Esterase activity of *Candida* species isolated from immunocompromised hosts

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Summary

A total of 149 clinical isolates of *Candida* species isolated from immunocompromised patients were examined to ascertain their esterase activity by the Tween 80 opacity test, which is a biochemical test used mainly to differentiate between *Candida albicans* and *Candida dubliniensis*. Our results showed that *C. albicans* (92.3%), *Candida tropicalis* (92.3%), *Candida parapsilosis* (25%), *C. dubliniensis* (16.6%), *Candida inconspicua* (100%), and *Candida lipolytica* (100%) produced opacity halos through the 10-day post-inoculation period. The remaining *Candida* species did not produce a positive test response. These findings indicate that Tween 80 opacity test cannot be used as the sole phenotypic trait in the differentiation of *C. albicans* and *C. dubliniensis*.

Key words *Candida*, Esterase activity, Tween 80 opacity test, Immunocompromised

Actividad esterasa en especies de *Candida* procedentes de pacientes inmunodeficientes

Resumen

Se ha evaluado la actividad esterasa de 149 aislamientos clínicos de *Candida* procedentes de pacientes inmunodeficientes, mediante el test de opacidad del Tween 80, prueba bioquímica utilizada principalmente para diferenciar entre *Candida albicans* y *Candida dubliniensis*. Nuestros resultados muestran que *C. albicans* (92,3%), *Candida tropicalis* (92,3%), *Candida parapsilosis* (25%), *C. dubliniensis* (16,6%), *Candida inconspicua* (100%) y *Candida lipolytica* (100%) produjeron halos de opacidad durante un periodo de 10 días post-inoculación. Por el contrario, el resto de las especies de *Candida* no produjeron una respuesta positiva. Estos hallazgos indican que el test de opacidad del Tween 80 no puede ser utilizado como el único rasgo fenotípico para diferenciar *C. albicans* y *C. dubliniensis*.

Palabras clave *Candida*, Actividad esterasa, Test de opacidad con Tween 80, Inmunodeficiencia

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Infections caused by *Candida* species, especially *Candida albicans*, ranging from mucosal diseases, usually observed in AIDS patients, to fatal systemic episodes in severely neutropenic subjects, have increased worldwide [3,10]. The wide spectrum of candidiasis and its importance in clinical settings has stimulated interest in the identification of these yeasts, and their mechanisms of pathogenicity.

Candida dubliniensis is considered to be an emerging opportunistic pathogen, closely related to *C. albicans* and sharing many phenotypic and genotypic characteristics, which may lead to misidentification [2,8]. Various phenotypic tests have been reported that enable differentiation between the two *Candida* species, one of which is the Tween 80 opacity test [6]. The objective of the present study was to ascertain the patterns of esterase activity of clinical isolates of *Candida* species and the applicability of the Tween 80 opacity test in differentiating between *C. albicans* and *C. dubliniensis*.

A total of 149 isolates of *Candida* maintained in our laboratory stock collection that comprised of 37 isolates from HIV-seropositive patients and 89 from patients with malignancies, 18 reference strains of *C. dubliniensis* and five reference strains of *C. albicans* were examined (Table 1).

All isolates were speciated based on the conventional scheme employed in our laboratory that consisted of germ tube production in pooled human serum, chlamydospore production on corn meal Tween 80 agar, carbohydrate fermentation and assimilation tests [11]. The cultures were maintained on Sabouraud dextrose agar (SDA) with chloramphenicol (50 µg/ml) and stored at 4 °C until they were examined.

The Tween 80 opacity medium [6] was prepared with 10 g peptone, 5 g NaCl, 0.1 g $CaCl_2$ 15 g agar and 1000 ml distilled water, with pH adjusted to 6.8. All ingredients were purchased from Hi Media (Mumbai, India) except $CaCl_2$, which was obtained from Sigma (St. Louis, USA). The medium was autoclaved at 121 °C for 15 min, allowed to cool to about 50 °C, mixed with 5 ml of pre-autoclaved and cooled Tween 80 (Hi Media), and dispensed into sterile 90 mm-diameter petri dishes (25 ml of agar per plate).

A loopfull of overnight culture of each *Candida* isolate grown on SDA was transferred to the Tween 80 opacity medium and spread over a circular inoculation site of approximately 10 mm diameter. The inoculated agar plates were incubated aerobically at 35 °C and were examined daily for up to 10 days. All strains were assayed in duplicate. Detection of esterase activity on the test plates was performed by observing halos of precipitation around the inocula under transmitted light.

C. albicans isolates that failed to produce opacity halos were presumptively identified as *C. dubliniensis*. Confirmation of these strains was done by studying the colony morphology on Staib's agar [8] and genotyping by the 25S rRNA gene (rDNA) [9].

Sixty of the 65 *C. albicans* (92.3%), 36 of the 39 *Candida tropicalis* (92.3%), three of the 18 *C. dubliniensis* (16.6%), one of the four *Candida parapsilosis* (25%) and one isolate each of *Candida inconspicua* and *Candida lipolytica* produced precipitation halos on the Tween 80 opacity medium around their inoculation sites. Eleven *C. albicans* (16.9%) and one *C. parapsilosis* (25%) yielded halos only four days after inoculation whereas the remaining esterase positive strains demonstrated activity in 2-3 days.

Fifteen of the 18 *C. dubliniensis* (83.3%), three of the four *C. parapsilosis* (75%) and all isolates of *Candida krusei*, *Candida glabrata*, *Candida guillermondii* and *Candida kefyr* failed to produce esterase activity on the Tween 80 opacity medium till the 10th day post-inoculation (Table 2). A distinctive pattern of double halo of precipitation was produced by strains of *C. tropicalis* (82.1%), *C. albicans* (75.4%) and *C. dubliniensis* (16.6%).

Previous studies have used Tween 80 opacity test for detection of lipolytic activity among various bacteria and fungi [5,6]. This test has also been reported to aid identification of dermatophytes and *Candida* [1,4,6,7]. The hydrolysis of the Tween compound is mediated by the lipolytic enzymes elaborated by the respective *Candida* species. Subsequent to cleavage of ester bonds, the released fatty acids bind with the calcium ions incorporated in the medium and form insoluble calcium complexes [4]. These complexes result in the characteristic precipitation halo seen around the colony. Esterase activity has also been demonstrated on calcium salt free medium containing Tween 40 or 60 [4].

In the present study, five out of 65 *C. albicans* (7.7%) and three out of 39 *C. tropicalis* (7.7%) isolates failed to produce esterase, contrary to previous reports of 100% esterase activity between these two *Candida* species [1,6]. Production of double halo of precipitation by *C. tropicalis* was reported in an earlier study to differentiate it

Table 1. Source of the reference *Candida* isolates used in the study.

C. dubliniensis (n=18)	
CD 36, CD 514, CD 519, I 47, Man 448, CM 1	DJ Sullivan School of Dental Science and Dublin Dental Hospital, Trinity College, University of Dublin. Ireland.
NRRL Y-17512, NRRL Y-17969, NRRL Y-17971	CP Kurtzman Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Peoria, USA.
Wü284, Wü294, Wü361	Joachim Morschhäuser Zentrum für Infektionsforschung, Institut für Molekulare Infektions biologie der Universität Würzburg, Germany.
IFM 49829	Yuzuru Mikami Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan.
ZW677, ZW678, ZW679, ZW680, ZW681	Wang Yue Microbial Collection and Screening Laboratory, Institute of Molecular and Cell Biology, Singapore.
C. albicans (n=5)	
IFM 40213 (ATCC 90028), IFM 40214 (ATCC 90029), IFM 52112, IFM 49826	Yuzuru Mikami Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan.
ATCC 10321	Mycology laboratory, Department of Microbiology, Dr ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, India.

Table 2. Esterase activity of various *Candida* species on Tween 80 opacity medium.

Species	No of isolates tested	No of isolates positive for the following time period (days)			No of isolates negative
		1-3	4-7	8-10	
C. albicans	65	49	11	–	5
C. tropicalis	39	36	–	–	3
C. dubliniensis	18	3	–	–	15
C. krusei	12	–	–	–	12
C. glabrata	7	–	–	–	7
C. parapsilosis	4	–	1	–	3
C. guillermondii	1	–	–	–	1
C. inconspicua	1	1	–	–	–
C. lipolytica	1	1	–	–	–
C. kefyr	1	–	–	–	1

from *C. albicans* on a medium containing Tween 60 [4]. In the present study, double halos were produced by either species on the Tween 80 agar.

An earlier study by Slifkin [6] showed Tween 80 opacity test to be an excellent means of differentiation of *C. albicans* and *C. dubliniensis*. In spite of the clear differentiation of these two yeasts on Tween 80 medium, the author recommended its use only as an adjunct to standard morphological and physiological tests. In the present study, three of the 18 reference strains of *C. dubliniensis* tested produced opacity halos. Likewise, five of the 65 *C. albicans* tested were found to be negative for esterase activity and were presumptively identified as *C. dubliniensis.* Nevertheless, these isolates were subsequently confirmed to be *C. albicans* by Staib's agar morphology and genotyping (data not shown). Thus, the findings of the present study were contrary to the observations of Slifkin [6], raising doubts over the utility of the test in the identification of *C. dubliniensis.* The disagreement observed in this study, regarding the esterase activity among *C. dubliniensis* and the previous report by Slifkin [6], could be attributed to the fact that major studies on this matter are yet to be performed. Perhaps centers with a larger repository of *C. dubliniensis* isolates could undertake an evaluation of these isolates using the esterase activity to validate the utility of this assay in identification of *C. dubliniensis.*

Although the Tween 80 opacity test appears to be a simple, economical and easy to perform method for use in smaller clinical laboratories, the finding of this study indicates that esterase activity cannot be used as a phenotypic trait for the proper identification of *C. albicans* and *C. dubliniensis*.

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