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Influence of Cultural Conditions on Lipase Production in *Candida albicans*

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Abstract: We studied the Lipase (LP) an enzyme which hydrolyses the ester bonds of triacylglycerols to yield glycerol and fatty acids in an opportunistic yeast human pathogen, *Candida albicans* (CA). The yeast was grown in Sabouraud dextrose broth and Lee synthetic medium at 25°C on a rotary shaker (100 rpm). At 24, 48 and 72 h of inoculation, cells were separated from the media and the intracellular and extracellular LP were measured from cell free homogenate and culture media, respectively. Lipase activity was determined by the rate of hydrolysis of olive oil emulsion by potentiometric titration. The influence of various factors such as growth, pH, temperature and media on the production of extra and intracellular Lipases (LP) has been studied. All the experiments were conducted at least twice and the analyses were carried out in triplicates. *Candida albicans* produced both extra and intracellular lipases in both the tested media. Although, LP was produced throughout the growth phase, maximum enzyme activity was detected at 24 h of growth. Optimum pH and temperature for the LP activity were 7 and 37°C, respectively.

Key words: *Candida albicans*, opportunistic pathogen, lipase synthesis and activity, yeast cultural conditions, dimorphic yeast

INTRODUCTION

Candida albicans, a dimorphic fungus, is an important opportunistic pathogenic species of *Candida* causes oral thrush, esophageal infection and disseminated candidiasis. It has been realized that the phenotypic divergence of *C. albicans*, which is controlled by a host of factors, is associated with a successful infection and the evasion of anti-Candida drugs. This organism is capable of growing in either of two forms, the budding yeast form or the elongating hyphal form. While both morphological forms bud and hypha are found at the site of infection, the hyphal form is believed to have evolved primarily as the mechanism of tissue penetration (Prasad, 1991; Whiteway and Bachewich, 2007).

Lipases (glycerol ester hydrolases EC 3.1.1.3.) comprise a group of enzymes which catalyze the hydrolysis of triacylglycerols to give free fatty acids, diacylglycerols, monoacylglycerols and glycerol. Earlier studies have shown the correlation between lipolytic activity and pathogenesis in bacteria (Rollof *et al*., 1987; Cutler, 1991; Gácser *et al*., 2007). Although, its precise role in disease is poorly understood, lipase appears to contribute to the localization of infection. Occurrence of extracellular enzymes has been studied extensively in dermatophytes and other pathogenic fungi (Mushin *et al*., 1997; Whiteway and Bachewich, 2007). These enzymes probably play an important role in the pathogenesis of infections caused by these fungi (Okeke and Gugnani, 1989; Ibrahim *et al*., 1995; Chen *et al*., 1997; Kurnatowska, 1998; Whiteway and Bachewich, 2007). The study of lipase is also useful in the diagnosis of various diseases e.g. Pancreatitis (Chen *et al*., 1997).

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Lipase is naturally produced in many different organisms; however, the rate of production can be increased with the aid of inducers such as olive oil, complex nitrogen sources (Nesbit and Gunasekaran, 1993), soybean meal and calcium carbonate. Lipase activity varies widely depending upon the source and type of substrate (Vakhlu and Kour, 2006). The purpose of this investigation is to study the influence of cultural conditions and properties of the extra and intracellular lipases from *C. albicans*, opportunistic dimorphic yeast causes different types of candidiasis in humans.

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from Sigma Chemical Co., St Louis, MO. All solvents for analysis (HPLC grade) were bought from Fisher Scientific, Atlanta, GA.

Organism and Cultural Conditions

Stock cultures of *C. albicans* 3153A was maintained on Sabouraud Dextrose Agar (SDA) at 37°C. Inoculum was prepared by adding sterile distilled water aseptically to the cultures grown on SDA plates for 72 h. The cell suspension was adjusted to approximately to 10^7 mL^{-1} and 1 mL of inoculum was added in each flask containing 50 mL of Lee's synthetic media (Lee *et al*., 1975). The cultures were incubated for 72 h at 25°C on a rotary shaker (100 rpm).

Preparation of Intracellular Crude Extract

Cells were harvested from the media by centrifugation (7,000 rpm for 15 min) at 4°C, washed twice with 0.1 mM Tris-HCl buffer (pH 7.0) and centrifuged at 13,000 rpm for 30 min. Harvested cells were weighed to determine cell growth. The cells were ground with acid wash sand in pre-chilled mortar using 1.5 mL of Tris-HCl buffer for each gram of cell. The cell free homogenate was centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was used as the source of intracellular enzyme.

Preparation of Extracellular Crude Extract

The culture filtrate was concentrated by dialysis to 5-10 mL (more than 5-10 fold) at 4°C and used as the extracellular enzyme.

Lipase Assay

Two milliliters of the polyvinyl alcohol-emulsified substrate (PVA-emulsified substrate), 1 mL of 0.1 mM Tris-HC1 buffer (pH 7.4) and 1 mL of enzyme preparation were combined and incubated for 4 h at 37°C on a rotary shaker (100 rpm). After incubation, 6 mL of 1:1 v/v alcohol-acetone were added to the mixture to stop the reaction. Two drops of phenolphthalein indicator were added and the mixture was titrated against 0.05 N NaOH to determine the lipase activity (Nesbit and Gunasekaran, 1993).

Protein Determination

Protein content of the extracts was determined using the Coomassie Brilliant Blue method of Bradford (1976) with bovine serum albumin as standard. Absorbance was measured at 595 nm.

RESULTS AND DISCUSSION

It has been generally accepted that the production of microbial enzymes is remarkably affected by the conditions of cultivation. Lipolytic activity in *C. albicans* was assayed both in whole cells and in the culture supernatant. However, for the majority of the assays this enzymatic activity in whole cells was very low.

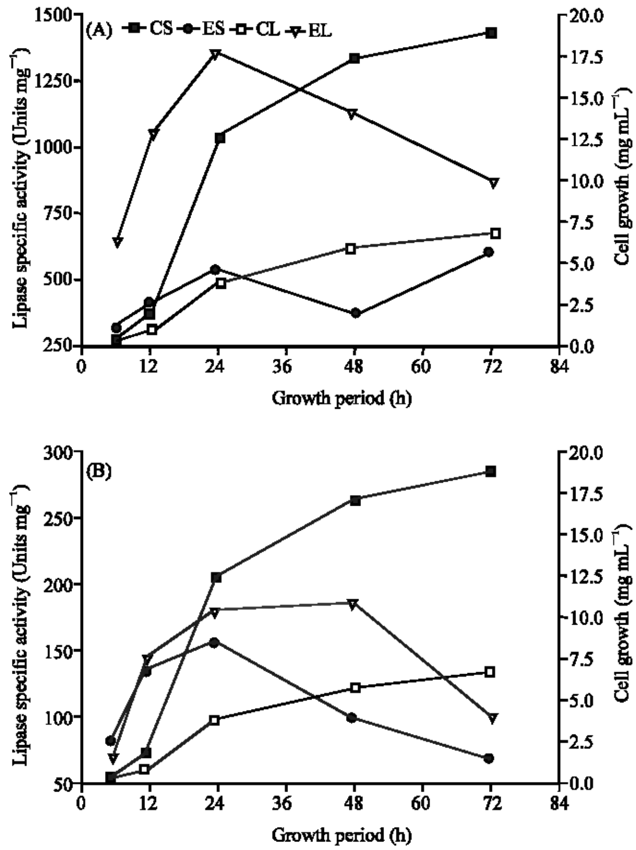

Fig. 1: Effect of growth on (A) extracellular (E) and (B) intracellular (I) lipase activities in yeast cells of *Candida albicans* grown in SDB (CS) or Lee's synthetic media (CL) at 25°C. In Fig. 1A, ES and EL represent the extracellular lipase activities of cells grown in SDB and Lee's media, respectively. In Fig. B, IS and IL represent the intracellular lipase activities of cells grown in SDB and Lee's media, respectively

Kinetics of Growth on Protease Activity of *C. albicans*

Candida albicans, cultures were grown in Sabouraud Dextrose Broth (SDB) and Lee media and kinetics of growth and lipase production was measured. The organism grew slowly and reached the maximum at 72 h of growth in both SDB and Lee media (Fig. 1A, B). However, the amount of growth was more in SDB than in Lee media at all growth periods. *C. albicans* produced both extracellular and intracellular lipases and these activities increased at the early stages of growth and reached a maximum at 24 h of growth regardless of the media (Fig. 1A, B). In Lee media, both intra and extracellular lipase activities were higher than the SDB media. After 24 h, the enzyme activity gradually declined. Higher activity at the earlier growth stages suggested that these enzymes may play a significant role in the establishment of pathogenesis of *C. albicans* (Cutler, 1991; Kurnatowska, 1998).

Influence of pH and Temperature on Lipase Activity of *C. albicans*

To study the influence of pH, buffers of different pH ranging from 4-10 were used in lipase assay. The maximum lipase activity was observed at pH 7 (Fig. 2). Although, the extracellular enzymes from both the media had the same optimal pH, the enzyme activity was higher in Lee media than in SDB.

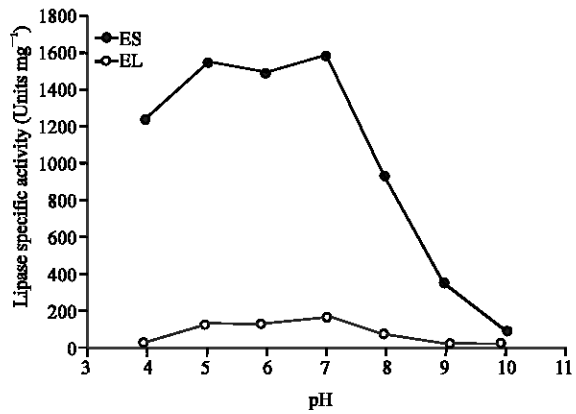

Fig. 2: Effect of pH on extracellular lipase activities in *Candida albicans* grown SDB and Lee's media. (ES) and (EL) represent the extracellular lipase activities of cells grown in SDB and Lee's media, respectively

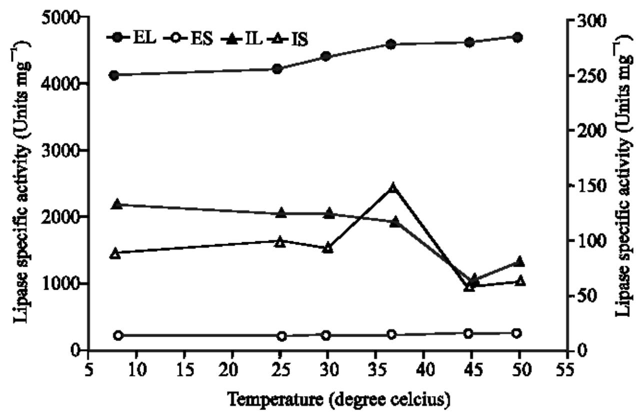

Fig. 3: Effect of temperature on intracellular and extracellular lipase activities in *Candida albicans* grown in SDB and Lee's media at 25°C. (ES) and (EL) represent the extracellular lipase activities of cells grown in SDB and Lee's media, respectively. (IS) and (IL) represent the intracellular lipase activities from cells grown in SDB and Lee's media, respectively

The enzyme was most active at pH 7.0, but retained over 75% of its activity at pH 4.0 (Fig. 2). Above pH 7.0 the activity dropped sharply. The enzyme extract was pretreated at various temperatures ranging from 8-50°C for 20 min and used in lipase assay. The enzyme was active in all tested temperatures. Intracellular enzyme from the SDB cells had a higher activity than the cells grown in Lee media regardless of the temperature (Fig. 3).

Extracellular lipase from Lee media had maximum activity between 30-50°C and also had higher activity as compared to the SDB media. These results suggest that the lipase of *C. albicans* is thermostable. Similar thermostable lipases were reported from other fungi (Dharmsthiti and Ammaranond, 1997; Mushin *et al*., 1997; Costa and Peralta, 1999; Sharma *et al*., 2001).

Among the several diagnostic tools that have been used to detect diseases, one significant diagnostic feature is the use of lipase to detect infection (Cutler, 1991; Rollof *et al*., 1987). The specific role of lipase in the disease is not completely understood. An attempt is being made to investigate the effects of various environmental factors that affect growth and lipase production in this organism in order to understand the basic mechanism of pathogenesis. The most obvious function of lipases is the hydrolysis of lipids in order to use fatty acids and/or glycerol as substrates. *C. albicans* is able to grow in different rates in synthetic and complex media containing lipids as the sole source of carbon (Nesbit and Gunasekaran, 1993). The fungus is able to hydrolyze the lipids and to transport the hydrolytic products into the cell. Possible lipid substrates may be found on human tissue such as the skin or the intestinal tract. The release of fatty acids may also have additional advantages for the fungus. In addition the release of fatty acids may change the pH in the micro environment of the cells. An active alteration of the pH in the micro environment to allow the production and activity of extracellular pretenses has been shown for the entomopathogenic fungus *Metarhizium anisopliae* (St Leger *et al*., 1999). In addition, the release of fatty acids may enhance the adherence of *C. albicans* to host surfaces, as shown for *P. acnes* (Gribbon *et al*., 1993). Lipases may also develop lipolytic activities towards phospholipids. Such non-specific hydrolysis of phospholipids may damage host cell membranes, as previously shown for phospholipase B activities (Ibrahim *et al*., 1995). In addition, *C. albicans* lipases, with or without the synergistic action of other phospholipases, may affect the host immune system by inhibiting cell-mediated chemotaxis or by damaging phagocytotic cells directly (Stehr *et al*., 2003).

CONCLUSION

In conclusion, the growth and lipase production in *C. albicans* were found to be influenced by various nutritional and environmental factors such as culture media, pH, temperature and growth periods. Maximal growth was observed at 72 h, whereas maximum extracellular and intracellular lipase activities were observed at 24 h after inoculation regardless of the media. Optimum temperature for both extra and intracellular enzymes was found to be 37°C.

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REFERENCES

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Chen, S.C., M. Muller, J. Zhou, Z. Wright, L.C. Wright and T.C. Sorrell, 1997. Phospholipase activity in *Cryptococcus neoformans*: A new virulence factor? J. Infect Dis., 175: 414-420.

Costa, M.A. and R.M. Peralta, 1999. Production of lipase by soil fungi and partial characterization of lipase from a selected strain (*Penicillium wortmanii*). J. Basic Microbiol., 39: 11-15.

Cutler, J.E., 1991. Putative virulence factors of *Candida albicans*. Ann. Rev. Microbiol., 45: 187-218.

Dharmsthiti, S. and P. Ammaranond, 1997. Purification and characterization of lipase from a raw-milk yeast (*Trichosporon asteroids*). Biotechnol. Applied Biochem., 26: 111-116.

Gácser, A., F. Stehr, C. Kröger, L. Kredics, W. Schäfer and J.D. Nosanchuk, 2007. Lipase 8 affects the pathogenesis of *Candida albicans*. Infect. Immun., 75: 4710-4718.

Gribbon, E.M., W.J. Cunliffe and K.T. Holland, 1993. Interaction of *Propionibacterium acnes* with skin lipids *in vitro*. J. Gen. Microbiol., 139: 1745-1751.

Ibrahim, A.S., F. Mirbod, S.G. Filler, Y. Banno and G.T. Gole *et al*., 1995. Evidence implicating phospholipase as a virulence factor of *Candida albicans*. Infect. Immunol., 63: 1993-1998.

Kurnatowska, A.J., 1998. Activity of hydrolytic enzymes of *Candida albicans* strains isolated from patients with periodontal and membrane mucosae of oral cavity diseases. Mycopathologia, 141: 105-109.

Lee, K.L., H.R. Buckley and C.C. Campbell, 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia, 13: 148-153.

Mushin, T.M., A.H. Aubaid and A.H. Al-Duboon, 1997. Extracellular enzyme activities of dermatophytes and yeast isolates on solid media. Mycoses, 40: 465-469.

Nesbit, E.A. and M. Gunasekaran, 1993. Influence of cultural conditions on growth and lipolytic activity in *Nocardia asteriodes*. Folia Microbiol., 38: 451-455.

Okeke, C.N. and H. Gugnani, 1989. Lipases of *Fonsecaea pedrosoi* and *Phialophora verrucosa*. Antonie Van Leeuwenhoek, 55: 313-324.

Prasad, R., 1991. Candida Albicans: Cellular and Molecular Biology. 1st Edn., Springer-Verlag, New York.

Rollof, J., S.A. Hedstrom and P. Nilsson-Ehle, 1987. Lipolytic activity of *Staphylococcus aureus* strains from disseminated and localized infections. Acta Pathol. Microbiol. Immunol. Scand. Sec. B, 95: 109-113.

Sharma, R., Y. Chisti and U.C. Banerjee, 2001. Production, purification, characterization and applications of lipases. Biotechnol. Adv., 19: 627-662.

St Leger, R.J., J.O. Nelson and S.E. Screen, 1999. The entomopathogenic fungus *Metarhizium anisopliae* alters ambient pH, allowing extracellular protease production and activity. Microbiology, 145: 2691-2699.

Stehr, F., M. Kretschmar, C. Kröger, B. Hube and W. Schäfer, 2003. Microbial lipases as virulence factors. J. Mol. Catalysis. B, Enzymatic, 22: 347-355.

Vakhlu, J. and A. Kour, 2006. Yeast lipases: Enzyme purification, biochemical properties and gene cloning. Elect. J. Biotechnol.

Whiteway, M. and C. Bachewich, 2007. Morphogenesis in *Candida albicans*. Ann. Rev. Microbiol., 61: 529-553.