Adherence of *Candida albicans* and *Candida dubliniensis* to buccal and vaginal cells

Valerio Vidotto[1], Barbara Mantoan[1], Agostino Pugliese[1], José Pontón[2], Guillermo Quindós[2], Shigeji Aoki[3] & Shoko Ito-Kuwa[3]

[1]Laboratorio Micologia Medica, Dipartimento Discipline Medico-Chirurgiche, Sezione Microbiologia Clinica, Università di Torino, Torino, Italy; [2]Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Bilbao, Spain; [3]Advanced Research Center, Nippon Dental University, Niigata, Japan

Summary

Twenty-seven *Candida albicans* strains and 26 *Candida dubliniensis* strains, isolated from HIV patients, were tested for their adherence to buccal and vaginal epithelial cells. Both species showed important levels of adhesion to buccal and vaginal epithelial cells, although *C. albicans* showed the highest levels of adhesion. These results suggest that both *Candida* species are well adapted, in terms of adhesion capability, to the oral and vaginal environment.

Key words *Candida dubliniensis*, *Candida albicans*, adherence, buccal and vaginal cells.

Adhesión de *Candida albicans* y *Candida dubliniensis* a células epiteliales bucales y vaginales

Resumen

Se ha estudiado la capacidad de 27 cepas de *Candida albicans* y 26 de *Candida dubliniensis* aisladas de pacientes con SIDA para adherirse a células del epitelio bucal y vaginal. Ambas especies mostraron niveles importantes de adhesión a células del epitelio bucal y vaginal, aunque *C. albicans* mostró los niveles más altos de adhesión. Estos resultados sugieren que las dos especies de *Candida* están adaptadas, en cuanto a su capacidad de adherencia, al ambiente oral y vaginal.

Palabras clave *Candida dubliniensis*, *Candida albicans*, Adherencia, Epitelio bucal y vaginal

Adhesion of *Candida* sp. to mucosa, and particularly in *Candida albicans*, is probably an important initial step in the pathogenesis of infections caused by these yeasts [1,2]. This adhesion occurs by the interaction between yeast and epithelial cell receptors [1-3].

It is well known that *C. albicans* adhesion to mucosal cells is enhanced by several factors such as germ tube production, phospholipase, protease, other extracellular enzymatic activities, carbohydrates, pH and temperature [4-10]. On the other hand, some antimycotics seem to inhibit this adherence [8,11,12]. It is possible that these factors together may contribute not only to the virulence and pathogenicity in *C. albicans*, but also in *Candida dubliniensis*. *C. dubliniensis* is very similar to *C. albicans*, in terms of genotypic and phenotypic characteristics [13,14]. It is becoming a clinically relevant yeast due to its world wide distribution and its association to both mucosal and systemic candidiasis [15,16]. The yeast has been isolated not only in the oral cavities of immunocompromised patients but also in lungs, vagina, blood, sputum, and gastrointestinal tract [17,18].

The majority of the studies focused on the adherence to mucosal surfaces (oral and vaginal) refer to *C. albicans* [3,12]. Very few studies have focused on the adherence of *C. dubliniensis* to oral mucosa [19-21] and none to vaginal mucosa. The aim of this study was to examine the adherence *in vitro* of *C. dubliniensis* and *C. albicans* to oral and vaginal mucosa.

Materials and methods

Fungal strains and culture conditions. Two *C. albicans* reference strains from the National Collection of Pathogenic Fungi (NCPF, Bristol, UK), 25 *C. albicans* from the Infectious Disease Institute of Torino University collection, and 26 *C. dubliniensis* from the Universidad del País Vasco culture collection were used in this study. With the exception of the NCPF strains, the rest were isolated from HIV patients with oral candidiasis. They were transferred onto fresh Malt agar (Difco, USA) slants and stored at 4 °C.

Dirección para correspondencia:
Dr. Valerio Vidotto
Laboratorio Micologia Medica
Dipartimento Discipline Medico-Chirurgiche
Sezione Microbiologia Clinica
Università diTorino
Corso Svizzera 164
10149 Torino, Italy
E-mail: valerio.vidotto@libero.it

Aceptado para publicación el 6 de Junio de 2003

1130-1406/01/10.00 Euros

Adherence test. The *in vitro* adherence test for the buccal epithelial cells (BEC) and vaginal epithelial cells (VEC) was performed as described by Macura & Tondyra [8] and Wellmer & Bernhardt [22]. Each *C. albicans* and *C. dubliniensis* strain was inoculated into 400 ml of Malt extract broth (Difco) containing 50 nmol of D-galactose (Sigma, USA). After an incubation period of 24 h at 37 °C, the cells were harvested by centrifugation, washed three times in phosphate-buffered saline (PBS, Sigma) and turbidimetrically adjusted to a concentration of 10^8 cells/ml. Buccal epithelial cells were obtained from healthy volunteers by gently scrap ing their cheeks with a wooden spatula. Vaginal epithelial cells were also obtained in the same way from healthy volunteers. These cells were then suspended in PBS, washed three times in the same buffered solution and adjusted to a concentration of 10^6 cells/ml by using a Bürker hemocytometer. Two hundred microlitres of the *C. albicans* and the *C. dubliniensis* suspensions and BEC and VEC (average 300 cells/200 µl) were mixed and incubated for 1 h at 37 °C. The non-adhering fungal cells were washed off through a 12 µm polycarbonate filter (Schleicher & Schuell, Germany) with 5 ml of PBS. The filter was stained with trypan blue and the number of fungal cells stained with trypan blue attached to 25 BEC or VEC was recorded. The number of adherent cells with respect to each BEC and VEC and the percentage of *C. dubliniensis* and *C. albicans* adherent cells were calculated.

Statistical analysis. The Mann-Whitney and chi-square tests were performed to compare data from *C. dubliniensis* and *C. albicans* adhesion to BEC and VEC.

Results

Among the 25 BEC considered, the average adherence of *C. dubliniensis* was 15.2 ± 0.8 (60.8%). The average number of *C. dubliniensis* adhered to each BEC was 1.99 ± 0.20 (Table). *C. dubliniensis* isolates showed a higher adhesion to VEC than to BEC, since the average adherence of *C. dubliniensis* to the 25 VEC was 21.4 ± 0.7 (85.6%). The average number of *C. dubliniensis* adherent to each VEC was 4.12 ± 0.48 (Table).

Table. Adherence of *C. dubliniensis* and *C. albicans* to buccal and vaginal cells.

Species	Cells	No. of cells showing yeast adherence among 25 cells (%)*	No. of yeasts adhered per cell*
C. dubliniensis (n = 26)	Buccal	15.2 ± 0.8 (60.8)	1.99 ± 0.20
	Vaginal	21.4 ± 0.7 (85.6)	4.12 ± 0.48
C. albicans (n = 27)	Buccal	17.9 ± 0.6 (71.6)	2.36 ± 0.21
	Vaginal	23.0 ± 0.3 (92.0)	4.88 ± 0.35

*Average values obtained with 26 *C. dubliniensis* and 27 *C.albicans* strains

The average adherence of *C. albicans* to the 25 BEC was 17.9 ± 0.6 (71.6%) with an average number of *C. albicans* adherent to each BEC of 2.36 ± 0.2 1 (Table). As it was observed in *C. dubliniensis*, *C. albicans* also showed a higher adherence to VEC than to BEC (average adhesion to VEC 23.0 ± 0.3 [92.0%], average number of *C. albicans* adherent to each VEC 4.88 ± 0.35).

When the adhesion of both species was compared, the adherence of *C. dubliniensis* clinical isolates to both BEC and VEC was lower than that showed by the clinical isolates of *C. albicans* (Table).

According to the Mann-Whitney test differences in adhesion between of *C. dubliniensis* and *C. albicans* to BEC were statistically significant (p = 0.01). Although the same trend was observed when we compared the adhesion of *C. dubliniensis* and *C. albicans* to VEC, differences were not statistically significant.

Differences in adhesion of *C.albicans* to BEC and VEC, as well as differences in adhesion of *C. dubliniensis* to buccal and vaginal cells, were statistically significant (p = 0.01).

Discussion

Candida albicans virulence and pathogenicity is complex and it is believed to be correlated to different factors such as germ tube production, adherence, phospholipase, protease and other different extracellular enzymatic activities recently described [1,6,7,9,10]. It is known that in this yeast the activity of extracellular enzymes is particularly concentrated at the tip of the hyphae [7,23].

According to the literature, genetic, environmental and phenotypic factors such as pH, temperature, anaerobic conditions and nutritional factors can contribute to tissue digestion enhancing *C. albicans* penetration through mucosal cells [24,25]. All these genetic, environmental and phenotypic factors could also play a role in *C. dubliniensis* virulence and pathogenicity. The new and recently recognized *C. dubliniensis* species is genetically and phenotypically very similar to *C. albicans* [13,14,24].

All the C. *dubliniensis* and the *C. albicans* strains tested in this study produced high levels of adherence to BEC. A similar finding has been reported in *C. albicans* by Al Rawi y Kawanagh [26]. When the ability of *C. albicans* and *C. dubliniensis* strains to adhere to both BEC and VEC was compared, the adherence of *C. albicans* was higher than that of the *C. dubliniensis* strains. These results are in agreement with our work on the adhesion of *C. albicans* and *C. dubliniensis* to a resin composite restorative dental material [27,28] but disagree with those described by Gilfillan *et al*., [13] who showed that oral *C. dubliniensis* isolates were more adherent to BEC than *C. albicans* when grown in glucose, and equally adherent when grown in galactose.

Althought *C. albicans* isolates were more adherent to VEC (p = 0.049), it is also interesting to mention the high levels of adherence that *C. dubliniensis* showed to this epithelium. The adherence of *C. albicans* and in particular *C. dubliniensis* to the vaginal epithelium, according to Boris *et al*.[29], could be attributed to the interaction with receptors of the vaginal cells such as glycolipids, glycoproteins and carbohydrates. In this habitat *C. albicans* and *C. dubliniensis* can easily compete and prevail with lactobacilli and other pathogenic microrganisms which also target the epithelial vaginal cells.

The greater adherence of *C. albicans* with respect to *C. dubliniensis* to buccal and vaginal epithelial cells, is in agreement with the fact that *C. albicans* is usually considered more virulent than *C. dubliniensis* [10,20,25,29]. However, it is important to point out that the ability of *C. dubliniensis* to adhere to BEC and VEC is similar to that of *C. albicans*, as demonstrated during this study. Further and in depth research should be performed in this field to identify the main adhesive differences between both *Candida* species.

References

1. King RD, Lee JC, Morris AL. Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. Infect Immun 1980; 27: 667-674.
2. Sturtevant J, Calderone R. *Candida albicans* adhesins: Biochemical aspects and virulence. Rev Iberoam Micol 1997; 14: 90-97.
3. Enache E, Eskandari T, Borja L, Wadsworth E, Hoxter B, Calderone R. *Candida albicans* adherence to human oesophageal cell line. Microbiology 1996; 142: 2741-2746.
4. Barrett-Bee K, Hayes Y, Wilson RG, Ryley JF. A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. J Gen Microbiol 1985; 131: 1217-1221.
5. Cardaropoli S, Di Fraia D, Menegatti F, Aoki S, Vidotto V. Correlation between chlamydosporulation, germ tube, phospholipase and proteinase production in *Candidaalbicans.* J Mycol Med 1997; 7: 169-170.
6. Ghannoum M, Abu Elteen K. Correlative relationship between proteinase production, adherence and pathogenicity of various strains of *Candida albicans*. J Med Vet Mycol 1986; 24: 407-413.
7. Hube B. *Candida albicans* secreted aspartyl proteinases. Curr Top Med Mycol 1996; 7: 55-69.
8. Macura AB, Tondyra E. Influence of some carbohydrates and concanavalin A on the adherence of *Candida albicans* in vitro to buccal epithelial cells. Zbl Bakt 1989; 272: 196-201.
9. Vidotto V, Koga-Ito CY, Milano R, Fianchino B, Pontón J. Correlation between germ tube production, phospholipase activity and serotype distribution in *Candida albicans.* Rev Iberoam Micol 1999; 16: 208-210.
10. Gale CA, Bendel CM, McClellan M, Becker JM, Berman J, Hostetter MK. Linkage of adhesion, filamentous growth and virulence in *Candida albicans* to a single gene, INTI. Science 1998; 279: 1355-1358.
11. Segal E, Trygerman O, Gov Y, Sandovsky-Losica H, Berdicevsky I. Adhesion of *Candida albicans* to epithelial cells: effect of antimycotics. J Mycol Med 1997; 7: 71-76.
12. Ellepola ANB, Samaranayake LP. The effect of limited exposure to antimycotics on the relative cell-surface hydrophobicity and the adhesion of oral *Candida albicans* to buccal epithelial cells. Arch Oral Biol 1998; 43: 879-887.
13. Gilfillan GD, Sullivan DJ, Haynes K, *et. al*. *Candidadubliniensis*: phylogeny and putative virulence factors. Microbiology 1998; 144: 829-838.
14. Sullivan DJ, Westerneng TJ, Haynes KA, Bennett DE, Coleman DC. *Candida dubliniensissp* nov: phenotypic and molecular characterization of novel species associatedwith oral candidosis in HIV infected individuals. Microbiology 1995; 141: 1507-1521.
15. Sullivan D, Haynes K, Bille J, *et al.* Widespread geographyc distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. J Clin Microbiol 1997; 35: 960-964.
16. Jabra-Rizk MA, Baqui AAMA, Kelley Jl, Falkler Jr WA, Merz WG, Meiller TF. Identification of *Candida dubliniensis* in a prospective study of patients in the United States. J Clin Microbiol 1999; 37: 321-326.
17. Sullivan DJ, Moran G, Donnelly S, *et al*. *Candida dubliniensis*: an update. Rev Iberoam Micol 1999; 16: 72-76.
18. Meis JF, Ruhnke M, De Pauw BE, Odds FC, Siegert W, Verweij PE. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. Emerg Infect Dis 1999; 5: 150–153.
19. Ramage G, Vande Walle K, Wickes BL, Lopez-Ribot JL. Biofilm formation by *Candida dubliniensis*. J Clin Microbiol 2001; 39: 3234-3240.
20. Pereiro M Jr, Losada A, Toribio J. Adherence of *Candida albicans* strains isolated from AIDS patients. Comparison with pathogenic yeasts isolated from patients without HIV infection. Br J Dermatol 1997; 137: 76-80.
21. Jabra-Rizk MA, Falkler Jr WA, Merz WG, Baqui AAMA, Kelley JI, Meiller TF. Retrospective identification and characterization of *Candida dubliniensis* isolates among *Candida albicans* clinical laboratory isolates from human immunodeficiency virus HIV infected and non HIV infected individuals. J Clin Microbiol 2000; 38: 2423-2426.
22. Wellmer A, Bernhardt H. Adherence on buccal epithelial cells and germ tube formation in the continuous flow culture of clinical *Candida albicans* isolates. Mycoses 1997; 40: 363-368.
23. Pugh D, Cawson RA. The cytochemical localization of phospholipase in *Candida albicans* infecting the chick-allantoic membrane. Sabouraudia 1977; 15: 29-35.
24. Staib P, Michel S, Koheler G, Morshauser J. A molecular genetic system for the pathogenic yeast *Candida dubliniensis*. Gene 2000; 242: 393-398.
25. Calderone R, Suzuki S, Cannon R, *et. al*. *Candidaalbicans*: adherence, signalling and virulence. Med Myco. 2000; 38 (suppl1): 125-137.
26. Al Rawi N, Kawanagh K. Characterization of yeasts implicated in vulvovaginal candidosis in Irish women. Br J Biomed Science 1999; 56: 99-104.
27. Maza JL, Prado C, Vidotto V, Elguezábal N, Pontón J. Adherencia de diversas especies de *Candida* a composites híbridos. Rev Eur Odontoestomatol 2001; 13: 177-180.
28. Elguezábal N, Maza JL, Pontón J. Inhibition of adherence of *Candida albicans* and *Candida dubliniensis* to a resin composite restorative dental material by salivary secretory IgA and monoclonal antibodies. Oral Diseases 2003; *submitted.*
29. Boris S, Suarez JE, Vazques F, Barbes C. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infect Immun 1998; 66: 1985-1989.