

Extracellular Esterase Secretion by Vaginal Isolates of *Candida Albicans*

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Many of the pathogenic *Candida* species have the ability to secrete extracellular lipolytic enzymes such as esterases and phospholipases (1, 2). These enzymes are categorized into two important types: proteinases, which hydrolyze peptide bonds; and lipolytic enzymes (phospholipase and esterases), which hydrolyze lipid compounds in host cells tissues (3). Destroying or deranging constituents of host cell membranes due to hydrolytic enzymes causes dysfunction and/or cells disruption. Adherence and tissue penetration by *Candida* species will be facilitated by secreted extracellular enzymes and therefore, host invasion occurs (4, 5). Khosravi et al. examined esterase activity of *Candida albicans* as a potential virulence factor in animal model (5). The esterase activities of these yeasts were previously demonstrated with the application of the tween opacity test medium with different tween compounds (2) and colorimetry method (6).

The aim of the present study was to investigate esterase activities on vaginal isolates of *C. albicans*. A total of 85 vaginal isolates of *C. albicans* were used. The identities of the isolates were confirmed according to their morphology on CHROMagar *Candida* (CHROMagar *Candida*, Paris, France), cornmeal agar (HiMedia, India), and germ tube production (7). Esterase activity was determined by visual methods presented by Slifkin (8). The agar medium consisted of 1 g of peptone (Difco, USA), 0.5 g of NaCl (Merck, Germany), 0.01 g of CaCl₂ (Merck, Germany), and 1.5 g of agar (Merck, Germany). Medium was sterilized and cooled to about 50°C; then 0.5 mL of sterile tween 80 (Merck, Germany) was added.

An overnight culture of each isolate was inoculated into medium by touching the center of the agar medium with a cotton swab in triplicate. The inoculated culture plates were incubated at 29°C and were examined during ten days. The presence of a halo around an inoculated site

was indicative of a positive test and a sign of esterase production by the *Candida* isolate. Esterase activity (Ez value) was reported as follows: a Ez of 1.0 as negative (-), 0.90 to 0.99 as weak (+), 0.80 to 0.89 as mild (+ +), 0.70 to 0.79 as strong (+ + +), and < 0.69 as very strong (+ + + +) (2). The results were calculated from triplicate tests for each isolate. In the present study, 23.5% of isolates showed esterase activity after two days, 47.1% after three days, 14.1% after four days, 10.6% after five days, and 4.7% after seven days. The highest esterase activity (Ez value < 0.40) was observed in 21.2% of isolates (Table 1).

Aktas et al. (9) have shown that 98.3% of *C. albicans* strains yielded positive results for esterase activity in two to ten days. In addition, all tested isolates of *C. albicans* and *C. Candida tropicalis* showed esterase activity during two to three days by Slifkin (8). All of the Aktas et al. (9) tested *C. tropicalis* and *C. Candida guilliermondii* strains had positive results for esterase activity whereas most of the tested *C. Candida parapsilosis* and *C. Candida metapsilosis* by Ge et al. (2) failed to show esterase activity. Pakshir et al. tested *C. albicans* from onychomycosis and oral lichen planus patients for esterase activity (10). Most tested species produced esterase enzyme during three days and no significant difference was seen between the two groups of *C. albicans*. In the presents study, all *C. albicans* strains were isolated from patients with vulvovaginal candidiasis and were definitely considered as pathogen with Ez ≤ 60 in all strains; however, there was a difference among *Candida* strains with regard to the production of esterase (Table 1). Ge et al. in their study found Ez < 0.69 for esterase activity among tested *Candida* species, which was determined as 4⁺ (2). In conclusion, our results showed that all isolates yielded positive results; however, variable levels of esterase activity were observed during incubation period.

Table 1. Esterase Activity Among Eighty-Five Isolates of *Candida albicans* During One Week

Enzymatic activities, Ez	2 days	3 days	4 days	5 days	7 days	Total
< 0.40	0	15	0	3	0	18 (21.2)
0.41-0.45	13	23	8	4	4	52 (61.2)
0.46-0.50	6	2	3	2	0	13 (15.3)
0.51-0.55	0	0	1	0	0	1 (1.2)
0.56-0.60	1	0	0	0	0	1 (1.2)
Total	20 (23.5)	40 (47.1)	12 (14.1)	9 (10.6)	4 (4.7)	85 (100)

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