

# Enzymatic activities of *Candida tropicalis* isolated from hospitalized patients

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Secretion of hydrolytic enzymes is considered a virulence factor in *Candida* spp. Extracellular enzymatic activities in 29 clinical isolates of *Candida tropicalis* were analyzed by plate assays. *C. tropicalis*, similar to *Candida albicans*, showed elevated hemolytic and esterase activities. However, unlike *C. albicans*, low aspartyl protease and very low phospholipase activities were detected in *C. tropicalis* isolates.

**Keywords** *Candida tropicalis*, hemolysins, aspartyl proteinases, phospholipases, esterases

## Introduction

*Candida* infections have increased in recent years, and those caused by non-*C. albicans* *Candida* species are rising proportionally more than *Candida albicans*; *Candida tropicalis* is one of the most commonly isolated of these non-*C. albicans* *Candida* species [1,2]. Secretion of hydrolytic enzymes is considered an important virulence factor [3,4], but in non-*C. albicans* *Candida* species, these determinants of pathogenicity are largely undescribed [5]. The aim of this study was to evaluate hemolytic, aspartyl proteinase, phospholipase and esterase activities of *C. tropicalis* isolates recovered from clinical samples.

## Material and methods

Extracellular enzymatic activities of a total of 29 clinical isolates of *C. tropicalis* collected between 2003 and 2007 from different patients at the Infanta Cristina University Hospital (Badajoz, Spain), as well as *C. tropicalis* CECT1440 (Spanish Collection Culture Type, Burjasot,

Spain) were analyzed. The isolates were identified through the use of the API ID 32C system (bioMérieux, Marcy l'Etoile, France). The origins of the strains were 51.7% from urinary tract, 24.1% from respiratory tract, and 24.2% from skin and biological fluids.

Assessments of enzymatic activity were made by plate assays with different test media (10 ml into sterile 90-mm Petri dishes). The plates were incubated for 24, 48 and 72 h at 22°C and 37°C. The activity was expressed according to the Pz index (colony diameter/total diameter of the colony plus the precipitation halo) [6].

Hemolytic activity was determined using a method described by Luo *et al.* [7], employing Sabouraud dextrose agar (SDA; Difco, Madrid Spain) supplemented with 3% glucose and 7% fresh sheep blood (Oxoid, Madrid, Spain), and incubated in 5% CO<sub>2</sub>. A translucent halo around the inoculum site indicated positive hemolytic activity.

Secreted aspartyl proteinases (Saps) activity was assayed using a test medium with 1.17 g of yeast carbon base (YCB; Difco), 0.01 g yeast extract (BBL, Madrid, Spain) and 0.2 g bovine serum albumin (BSA; Sigma, Madrid, Spain) as the nitrogen source. The pH was adjusted to pH 5.0 in 10 ml of water, sterilized by filtration, and added to 90 ml of autoclaved water containing 1.5 g of agar [8]. The cleavage of BSA by Saps results in zones of clearance.

Phospholipase activity was evaluated using SDA supplemented with 3% glucose, 1 M NaCl, and 0.005 M CaCl<sub>2</sub> [6]. The solution was autoclaved, and 2%

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sterile egg yolk emulsion (Oxoid) was added later. Phospholipase activity produces a dense zone of precipitation around the colony [9].

Esterase activity was determined using the Tween 80 opacity test medium [10], prepared with 10 g Bacto Peptone (Difco), 5 g of NaCl, 0.1 g CaCl<sub>2</sub>, and 15 g agar in 1 l of distilled water, adjusted to pH 6.8 and autoclaved. When the medium cooled (50°C), 5 ml of Tween 80 (Sigma) was added. Lipolytic enzymes hydrolyze the medium, liberating fatty acids which bind with calcium, forming a precipitation halo around the inoculation site [11].

Each study was repeated at least three times. Portions of growth from stock cultures stored at -20°C were transferred to and grown on SDA for 24 h and then subcultured to the same medium at 22°C and 37°C, depending on the temperature used later in the enzymatic evaluation. Ten µl of a suspension with 0.7 optical density at 590 nm (10<sup>7</sup> cfu/ml) was dropped onto each test medium. The following ranges of activity according to Pz index were established: high, Pz < 0.40; medium, Pz = 0.41–0.60; low, Pz = 0.61–0.80; very low, Pz = 0.81–0.99; none, Pz = 1. The Pz index is a reproducible semi-quantitative technique used widely [12,13].

## Results and discussion

The results obtained in this study are summarized in Table 1.

In our investigation, all isolates of *C. tropicalis* produced hemolytic activity at 37°C within 24 h; hemolysis at 72 h was more evident in the plates, although the majority of the strains remained in the same range of activity. At 22°C, all strains were positive at 48 h. While hemolysin production has been extensively studied in *C. albicans* [14], there have been few investigations with *C. tropicalis*. Our results agree with those observed by Luo *et al.* [7].

At 37°C and 24 h, the majority of strains (28/29) presented very low Saps activity, whereas at 72 h, half of the strains showed medium activity. In contrast, low activity was noted at 22°C. Saps activity in *Candida* spp. has been widely studied [15,16]. Our results were similar to those obtained by Kantarcioglu and Yucel [17] who also found activity in all the strains, while Yamamoto *et al.* [18] identified proteolytic activity in 13 of 18 strains.

Very low or no phospholipase activity was detected in the 28 strains at 24 h and 37°C. The low and very low activity was transformed progressively into no activity at

**Table 1** Enzymatic activities of 29 clinical strains of *Candida tropicalis* at different periods of time (24, 48 and 72 h) and two incubation temperatures (37 and 22°C) measured by plate assays according to Pz index.

| Enzymatic activities of 29 strains |           | Number of strains according to Pz index <sup>a</sup> |      |      |      |      |      |
|------------------------------------|-----------|--|------|------|------|------|------|
|                                    |           | 37°C   |      |      | 22°C |      |      |
| Pz index                           |           | 24 h   | 48 h | 72 h | 24 h | 48 h | 72 h |
| <b>Hemolysins</b>                  |           |  |      |      |      |      |      |
| High                               | <0.40     | 0  | 0    | 1    | 0    | 0    | 2    |
| Medium                             | 0.41–0.60 | 26   | 26   | 26   | 0    | 28   | 27   |
| Low                                | 0.61–0.80 | 3  | 3    | 2    | 8    | 1    | 0    |
| Very low                           | 0.81–0.99 | 0  | 0    | 0    | 17   | 0    | 0    |
| None                               | 1         | 0  | 0    | 0    | 4    | 0    | 0    |
| <b>Aspartyl proteases</b>          |           |  |      |      |      |      |      |
| High                               | <0.40     | 0  | 0    | 0    | 0    | 0    | 0    |
| Medium                             | 0.41–0.60 | 0  | 8    | 15   | 0    | 0    | 0    |
| Low                                | 0.61–0.80 | 1  | 8    | 7    | 0    | 1    | 13   |
| Very low                           | 0.81–0.99 | 28   | 13   | 7    | 23   | 28   | 16   |
| None                               | 1         | 0  | 0    | 0    | 6    | 0    | 0    |
| <b>Phospholipases</b>              |           |  |      |      |      |      |      |
| High                               | <0.40     | 0  | 0    | 0    | 0    | 0    | 0    |
| Medium                             | 0.41–0.60 | 0  | 0    | 0    | 0    | 0    | 0    |
| Low                                | 0.61–0.80 | 1  | 0    | 0    | 0    | 6    | 16   |
| Very low                           | 0.81–0.99 | 20   | 17   | 6    | 0    | 22   | 12   |
| None                               | 1         | 8  | 12   | 23   | 29   | 1    | 1    |
| <b>Esterases</b>                   |           |  |      |      |      |      |      |
| High                               | <0.40     | 0  | 0    | 6    | 0    | 4    | 19   |
| Medium                             | 0.41–0.60 | 0  | 20   | 22   | 0    | 24   | 10   |
| Low                                | 0.61–0.80 | 19   | 9    | 1    | 1    | 1    | 0    |
| Very Low                           | 0.81–0.99 | 9  | 0    | 0    | 6    | 0    | 0    |
| None                               | 1         | 1  | 0    | 0    | 22   | 0    | 0    |

<sup>a</sup>Pz index: colony diameter/total diameter of the colony plus the precipitation zone/halo.

48 and 72 h in many strains. This change could be due to the masking of the small halo of lysis by the growing colony. At 22°C and 24 h, activity was not detected but by 72 h, low and very low activity was noticed in 97% of the strains, which is in agreement with Tamura *et al.* [19]. However, other authors did not detect phospholipase activity in *C. tropicalis* [17,20]. This discrepancy could be attributed to the plate method used because it does not detect activity in low-phospholipase-producing strains [9]. Effectively, results obtained with *C. albicans*, which is a good-phospholipase-producing species, are concordant [9,13,16,21]. The greater sensitivity found in our study was likely due to the measurement at 24 h, the very thin layer of medium used, and the different preparation of the egg yolk agar plates.

At 37°C, esterase activity was low or very low in 28 strains at 24 h but by 72 h, the strains showed higher activity. At 22°C and 24 h, no activity was detected for the majority of strains but by 72 h, 66% reached high activity. At 48 and 72 h, the strains had higher esterase activity than those incubated at 37°C. This result could be due to the slower growth at 22°C. Our results coincide with those described by other authors [11,22], who also found that all of their isolates of *C. tropicalis* had activity at two days after inoculation. Yucesoy and Marol [23] found positive activity in 93% of their strains, and Kumar *et al.* [10] in 92.3%.

Very few studies have analyzed the hemolytic, proteinase, phospholipase, and esterase activities on the whole. In our study, *C. tropicalis* presented, similar to *C. albicans*, elevated hemolytic and esterase activities. However, low aspartyl protease and very low phospholipase activities were detected which is dissimilar to the situation noted with *C. albicans*. We obtained variations depending on time and temperature; however, no significant differences were observed in relation to the origin of the isolates. Further investigations are needed to explain the increase of *C. tropicalis* infections and the role of enzymatic activities in the establishment and progression of the infection.

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