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Occurrence of killer yeasts in isolates of clinical origin

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Abstract. A total of 1 025 strains belonging to different *Candida* species of clinical origin were evaluated for their killer activity against sensitive strains of Saccharomyces *cerevisiae*. Isolates were identified by standard morphological and biochemical analyses. For the evaluation of the killer activity, potential killer isolates were streaked on plates previously seeded with the sensitive strain. A total of 52 *Candida* isolates (5%) exhibited killer activity against both sensitive yeast strains. The occurrence of the killer phenomenon was proportionally higher in isolates recovered from closed cavities. *Candida glabrata* was the species with the most occurrences of killer strains, but a bigger proportion of killer activity was observed in *Candida utilis*. Secretion of killer toxins could represent at least partially, an advantage against other candida and non-*Candida* strains in the colonization process, especially for uncommon *Candida* species.

INTRODUCTION

The killer phenomenon of yeasts was described for the first time in 1963 by Bevan & Makover in strains of *Saccharomyces cerevisiae*. Since then, many different yeast species have been reported to show the killer phenomenon and that number increases continuously. The killer toxins, known also as mycocins, are of proteinaceous nature and are not only active against other yeasts but also against filamentous fungi, for which they have been suggested as a tool for biocontrol strategies (Santos *et al*., 2004; Bleve *et al*., 2005; de Souza *et al*., 2009). Killer yeasts are commonly isolated from natural occurring communities (Buzzini & Martini, 2000), thus, their ecological role as a community preservation mechanism has been discussed previously (Starmer *et al*., 1987). In the present study, we investigated the presence of the killer phenomenon in a collection of 1025 isolates of *Candida* spp. strains of diverse clinical origin using 2 reference *S. cerevisiae* susceptible strains.

MATERIALS AND METHODS

Strains were isolated from patients at Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, in Monterrey, México.

A total of 1 025 strains belonging to different *Candida* species (*Candida albicans*, *Candida boidinii*, *Candida famata*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida rugosa*, *Candida* sp., *Candida tropicalis*, *Candida utilis*, *Candida zeylanoides*) were tested as potential killer yeasts. Although some of the strains are not commonly considered as medically important, all strains used in this study have a clinical origin (blood, sputum, vaginal exudates, semen,

faeces, nail scrapings and skin scrapings, urine, nasal sinuses, and closed cavities).

The isolates were identified by standard morphological and biochemical analyses. Two reference strains of *S. cerevisiae* were used as susceptibility targets (ATCC 26609 and ATCC 38527).

The investigation of killer activity was performed using YEPD-MB agar (0.3% Yeast extract, 0.3% Malt extract, 0.5% Peptone, 1%-2% Glucose, Agar and 0.003% methylene blue, buffered to pH 4.5 with 0.1 M citrate-phosphate buffer). 24-hour old cultures of susceptible strains were resuspended in sterile saline solution and adjusted to a density of $1x10^6$ cells/ml. From the adjusted inocula, 400 µl were poured into YEPD-MB Petri dishes respectively and spread as a uniform lawn. Overnight cultures of the potential killer strains were inoculated over the susceptible lawn and plates were incubated at 25ºC for up to 72h. Killer activity was considered as positive when a clear inhibition zone surrounding the potential killer strain appeared and a zone of dark blue stained cells was visible. If a zone of inhibition was present without blue stained cells, the interaction was considered as antagonism and not reported as killer activity.

RESULTS

A total of 52 *Candida* isolates exhibited killer activity against both *S. cerevisiae* strains (Table 1), although the strain ATCC 26609 showed higher susceptibilities (*i.e.* bigger inhibition zones, data not shown) than ATCC 38527 strain. While most of the killer strains were isolated from blood cultures, the number of killer yeasts isolated from closed cavities was proportionally the biggest. Blood culture isolates represented the broadest number of species with killer activity. *Candida glabrata* was the species with the most occurrences of killer strains, but a bigger proportion of killer activity occurrence was observed in *C. utilis* (1 positive out of 4 analyzed).

Since the largest amount of isolates came from blood cultures, we compared the proportions of killer activity in each of the four most abundant species obtained from blood (*C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*) against each other as independent groups using the Z-test (confidence level 95%). *Candida glabrata* resulted in significant difference compared to any of the other groups, while none of the other combinations was statistically different.

Tabla 1. Killer activity against *S. cerevisiae* strains

Candida species	Isolate origin (No. of isolates)							No. of isolates with killer activity
	Blood culture	Closed cavities	Urine	Skin and mucous membranes	Vaginal exudates	Others	**Total**	
Candida albicans	172	48	53	63	22	22	380	7
Candida parapsilosis	202	18	2	37	0	7	266	7
Candida glabrata	44	22	62	2	21	10	161	28
Candida tropicalis	94	22	20	1	2	9	148	6
Candida krusei	12	3	1	2	0	1	19	
Candida zeylanoides	2	1	0	2	0	1	6	
Candida utilis	3	0	0	1	0	0	4	1
Candida guilliermondii	7	0	1	5	0	0	13	2
Candida boidinii	1	0	0	0	0	0	1	
Candida famata	4	1	2	0	0	1	8	1
Candida rugosa	2	0	0	0	0	0	2	
Candida lusitaniae	2	0	1	0	0	0	3	
Candida mangoliae	0	0	0	1	0	0	1	
Candida sp.	0	0	7	6	0	1	13	
TOTAL	**545**	**115**	**149**	**120**	**45**	**52**	**1025**	**52**

DISCUSSION

Many reports have investigated the killer phenomenon in pathogenic yeasts from a susceptibility perspective (Walker *et al*., 1995; Fuentefria *et al*., 2006; Vadkertiová & Sláviková, 2007). In this report, we investigated the opposite *i.e.* the killer potential of pathogenic and not strictly pathogenic yeasts of clinical origin. While previous reports have described a similar approach (Kandel & Stern, 1979; Baeza *et al*., 2008), this is to the best of our knowledge, the study with the largest strain number and also the broadest amount of *Candida* species to be analyzed for their killer potential. A couple of observations are noteworthy: the killer phenotype of *C. utilis* has been reported recently by Antunes & Aguiar, who described a broad killer spectrum for this species against *Candida* and non-*Candida* species (Antunes & Aguiar, 2011). Secondly, the occurrence of the killer phenotype in *C. glabrata* is remarkable. While this species is well known as a killer yeast, we could not find any reports describing such high incidence as that observed in the present study. The hypothesis of the killer phenotype as a virulence factor, favouring the killer yeast in the colonization of the host, has been investigated before in *Pichia anomala* and *C. albicans* mixed cultures (Conti *et al*., 1996). The authors' findings suggested that for *P. anomala*, the killer factor was not likely to represent an advantage against *C. albicans* in physiological conditions. Nevertheless, Buzzini & Martini more recently reported the results of a large screening of various killer yeasts (including one of *P. anomala*) against pathogenic strains at human body temperature, demonstrating the availability of killer toxins that exhibit stable activity at this less strict condition (Buzzini & Martini, 2001). We consider that this observation together with the level of occurrence reported in our study, suggests that the killer factor could represent at least partially, an advantage against other candida and non-*Candida* strains in the colonization process.

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