Original article

Phospholipase activity of *Candida albicans* isolated from vagina and urine samples

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Abstract

Introduction and objective: *Candida albicans* is the most virulent among the *Candida* species, and can cause several forms of candidiasis in human. Extracellular phospholipases in *C. albicans* is discussed as one of the virulence factors. The present study, focused on extracellular phospholipase activities in different isolates of *C. albicans* isolated from vagina and urine samples from Ahvaz, Iran. In addition, phospholipase activities were compared in *C. albicans* isolated from two different sources.

Materials and methods: In the present study, phospholipase activity of 100 isolates of *C. albicans* with urine and vaginitis origin was demonstrated using Sabouraud's dextrose agar supplemented with egg yolk.

Results: The phospholipase activity was detected in all tested isolates with a high level in Pz<0.70. In the present study phospholipase activity with higher Pz values was more common in vaginal isolates (84.7% isolates with Pz value <0.70) compared with 75% in urine isolates.

Conclusion: In the present study, 100% clinical isolates of *C. albicans* from vaginitis and urine samples demonstrated phospholipase activity.

Keywords: *Candida albicans*, Phospholipases activity, *Candida* vaginitis, Iran

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Introduction

Candida albicans is the most virulent among the *Candida* species, and can cause several forms of candidiasis in debilitated human. Several factors were described as virulent for pathogenesis of *C. albicans*. The secreted aspartyl proteinases, phospholipases, germ tube formation, adherence to host tissues and phenotypic switching have been more discussed in literatures [1-3]. Both hydrolytic enzymes can cause cellular membranes destruction. Four types of phospholipases have been reported in *C. albicans* including phospholipase A, B, C, and D. The extracellular phospholipases of *C. albicans* have a significant role in the pathogenesis of infections and invasion to mucosal epithelia [1,2]. In addition, several studies have shown that clinical isolates of *C. albicans* have higher levels of extracellular phospholipase activity [2].

Vidotto *et al.* [3] believe that the correlation between phospholipase activity and high germ tube formation can facilitate the mucosa penetration. Vulvovaginal candidosis is an opportunistic yeast infection that presents as a spectrum of very different clinical variants (e.g. acute candidosis, recurrent and chronic candidosis). Disease is caused by an overgrowth or infection of the vagina by a *Candida* species. However, *C. albicans* represent more than 80% of isolates from clinical infection [4]. Candiduria is a common nosocomial infection, which involves the urinary tract system as asymptomatic candiduria. The disease is most commonly caused by *C. albicans* [5].

Pinto *et al.* [6] compared the level of production of phospholipase in *C. albicans* isolated from the cases of infection and commensals. They observed the higher-level phospholipase activity in *C. albicans* with infection sources. In a study conducted by Farina *et al.* [7], 14.5% of vaginal strains of *C. albicans* were without phospholipase activity, whereas 40.6% of isolates have large production of phospholipase. Basu *et al*. [8] compared phospholipase activity of *C. albicans* isolated from vagina and urine samples. They found that 66.6% of vaginal isolates have phospholipase activity (Pz value) 0.82-0.86 and 60% urinary isolates have Pz value 0.84-0.89. Although there are several papers in *Candida* and candidiasis in Iran [4,5,9,10] there is little knowledge about their phospholipase activities.

The present study focused on extracellular phospholipase activities in different isolates of *C. albicans* isolated from vagina and urine samples from Ahvaz, Iran. In addition, phospholipase activities were compared in *C. albicans* isolated from two different sources.

Materials and methods

Isolates and identification

Seventy-two *C. albicans* strains isolated from patients with vulvovaginal candidiasis and 28 isolates from urine samples from hospitalized patients were studied. The strains were stored in medical mycology laboratory, Ahvaz Jundishapur University of medical sciences. All strains were transferred onto fresh Sabouraud's dextrose agar, SDA (Merck Germany) plates and incubated at 37°C for 24h. Then isolates were re-identified by germ tube test, production chlamydoconidia on Corn meal agar (Difco, USA) and green colour colonies on CHROMagar Candida (CHROMagar Candida, France) [2].

Suspension preparing

Each isolate was inoculated onto test tubes contained 10ml SDA and incubated at 37°C for 18h shaking. Each tube centrifuged for

30min and the sediment was washed by PBS for 30min. Supernant was removed and sediment was re-suspended in sterile distilled water. A suspension with turbidity according to the McFarland standard #2 of yeast cells was prepared in distilled water [11].

Phospholipase activity assays

Phospholipase activity assays were performed according to Price *et al*. [11]. The test medium contained 65g SDA, 58.4g NaCl and 5.5g $CaCl_2$. Medium was dissolved in 980ml distilled water and sterilized at 121°C for 12min. Egg yolk was centrifuged at 5000g for 30 minutes. The supernatant was removed and added to cooled medium (45-50°C) (2%), mixed and dispensed in plates. An aliquot (10µl) of the yeasts suspension was inoculated onto test medium which was then incubated at 37°C for four days. Colony diameter and colony diameter plus precipitation zone were measured for each isolate (Fig. 1). Calculation of the zone of phospholipase activity was performed according to Price *et al.* method [11].

$$\frac{\text{Colony diameter}}{\text{Colony diameter} + \text{Zone of precipitation}} = Pz$$

Five classes were described for phospholipase activity including; Pz value = 1 means that the test strain is negative for phospholipase, while a value of Pz <0.90-0.99 = weak phospholipase activity (+), 0.80-0.89 = poor phospholipase activity (++); 0.70-0.79= moderate phospholipase activity (+++) and <0.70 = large phospholipase activity (++++).

Results and discussion

The present study aimed at determining *in vitro* phospholipase activity in several isolates of *C. albicans* from urogenital samples. As shown in table one, all tested isolates (100 isolates) were found to be positive for the production of extracellular phospholipase. *C. albicans* is the common *Candida* species that is most frequently associated with the mucosal colonization, oropharyngeal, esophageal, vulvovaginal or urinary system candidiasis among debilitated patients [3,12]. Several studies show that phospholipase [2,8,13], proteinases [3,8], and germ tube production [3] have been implicated in the invasion and destruction of host tissue as pathogenic factors for *C. albicans*.

Fig. 1: Phospholipase activity of *C. albicans* in Sabouraud's dextrose agar supplemented with egg yolk

Table 1: Phospholipase activity (mm) exhibited by *C. albicans* isolated from vagina and urine samples

Pz Value		Vaginal samples (72)	Urine samples (28)
1	Negative	0 (0%)	0 (0%)
0.90-0.99	+	1 (1.39%)	0 (0%)
0.80-0.89	++	3 (4.18%)	0 (0%)
0.70-0.79	+++	7 (9.73%)	7 (25%)
<0.70	++++	61 (84.7%)	21 (75%)

Several studies have shown that phospholipase activity is observed only in *C. albicans* strains [6]. Pnito *et al*. [6] reported 99.4% of isolates of *C. albicans* with phospholipase activity. However, other researchers described that other *Candida* species such as *C. glabrata*, *C. guilliermondii*, *C. tropicalis*, *C. famata* and *C. inconspicua* secreted smaller amounts of phospholipase [7,14,15]. In the present study, 100% isolates of *C. albicans* had detectable phospholipase activity (Table 1). Basu *et al*. [8] documented that 48.7% clinical isolates of *C. albicans* and only two isolates from normal healthy people were positive for phospholipase activity. In another study conducted by Samaranayake *et al*. [2] 80% isolates of *C. albicans* recovered from HIV patients were phospholipase-positive. Reports from different countries show that there are more different phospholipase activities in different regions. Borst and Fluit [16] believed that virulence factors could be associated with geographical region and infection type.

As shown in table 1, 100% of urine and 94.43% of vaginalis isolates were produced the higher amount of phospholipase. In our study phospholipase activity with higher Pz values seems to be more commonly present in vaginal isolates (84.7% isolates with Pz value <0.70) compared with 75% in urine isolates. In the present study, we tested *C. albicans* isolated from vaginal candidiasis with acute, chronic or relapse disease. Probably variable levels of phospholipase activity in our study are related to source of *C. albicans*. Oksuz *et al*. [15] detected phospholipase activity in 53.08% of tested *C. albicans* that originated from healthy adults. In addition this activity was found more frequently in oral yeasts followed by fecal yeasts. Borst and Fluit [16] also found difference phospholipase activity between samples originated from urine, blood and wound.

Conclusion

There is no previous study of phospholipase activity of Iranian isolates of *Candida* species. In the present study, 100% clinical isolates of *C. albicans* from vaginitis and urine samples demonstrated phospholipase activity.

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