggregation (CoAg) was observed between *C. dubliniensis* and the oral anaerobic bacterium *Fusobacterium nucleatum* (14). It was observed that when suspensions of *C. dubliniensis* cells grown at either 37 or 25°C were mixed with suspensions of *F. nucleatum* cells, all the *C. dubliniensis* isolates tested coaggregated with cells of *F. nucleatum*. However, when *C. albicans* cells grown at 37°C under the same conditions were mixed with an *F. nucleatum* cell suspension, all isolates tested failed to coaggregate with *F. nucleatum*. When the same *C. albicans* isolates were rendered hydrophobic by growing them at 25°C and suspensions of the cells were mixed with an *F. nucleatum* cell suspension, *C. albicans* cells coaggregated in a manner similar to that of the *C. dubliniensis* isolates (14).

The results of this previous study established that a specific CoAg relationship exists between *F. nucleatum* and *C. dubliniensis* and other yeast cells known to be hydrophobic. This suggests that CoAg assay results, in addition to species differentiation, may correlate with HMA-determined CSH levels and therefore can be used to determine yeast CSH. To confirm this hypothesis, a study was designed to compare the performance of the CoAg assay in detecting hydrophobic *C. albicans* and *C. dubliniensis* isolates to that of the HMA, an established assay for detection of hydrophobic yeast cell populations based on the attachment of microspheres to hydrophobic yeast cells (8–11).

**MATERIALS AND METHODS**

**Yeast and bacterial cultures.** Yeast strains and culture conditions. Forty-two *C. albicans* isolates including strains ATCC 18804 and LGH1095, 40 *C. dubliniensis* isolates including the reference strain (CD36 NCPF 3949), and a *C. albicans*
TABLE 1. CSH and CoAg of C. albicans isolates grown at room temperature or 37°C

| Strain       | % CSH 37°C | % CSH 25°C | CoAg 37°C | CoAg 25°C |
|--------------|------------|------------|-----------|-----------|
| LGH1095      | 3.6        | 83         | 0         | 3+        |
| ATCC 18804   | 4          | 77         | 0         | 3+        |
| A9V10        | 98         | 98         | 4+        | 4+        |

TABLE 2. CSH of C. dubliniensis isolates grown at room temperature or 37°C

| Isolate | % CSH 37°C | % CSH 25°C |
|---------|------------|------------|
| CD 36   | 99         | 99         |
| J-R 01  | 72         | 96         |
| J-R 02  | 93         | 76         |
| J-R 03  | 62         | 63         |
| J-R 04  | 85         | 83         |
| J-R 05  | 75         | 83         |
| J-R 06  | 67         | 72         |
| J-R 07  | 71         | 46         |
| J-R 08  | 80         | 83         |
| J-R 09  | 72         | 67         |
| J-R 10  | 69         | 68         |
| J-R 11  | 87         | 60         |
| J-R 12  | 69         | 83         |
| J-R 13  | 96         | 94         |
| J-R 14  | 62         | 60         |
| J-R 15  | 97         | 62         |
| J-R 16  | 91         | 98         |
| J-R 17  | 79         | 90         |
| J-R 18  | 93         | 93         |
| J-R 19  | 97         | 88         |
| J-R 20  | 97         | 88         |
| J-R 21  | 97         | 99         |
| J-R 22  | 92         | 82         |
| J-R 23  | 92         | 73         |
| J-R 24  | 96         | 89         |
| J-R 25  | 58         | 79         |
| J-R 26  | 98         | 79         |
| J-R 27  | 81         | 88         |
| J-R 28  | 92         | 87         |
| J-R 29  | 98         | 68         |
| J-R 30  | 92         | 91         |
| J-R 31  | 85         | 90         |
| J-R 32  | 74         | 77         |
| J-R 33  | 70         | 73         |
| J-R 34  | 73         | 80         |
| J-R 35  | 77         | 89         |
| J-R 36  | 76         | 60         |
| J-R 37  | 75         | 64         |
| J-R 38  | 70         | 82         |
| J-R 39  | 90         | 95         |

hydrophobic variant A9V10 (21) were included in this study. C. dubliniensis strains were identified using established criteria (12). Suspensions were prepared for C. albicans and C. dubliniensis isolates from colonies grown at 25 or 37°C for 24 or 48 h on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.).

Bacterial cultures. F. nucleatum (ATCC 49256) was grown on brucella blood agar in a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) at 37°C for 2 to 4 days. Organisms were harvested by scraping the plates, and cells were washed twice in CoAg buffer (20 mM Tris-HCl [pH 7.8], 0.1 mM CaCl₂, 0.1 mM MgCl₂, 0.15 M NaCl, 0.02% NaN₃) (4). Suspensions of bacterial cells were packed after centrifugation at 3,000 × g for 10 min and resuspended into a 1% (vol/vol) cell suspension with CoAg buffer. Bacterial cells were used immediately or stored at 4°C until use.

HMA. CSH levels for 40 C. dubliniensis clinical isolates including the type strain CD36 were determined by the method of Hazen et al. (10). For comparison, the CSH levels of two C. albicans strains, ATCC 18804 and LGH1095, were also determined. For this assay, yeast cells were grown on SDA for 48 h at 25 or 37°C. Cells were then harvested by centrifugation and washed three times with cold, sterile distilled H₂O, and the pellet was suspended in cold, sterile distilled H₂O. From the suspension, 3 μl was added to 3 ml of water (1/1,000 dilution), while the original cell suspension was repelleted and held on ice. The cell concentration was determined by loading 10 μl of the second suspension (1/1,000) in a hemocytometer, the cells were counted, and cell concentration was then adjusted to 2 × 10⁶/ml in sodium phosphate buffer (0.05 M, pH 7.2). The cells in suspension were repelleted and placed on ice. In a separate clean glass tube, 6 μl of bead suspension (low-sulfate white polystyrene microspheres; 0.825-μm diameter; Bangs Laboratories, Inc., Fishers, Ind.) was added to 2 ml of cold HMA buffer and then mixed by vortexing (the final bead concentration of 1,000 μl was added to 8 ml of 0.825 mM MgCl₂, 0.15 M NaCl, 0.02% NaN₃) (4). Suspensions of bacterial cells were vortexed for 10 s, shaken on a rotary platform shaker for 3 min, and left undisturbed at room temperature for an additional 2 min. The degree of CoAg reactions were performed in duplicate.

RESULTS

HMA results. CSH values obtained with the HMA for the C. albicans variant A9V10 (Table 1) and all 40 C. dubliniensis strains (Table 2) were consistent with a hydrophobic yeast cell status for all isolates grown at 25 and 37°C. However, CSH values for the C. albicans ATCC 18804 strain and LGH1095 strain used as controls differed between growth temperatures, as previously reported (8, 9). Both strains were hydrophobic when grown at room temperature and hydrophilic at 37°C (Table 1).

CoAg results. Suspensions in CoAg buffer of 40 strains of C. dubliniensis, 42 strains of C. albicans, and the C. albicans hydrophobic variant A9V10 grown at 37°C on SDA plates were tested with strains of F. nucleatum. Visual CoAg with F. nucleatum was observed only with the 40 C. dubliniensis strains, grown at either temperature, with the 25°C-grown 42 C. albicans isolates, and with the C. albicans hydrophobic variant A9V10. No visual CoAg was observed between F. nucleatum and these 42 C. albicans strains grown at 37°C. The CoAg reaction between all 40 C. dubliniensis strains grown at either temperature and F. nucleatum was the maximum 4+. Similarly, the CoAg reaction with the C. albicans hydrophobic variant and F. nucleatum was 4+ regardless of growth temperature. The CoAg reactions between the 40 25°C-grown clinical C. albicans isolates and the C. albicans ATCC 18804 and
LGH1095 strains (Table 1) and *F. nucleatum* ranged between 3+ and 4+.

**DISCUSSION**

The similarities between the interactions of hydrophobic yeast cells with both microsphere beads and *F. nucleatum* support the hypothesis that the CoAg assay detects CSH and is similar to the HMA in function (14). In our study, 40 *C. dubliniensis* isolates were grown at 25 and 37°C and tested with the HMA. In addition, the hydrophobic variant *C. albicans* A9V10 was also included for comparison along with the *C. albicans* LGH1095 strain, which has been extensively used by Hazen et al. (8, 10) in hydrophobicity studies. *C. albicans* LGH1095 is reported to typically have a hydrophobicity value of >70% when grown at 25°C and a value of <10% when grown at 37°C (8, 10).

Our CSH values for *C. albicans* LGH1095 correlated with the previously reported values (Table 1) (8, 10). CSH values for each *C. dubliniensis* strain tested (Table 2), as well as the *C. albicans* hydrophobic variant A9V10 (Table 1), were similar (i.e., constant hydrophobicity) for 25°C- and 37°C-grown cells. Unlike typical *C. albicans* strains, which demonstrate <10% hydrophobicity at 37°C, *C. dubliniensis* isolates and the *C. albicans* hydrophobic variant V9A10 had CSH values at the 37°C growth temperature that demonstrated hydrophobicity (9, 10).

With a variety of previously published methods, the CoAg reaction was characterized as involving a heat-labile (protein) receptor on *F. nucleatum* and a heat-stable or a polysaccharide component on *C. dubliniensis* (14). Although the specific mediators of adherence between *Candida* and *F. nucleatum* in the CoAg assay or *Candida* and the microsphere beads in the HMA are not fully known, both types of interactions seem to be consistently dependent on a hydrophobic cell surface topography of the yeast.

In the CoAg assay, cells of the bacterium *F. nucleatum*, like the microsphere beads in the HMA, are able to penetrate the spaced-out, short fibrils of the fibrillar layer of hydrophobic cells, whether 25°C-grown *C. albicans*, strain A9V10, or *C. dubliniensis*, and adhere to receptors embedded in the cell wall of the yeast (13, 14). In the case of the CoAg, however, due to their enhanced ability to adhere, *F. nucleatum* cells are able to adhere to multiple yeast cells, causing linking between the yeast cells, which results in CoAg. This resulting visual CoAg in the test tube can be used to determine the hydrophobicity of a yeast cell suspension, similarly to the HMA process of counting microspheres attached to yeast cells.

The parallel results obtained with the two assays for all 40 *C. dubliniensis* isolates used in this study support the assertion that the CoAg assay, in addition to being used to differentiate between *C. dubliniensis* and *C. albicans*, can also be used to evaluate CSH. The HMA gives the average CSH level for the cell population, and therefore CSH values for a hydrophobic population of cells at different stages of growth may vary.

Its simplicity and rapidity make the CoAg assay preferable for use in clinical laboratories for differentiation between *C. albicans* and *C. dubliniensis*, as well as in research laboratories for yeast CSH studies.

**REFERENCES**

1. Antley, P. P., and K. C. Hazen. 1988. Role of yeast cell growth temperature on *Candida albicans* virulence in mice. Infect. Immun. 56:2884–2890.
2. Calderone, R. A., and P. C. Braun. 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. 55:1–20.
3. Ellepola, A. N. B., G. J. Panagoda, and L. P. Samarawayake. 1999. Adhesion of oral *Candida* species to human buccal epithelial cells following brief exposure to nystatin. Oral Microbiol. Immunol. 14:358–363.
4. George, K. S., and W. A. Falkler, Jr. 1992. Coaggregation studies of the *Eubacterium* species. Oral Microbiol. Immunol. 7:285–290.
5. Gibbons, R. J., and M. Nygaar. 1970. Interbacterial aggregations of plaque bacteria. Arch. Oral Biol. 15:1397–1400.
6. Glee, P. M., P. Sundstrom, and K. C. Hazen. 1995. Expression of surface hydrophobic proteins by *Candida albicans* in vivo. Infect. Immun. 63:1373–1379.
7. Grimaudo, N. J., and W. E. Nesbitt. 1997. Coaggregation of *Candida albicans* with oral *Fusobacterium* species. Oral Microbiol. Immunol. 12:168–173.
8. Hazen, B. W., and K. C. Hazen. 1988. Dynamic expression of cell surface hydrophobicity during initial yeast cell growth and before germ tube formation of *Candida albicans*. Infect. Immun. 56:2521–2525.
9. Hazen, K. C. 1989. Participation of yeast cell surface hydrophobicity in adherence of *Candida albicans* to human epithelial cells. Infect. Immun. 57:1894–1900.
10. Hazen, K. C., D. L. Brawner, M. H. Riesselman, M. A. Jutila, and J. E. Cutler. 1991. Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. Infect. Immun. 59:907–912.
11. Hazen, K. C., and B. W. Hazen. 1992. Hydrophobic surface protein masking by the opportunistic fungal pathogen *Candida albicans*. Infect. Immun. 60:1499–1508.
12. Jabara-Rizk, M. A., A. A. A. M. Baqui, J. I. Kelley, W. A. Falkler, Jr., W. G. Merz, and T. F. Meiller. 1999. Identification of *Candida dubliniensis* in a prospective study of patients in the United States. J. Clin. Microbiol. 37:321–326.
13. Jabara-Rizk, M. A., W. A. Falkler, Jr., W. G. Merz, J. I. Kelley, A. A. A. M. Baqui, and T. F. Meiller. 1999. *Candida dubliniensis* and *Candida albicans* display surface variations consistent with observed intergeneric coaggregation. Rev. Iberoam. Microl. 16:18–44.
14. Jabara-Rizk, M. A., W. A. Falkler, Jr., W. G. Merz, J. I. Kelley, A. A. A. M. Baqui, and T. F. Meiller. 1999. Coaggregation of *Candida dubliniensis* with *Fusobacterium nucleatum*. J. Clin. Microbiol. 37:1464–1468.
15. Klotz, S. A., D. J. Drutz, J. L. Harrison, and M. Hupert. 1983. Adherence and penetration of vascular endothelium by *Candida* yeasts. Infect. Immun. 42:374–384.
16. Kolenbrander, P. E., and R. N. Andersen. 1989. Inhibition of coaggregation between *Fusobacterium nucleatum* and *Porphyromonas* (Bacteroides) gingivalis by lactose and related sugars. Infect. Immun. 57:3204–3209.
17. Kolenbrander, P. E., and R. N. Anderson. 1986. Multigeneric aggregations among oral bacteria: a network of cell-to-cell interactions. J. Bacteriol. 168:851–859.
18. Sturtevant, J., and R. Calderone. 1997. *Candida albicans* adhesins: biochemical and virulence. Rev. Iberoam. Microl. 14:49–97.
19. Sullivan, D., and D. Coleman. 1998. *Candida dubliniensis*: characteristics and identification. J. Clin. Microbiol. 36:329–334.
20. Sullivan, D. J., G. Moran, S. Donnelly, S. Gee, E. Pinjon, B. McCant, D. B. Shalney, and D. C. Coleman. 1999. *Candida dubliniensis*: an update. Rev. Iberoam. Microl. 16:72–76.
21. Whelan, W. L., J. M. Delga, E. Wadsworth, T. J. Walsh, K. J. Kwon-Chung, R. Calderone, and P. N. Lipke. 1990. Isolation and characterization of cell surface mutants of *Candida albicans*. Infect. Immun. 58:1552–1557.

Downloaded from http://cvi.asm.org/ on May 1, 2019 by guest