Endo and exocellular Phospholipase Activity Secreted By *Candida albicans* in vitro.

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Abstract

The enzymatic activity (exo and endo cellular) of *Candida albicans* was studies in vitro. The yeast was grown in SDA and the phospholipase activity was measured by plate method, and assayed by use lecithin extracted from egg yolk one of the phosholipid as substrate. The reaction products depend on fatty acid librated from lecithin degradation by enzyme.

الخلاصة

درست الفعالية الأنزيمية الداخل والخارج خلوية الخميرة *Candida albicans* مختبرياً، حيـث تـم تنمية الخميرة في وسط زرعي SDA وقيست فعالية أنزيم الفوسفولايبيز باستخدام طريقة الصفيحة، بعدها تـم فحص الانزيم باستخدام اللسثين المستخرج من صفار البيض كأحد الدهون المفسفرة والذي يعد كمـادة اسـاس (Substrate). يعتمد ناتج التفاعل يعتمد على الأحماض الدهنية الناتجة من تحلـل اللـسثين بواسـطة انـزيم الفوسفولايبيز.

Introduction

The production of extracellular phospholipase has been shown to be important in the pathogenesis of several microorganism such as bacteria, yeasts and fungi[1]. Increased phospholipase activity has also been correlated to increased mucosal pathogenicity in the opportunistic yeast *Candida albicans* [2]. Recently, blood isolated of *Candida albicans* have been shown to produce increased extracellular phospholipases compared with mucosal isolates [3]. Exogenous phospholipase has been shown to cause transient permeability in mammalian cells sufficient to allow entry of the protein toxin alpha-sarcin[4].

Phospholipases are a heterogeneous group of enzymes that are able to hydrolyze one or more ester linkages in glycerophospholipids. The actions of phospholipases can result in the destabilization of membranes cell lysis and release of lipid second messengers [5]. These enzymes are categorized according to the specificity of the ester link that is cleaved.

The nomenclature is confusing and the appropriateness of the use of the term phospholipase B is controversial. Strictly speaking phospholipase B refers to an enzyme that can remove either sn-

1 or sn-2 fatty acids from glycerophospholipid. However, Microorganisms can have a single enzyme that not only has the hydrolase (fatty acid release) activities of phospholipase [6]. The finding of a single anzyme having these multiple and seemingly paradoxical functions can be confusing. It is known that a single gene product has all three activities (phospholipase B, Lysophospholipase and Lysophospholipase transacylase in *Candida albicans* [7]. There is substantial evidence supporting the role of extra cellular phospholipase as a virulence factor in experimental of some Microorganisms.

The overall incidence of *Candida albicans* infections has increased significantly in the last two decades [8]. Raging from 75% increase in small hospitals to an over 400% increase in some large tertiary-care centers. This increase led to a tremendous interest in the study of Candidal pathogenesis and strategies for control and prevention of this clinically important fungus-Candidal virulence factors have also attracted interest as a possible means for developing novel therapeutic interventions against candidiasis [9]. Such virulence factors include adherence, germination, extracellular proteinases [10] and phospholipase.

The secretion of extracellular proteinases by *Candida albicans* was first reported in the 1960_s and Werner [11] by growing the yeast on solid media containing egg yolk or lecithin and analyzing the lipid breakdown products. Later, *C. albicans* strains by using media containing blood serum and sheep erythrocytes [12].

The egg ylolk contains substrates for both phospholipase (phospholipids) and Lipases (Triglycerides). The egg yolk based assay is not specific and therefore its use should be limited to initial screens only [13]. Furthermore, the assay is not levels of phospholipase-conformation of phospholipase activity necessitates the use of specific radiometric or colorimetric assay and the use of concentrated culture filtarate, particulary in poorly phospholipase-producing strains [14].

Materials and Methods

Determination of phospholipase production was performed essentially according to Price et al [15] using the egg-yolk plate method, the inoculated plates were incubated at 37°c . After 6 days of incubation, the dimeter of the colony (a) and that of the colony plus precipitation zine (b) were measured. An isolate of *Candida albicans*, obtained from the Department of Biology, college of science, Thi-Qar University, this isolate was isolated from patients with candidiasis, cells grown on sabouraud glucose (2% w:v) agar were used as the inoculum and were grown in modified Sabouraud broth (medium A; 30 gm glucose, 10 gm polypeptone and 10 gm yeast extract (w:v) in 1L distilled water) at 37°c with shaking, at an initial concentration of 1.5×10^5 cells ml^{-1}. The *C. albicans* cells was inoculated into 200 ml of medium A and grown at 37°c for 10h with shaking. After removing cells at the stationary phase by centerfugation at 1500g for 10 min, the supernatant was collected by filtration (0.45 um pore diameter filter) and concentrated to approximately 10 ml by Freez dryer apparatus (Edwards pirani 50L 283-England).

Enzyme assay:

Phospholipase activity was assayed using lecithin from egg yolk as substrate. The assay of enzyme activity of was based on the rate of production of fatty acids from lecithin, The reaction mixture (2 ml) contained 0.1 M citrate buffer (pH 6) and 1 ml of lecithin solution and enzyme solution (0.1 ml). The reaction was carried out at 37°c for 30 min and was stopped by adding 2 ml of chloroform methanol solution (1:2 v:v). The reaction products were extracted by the method of Bligh & Dyer [16] and evaporated, the residue was redissolved in an appropriate volume of chloroform-methanol (6:1 v:v). An aliquot of the lipid extract was applied onto a silica gel thin-layer plate, which was the developed with chloroform-methanol-water (65:25:4 v:v:v). fatty acids were identified by comparisim with authentic standard fatty acids, using TLC technic.

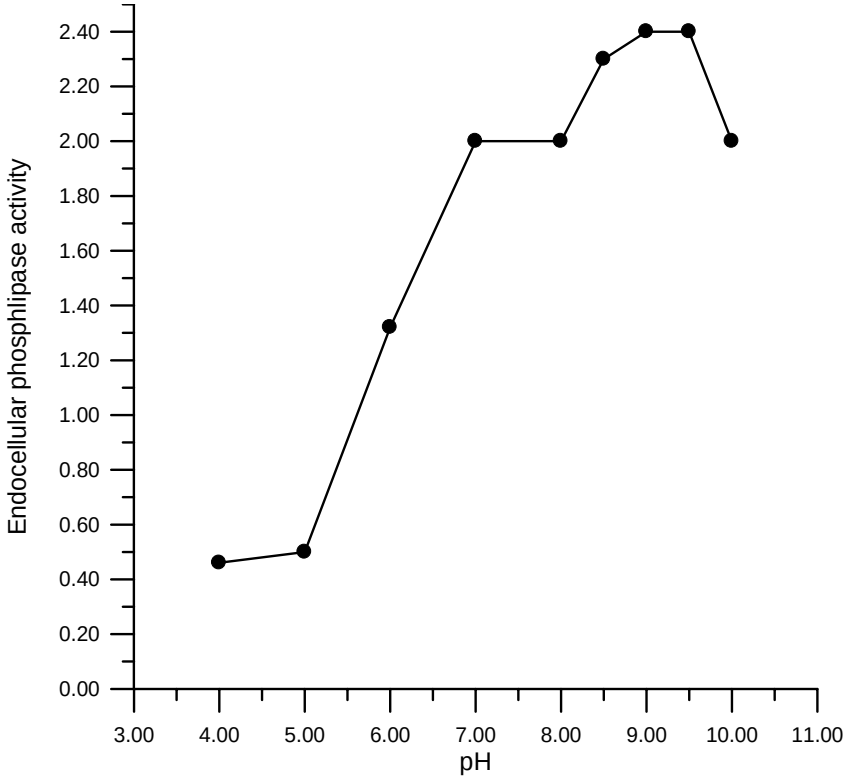
Results and Discussion

The enzyme activity was expressed by of Pz=a/b=0.652 a low Pz value means production of phospholipase in solid media.

The cell free extract and culture filtarates of *Candida albicans* in saboaraud's glucose-pepton broth contained phospholipase activity. The pH activity curves (measured with lecithin as substrate (Fig1,2) showed that both endo and exo-cellular enzyme activities did not have sharp pH optima. This profile suggests the probability of existence of more than one phospholipase enzyme within the cells and in the exocellular medium of this organism, this result is agree with Samaranayake et al [7]. A measurable amount of lecithin substracte was hydrolysed between pH 5 and 10. The pH activity curves for endo and exocellular phospholipase were not paralled throughout the pH range, both endo and exocellular enzyme activities were conspicuously higher at alkaline pH values than those at pH below 7. The content mycelium or culture fluid increased with increase in the age of the *Candida albicans* (Fig 3, 4, 5), the growth and phospholipase activity versus culture age curves were parallel. The synthesis of phosphorlipase enzymes may be stimulated by partial depletion of fatty acids in the growth medium.

Yan et al (2004), [18] explain that exocelluiar enzyme in rat, this enzyme (PLD) is a wide variety of neuronal and glial cells can be stimulated in response to various signals and isoform of this enzyme possess different regulatory properties and undertake different cell biological roles. Phospholipase has also been shown to participate in exo cytosis and regulated secretory processes in epithelial cells and mast cells [19].

Banno et al (1985), [20] shows that the yeast form of *Candida albicans* secrets three kinds of phospholipase into the culture filtarate (Lysophospholipase transacylase and phospholipase B, by comparison, all phospholipase activities in mycelial from cells were extremely low as compared to those of yeast form cells-It remains to be proven whether these different activities relate to the different culture media and growth periods used to obtain the two morphological forms or wether they reflect physiological forms or wether they reflect physiological differences between the forms. Barrett-Bee et al [2] was the first to evaluate the role of extracelluar candidal phospholipasees in virulence by using a murine model of candidiasis.



Fig(1): pH-activity profiles of crude endo cellular phospholipase of *Candida albicans.*

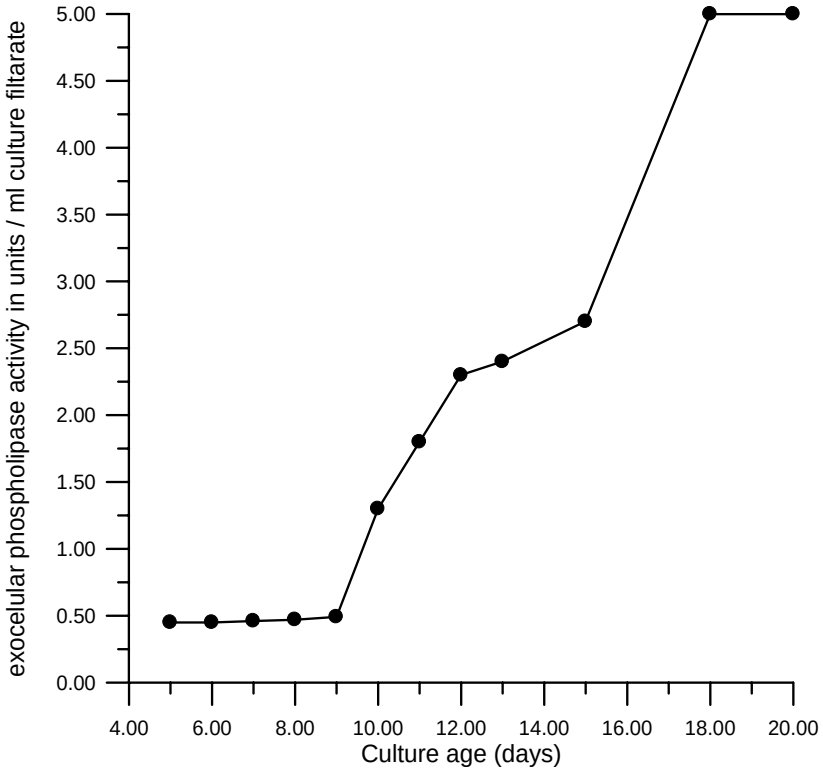


Fig (2): pH-activity profiles of crude exocoellular phosoholipase of *Candida albicans*

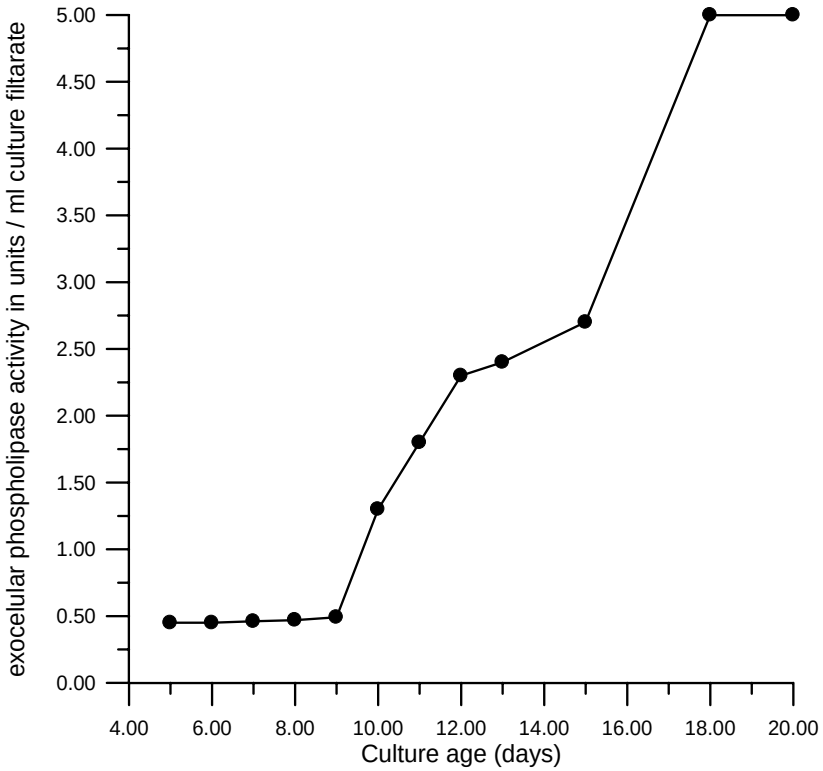


Fig (3): Exocellular phospholipase activity of *candida albicans* at different culture.

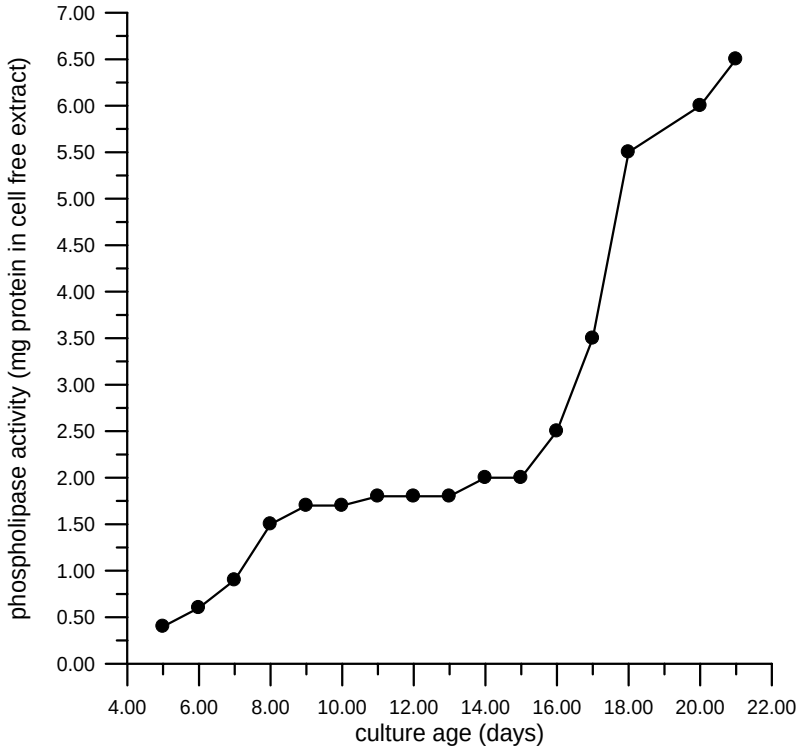


Fig (4): Exocellular phospholipase activity of *candida albicans* at different culture age.

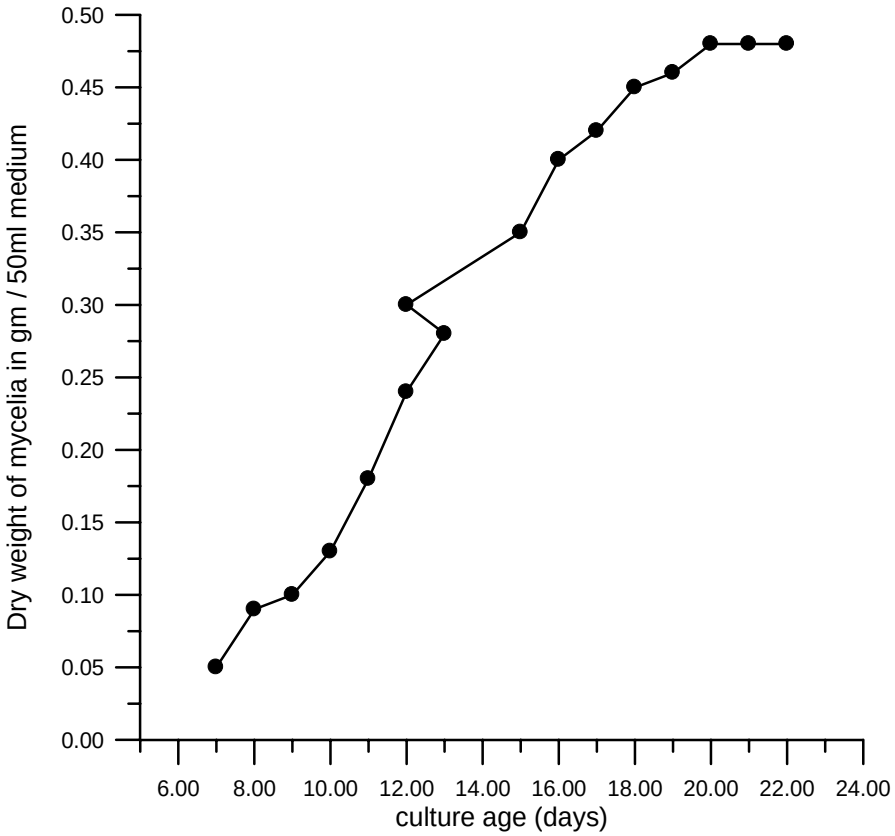


Fig (5): growth of *Candida albicans* mycelium (dry weight of cell).

Reference

1- Titball, R. W., S. E. C. Hunter, K. L. Martin, B. C. Morris, A. D. Shuttle-Worth, T. Rubidge, D. W. Anderson, and D. C. Kelly., ***Infec. Immun***., 1989, **57**, 367.

2-Barrett-Bee, K., Y. Hayes, R. G. Wilson, and J. F. Ryley., ***J. Gen. Microbil***., 1985, **131**, 1217.

3-Ibrahim, A. S., F. Mirbod, S. G. Filler, Y. Banno, G. T. Cole, Y. Kitajima, J. E. Edwards, Y. Nozawa, and M. A. Ghannoum., ***Infect. Immun***., 1995, **63**, 1993.

4- Otero, M. J., and L. Carrasco., ***Exp. Cell Res***., 1988, **177**, 154.

5- Ghannum, M. A., ***Clin. Micro Rev.***, 2000, **13**,122.

6- Chen, S. C., Muller, M., Zhou, J. Z., Wright, L. C. and Sorrell, T. C., ***J. Infec Dis***., 1997, **175**, 414.

7- M. Takahashi, Y. Banno and Y. Nozawa., 1991. Secreted *Candida albicans* phospholipases: Purification and characterization of two forms of lysophospholipase-transacylase.

8- Banerjee, S. N., T. G. Emori, D. H. Culver, R. P. Gaynes, W. R. Jarvis, T. Horan, J. E. Edwards, J. Tolson, 1995. Secular trends in nosccomical primary bloodstream infections in the united states, 1980-1989. P. 3-8. In J. Rex and M. D. Anderson, Serious *Candida* infections : risk factors, treatment, and prevention.

9- Perfect, J. R. ,A***gents chemothor***, 1996, **40**, 1577.

10- Hube, B., ***Rev. Iberoam-Mical,*** 1998, **15**, 65.

11- Werner, H. , ***Hyg. I Abt orig. A***., 1966, **200**,113.

12- Costa, A., C. Costa, A. Misefari, and A. of certain fungi. VII. Phosphatidase activity on media containing sheep's blood of pathogenic strains of *Candida albicans*, ***Atti Soc. Sci.*** Fis. Mat. Nat. **XIV** ,93.
13- Fu, Y., A. Ibrahim, F. Fonzi, X. Zhou, C. Ramos and M. Ghannoum, ***Microbiology***, 1997, **143**, 340.
14- Mirbod, F., Y. Banno, M. A. Ghannoum, A. S. Ibrahim, S. Nakashima, Y. Kitajima, G. T. Cole, and Y. Nozawa, ***Biochem, Biophys. Acta*** , 1995, **1257**, 181.
15- Price MF, Wilkinson ID, Gentry Lo-Plate method for detection of phospholipase activity in *Candida albicans*. ***Sabouraudia***, 1982, **20**, 7.
16- Bligh, E. C. & Dyer, W. J., ***Canadian Journal of Biochemistry and physiology***, 1959, **37**, 911.
17- Samaranayake, L. P., J. M. Rawsida, and T. W. Macfaralane, ***Sabouraudia, 1984,*** **22**, 201[Medline].
18- Yan Zhang, Ping Huang, Guangwei Du, Yansunori Kanaho, Michael A. Frohman and Stella E. Tsirka, 2004, In creased Sxpression of two phosphlipase D isoforms During experimentally Induced Hippocampal Mossy Fiber outgrowth GLIA **46**, 74.
19- Cho:, Ws, Kim Ym, Combs C. Frohman, MA, Beaven MA. , ***J. Imununol***, 2002, **168**, 5682.
20- Y. Banno, T. Yamada and Y. Nozawa., ***Sabouraudia***, 1985, **23**, 47.