INFECTION AND IMMUNITY, Aug. 1996, p. 2936–2940 Vol. 64, No. 8
0019-9567/96/$04.00+0

Induction of an Extracellular Esterase from *Candida albicans* and Some of Its Properties

RYOJI TSUBOI,* HISANO KOMATSUZAKI, AND HIDEOKI OGAWA

Department of Dermatology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo 113, Japan

Received 20 February 1996/Returned for modification 21 March 1996/Accepted 2 May 1996

An extracellular esterase from *Candida albicans* A-714 was found to be induced in a medium containing 0.7% yeast nitrogen base and 2.5% Tween 80 (polyoxyethylenesorbitan compounds). Enzyme activity, which exists predominantly in the extracellular space, was measured by a colorimetric method using α-naphthyl palmitate as a substrate. The induction level of the esterase activity was found to be well correlated with fungal growth and was dependent on the Tween 80 concentration. Such esterase activity was observed only in medium containing Tween 80 or other Tweens as the sole carbon source and therefore was not observed in either peptone-glucose medium or peptone-glucose medium supplemented with Tween 80. The induced esterase was heat labile and had maximum activity at pH 5.5. Enzyme activity was stimulated by the addition of sodium taurocholate, an activator of lipase. Thin-layer chromatography revealed that this enzyme does not hydrolyze triolein and L-α-lecithin, suggesting that it is a monoester hydrolase (not a lipase in the strict sense of the word). Esterase activity was examined in 85 clinical isolates of *Candida* species; *C. albicans*, *C. tropicalis*, and *C. parapsilosis* tended to have higher enzyme activities than *C. kefyr*, *C. krusei*, *C. glabrata*, and *C. guilliermondii*. Although the physiological properties of this esterase are not clear at present, it was found to be crucial for fungal growth under specific conditions.

Candida albicans is a pathogenic fungus which frequently causes superficial and deep mycoses. Many investigations have listed the pathogenic factors of *C. albicans*, among which hydrolytic enzymes, in particular the secreted aspartyl proteinases, have recently attracted the interest of mycologists (11, 13, 21–23). We previously demonstrated that *C. albicans* proteinase is a key enzyme for fungal growth in stratum corneum-containing medium (10, 20, 31) and that *C. albicans* α-glucosidase is crucial in maltose-containing medium (6). Destruction of the skin surface by enzymatic digestion is expected to be important for fungal adhesion, invasion, and growth. Considering that 25% of the total lipid content in human stratum corneum has been found to be triacylglycerol (13), we expected that pathogenic fungi may secrete lipolytic enzymes.

Triacylglycerol lipase (EC 3.1.1.3) is an enzyme which hydrolyzes the ester bonds in triacylglycerol (4). Within the mycological field, little attention has been paid to lipase; only industrial applications of lipases from *C. lipolytica* (14, 17) and *C. rugosa* (formerly *C. cylindracea*) (3, 5, 9, 12, 15, 30, 33) have been reported on. With respect to the pathogenic *Candida* species, esterase activity has been found when they were biotyped (7, 24, 27). During the process of searching for lipolytic enzymes, we recently detected lipase activity in the insoluble fraction of cell extracts from *Malassezia furfur* (26). As a continuation of this series of studies, we report here that *C. albicans* secreted an extracellular esterase (not a lipase in the strict sense of the word) in medium containing Tween 80 as the sole carbon source. Some biochemical properties of the esterase and its activities in various *Candida* species are further described.

MATERIALS AND METHODS

Strains and culture conditions. *C. albicans* serotype A (A-714) and fresh clinical isolates of *Candida* species were used for this study after identification by morphological and immunoantigenic analyses (Candida Check; IATRON, Tokyo, Japan). These strains were maintained in 1% peptone (Difco Laboratories, Detroit, Mich.)–2% glucose agar and precultivated in 1% peptone–4% glucose broth at 37°C for 24 h. The organisms were then washed with phosphate-buffered saline (PBS) and adjusted to give 2×10^5 cells in the growth medium. For the induction of an esterase, a growth medium containing 0.7% yeast nitrogen base (YNB; Difco) and 2.5% Tween 80 (polyoxyethylenesorbitan monooleate; Sigma Chemical Co., St. Louis, Mo.) was used after sterilization. For some experiments, filtrated 0.7% YNB mixed with Tween 20 (polyoxyethylenesorbitan monolaurate), Tween 40 (polyoxyethylenesorbitan monopalmitate), Tween 60 (polyoxyethylenesorbitan monostearate), Tween 85 (polyoxyethylenesorbitan trioleate), Triton X-100 (*t*-octylphenoxypolyethoxyethanol), tripalmitin, or triolein (all reagents were purchased from Sigma) was tested to ascertain the induction of an esterase. A medium containing 0.7% yeast carbon base (YCB; Difco) and 0.5% bovine serum albumin (BSA; Sigma) was also used as a growth medium. After addition of the organisms to a flask containing 100 ml of growth medium, the flask was shaken at 37°C for a specified period. The organisms were then counted by a hemacytometer, and the culture filtrate was collected. In an experiment to examine the localization of the enzyme, cell extracts from *C. albicans* were prepared. The cells, mixed with Glasperlen (B. Braun Melsungen AG, Melsungen, Germany), were homogenized and then centrifuged at 10,000 × *g* for 30 min. Enzyme activities of the supernatant and precipitate were measured.

Esterase assay. Esterase activity was measured by a colorimetric method (34), using a lipase assay kit (Toyobo, Osaka, Japan). Culture filtrate (0.2 ml) was reacted with 1.12 mM α-naphthyl palmitate in 1.0 ml of 14 mM citric acid–74 mM Tris buffer (pH 5.5) at 37°C for 1 h. The mixture contained 0.1 μM eserine to inhibit cholinesterase activity. The reaction was terminated by the addition of 0.5 ml of 0.5 N sodium hydroxide solution. Released α-naphthol was reacted with fast violet B, and A_{520} was measured.

Assays to examine the biochemical properties of the enzyme. The optimum pH of esterase activity was examined by the aforementioned esterase assay method, using 14 mM citric acid–74 mM Tris buffer (pH 3.0 to 7.0) or 74 mM Tris-hydrochloride buffer (pH 7.0 to 9.0). The stimulatory or inhibitory effect of the various reagents on esterase activity was examined by preincubating the enzyme solution with the following reagents at 37°C for 15 min prior to the esterase assay: phenylmethylsulfonyl fluoride, *N*-ethylmaleimide, EDTA, $CaCl_2$, $MgCl_2$, $FeCl_2$, taurodeoxycholic acid, Tween 20, Tween 80, trypsin (all from Sigma), pepstatin (Peptide Institute, Tokyo, Japan), ebelactone B (Peptide Institute) (32), and sodium taurocholate (Difco).

Thin-layer chromatography. To determine the substrate specificity, enzyme solution (0.1 ml) was reacted with 1 mg of triolein, diolein, or L-α-lecithin (Sigma) in buffer (0.4 ml, pH 5.5) containing 50 μl of arabic gum (Sigma) at 37°C for 2 h. A commercial lipase from *C. cylindracea* (Sigma) was used for comparison. Two microliters of the reaction mixture was applied on a silica gel plate (Polygram, Macherey-Nagel, Germany) and separated by the solvent mixture, hexane-ether-acetic acid (80:20:1 [vol/vol/vol]). Lipids were visualized by the

* Corresponding author. Mailing address: Department of Dermatology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. Phone and fax: (81)-3-3813-2205.

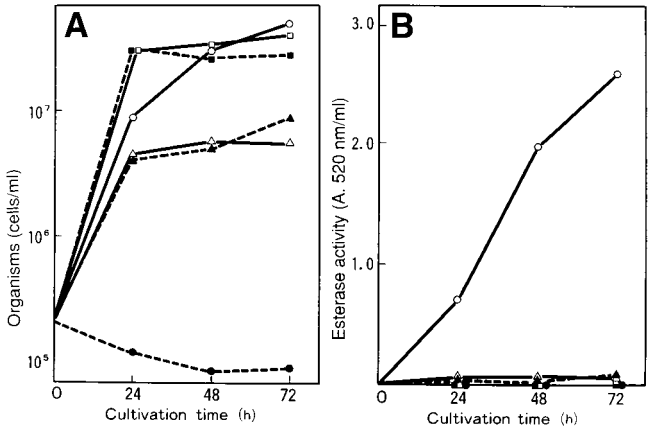

FIG. 1. Cell growth and esterase activity in various media. *C. albicans* (2×10^5 cells per ml) was inoculated in various media at 37°C. Cell growth and esterase activity in the medium were measured during a 72-h incubation period as described in the text. (A) Cell growth; (B) esterase activity (A_{520} per milliliter of enzyme solution). The data shown are the means of two values and representative of experiments repeated four times. Symbols: ■, 1% peptone–4% glucose; ▲, 0.7% YCB–0.5% BSA; ●, 0.7% YNB; □, 1% peptone–4% glucose–2.5% Tween 80; △, 0.7% YCB–0.5% BSA–2.5% Tween 80; ○, 0.7% YNB–2.5% Tween 80.

spraying of 10% molybdenum alcohol and subsequent heating of the plate at 150°C for 5 min.

RESULTS

Induction of an extracellular esterase from *C. albicans*. Various media were tested to determine the appropriate conditions for inducing an extracellular esterase. The data shown in Fig. 1 demonstrate the correlation between esterase activity and cell growth in six different media. Prominent cell growth was observed in all media tested except for the medium containing YNB alone. However, extracellular esterase activity was detected only in the medium containing YNB and Tween 80, and its activity in this medium was found to correlate well with cell growth. Esterase activity was high in the logarithmic growth phase, plateaued, and then decreased at the stationary growth phase (data not shown). Peptone-glucose medium and a medium prepared for the induction of extracellular proteinase (YCB-BSA) did not induce esterase activity. Since Tweens (polyoxyethylenesorbitan compounds) are usually used as detergents, the possibility that Tweens acted to reveal the enzyme activity arose. As shown in Fig. 1, neither peptone-glucose-Tween 80 medium nor YCB-BSA-Tween 80 medium induced any esterase activity, suggesting that Tween 80 worked as a substrate which is essential for the induction of an esterase. Figure 2 further indicates that the levels of cell growth and esterase activity were dependent on the concentration of Tween 80 in the medium. Absence of Tween 80 in YNB medium did not induce any esterase activity. In subsequent experiments, Tween 80 was replaced by other Tweens. As shown in Fig. 3, cell growth and esterase activity were more prominent in media containing Tween 85, 80, or 60 than in those containing Tween 40 or 20. However, some strains showed the highest esterase activity in the medium containing Tween 40 but no enzyme activity in the medium containing Tween 20 (data not shown). This result implies that esterase induction varies according to the strain and the kind of Tween. In repeated experiments, cultivation in medium containing 0.7% YNB supplemented with 2.5% Triton X-100, 2.5% triolein, or 2.5% tripalmitin did not result in any cell growth or esterase

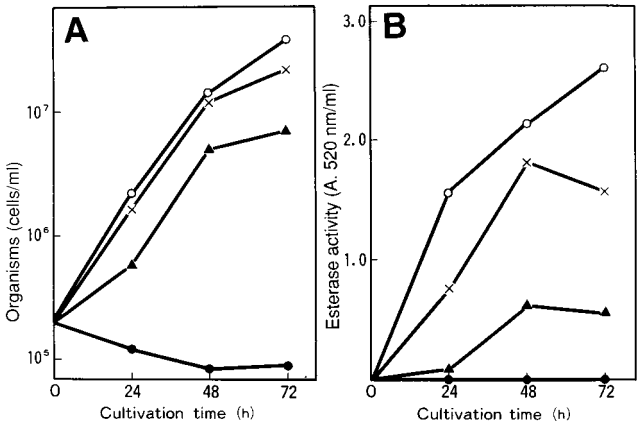

FIG. 2. Concentration effect of Tween 80 on cell growth and esterase activity. (A) Cell growth; (B) esterase activity (A_{520} per millliter of enzyme solution). The data shown are the means of two values and representative of experiments repeated four times. Symbols: ○, 0.7% YNB–2.5% Tween 80; ×, 0.7% YNB–0.5% Tween 80; ▲, 0.7% YNB–0.1% Tween 80; ●, 0.7% YNB.

activity (data not shown). Nevertheless, the possible induction of triacylglycerol hydrolase (lipase) should not necessarily be ruled out, since the layer of triolein or tripalmitin was completely separated from the water layer during the cultivation period. The aforementioned results suggest that the lack of a carbohydrate source (YNB) combined with the presence of a soluble monoester with a long-chain fatty acid appear to be crucial for the induction of esterase activity.

The localization of the enzyme in the cell was investigated by preparing cell extracts. *C. albicans* was inoculated in 500 ml of 0.7% YNB–2.5% Tween 80 medium at 37°C for 48 h. The collected cells (2.5 g) were then homogenized in PBS and centrifuged at $10{,}000 \times g$ for 30 min. The ratios of the esterase activity in the three fractions, culture filtrate, supernatant of the cell extract, and the precipitate, were 97.3, 0.2, and 2.5%, respectively. These results suggest that synthesized esterase is quickly secreted and exists primarily in the culture medium.

Biochemical properties of the esterase. Some biochemical

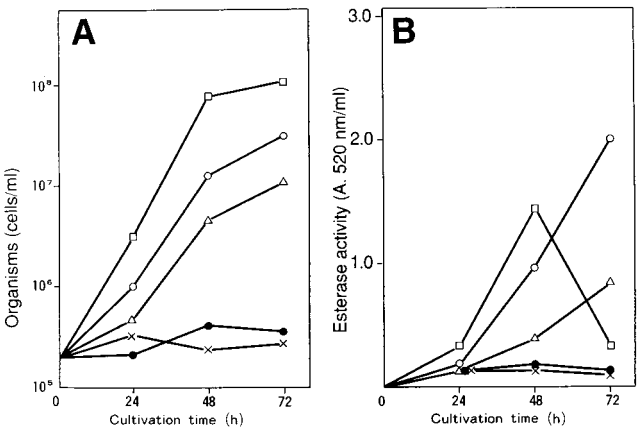

FIG. 3. Effects of the various Tweens on cell growth and esterase activity. Different kinds of Tweens were added as the sole carbon sources to the growth medium. (A) Cell growth; (B) esterase activity (A_{520} per milliliter of enzyme solution). The data shown are the means of two values and representative of experiments repeated five times. Symbols: □, 0.7% YNB–2.5% Tween 85; ○, 0.7% YNB–2.5% Tween 80; △, 0.7% YNB–2.5% Tween 60; ×, 0.7% YNB–2.5% Tween 40; ●, 0.7% YNB–2.5% Tween 20.

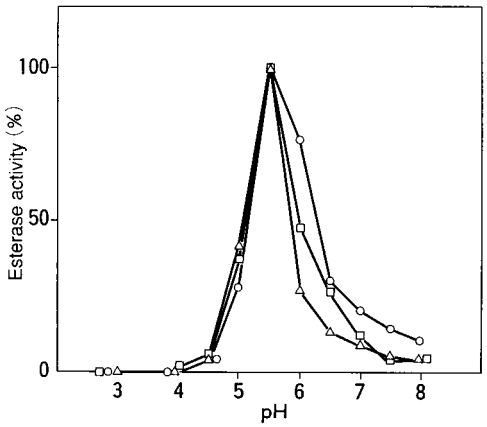

FIG. 4. pH profile of esterase activity. pH-dependent activity was examined in the range shown, using the culture supernatant obtained from the different growth media. Maximal esterase activity in each preparation was expressed as 100%. The data shown are the means of two values and representative of experiments repeated three times. Symbols: □, 0.7% YNB–2.5% Tween 85; ○, 0.7% YNB–2.5% Tween 80; △, 0.7% YNB–2.5% Tween 60.

properties of the enzyme were investigated by using the culture filtrate as a crude enzyme preparation. As shown in Fig. 4, enzyme solutions obtained from induction media containing Tween 85, 80, or 60 as the sole carbon source showed similar pH profiles, with a maximum enzyme activity at pH 5.5, which implies that similar or identical enzymes were induced from these media. Esterase activity was lost after boiling of the enzyme solution for 10 min. The effects of various reagents on the enzyme activity were then examined, using the enzyme solution obtained from YNB-Tween 80 medium. As shown in Table 1, enzyme activity was found not to be affected by the addition of proteinase inhibitors, metal ions, or an esterase inhibitor, ebelactone B. Sodium taurocholate, an activator of lipase, increased the enzyme activity, but trypsin, a proteinase which activates zymogen, did not. The addition of Tween 20 or 80 prior to the reaction led to a twofold increase in esterase activity.

As is standard for a biochemical study, purification of the esterase was then attempted. However, biochemical procedures, including condensation of the culture filtrate by ultrafiltration and ammonium sulfate precipitation, gel filtration,

TABLE 1. Effects of various reagents on enzyme activity

Reagent	Concn	Residual activity (%)[a]
None		100
Phenylmethylsulfonyl fluoride	1 mM	96
N-Ethylmaleimide	1 mM	94
Pepstatin	100 μg/ml	93
EDTA	1 mM	97
Ebelactone B	100 μg/ml	83
Ca^{2+}	1 mM	87
Mg^{2+}	1 mM	85
Fe^{2+}	1 mM	99
Sodium taurocholate	10 mM	183
Taurodeoxycholic acid	10 mM	103
Tween 20	5%	188
Tween 80	5%	215
Trypsin	100 μg/ml	98

[a] Mean of two values. The experiments were repeated five times, with similar profiles being obtained.

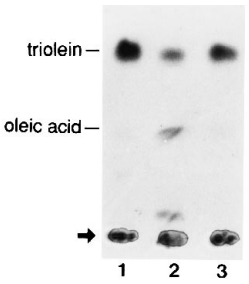

FIG. 5. Thin-layer chromatography. Triolein (1 mg) in buffer (pH 5.5) containing arabic gum was reacted with the enzyme solution at 37°C for 2 h. Two microliters of the reaction mixture was applied on a silica gel plate and then separated by the solvent mixture, hexane-ether-acetic acid (80:20:1 [vol/vol/vol]). Lipids were visualized by spraying of 10% molybdenum alcohol followed by heating of the plate. Lanes: 1, nontreated triolein; 2, triolein reacted with a commercial lipase from *C. cylindracea*; 3, triolein reacted with the enzyme solution from *C. albicans*. The arrow indicates the blotted point.

and electrophoresis, failed to isolate the enzyme. This failure was due to the enzyme activity remaining in the Tween 80 solution, which could not be extracted into aqueous solution.

In this experiment, esterase activity was measured by a lipase assay kit using α-naphthyl palmitate. The substrate was a monoester conjugated with a long-chain fatty acid. In the strict sense of the word, lipase is defined as a triacylglycerol hydrolase, and therefore hydrolysis of a triacylglycerol by the enzyme solution was analyzed by thin-layer chromatography. A commercial *C. cylindracea* lipase was used as a positive enzyme. As shown in Fig. 5, *C. cylindracea* lipase degraded a quantity of the triolein into oleic acid and diolein, while the enzyme solution from *C. albicans* did not. Neither diolein nor L-α-lecithin was hydrolyzed by the enzyme solution from *C. albicans* (data not shown). The same results were obtained by gas chromatography analysis (data not shown). These results suggest that under the conditions tested, the extracellular enzyme from *C. albicans* was an esterase (monoester hydrolase) and not a lipase or a phospholipase.

Esterase activities in *Candida* species. To demonstrate the widespread existence of the inducible esterase, we measured the esterase activity from 85 clinical isolates of *Candida* species (Fig. 6). Although there was a relatively large variation among the strains, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* tended to have higher enzyme activities than the other species. The data shown in Figure 6 imply that the extracellular esterase is induced in all of the *Candida* strains.

DISCUSSION

Lipase is a family of enzymes which hydrolyze the ester bonds in triacylglycerol to yield free fatty acids (4). In the broad sense of the term, an enzyme which hydrolyzes monoacylglycerol with a long-chain fatty acid (C_{12} or more) is classified as a monoacylglycerol lipase (7). The extracellular enzyme described here was considered an esterase, in the strict sense of the word, because of its hydrolytic activity with respect to water-soluble monoesters with a long-chain fatty acid (27).

As an example of a well-known lipase, pancreatic lipase is known to be activated from zymogen by the proteolytic activity of trypsin and to then hydrolyze lipids at pH 7 in the presence of cholic acid and Ca^{2+} (4). Since lipase works at the water/lipid interface, cholic acid, acting as a solvent, provides easier access for the enzyme to the substrate and induces conformational rearrangements of the enzyme (9). The activity of the enzyme that we characterized was also activated by the addi-

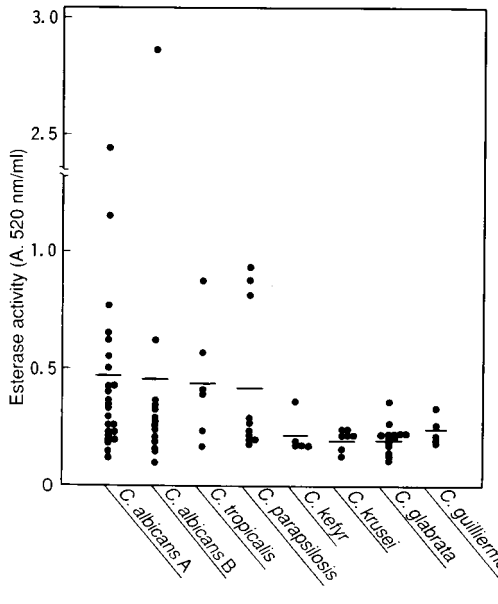

FIG. 6. Esterase activity of the clinical isolates of *Candida* species. Fresh clinical isolates of *Candida* species (2×10^5 cells per ml) were inoculated in 0.7% YNB–2.5% Tween 80 medium at 37°C for 48 h, and the esterase activity of the medium was measured. Horizontal lines in each column indicate the mean of the values.

tion of sodium taurocholate. However, unlike the activity of pancreatic lipase, its activity was not affected by either trypsin or Ca^{2+}. The optimal enzyme activity at weakly acidic pH coincided with those of lipases from *M. furfur* (26) and *C. rugosa* (30).

For the detection of enzyme activity, we performed a colorimetric assay using α-naphthyl palmitate (C_{16}), rather than a titrimetric assay using triacylglycerol. The colorimetric assay kit, described by Whitaker (34), is used for the detection of pancreatic lipase activity, as cholineesterase activity is designed to be inhibited by the presence of eserine in the assay kit. We later verified by thin-layer chromatography that the enzyme was an esterase, since it did not hydrolyze triacylglycerol.

For the induction of extracellular esterase, lack of a carbohydrate source (YNB) combined with Tween 80 was found to be crucial. Tween consists of a mixture of polyoxyethylenesorbitan compounds and is thought to be suitable for broth media because it is water soluble. The possibility that Tween acted as a solvent in this investigation was ruled out by subsequent experiments. Major fatty acids known to be incorporated in Tweens are laurate (C_{12}) in Tween 20, palmitate (C_{16}) in Tween 40, stearate (C_{18}) in Tween 60, and oleate (C_{18}) in Tweens 80 and 85. We found that esterase activity was highly induced in medium containing Tween 80 or 85 but absent in medium containing Tween 20. It is unclear at present whether this result was dependent on such factors as the number of carbons or double bonds in the incorporated fatty acid. Casal and Linares (7) examined biotypes of *Candida* species by measuring 20 hydrolytic enzyme profiles. Their qualitative data for 186 *C. albicans* strains indicate that more than 95% of the strains hydrolyze α-naphthyl butyrate (C_4) and α-naphthyl caprylate (C_8) but that none hydrolyze α-naphthyl myristate (C_{14}). Myristate monoester was not hydrolyzed probably because the organisms were preincubated in Sabouraud-dextrose broth, in which the presence of dextrose would have prevented the induction of extracellular esterase.

The enzyme that we characterized should be differentiated from other hydrolytic enzymes related to lipid metabolisms. Phospholipases from *C. albicans* (1, 2, 16, 18, 25, 28, 29) have been well investigated and purified to homogeneity. Evidence that phospholipases and the extracellular esterase described here are distinct enzymes includes the following: (i) phospholipase activities, detected extracellularly (1, 2, 18, 25, 28, 29) or intracellularly (2, 16), were observed in organisms grown in Sabouraud-dextrose medium, (ii) phospholipases existed in aqueous solution and were purified by standard purification procedures, (iii) extracellular esterase did not hydrolyze L-α-lecithin, and (iv) extracellular esterase was induced only in the medium containing Tween as the sole carbon source. Although we failed to purify the enzyme, these results suggest that *C. albicans* extracellular esterase is an enzyme that differs from phospholipases.

Barrett-Bee et al. (2) suggested that *C. albicans* strains, having higher phospholipase activities, adhere more strongly to buccal epithelial cells and are more pathogenic in mice. In this investigation, we measured the extracellular esterase activities of the 85 clinical isolates of *Candida* species and found that they weakly paralleled the relative pathogenic ranking of *Candida* species in animals (21). Although there have been no other reports describing the relationship between lipolytic activity and pathogenicity, these data encourage further investigation to clarify the role of lipolytic enzymes.

Although the stratum corneum of human skin is rich in triacylglycerol (13), monoacylglycerol, a substrate of extracellular esterase, may be difficult to find under physiologically normal conditions. There remains a possibility that extracellular esterase hydrolyzes some other substrates in vivo, as it is relatively difficult to provide emulsified lipid/water phase in an in vitro culture system. Nevertheless, our findings indicate that under specific in vitro growth conditions, an inducible extracellular esterase was crucial for fungal growth.

REFERENCES

1. **Banno, Y., T. Yamada, and Y. Nozawa.** 1985. Secreted phospholipases of the dimorphic fungus, *Candida albicans*: separation of three enzymes and some biological properties. J. Med. Vet. Mycol. **23:**47–54.
2. **Barrett-Bee, K., Y. Hayes, R. G. Wilson, and J. F. Ryley.** 1985. A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. J. Gen. Microbiol. **131:**1217–1221.
3. **Benzonana, G., and S. Esposito.** 1971. On the positional and chain specificities of *Candida cylindracea* lipase. Biochim. Biophys. Acta **231:**15–22.
4. **Bier, M.** 1955. Lipases. Methods Enzymol. **1:**627–642.
5. **Brahimi-Horn, M.-C., M. L. Guglielmino, L. Elling, and L. G. Sparrow.** 1990. The esterase profile of a lipase from *Candida cylindracea*. Biochim. Biophys. Acta **1042:**51–54.
6. **Bramono, K., R. Tsuboi, and H. Ogawa.** 1995. A carbohydrate-degrading enzyme from *Candida albicans*: correlation between α-glucosidase activity and fungal growth. Mycoses **38:**349–353.
7. **Casal, M., and M. J. Linares.** 1983. Contribution to the study of the enzymatic profiles of yeast organisms with medical interest. Mycopathologia **81:**155–159.
8. **Cutler, J. E.** 1991. Putative virulence factors of *Candida albicans*. Annu. Rev. Microbiol. **45:**187–218.
9. **Grochulski, P., Y. Li, J. D. Schrage, F. Bouthillier, P. Smith, D. Harrison, B. Rubin, and M. Cygler.** 1993. Insights into interfacial activation from an open structure of *Candida rugosa* lipase. J. Biol. Chem. **268:**12843–12847.
10. **Hattori, M., K. Yoshiura, M. Negi, and H. Ogawa.** 1984. Keratinolytic proteinase produced by *Candida albicans*. Sabouraudia **22:**175–183.
11. **Hube, B., M. Monod, D. A. Schofield, A. J. P. Brown, and N. A. R. Gow.** 1994. Expression of seven members of the gene family encoding secretory aspartate proteinase in *Candida albicans*. Mol. Microbiol. **14:**87–99.
12. **Kawaguchi, Y., H. Honda, J. Taniguchi-Morimura, and S. Iwasaki.** 1989. The codon CUG is read as serine in an asporogenic yeast *Candida cylindracea*. Nature (London) **341:**164–166.
13. **Lampe, M. A., M. L. Williams, and P. M. Elias.** 1983. Human epidermal lipids: characterization and modulations during differentiation. J. Lipid Res. **24:**131–140.
14. **Lloyd, G. I., E. O. Morris, and J. E. Smith.** 1971. A study of the esterases and

their function in *Candida lipolytica*, *Aspergillus niger* and a yeast-like fungus. J. Gen. Microbiol. **63:**141–150.
15. **Longhi, S., F. Fusetti, R. Grandori, M. Lotti, M. Vanoni, and L. Alberghina.** 1992. Cloning and nucleotide sequences of two lipase genes from *Candida cylindracea*. Biochim. Biophys. Acta **1131:**227–232.
16. **Mago, N., and G. K. Khuller.** 1990. Subcellular localization of enzymes of phospholipid metabolism in *Candida albicans*. J. Med. Vet. Mycol. **28:**355–362.
17. **Mattey, M., and D. Morgan.** 1978. Secretion of extracellular lipases by *Candida lipolytica*. Biochem. Soc. Trans. **6:**426–428.
18. **Mirbod, F., Y. Banno, M. A. Ghannoum, A. S. Ibrahim, S. Nakashima, Y. Kitajima, G. T. Cole, and Y. Nozawa.** 1995. Purification and characterization of lysophospholipase-transacylase (h-LPTA) from a highly virulent strain of *Candida albicans*. Biochim. Biophys. Acta **1257:**181–188.
19. **Mishra, P., J. Bolard, and R. Prasad.** 1992. Emerging role of lipids of *Candida albicans*, a pathogenic dimorphic yeast. Biochim. Biophys. Acta **1127:**1–14.
20. **Negi, M., R. Tsuboi, T. Matsui, and H. Ogawa.** 1984. Isolation and characterization from *Candida albicans*: substrate specificity. J. Invest. Dermatol. **83:**32–36.
21. **Odds, F. C.** 1988. Pathogenesis of candidosis, p. 252–278. *In* F. C. Odds (ed.), Candida and candidosis, 2nd ed. Bailliere Tindall, London.
22. **Ogawa, H., Y. Nozawa, V. Rojanavanich, R. Tsuboi, T. Yoshiike, Y. Banno, M. Takahashi, C. Nombela, E. Herreros, M. I. Garcia-Saez, A. I. Santos, and M. Sanchez.** 1992. Fungal enzymes in the pathogenesis of fungal infections. J. Med. Vet. Mycol. **30**(Suppl. 1)**:**189–196.
23. **Ogawa, H., and R. Tsuboi.** 1996. Fungal enzymes related to the pathogenesis of mycoses, p. 191–207. *In* Jacobs and Nall (ed.), Biomedical mycology. Marcel Dekker, Inc., New York.
24. **Pospisil, L., and A. Kabatova.** 1976. Lipolytic activity in some *Candida* strains. Zeutralbl. Bakteriol. Abt. II Bd. **131S:**692–696.
25. **Price, M. F., I. D. Wilkinson, and L. O. Gentry.** 1982. Plate method for detection of phospholipase activity in *Candida albicans*. J. Med. Vet. Mycol. **20:**7–14.
26. **Ran, Y., T. Yoshiike, and H. Ogawa.** 1993. Lipase of *Malassezia furfur*; some properties and their relationship to cell growth. J. Med. Vet. Mycol. **31:**77–85.
27. **Rudek, W.** 1978. Esterase activity in *Candida* species. J. Clin. Microbiol. **8:**756–759.
28. **Takahashi, M., Y. Banno, and Y. Nozawa.** 1991. Secreted *Candida albicans* phospholipases: purification and characterization of two forms of lysophospholipase-transacylase. J. Med. Vet. Mycol. **29:**193–204.
29. **Takahashi, M., Y. Banno, Y. Shikano, S. Mori, and Y. Nozawa.** 1991. Purification and characterization of lysophospholipase-transacylase of pathogenic fungus *Candida albicans*. Biochim. Biophys. Acta **1082:**161–169.
30. **Tomizuka, N., Y. Ota, and K. Yamada.** 1966. Studies on lipase from *Candida cylindracea*. Part I. Purification and properties. Agric. Biol. Chem. **30:**576–584.
31. **Tsuboi, R., Y. Kurita, M. Negi, and H. Ogawa.** 1985. A specific inhibitor of keratinolytic proteinase from *Candida albicans* could inhibit the cell growth of *C. albicans*. J. Invest. Dermatol. **85:**438–440.
32. **Umezawa, H., T. Aoyagi, K. Uotani, M. Hamada, T. Takeuchi, and S. Takahashi.** 1980. Ebelactone, an inhibitor of esterase, produced by *Actinomycetes*. J. Antibiot. **33:**1594–1596.
33. **Veeraragavan, K., and B. F. Gibbs.** 1989. Detection and partial purification of two lipases from *Candida rugosa*. Biotechnol. Lett. **11:**345–348.
34. **Whitaker, J. F.** 1973. A rapid and specific method for the determination of pancreatic lipase in serum and urine. Clin. Chim. Acta **44:**133–138.

Editor: T. R. Kozel