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

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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.

**Summary**

**Key words**

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

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

Se ha estudiado la capacidad de 27 cepas de *Candida albicans* y 26 de *Candida dubliniensis* aisladases 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.
Adherence test. The in vitro adherence test for the buccal epithelial cells (BEC) and vaginal epithelial cells (VEC) was performed as described by Macura & Tondrya [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 10^6 cells/ml. Buccal epithelial cells were obtained from healthy volunteers by gently scraping 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 10^8 cells/ml by using a Bürker hemocytometer. Two hundred microliters 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>
<thead>
<tr>
<th>Species</th>
<th>Cells</th>
<th>No. of cells showing yeast adherence among 25 cells (%)</th>
<th>No. of yeasts adhered per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. dubliniensis</td>
<td>Buccal</td>
<td>15.2 ± 0.8 (60.8)</td>
<td>1.99 ± 0.20</td>
</tr>
<tr>
<td>(n = 26)</td>
<td>Vaginal</td>
<td>21.4 ± 0.7 (85.6)</td>
<td>4.12 ± 0.48</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Buccal</td>
<td>17.9 ± 0.6 (71.6)</td>
<td>2.36 ± 0.21</td>
</tr>
<tr>
<td>(n = 27)</td>
<td>Vaginal</td>
<td>23.0 ± 0.3 (92.0)</td>
<td>4.88 ± 0.35</td>
</tr>
</tbody>
</table>

*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 ± 0.21 (Table). As it was observed in C. dubliniensis, C. albicans also showed a higher adherence to VEC than to BEC (average adherence 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, adhesion, 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 & 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.

Although 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 glycoproteins, glycoproteins and carbohydrates. In this habitat C. albicans and C. dubliniensis can easily compete and prevail with lactobacilli and other pathogenic microorganisms 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