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Esterase Activity in *Candida* Species

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The purpose of this investigation was to ascertain the lipolytic activities (specifically, esterase) of those species of *Candida* that are most commonly isolated from human infections. Eight species of *Candida* were surveyed for their ability to hydrolyze various polyoxyethylene sorbitan compounds (Tweens). Of the 64 isolates tested, each had activity for at least one of the substrates. Most of the isolates hydrolyzed Tweens 40, 60, and 85. In contrast, none hydrolyzed Tween 80. Only one species hydrolyzed Tween 20. The patterns of precipitation resulting from reactions of fatty acids hydrolyzed from Tweens 40, 60, and 85 with calcium ions in the media were also useful in distinguishing some of the species. In the past, such reactions have been reported as being dependent on esterase activity.

Lipolytic activities in some species of *Candida* have been reported (4, 10, 11). However, relatively little information is available concerning the lipolytic activities of those isolates that are consistently associated with human infection. Since lipolytic activity has been correlated with the virulence of certain organisms (6, 7), it is of interest to determine whether these factors are also present in pathogenic strains of *Candida*. Although some earlier investigators (1) have reported negative findings after testing for lipolytic activity, more recently Price and Cawson (5) have shown phospholipase A and lysophospholipase in *Candida albicans*. Pospisil and Kabatova (4) also found a lipolytic activity in some strains of *Candida* by using a Tween compound as the substrate. It was the purpose of this investigation to ascertain the patterns of lipolytic activity of various clinically significant strains of *Candida* on a variety of Tween compounds to determine whether certain patterns could be useful in identification and possibly as indicators of virulence.

Since Tween compounds were used as the substrates in this investigation, esterase activity was the specific lipolytic activity to be tested. Wills (9) states that "whenever a water soluble substrate is used it appears that an esterase and not a lipase was actually under investigation." Since our preliminary studies have shown that water-soluble substrates were acted upon by some members of the genus *Candida*, this investigation was intended to detect esterase activity.

MATERIALS AND METHODS

Organisms. Sixty-four cultures, representing eight species of *Candida*, were examined. Reference strains of seven of the species were obtained from the American Type Culture Collection (ATCC). These were *C. albicans* (ATCC 10231), *C. tropicalis* (ATCC 750), *C. pseudotropicalis* (ATCC 4135), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019), *C. viswanathii* (ATCC 22981), and *C. stellatoidea* (ATCC 11006). The remainder of the cultures were clinical isolates obtained from Ravenswood Hospital, Belmont Community Hospital, or Hektoen Institute, Cook County Hospital, Chicago, Ill. Upon reception, all strains were recharacterized and identified, using chlamydospore formation, sugar fermentation, and assimilation tests as major taxonomic criteria (3).

Media. Subcultures of the test organisms were maintained on Sabouraud dextrose agar (Difco). For detection of esterase activity, a modified-substrate agar plate was used (6). The polyoxyethylene sorbitan substrates (Tweens 20, 40, 60, 80, and 85, obtained from Sigma Chemical Co.) were separately added to Sabouraud liquid broth modified medium (Difco) to a final concentration of 1%. One percent agarose (Colab) was also added as a solidifying agent. The mixture was autoclaved at 121°C for 15 min.

Overnight cultures were transferred to the substrate test medium via stab inoculation. The plates were incubated at 35°C in a humid environment and observed at 3, 5, and 7 days. Detection of lipolytic activity on all substrate plates was performed by observing zones of precipitation around the colonies when viewed by transmitted light. These precipitates are formed as a result of hydrolysis of the Tween compound. Subsequent to the cleavage of ester bonds, the fatty acids released combine with calcium ions in the medium to form insoluble calcium salts (6).

RESULTS

Results are summarized in Table 1. With the exception of Tweens 20 and 80, hydrolysis of the Tween compounds was a common feature of

these organisms. In addition, characteristic patterns of precipitation were evident within 5 days for some of the isolates.

With the exception of *C. albicans* on Tween 40, the ability to hydrolyze various Tween compounds was consistent within a given species. For instance, most of the isolates of *C. parapsilosis* were able to hydrolyze Tween 20, whereas none of the other *Candida* species had any effect on this substrate even when incubation was extended to 7 days.

Tween 40 hydrolysis was much more common within this genus. All of the isolates of *C. tropicalis, C. krusei,* and *C. viswanathii* as well as the majority of *C. parapsilosis* and *C. stellatoidea* were positive on this substrate at 5 days. *C. albicans* was the only species that gave inconsistent results. Only two species, the one isolate of *C. pseudotropicalis* and the one isolate of *C. guilliermondii,* failed to hydrolyze Tween 40.

The reactions on Tween 60 were the most consistent. With the exception of the one strain of *C. pseudotropicalis,* all *Candida* species tested were positive on this substrate. This is in contrast to Tween 80 hydrolysis, which showed no activity even after 7 days of incubation for all of the isolates tested.

Hydrolysis of Tween 85 could be demonstrated in each species represented. This was the only substrate hydrolyzed by *C. pseudotropicalis.* The other species showed activities similar to those found on Tween 40 or 60.

Distinctive patterns of precipitation were also evident on the various substrates. The most common pattern was that of diffuse precipitation extending several millimeters from the edge of the colony (Fig. 1). A notable exception to this pattern was found for only one species on only one of the substrates. *C. tropicalis* gave double zones of precipitation on Tween 60. This unique feature was consistent for all of the isolates of *C. tropicalis* tested. No other organism in the study gave this reaction. *C. tropicalis* could thus be easily distinguished from the other species of *Candida* on this medium (Fig. 2).

The patterns on Tween 85 were also distinctive. Most strains produced a narrow, dense zone of precipitation around the colony, which extended only 2 to 3 mm. *C. tropicalis* produced wide zones on this medium, which extended 8 to 10 mm from the colony.

DISCUSSION

Judging from the data presented in this paper, esterase activity would appear to be a common feature of those *Candida* species that are frequently isolated from clinical specimens. The results of this study indicate that each of the isolates tested had this lipolytic activity for at least one of the substrates. Most were active on at least three Tween compounds. None of the cultures, however, were active when tested on Tween 80.

The lack of lipolytic activity on Tween 80 is not unusual. Tirunarayanan and Lundbeck (6) reported similar findings from testing *Staphylococcus aureus* on various Tween compounds. Although they found activity with the other substrates, Tween 80 was negative under certain conditions. Since hydrolysis of the Tween compound is evident only when calcium salts are formed with the fatty acids released, these investigators postulated that the lack of activity was due to either low concentrations of substrate or limiting amounts of calcium. Earlier studies of Delmotte (2) also showed this lack of reaction on Tween 80. Pospisil and Kabatova (4), however, reported lipolytic activity in *Candida* with Tween 80 as the substrate. They supplemented their media with additional $CaCl_2$ and used 10-fold higher concentrations of Tween 80 in their media. Since our medium was designed after that of Tirunarayanan and Lundbeck, which required only the addition of the substrate, it is not surprising that Tween 80 activity was absent in our results.

Even with the absence of activity on Tween

TABLE 1. *Esterase activity of selected strains of Candida*

Organism	No. of strains	No. of strains positive at 5 days on:				
		Tween 20	Tween 40	Tween 60	Tween 80	Tween 85
C. albicans	32	0	18	32	0	29
C. tropicalis	15	0	15	15	0	13
C. krusei	5	0	5	5	0	5
C. parapsilosis	5	4	4	5	0	5
C. stellatoidea	3	0	2	3	0	2
C. viswanathii	1	0	1	1	0	1
C. pseudotropicalis	1	0	0	0	0	1
C. guilliermondii	1	0	0	1	0	1

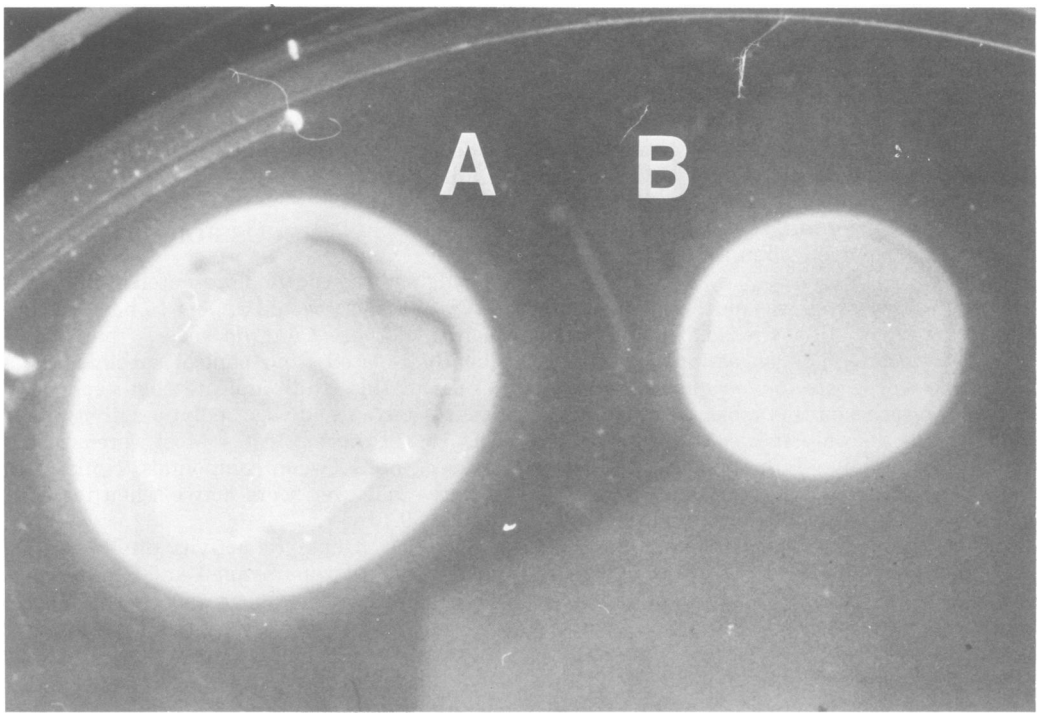

FIG. 1. *Typical zones of precipitation around colonies of Candida on Tween 40. (A) C. tropicalis; (B) C. krusei.*

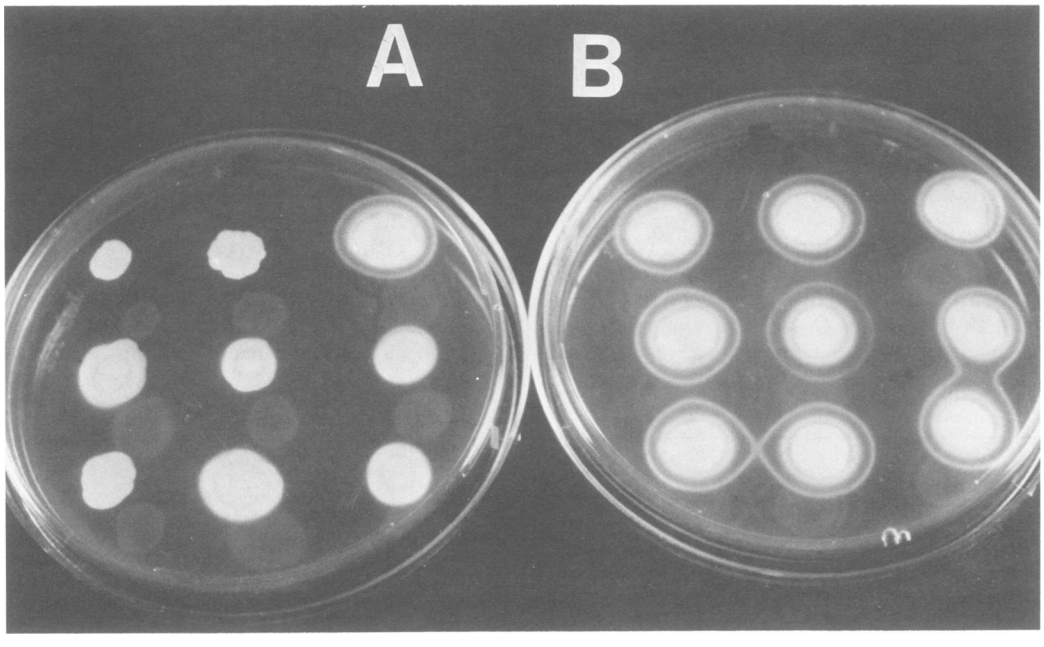

FIG. 2. *Differentiation of C. tropicalis from other species of Candida on Tween 60. (A) Single colony of C. tropicalis in upper right corner, showing double zones of precipitation; (B) plate with only colonies of C. tropicalis, all showing double zones of precipitation.*

80, the results of the remaining four Tween compounds showed patterns which could be useful for taxonomic purposes once the organism was identified as belonging to the genus *Candida*.

Within this study, *C. tropicalis* and *C. parapsilosis* could be easily distinguished from the other species by the patterns and zones of hydrolysis produced. *C. tropicalis* could be readily identified as the only species producing double zones of precipitation on Tween 60, and *C. parapsilosis* could be identified as the only species hydrolyzing Tween 20.

The ease in distinguishing *C. tropicalis*, especially from *C. albicans*, is of special interest. When incubated in horse serum, *C. tropicalis* produces beginning pseudohyphae that are difficult to distinguish from germ tubes of *C. albicans* (8). With the use of our modified-substrate medium, *C. tropicalis* would be easily identified by the double zones of precipitation around the colony.

The patterns of precipitation, however, failed to elucidate a pattern which could be utilized as an indicator of virulence. *C. albicans*, the most frequently isolated species, showed activity similar to or even less than some of the other species which are isolated less frequently. Since differences in the patterns of hydrolysis were evident for some species, such as *C. tropicalis*, current studies are directed toward the specificities of these esterases and their role in the ecology of *Candida*.

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