Comparism between phospholipase activity in (*Candida albicans)* by use two substrates (lecithin, L-α-phosphatidyl inositol)

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Abstract

Comparative study on the activity of phospholipase enzyme which is produced by yeast *candida albicans* into different culture media that contain phospholipids as substrates. The agar containing substrate (L-α-phosphatidyl inositol) was used .

The agar containing lecithin as other substrate for comparision was also used .

The second culture media was found the best for enzyme activity which was measured by precipitation zone .

Introduction

Secretory phospholipase, well- known for their destructive effects on biological tissue , have been implicated in the pathogenesis of micro organism infections,(1). Studies of fungal phospholipases, however, have mainly focused on the characterization of phospholipase , of the medically important fungi, extra cellular phospholipase activities have been described in *Candida albicans* and *Aspergillus fumigates* but characterized only in the former (2,3). In *Candida albicans Lysophospholipase* ,(LPL) and Lysophspholipase transacylase (LPTA) and phospholipase B (PLB)activities were identified in crude culture

filtrates of the organism, but only enzymes with LPTA activity were purified (4)

Candida albicanse is a commonly isolated human opportunistic pathogen capable of causing both superficial and systemic candidoses (5) . Its ability to produce phospholipase is considered to be an important pathogenic feature .(6) There have been suggestions that the ability of *Candida albicans* to produce cytolytic enzymes such as proteinases (7) and phospholipase may be associated with the pathogenicity of this fungus (8). The presence of phospholipase activity in *Candida albicans* was first detected by growing the fungus on media containing egg yolk and lecithin. The quantitative detection of phospholipase activity in *Candida albicans* by plate assay was described by (9), Extra cellular enzymatic activity in *Candida aibicans* has been well known for along time with great number of published reports(10).

Materials and Methods

Detection of phospholipase by use TY agar (1% peptone, 0.5% yeast extract, 1% Nacl,1% agar) and incubated at 37°C for 24 to 48h. There after, the agar plates (9-cm diameter) were overlayed with L-α-phosphatidylionsitol substrate in agarose and incubated at different temperature. The plates were observed for turbid halos around colonies for up to 6 days of incubation.

(The L-α-phosphatidylinositol substrate is prepared As follows:- Add L-α-phosphatidylinositol to 20mM Tris-Hcl buffer, pH 7.0 at a concentration of 20 mg/ml. The resulting turbid solution is Subjected to ultrasonicator, with 15-5 cooling intervals).prewarm the optained solution to 55 °C in a waterbath .Dissolve agarose in 20mM Tris-Hcl buffer pH 7.0 ,to a concentration of 1.4% , and cool to 55ml , especially if plates are incubated for several days, Mix the α-phosphatidylinositol solution and the agarose solution at equal volumes),(9).

phospholiase production by use egg yolk plates (Containe lecithin as substrate)

Determination of phospholipase production was performed essentially according to (11) using the egg-yolk plate method of (9).

The inoculated egg-yolk plates were incubated at different temperature .After 6 days of incubation ,the diameter of the colony (a) and that of the colony plus precipitation zone (b) were measured . The phospholipase activity was expressed by Pz=a/b. Thus ,a high Pz value means low production of phospholipase. The average Pz value was obtained .

Results and Discussion

An assay consisting simply of overlaying TY agar plates with L-α- phasphatidylinositol was used to detect the precence of phasopholipase activity in *Candida albicans.* The table No. (1) explains the activity of phospholipase enzyme
in the solid media (TY agar) at different temperatures. The maximum activity of the enzyme is at 30 temperature which is considered as the optimum to the enzyme in the solid media (TY). This agrees with what Serve and others have achieved (13) in a study of *Lesteria*. As for table no. (2) it explains the activity of the enzyme in the solid media which contains lecithin as substrate . The enzyme activity showed the maximum at temperature 37°C . This is completed different from the first state. (Tablet no.1). The difference is due to substrate. Rosmary *et al* (1990) Showed that lecithin in one of more inciting materials of the enzyme activity .In addition to that it is more usable in most researches because it is available . We mention that the reason behind the increase of lecithin activity is the chemical structure if this substrate makes the enzyme more active compared with the inositol which has cyclic structure which may cause steric effect and make the enzyme low activitiy.

In table No (3) notict enzyme activity in creased parallel in the first ten days of the incubation period that the activity of the sixth days and after that begane to decrease indivigually up to the tenth day with the constant of the temperature (30°C) that equal to optima temperature as we are given the example in table No (1) above by use (L-α- phosphatidyl inositol) as substrate.

If we are compared the same mothod by using (Lecithin) in that we notict that the activity of enzyme was at the top. In the table No (4) at the temperature 37°C , if we tested the enzyme activity for the ten days at constant temperature that we notict oviuasly in the seven day the enzyme activity has the maximum activity were the value of P_Z was the best when

we are using (lecithin) compared with the same terms and condition that we used at (L-α- phosphatidyl inositol) as the main substrance .

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Table (1):Activity of phospholipase enzyme(p_z value) in Cultures media (TY agar) at different temperatures.

Temp.	P_z value
20°C	0.973
25°C	0.836
30°C	0.517
37°C	0.622
40°C	0.700

Table(2):Activity of phospholipase, enzyme(p_z value) in Cultures media by use lecithin at different temperatures.

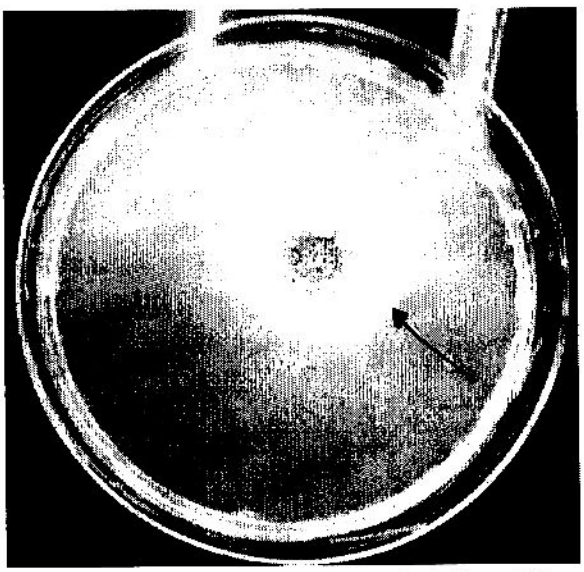
Temp.	Pz Value
20°C	0.811
25°C	0.762
30°C	0.666
37°C	0.500
40°C	0.590

Table (3):Phospholipase activity of diffrernt times in 30°C by use L-α- phophatidyl inositol as substrate.

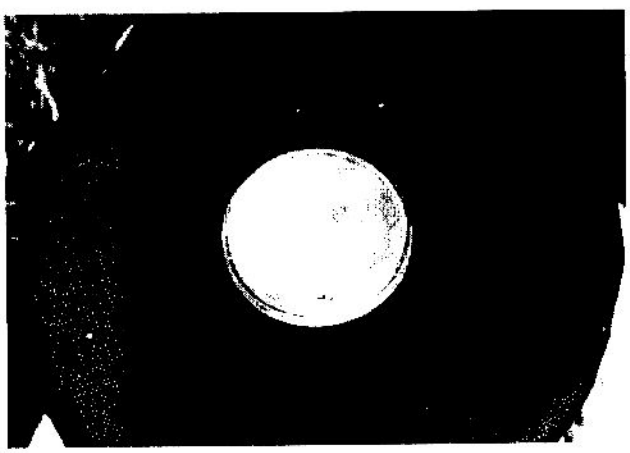
Days.	Pz Value
1	0.822
2	0.800
3	0.776
4	0.698
5	0.600
6	0.658
7	0.688
8	0.690
9	0.790
10	0.711

Table(4): Phospholipase activity (P_Z value) at different times in 37°C by use lecithin as substrate.

Days	Pz Value
1	0.788
2	0.712
3	0.621
4	0.698
5	0.577
6	0.501
7	0.432
8	0.489
9	0.400
10	0.453



Picture (1) :phospholipase activity (p_z value) in solid media by use lecithin as substrate the halos around the colonies that means enzyme.



Picture (2):phospholipase activity (p_z value) in solid media by use (L - α – phosphatidyl inositol) as substrate.

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مقارنة لفعالية إنزيم الفسفولايبيز الذي تفرزه الخميرة (*Candida albicans*) باستخدام وسطين زرعيين lecithin و L-α-phosphatidyl inositol

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الخلاصة

أجريت دراسة مقارنة لفعالية إنزيم الفسفولايبيز الذي تفرزه الخميرة (*Candida albicans*) في وسطين زرعيين مختلفين يحتويان على الدهون المفسفرة موادا" أساسية، اذ استعمل الاكار المحتوي على المادة (L- α - phosphatidyl inositol) وكذلك استعمال الاكار المحتوي على اللسثين (lecithin) مادة" أساسية أخرى للمقارنة اذ وجد أن الوسط الزرعي الثاني هو الأفضل لفاعلية الإنزيم التي قيست بوساطة الزيادة في قطر منطقة الترسيب (precipitiation zone)