ENZYMATIC AND HEMOLYTIC ACTIVITIES OF Candida dubliniensis STRAINS

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SUMMARY

Candida dubliniensis is an opportunistic yeast that has been recovered from several body sites in many populations; it is most often recovered from the oral cavities of human immunodeficiency virus-infected patients. Although extensive studies on epidemiology and phylogeny of C. dubliniensis have been performed, little is known about virulence factors such as exoenzymatic and hemolytic activities. In this study we compared proteinase, hyaluronidase, chondroitin sulphatase and hemolytic activities in 18 C. dubliniensis and 30 C. albicans strains isolated from AIDS patients. C. albicans isolates produced higher amounts of proteinase than C. dubliniensis (p < 0.05). All the tested C. dubliniensis strains expressed hyaluronidase and chondroitin sulphatase activities, but none of them were significantly different from those observed with C. albicans (p > 0.05). Hemolytic activity was affected by CaCl₂; when this component was absent, we did not notice any significant difference between C. albicans and C. dubliniensis hemolytic activities. On the contrary, when we added 2.5 g% CaCl₂, the hemolytic activity was reduced on C. dubliniensis and stimulated on C. albicans tested strains (p < 0.05).

KEYWORDS: Candida dubliniensis; Proteinase; Hyaluronidase; Chondroitin sulphatase; Hemolytic activity.

INTRODUCTION

Candida dubliniensis has recently been added up to the growing list of potential opportunistic pathogen yeasts. This species shares many phenotypic characteristics with C. albicans, such as production of chlamydospores and germ tube. The pathogenesis of diseases caused by this species is partially known and well-studied virulence factors in C. albicans must also be assessed in C. dubliniensis. In C. albicans the widely advocated virulence traits include dimorphism, adherence, enzyme production, rapid phenotypic switch, antigenic variation and other several immunoevasion mechanisms. Regarding enzyme production, hydrolytic enzymes such as proteinase, phospholipase, hyaluronidase and chondroitin-sulphatase are putative virulence factors that help C. albicans to invade tissues.

Secreted aspartic proteinases (Saps), encoded by the SAP gene family, appear to play a major role in C. albicans virulence. Seven homologues genes (SAP) were also detected in C. dubliniensis by Southern analysis but scarce studies were performed focusing proteinase activity in this species. Hyaluronidase and chondroitin-sulphatase are considered to be important virulence factors in oral bacteria that cause oral infectious diseases. SHIMIZU et al. were the first researchers that described these exoenzymes in Candida species; as far as we know, these enzymes have not yet been studied in C. dubliniensis isolates.

Hemolytic activity is another virulence factor exhibited by pathogenic microorganisms which permits growth in the host using several iron-binding proteins as a source of iron. Hemoglobin is an important iron-source for pathogenic microorganisms, and the hemolytic activity and the hemoglobin utilization have been considered as a pathogenic factor. Studies to evaluate this virulence factor in C. dubliniensis have not been carried out yet.

The purpose of the present study was to determine whether there are differences in the expression of proteinase, hyaluronidase, chondroitin sulphatase and hemolytic activity between C. albicans and C. dubliniensis. The interference of CaCl₂ in the hemolytic activity was also evaluated.

MATERIAL AND METHODS

Candida isolates: We have studied eighteen clinical strains of C. dubliniensis and thirty of C. albicans, both recovered from oral candidiasis of AIDS patients. Phenotypic identification tests of C. dubliniensis were confirmed by genotypic methods as randomly amplified polymorphic DNA (RAPD) using the primers CDU (5’ GCGATCCCC3’) and B-14 (5’GATCAAGT3’). C. albicans isolates were identified by classical methods. All the cultures were maintained at -80 °C as stock collection of Laboratório de Pesquisas Micológicas, Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil.

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**RESULTS**

All analyzed strains showed proteinase, hyaluronidase and chondroitin sulphatase activities.

Proteinase activity of *C. dubliniensis* resulted in Pz range from 0.61 to 0.84 (mean = 0.75) and 0.60 - 0.79 for *C. albicans* (mean = 0.68); these differences were significant (*p* < 0.02) (Table 1).

Hyaluronidase and chondroitin sulphatase activities in *C. dubliniensis* and *C. albicans* are shown in Table 1 and no statistical differences were detected when we compared both species.

In *vitro* hemolytic activities of *C. dubliniensis*, expressed as hemolysis index (Hi), changed from 0.55 (well-defined hemolysis zone) to 1.0 (absence of hemolytic activity). Only two *C. dubliniensis* isolates showed Hi = 1.0. In *C. albicans*, the Hi changed from 0.5 to 0.85 (Table 2). No significant differences were observed between both species (*p* > 0.05).

Adding CaCl\(_2\) 2.5% to Sabouraud glucose agar supplemented with sheep blood, the hemolytic activities declined in *C. dubliniensis* and rose in *C. albicans*. Hi in the *C. albicans* group, was significantly more expressive than that obtained for *C. dubliniensis* (*p* < 0.01). Furthermore, when we compared the *C. albicans* Hi between the results of the two media (with and without CaCl\(_2\)), we detected hemolytic activity of *C. albicans* was better expressed by CaCl\(_2\) addition (*p* < 0.01) (Table 2).

**DISCUSSION**

The most studied virulence factors of *C. dubliniensis* have been hydrophobicity, adhesion and those observed by experimental infections\(^{10}\).

We noticed that *C. albicans* produced higher amounts of proteinases than *C. dubliniensis*. This finding is divergent from that reported by McCULLOUGH et al.\(^{10}\) which suggested that *C. dubliniensis* produced higher levels of proteinase activity than *C. albicans* reference isolates. When studying phylogeny and putative virulence factors of *C. dubliniensis*, GILFILLAN et al.\(^{5}\) detected seven genes for secretory aspartyl proteinase (SAP), as occurs in *C. albicans*, but they did not confirm the findings of McCULLOUGH et al.\(^{10}\).

Among the 18 *C. dubliniensis* isolates tested all produced hyaluronidase and chondroitin sulphatase, but these activities were not

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**Table 1**

Production of proteinase, hyaluronidase and chondroitin sulphatase by *C. dubliniensis* and *C. albicans*

<table>
<thead>
<tr>
<th>Species</th>
<th>Proteinase (Pz)*</th>
<th>Hyaluronidase (Hz)*</th>
<th>Chondroitin sulphatase (Cz)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. dubliniensis</em> (n = 18)</td>
<td>0.61 - 0.84(^{a})</td>
<td>0.70 - 0.85(^{c})</td>
<td>0.55 - 0.81(^{b})</td>
</tr>
<tr>
<td><em>C. albicans</em> (n = 30)</td>
<td>0.60 - 0.79(^{b})</td>
<td>0.80 - 0.86(^{d})</td>
<td>0.60 - 0.92(^{f})</td>
</tr>
</tbody>
</table>

*Value obtained by dividing the diameter of the colony by the total diameter of the colony including precipitation or clear zone. a < b (*p* < 0.02)

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**Table 2**

Hemolytic activity of *C. dubliniensis* and *C. albicans* considering the effect of CaCl\(_2\)

<table>
<thead>
<tr>
<th>Species</th>
<th>Hemolysis index (Hi)*</th>
<th>Without CaCl(_2)</th>
<th>With 2.5% CaCl(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. dubliniensis</em> (n = 18)</td>
<td>0.55 - 1.0</td>
<td>0.78 - 1.0</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em> (n = 30)</td>
<td>0.5 - 0.85</td>
<td>0.44 - 0.63</td>
<td></td>
</tr>
</tbody>
</table>

*Value obtained by dividing the diameter of the colony by the total diameter of the colony plus the translucent halo. d > b (*p* < 0.01). d > c (*p* < 0.01)
different from those observed with *C. albicans*. Hyaluronidase and chondroitin sulphatase are involved in bacterial virulence and the substrates of these enzymes are among the major constituents of connective tissue and gingival epithelium. Hyaluronidase and chondroitin sulphatase can affect the permeability of epithelium in the intercellular spaces by attacking the intercellular cementing substances of tissue. Because *C. dubliniensis* has been isolated mainly from mouth, we judged important to evaluate these exoenzymes. As far as we know, hyaluronidase and chondroitin sulphatase activities from *C. dubliniensis* are here reported by the first time.

Since there is essentially no free iron in the human host, most pathogens acquire this indirectly from commonly available iron-containing compounds such as hemoglobin. The enzymes involved in this activity are classified as hemolysins. We have found that onto blood agar medium without CaCl₂, the hemolytic activity of *C. dubliniensis* was similar to that of *C. albicans*. However, when CaCl₂ 2.5% was added, the hemolytic activity of *C. dubliniensis* decreased while it increased for *C. albicans*. The hemolytic activity of medically important yeasts like genus Candida and Cryptococcus has been scarcely explored. A complement-mediated hemolysis induced by *C. albicans* was reported by MANNS et al. and *C. dubliniensis* among others showed alpha and beta hemolysis; this was the first study to demonstrate the variable expression profiles of hemolysins by different Candida species. However, LUO et al. have studied only two isolates of *C. dubliniensis*. The CaCl₂ has been included in culture media as Calcium donor in the media proposed by PRICE et al. and more recently in the media proposed by SLIFKIN, named Tween 80 opacity test. In both methods, after enzymatic activity action on distinct substrates, fatty acids are released and the formation of a calcium complex occurs producing a distinct, well-defined, dense white zone of precipitation around the colony. In the Tween 80 opacity test none of the Candida species showed a halo response when CaCl₂ was omitted from the medium. Based on these facts, we supplemented the sugar-enriched sheep blood agar medium with growing CaCl₂ concentrations in order to obtain a better reading of the hemolytic activity. In a previous assay we have observed that CaCl₂ concentrations > 2.5 g% were step by step inhibitory for *C. dubliniensis* (data not shown). So, we established CaCl₂ 2.5 g% as the more elevated concentration that did not inhibit the growth of *C. dubliniensis* and *C. albicans*. In general, our results showed that hemolytic activity of *C. dubliniensis* was inhibited by CaCl₂ 2.5 g% but the same concentration stimulated the hemolytic activity of *C. albicans* (Table 2). This finding is consistent with previous studies relating *C. dubliniensis* strains as more susceptible to physical and chemical agents than *C. albicans*. On the other hand, it was not possible to apply this finding as a screening test for differentiation between *C. albicans* and *C. dubliniensis*, because the more elevated *C. albicans* hemolytic activity was not absolute and some strains also showed inhibition.

In conclusion, our results suggest that *C. dubliniensis* seems to be less virulent than *C. albicans* because proteinase, the major putative virulence factors, was less expressed or absent. In addition, hyaluronidase, chondroitin sulphatase as well as hemolytic activity are virulence factors less studied and their importance requires new and more rigorous studies.

RESUMO

Atividade enzimática e hemolítica de *Candida dubliniensis*

*C. dubliniensis* é uma levedura oportunista que, embora já tenha sido isolada de vários sítios anatômicos é, com maior frequência, encontrada na boca de pacientes infectados pelo HIV. Embora tenham sido realizados numerosos estudos sobre a epidemiologia e filogenia, seus fatores de virulência como atividade enzimática e atividade hemolítica, são, ainda, pouco conhecidos. Neste estudo comparou-se a atividade in vitro de proteinase, hialuronidase, chondroitin sulfatase e atividade hemolítica de 18 cultivos de *C. dubliniensis* com 30 cultivos de *C. albicans*, todos isolados de pacientes com SIDA. Foi evidenciada maior atividade de proteinase em *C. albicans* em relação a *C. dubliniensis* (p < 0,05). Todos os isolados de *C. dubliniensis* evidenciaram atividade de hialuronidase e chondroitin-sulfatase de forma similar ao observado com *C. albicans* (p > 0,05). Constatou-se que a atividade hemolítica foi influenciada pelo CaCl₂; em sua ausência não foram observadas diferenças na atividade hemolítica das duas espécies; todavia, ao se agregar 2,5% de CaCl₂ a atividade hemolítica de *C. dubliniensis* foi reduzida enquanto a de *C. albicans*, estimulada (p < 0,05).

REFERENCES


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