



## Communication/Comunicação

### *Candida dubliniensis* does not show phospholipase activity: true or false?

*Candida dubliniensis* não evidencia atividade fosfolipásica: verdadeiro ou falso?

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#### ABSTRACT

**Introduction:** The phospholipase activity in *Candida albicans* and *Candida dubliniensis* isolated from oral candidiasis cases were studied. **Methods:** The phospholipase activity was evaluated in egg yolk agar. **Results:** All the *C. albicans* isolates (n = 48) showed phospholipase activity (mean Pz = 0.66). However, none of the *C. dubliniensis* isolates (n = 24) showed this activity. **Conclusions:** The authors discuss whether these findings are a true characteristic of *C. dubliniensis* or a consequence of the methodology employed, which includes the possibility that NaCl may have inhibited the enzymatic activity of *C. dubliniensis*.

**Key-words:** *Candida albicans*. *Candida dubliniensis*. Phospholipase activity.

#### RESUMO

**Introdução:** Avaliou-se a atividade fosfolipásica em *Candida albicans* e *Candida dubliniensis* isoladas de casos de candidíase oral. **Métodos:** A atividade de fosfolipase foi avaliada em ágar gema de ovo. **Resultados:** Todos os isolados de *C. albicans* (nº = 48) evidenciaram atividade fosfolipásica (média Pz = 0.66). Todavia, nenhum isolado de *C. dubliniensis* (nº = 24) demonstrou esta atividade. **Conclusões:** Os autores discutem se estes achados são uma característica verdadeira de *C. dubliniensis* ou uma consequência da metodologia empregada, a qual inclui a possibilidade de que o NaCl seja inibidor da atividade enzimática de *C. dubliniensis*.

**Palavras-chaves:** *Candida albicans*. *Candida dubliniensis*. Atividade de fosfolipase.

The virulence of yeast species is a multifactorial property dependent on many different virulence factors, including adhesion to the host cells, formation of hyphae, phenotypic switching and production of hydrolytic enzymes such as proteinases and phospholipases<sup>1-3</sup>. *Candida albicans* shows these putative virulence factors, but there has been less investigation of these phenotypic characteristics in *Candida dubliniensis*, particularly its phospholipase activity. So far, only two studies have reported the production of

phospholipases, and only in a limited quantity of *C. dubliniensis*<sup>2,4</sup>. This study aimed to compare the phospholipase activity of *C. albicans* and *C. dubliniensis* that were isolated from clinical specimens.

We studied twenty-four clinical strains of *C. dubliniensis* and forty-eight strains of *C. albicans*, both recovered from oral candidiasis lesions in AIDS patients. The *C. dubliniensis* isolates were obtained from different medical centers in Brazil between 1998 and 2007. The *C. albicans* isolates were obtained from the University Hospital of Santa Maria (Santa Maria, Rio Grande do Sul, Brazil) between 1995 and 2005. All the isolates were maintained at -70 °C. The results from phenotypic identification tests on *C. dubliniensis* were confirmed by genotypic methods using randomly amplified polymorphic DNA (RAPD) and using the primers CDU (5' GCGATCCCC3') and B-14 (5' GATCAAGTC3') (Bauer et al<sup>5</sup>). *C. albicans* isolates were identified using classical methods. The production of phospholipases by *C. albicans* and *C. dubliniensis* was investigated by means of culture media<sup>6</sup> and techniques described by Price et al<sup>7</sup> and Saramanayake et al<sup>8</sup>. *C. albicans* CBS 2730, *C. albicans* 2630 and *C. dubliniensis* CBS 7987 were included as controls.

Phospholipase activity of *C. albicans* was detected in all 48 of our assayed strains, which resulted in Pz ranges from 0.41 to 0.80 (mean = 0.66). However, phospholipase activity for *C. dubliniensis* was absent. The colonies of *C. albicans* developed dense growth, which resulted in a cream-like opaque zone around the colonies in the medium. In contrast, *C. dubliniensis* colonies scarcely developed, and no opaque halo surrounding the colonies was observed. None of the *C. dubliniensis* strains showed phospholipase activity.

Phospholipase exoenzymes are considered to play an important role in the pathogenesis of opportunistic fungi, as well as an active role in the invasion of host tissue during candidiasis<sup>9</sup>. By cleaving phospholipids, phospholipase destabilizes the membrane and promotes cell lysis<sup>3</sup>. Since Price et al<sup>7</sup> described a plate method for detection of phospholipase activity in *C. albicans*, it has become the traditional screening method for phospholipase activity among *Candida* species, as well as for other yeast-like fungi such as *Cryptococcus neoformans*<sup>3</sup> and *Malassezia pachydermatis*<sup>6</sup>. In the present study, we demonstrated that *C. dubliniensis* did not have any extracellular phospholipase activity. In our opinion, this result requires new studies, because the medium indicated by Price et al<sup>7</sup> is composed of 5.73 gram % NaCl, which seems to be an inhibitory factor for *C. dubliniensis* growth. We previously demonstrated that Sabouraud broth with 6.5% NaCl was inhibitory for *C. dubliniensis*, and then proposed a simple test for *C. dubliniensis* screening<sup>1</sup>.

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We therefore assume that the phospholipase activity of *C. dubliniensis* cannot be assayed using Price's method<sup>7</sup>, which could explain the scarcity of studies focusing on this virulence factor in relation to the biology of *C. dubliniensis*. Results similar to ours were reported by Hannula et al<sup>4</sup>, applying the same methodology that we used; however, those authors did not emphasize this finding. More recently, Fotedar & Al-Hedaithy<sup>2</sup> studied 87 *C. dubliniensis* strains and observed that none showed phospholipase activity, even after prolonging the incubation for seven days. Under those conditions, they reported that colonies with dense growth formed but did not have any enzymatic activity in Price's medium<sup>7</sup>. The relationship between NaCl and scarce growth was also observed for *C. albicans* in a hypertonic medium containing 6.5% NaCl, which explains why incubation took four days in that test<sup>1</sup>. *C. dubliniensis*, which is more sensitive, showed no growth under these conditions. Studies focusing on virulence factors have shown that *C. dubliniensis* is less virulent than *C. albicans*<sup>2,9</sup>. Would the phospholipase be the main cause of this characteristic? Based on our observations, new and more rigorous studies, such as biochemical analysis using radiometric or colorimetric assays, are needed in order to answer this question. In addition, gene cloning will elucidate the presence, activity and role of extracellular phospholipase as a virulence factor relating to *C. dubliniensis*<sup>3</sup>.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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