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Discrimination between *Candida albicans* and Other Pathogenic Species of the Genus *Candida* by Their Differential Sensitivities to Toxins of a Panel of Killer Yeasts

P. BUZZINI* AND A. MARTINI

Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Sezione di Microbiologia Applicata, University of Perugia, 06100 Perugia, Italy

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The differential sensitivities to toxins produced by a short panel of four killer yeasts allowed discrimination between 91 strains of the yeast *Candida albicans* and 223 non-*C. albicans Candida* strains. One hundred percent of *C. albicans* isolates exhibited negative results to the toxin panel, while 100% of non-*C. albicans* cultures gave well-defined and reproducible positive results to at least one of the four killer toxins. Among *C. albicans* strains only 96 and 87% gave germ tube (GT)- and chlamydospore-positive results, respectively. In addition a few GT-false-positive strains were detected among non-*C. albicans* isolates. Susceptibility to the toxin panel is apparently expressed more consistently than either GT or chlamydospore production and may constitute a promising basis for a new simple and easy-to-use procedure for routine discrimination between the species *C. albicans* and other species of the genus *Candida*.

The observation of germ tube (GT) production as a method for the presumptive identification of *Candida albicans* isolates from clinical samples has been used successfully for many years (1, 7). Recent investigations have reported that more than 5% of *C. albicans* strains may be GT negative (7), while false-positive results can occur with other species of the genus (13). As a consequence, in order to reach an unequivocal identification of *C. albicans*, additional morphological and physiological tests may be required (1). Several recently developed alternative diagnostic tests, such as chromogenic agar media or commercial rapid identification kits, are considered too expensive for routine use and/or are seemingly characterized by often inconsistent results (2, 6–8, 10, 11, 13).

Because of the recent success of using killer systems for inter- and intraspecific discrimination of ascomycetous and basidiomycetous yeasts (4, 5, 9, 12, 14), we evaluated a killer toxin panel by using 314 members of the genus *Candida*, including 91 isolates of *C. albicans* and 223 isolates of non-*C. albicans* species.

Four killer yeast strains, all previously demonstrated to possess cureless killer phenotypes (3, 4), were used to create a short panel to detect *C. albicans*; these strains were *Candida maltosa* G7A, *Debaryomyces hansenii* P41, and *Williopsis saturnus* DBVPG 3127 and DBVPG 3671, indicated in Table 1 by the index numbers 1, 2, 3, and 4, respectively.

Yeasts used as sensitive strains were as follows (percentages indicated are those isolated from clinical sources): 42 strains of *C. albicans* (46%), 28 strains of *Candida glabrata* (74%), 26 strains of *Candida guilliermondii* (imperfect state of *Pichia guilliermondii*) (88%), 5 strains of *Candida inconspicua* (40%), 53 strains of *Candida kefyr* (imperfect state of *Kluyveromyces marxianus*) (62%), 41 strains of *Candida krusei* (imperfect state of *Issatchenkia orientalis*) (78%), 3 strains of *Candida norvegensis* (imperfect state of *Pichia norvegensis*) (33%), 26 strains of *Candida parapsilosis* (50%), 13 strains of *Candida tropicalis* (92%), and 18 strains of *Candida zeylanoides* (78%). All strains listed above came from the Industrial Yeast Collection of the Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali of Perugia. In addition, 49 strains of *C. albicans* (54%) and 10 strains of *C. glabrata* (26%) came directly from clinical specimens of local laboratory hospitals, and each species was represented by well-characterized type strains from reference collections. Both collected and newly isolated sensitive cultures were previously controlled through reisolation procedures to avoid the presence of mixed cultures and were classified in our laboratory by conventional identification methods (16). In addition, all sensitive strains were tested for GT (15) production.

YEPG (yeast extract, 10 g/liter; peptone, 10 g/liter; glucose, 20 g/liter; Difco agar, 20 g/liter) agar slants were used to store cultures at 4°C. Killer medium (KM) (yeast extract, 10 g/liter; peptone, 20 g/liter; glucose, 20 g/liter; methylene blue, 0.03 g/liter; Difco agar, 20 g/liter [buffered at pH 4.5 with citrate-phosphate buffer]) was used to test killer activity. Sensitive strains (24-h cells) were suspended in sterile distilled water to obtain about 10^5 cells/ml. One milliliter of suspension was mixed thoroughly with 19 ml of molten KM agar and poured into a petri plate (100 by 15 mm). Killer yeasts (24-h cells) were inoculated onto the solidified agar surface (3, 4), and plates were incubated at 25°C.

A clear inhibition zone appearing within 48 h at 25°C around the killer strain colony surrounded by a crown (halo) of dark-blue-stained cells was considered to be a positive indication of the presence of killer activity. No discrepant results were encountered in repeated experiments.

Similar to previous reports (4, 5), the differential killer toxin sensitivity patterns were condensed into a "killer sensitivity index," where each one of the four yeasts capable of inhibiting

* Corresponding author. Mailing address: Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Sezione di Microbiologia Applicata, University of Perugia, 06100 Perugia, Italy. Phone: 39 075 585 6455. Fax: 39 075 585 6470. E-mail: pbuzzini@unipg.it.

TABLE 1. Killer sensitivity indices produced by four killer toxins on strains from species of *C. albicans* and non-*C. albicans* species

Group	Species	No. of strains	Killer sensitivity index[a]				No. of strains positive for:	
							GT	CF[b]
1	*C. albicans*	91	0	0	0	0	87	79
2a	*C. glabrata*	32	0	2	3	4	1	0
2b	*C. glabrata*	4	0	2	0	0	0	0
2c	*C. glabrata*	2	0	2	3	0	0	0
3a	*C. guilliermondii*	9	0	2	0	0	0	0
3b	*C. guilliermondii*	8	1	2	3	4	0	0
3c	*C. guilliermondii*	5	0	0	3	4	0	0
3d	*C. guilliermondii*	4	0	2	3	4	0	0
4	*C. inconspicua*	5	0	2	3	4	0	0
5a	*C. kefyr*	1	1	2	3	4	0	0
5b	*C. kefyr*	50	0	2	3	4	1	0
5c	*C. kefyr*	1	0	2	0	0	0	0
5d	*C. kefyr*	1	0	0	3	4	0	0
6a	*C. krusei*	29	0	2	3	4	1	0
6b	*C. krusei*	5	1	2	3	4	0	0
6c	*C. krusei*	3	0	2	0	0	0	0
6d	*C. krusei*	2	0	0	3	4	0	0
6e	*C. krusei*	1	0	2	3	0	0	0
6f	*C. krusei*	1	0	2	0	4	0	0
7	*C. norvegensis*	3	0	2	3	4	0	0
8a	*C. parapsilosis*	16	0	2	0	0	1	0
8b	*C. parapsilosis*	3	0	2	3	4	0	0
8c	*C. parapsilosis*	3	0	0	3	0	0	0
8d	*C. parapsilosis*	3	0	0	0	4	0	0
8e	*C. parapsilosis*	1	1	2	3	4	0	0
9	*C. tropicalis*	13	1	0	0	0	1	0
10a	*C. zeylanoides*	10	1	2	3	4	0	0
10b	*C. zeylanoides*	5	0	0	3	4	0	0
10c	*C. zeylanoides*	2	0	2	3	0	0	0
10d	*C. zeylanoides*	1	0	2	0	0	0	0

[a] Numbers 1 to 4 indicate sensitivity to killer strains 1 to 4, respectively (see text). Lack of sensitivity is indicated by 0.

[b] CF, chlamydospore formation (number of strains forming chlamydospore on corn meal agar).

the growth of a given strain was indicated by its reference number, while absence of activity was recorded as 0.

The killer yeast panel expressed a wide-spectrum killing activity against non-*C. albicans* species (Table 1). All non-*C. albicans* cultures gave well-defined and reproducible positive results to at least one of the four killer toxins. Because of their resistance to all members of the panel, all 91 *C. albicans* strains could be clearly discriminated from the remaining strains (Table 1).

Eight killer sensitivity indices were observed in non-*C. albicans* strains, including one index monospecific for *C. tropicalis* (Table 1) and seven aspecific indices casually distributed in strains of eight species: *C. glabrata* (groups 2a to 2c), *C. guilliermondii* (groups 3a to 3d), *C. inconspicua* (group 4), *C. kefyr* (groups 5a to 5d), *C. krusei* (groups 6a to 6f), *C. norvegensis* (group 7), *C. parapsilosis* (groups 8a to 8e), and *C. zeylanoides* (groups 10a to 10d) (Table 1).

Of the 91 *C. albicans* strains tested, 96% were GT positive whereas a few *C. glabrata*, *C. kefyr*, *C. krusei*, *C. parapsilosis,* and *C. tropicalis* isolates were GT false positive (Table 1). Only 87% of *C. albicans* isolates produced chlamydospore on corn meal agar (Table 1).

The cost of the above killer yeast method (approximately $0.40/identification, calculated on the basis of the list price of all medium ingredients and supplies used) was on the same order as that calculated for our in-house-made GT test (approximately $0.55/identification), but the efficiency of the presumptive identification was considerably improved (Table 1). Compared with data obtained by the use of several commercial identification kits, as reported in the literature (7, 8, 10, 11, 13), the identification efficiency of the killer yeast method was also on the same order, but at a significantly lower cost per test.

As previously demonstrated (4, 5), the method proposed here is extremely sensitive and accurate, the interpretation of results is generally easy and unequivocal, and the method is definitely less subjective than the GT assay (7) and is applicable in the laboratory by technicians untrained in mycology and lacking skill in microscopy. In order to reduce the 48-h incubation time, the procedure may be revised with only a reasonable increase in total cost. This could be done by producing crude toxin preparations in liquid medium and using them in agar diffusion well bioassays (5), whereby reproducible results are obtained after 24 h of incubation.

In the present study, all sensitive strains were identified by currently accepted morphological, physiological, and biochemical tests (16) and are well defined from a taxonomic viewpoint. Therefore, the resistance of *C. albicans* isolates to the actions of the killer toxin panel can be considered an objective basis on which to differentiate the isolates from those belonging to other species of the same genus. In addition, in agreement with previous reports (4, 5, 12), the presence of different clusters of non-*C. albicans* strains characterized by different sensitivity patterns may also represent an alternative approach in the identification of subspecific entities (Table 1).

The absence of discrepant results in repeated experiments seems to underline the absolute reproducibility of the above method.

Consequently, it is reasonable to assert that based on the above observations a novel, highly accurate, and easy-to-use test may be developed as a routine practice in clinical laboratories for preliminary discrimination between *C. albicans* and non-*C. albicans* isolates.

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