Note

Protease and phospholipase activities of Candida spp. isolated from cutaneous candidiasis

Lívia de Souza Ramos, Leonardo Silva Barbedo, Lys Adriana Braga-Silva, André Luis Souza dos Santos, Marcia Ribeiro Pinto, Diana Bridon da Graça Sgarbi

Aims: To determine the protease and phospholipase production from 58 human clinical isolates of Candida obtained from individuals with cutaneous candidiasis seen in the Human and Veterinary Diagnostic Mycology Sector from Universidade Federal Fluminense (UFF), Brazil, from November 2008 to August 2009.

Methods: Fungal identification was performed using biochemical tests. Proteolytic activity was detected on agar plates containing bovine serum albumin, and phospholipase production was determined on egg-yolk plates.

Results: The Candida species isolated were Candida parapsilosis (27.59%), Candida famata (18.96%), Candida albicans (15.52%), Candida haemulonii (12.06%), Candida ciferri (8.62%), Candida guilliermondii (6.90%), Candida tropicalis (5.17%) and Candida lipolytica (5.17%). All isolates of C. albicans produced both protease and phospholipase, as regards the isolates of non-C. albicans Candida species, 53.06% and 4.08% were able to produce protease and phospholipase, respectively. For example, the majority of isolates of C. parapsilosis (15/16) produced protease, while 40% of C. ciferri isolates (2/5) were phospholipase producers. This study shows, for the first time, that C. ciferri and C. haemulonii strains were able to produce protease.

Conclusions: Collectively, our results showed that different species of Candida isolated from cutaneous lesions were able to produce proteases and/or phospholipases, which are multifunctional molecules directly involved in the infective process of these fungi.

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Métodos: La identificación de las especies de *Candida* se realizó mediante pruebas bioquímicas, la actividad proteolítica se detectó en placas de agar que contenían albúmina de suero bovino y la actividad fosfolípasa se determinó utilizando el método de la placa de yema de huevo semi-cuantitativa.

Resultados: Las especies aisladas fueron *Candida parapsilosis* (27,59%), *Candida famata* (18,96%), *Candida albicans* (15,52%), *Candida haemulonii* (12,06%), *Candida ciferri* (8,62%), *Candida guilliermondii* (6,90%), *Candida tropicalis* (5,17%) y *Candida lipolytica* (5,17%). Todos los aislamientos de *C. albicans* produjeron tanto proteasa como fosfolipasa. El 53,06% de los aislamientos de *Candida no-C. albicans* fueron capaces de producir proteasa y el 4,08% fosfolipasa. La mayoría de los aislamientos de *C. parapsilosis* (15/16) produjo proteasa, mientras que el 40% de los aislamientos de *C. ciferri* (2/5) fueron productores de fosfolipasa. Se describe por primera vez en la literatura científica la producción de proteasas por cepas de *C. haemulonii* y *C. ciferri*.

Conclusions: Nuestros resultados muestran el potencial que tienen los aislamientos de *Candida* proveído para producir proteasas y fosfolipasas.

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data were analyzed statistically using Student’s t test. P values of 0.05 or less were considered statistically significant.

Results and discussion

The prevalence of superficial mycoses has tremendously risen in the last years, affecting around 20–25% of the world’s population. In this scenario, skin mycoses are the most frequent forms of infection and, consequently, constitute a major public health problem worldwide.15,25 Our results revealed that from the 58 Candida isolates recovered from cutaneous infections, C. parapsilosis was the most frequently isolated species (n = 16; 27.6%), followed by C. famata (n = 11; 19%), C. albicans (n = 9; 15.5%), C. haemulonii (n = 7; 12%), C. ciferri (n = 5; 8.6%), C. guilliermondii (n = 4; 6.9%), C. tropicalis (n = 3; 5.2%) and C. lipolytica (n = 3; 5.2%). Considering the site of the cutaneous infections, C. parapsilosis (20.5%) was the most prevalent Candida species isolated from finger followed by C. albicans (18.2%), C. famata (18.2%), C. haemulonii (13.6%), C. ciferri (11.4%), C. guilliermondii (9.1%), C. tropicalis (4.5%) and C. lipolytica (4.5%), while C. parapsilosis (70%) was again by far the most isolated species from skin followed by C. albicans (10%), C. famata (10%) and C. tropicalis (10%).

Epidemiological and etiological studies of superficial mycoses of the foot revealed that the major isolated causal agents were C. parapsilosis (25%) and Trichophyton rubrum (18.7%).3,6 C. parapsilosis was the leading cause of onychomycosis in a group of patients from Malta followed by C. tropicalis and C. guilliermondii.21 Similarly, a study performed in Brazil reported that the leading pathogen detected in samples from infected nails (n = 200) was C. parapsilosis (40.5%) followed by C. albicans (31.5%), C. tropicalis (26.0%) and C. guilliermondii (2.0%). Contrarily, C. albicans was the most common Candida species able to cause cutaneous/nail and vaginal candidiasis in Singapore,18 Jordan,1 Slovenia12 and the United States.8 Importantly, the increased tendency for non-C. albicans Candida infections poses a challenge in the disease management, since these species are often notorious to develop antifungal resistance that is correlated with routine fluconazole prophylaxis adopted in some patients and the intrinsic/acquired azole resistance of Candida spp.3

The production of proteases, especially secreted aspartic proteases (Saps), has been reported to be one of the most important determinants for pathogenicity of Candida.9 The culture of Candida yeasts in YCB-BSA at acidic pH is a recognized condition that induces the secretion of Saps.28 Under these conditions, we showed that protease activity was detected in 35 strains of all Candida species tested, among which all strains of C. albicans and almost all strains of C. parapsilosis were protease producers (Table 1). All C. albicans isolates exhibited high Pz values, which indicate low enzymatic production, whereas the majority (66.7%) of C. parapsilosis isolates displayed moderate Pz and the minority (33.3%) high Pz values. However, strains of C. famata showed lower Pz values when compared to the other Candida species (Table 1). In addition, the protease activity of C. famata was significantly higher than that of C. albicans (P < 0.001) or C. parapsilosis (P < 0.03). None of the C. lipolytica or C. guilliermondii isolates showed proteolytic activity (Table 1), under the employed experimental conditions. The analysis of the body location also revealed a significant difference (P < 0.05) in the protease production: there was a 90% of producers between the skin isolates, 55% between the finger nails’ isolates, 54.2% between the toe nails’ isolates, and 50% in the case of the sole of feet isolates.

Kantarcioglou and Yücel showed that 78.9% of all examined Candida strains (n = 95) were protease-positive.16 Those authors also interpreted their data by different viewpoints. For example, 95% of C. albicans produced proteases. However, considering only the site of fungal isolation, a new profile of protease producers could be seen, as follows: oral cavity (17/22 = 77.3%), respiratory tract (37/46 = 80.4%), urogenital system (21/23 = 91.3%), and blood (0/4 = 0%).16 These discrepancies in percentages of positivity may be considered as being relevant due to the number of the different species tested as well as the site of isolation. Corroborating these findings, De Bernardis and co-workers reported that all cutaneous isolates of C. parapsilosis had uniformly elevated secreted protease activity, more than four times higher than the enzyme activity of the blood isolates.4 Additionally, the cutaneous isolates of C. parapsilosis were highly vaginopathic in a rat vaginitis model when compared to the blood isolates.4 Cassone also detected a higher proteolytic activity in vaginal C. parapsilosis isolates when compared with blood isolates.2 Different published works revealed that 65–75% of clinical strains of C. tropicalis were able to yield aspartic proteases,17,22,26 which are in accordance with our findings (67%). We reported herein that cutaneous clinical isolates of C. famata were the most potent protease producers. For the first time, we emphasize that some clinical strains of C. ciferri and C. haemulonii are able to secrete aspartic proteases.

Phospholipases play an active role in the invasion of host tissue by Candida by disrupting the epithelial cell membranes and allowing the hyphal tip to enter the cytoplasm.16 Regarding phospholipase activity, all strains of C. albicans and only few strains (2/5) of C. ciferri were capable of producing phospholipase, while the great majority (81%) of the Candida strains isolated from cutaneous lesions was unable to yield it (Table 1). The 2 strains of C. ciferri and 5 strains of C. albicans able to produce phospholipase activity exhibited high Pz values, while the other 4 remaining strains of C. albicans showed moderate Pz values. Samaranyake and co-workers screened 41 Candida isolates for phospholipase activity and found no strains of C. tropicalis, C. glabrata and C. parapsilosis producing extracellular phospholipases, whereas 73% of the C. albicans isolates screened were found to be positive.24 Kumar and co-workers showed that 100% of clinical isolates of C. albicans isolated from HIV positive and cancer patients produced a pronounced phospholipase activity.1 The absence of protease and/or phospholipase in clinical isolates of Candida must be interpreted with caution. In this context, hydrolytic enzymes with activities against other substrates of relevance to human cutaneous infections, distinct to those used in the present study, can help in the detection of these enzymatic classes.

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Protease activity (%) Range (Pz)</th>
<th>Mean (Pz)</th>
<th>Phospholipase activity (%) Range (Pz)</th>
<th>Mean (Pz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>100 (9/9)</td>
<td>0.73−0.83</td>
<td>0.78 ± 0.04</td>
<td>0.73 ± 0.13</td>
</tr>
<tr>
<td>C. ciferri</td>
<td>20 (1/5)</td>
<td>0.78</td>
<td>0.78 ± 0.02</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>C. famata</td>
<td>63.6 (7/11)</td>
<td>0.45−0.85</td>
<td>0.57 ± 0.15</td>
<td>0.0/0.11</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>0 (0/4)</td>
<td>0</td>
<td>0.0/0.4</td>
<td>0/0</td>
</tr>
<tr>
<td>C. haemulonii</td>
<td>14.3 (1/7)</td>
<td>0.60</td>
<td>0.60 ± 0.05</td>
<td>0/0</td>
</tr>
<tr>
<td>C. lipolytica</td>
<td>0 (0/3)</td>
<td>0</td>
<td>0</td>
<td>0/0</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>93.8 (15/16)</td>
<td>0.57−0.90</td>
<td>0.69 ± 0.09</td>
<td>0/0</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>66.7 (2/3)</td>
<td>0.71−0.86</td>
<td>0.79 ± 0.11</td>
<td>0/0</td>
</tr>
<tr>
<td>Total</td>
<td>60.34% (35/58)</td>
<td></td>
<td>18.97% (11/58)</td>
<td></td>
</tr>
</tbody>
</table>
The comparison between C. albicans and non-C. albicans Candida species revealed that the frequency of isolation (15.5% versus 82.5%), as well as the production of both protease (100% versus 53.1%) and phospholipase (100% versus 4.1%) activities, was significantly different (P<0.05). Only 17.2% (10/58) of the Candida isolates produced both enzyme classes (all strains of C. albicans and one strain of C. ciferrii). Previous studies also reported that proteases and phospholipases are produced at high rates in C. albicans, whilst non-C. albicans Candida species usually present low rates of these enzymes.16,22 In this sense, the hydrolytic enzyme profiles provide some data about the potential virulence factors produced by Candida strains isolated from cutaneous infection, resulting in a great variability production in non-C. albicans Candida species (probably strain specific), in contrast to a homogeneous production of both protease and phospholipase by clinical isolates of C. albicans.

Conflict of interest

The authors report no conflict of interest.

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