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Killer Activity of Yeasts Isolated from Natural Environments against Some Medically Important *Candida* Species

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Abstract

Twenty-five yeast cultures, mainly of human origin, belonging to four pathogenic yeast species – *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* were tested for their sensitivity to ten basidiomycetous and eleven ascomycetous yeast species isolated from the water and soil environments and from tree leaves. The best killer activity among basidiomycetous species was exhibited by *Rhodotorula glutinis*, and *R. mucilaginosa*. The other carotenoid producing species *Cystofilobasidium capitatum*, *Sporobolomyces salmonicolor*, and *S. roseus* were active only against about 40% of the tested strains and exhibited weak activity. The broadest killer activity among ascomycetous yeasts was shown by the strains *Pichia anomala* and *Metschnikowia pulcherrima*. The species *Debaryomyces castellii*, *Debaryomyces hansenii*, *Hanseniaspora guilliermondii*, *Pichia membranifaciens*, and *Williopsis californica* did not show any killer activity. The best killer activity exhibited the strains isolated from leafy material. The lowest activity pattern was found among strains originating from soil environment.

Key words: *Candida* species, killer activity, yeasts

Introduction

Yeasts are known to secrete proteinaceous mycocins (killer toxins) lethal to susceptible yeasts and fungi (Golubev, 1998), but antibacterial activity of some yeast strains was also demonstrated (de Oliva Neto *et al*., 2004). The killer phenomenon is widely distributed among yeast strains – killer activity has been reported in almost 100 yeast species belonging to more than 20 genera and their number is increasing (Golubev, 1998; Buzzini and Martini, 2001). Killer toxins differ between species or strains, showing the diverse characteristics in terms of structural genes, molecular size, mature structure and immunity (Marquina *et al*., 2002).

Killer yeasts have been of technological importance. In the wine industry the interest is focused on the definition of killer yeasts in mixed cultures to determine which yeasts will prevail in fermentation process (Pasqual *et al.*, 1990); the use of the killer yeast with positive enological characteristics as a starter culture was discussed by Zagorc *et al.* (2001), and the activity of killer toxin against wild yeasts which predominates on grape surfaces and in freshly pressed juice was studied by Ciani and Fatichenti (2001).

Some yeasts demonstrated a potential as biological control agents against plant-pathogenic fungi. A natural inhabitant of the phyllosphere *Pseudozyma flocculosa* was found to produce unusual extracellular fatty acids with detrimental effect to powdery mildews (Avis and Bélanger, 2002), whereas *Pichia membranifaciens* might have the potential to control *Botrytis cinerea*, which causes the gray mold disease (Santos *et al.*, 2004).

The killer toxin producing yeasts has also a clinical significance due to the search for new antimycotic agents against medically important strains. Polonelli *et al.* (1983) developed the method for typing of *Candida albicans* isolates based on susceptibility to the killer-toxin produced by some species of the genus *Pichia* (*Hansenula*). The killer toxin activity of *Pichia anomala* was reported to be fungistatic for *Candida albicans* by Sawant and Ahearn (1990) and Theisen *et al.*, 2000. Buzzini and Martini (2001), who screened the killer activity of selected *Candida maltosa*, *Debaryomyces hansenii* and *Pichia anomala* strains against pathogenic yeasts, found the combinations of

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killer toxins to be more effective mainly against *Candida* strains than the certain killer toxin alone.

The aim of this work was to study the killer activity of ascomycetous and basidiomycetous yeast strains isolated from water, soil, and leafy materials against some medically important *Candida* species as well as to find if the strains originating from these environments have different or similar killer activity patterns.

Experimental

Materials and Methods

Reference strains for testing killer activity. Twenty-five yeast cultures, mainly of human origin, belonging to four pathogenic yeast species *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis,* maintained in the Culture Collection of Yeasts (CCY) in Slovakia were tested as sensitive strains (Table I).

Strains tested for killer activity. Fourty four strains isolated from the water environment (fresh-water lakes and rivers), soil environment (forest, grassy, and tilled soils) and from plant material were screened for their killer activity (Table II).

Medium and killer activity assay. The medium used was YEPD-agar supplemented with methylene blue (0.3% yeast extract, 1% peptone, 0.5% glucose, 2% agar, and 0.003% methylene blue, pH buffered to 4.5 with 0.1 M citrate-phosphate buffer). The killer activity was assayed by the methods described previously (Starmer *et al*., 1987; Vadkertiová and Sláviková, 1995; Buzzini and Martini, 2000). Strains tested for their sensitivity (approximately 10^5 cells/plate) were added into the assay medium. Potential killer yeasts were grown on agar slants for 48 h and spread onto the plates, which were then incubated at 28°C for 5 days and checked daily. If the inoculated strain was surrounded by bluish coloured cells and a clear zone <1 mm, or only surrounded by a blue zone, the reaction was recorded as "w" (weak killer reaction); if the inoculated strain was surrounded by bluish coloured cells and a clear zone ≥ 1 mm, it was designated as + (positive killer reaction).

Results

Ten basidiomycetous and eleven ascomycetous yeast species were tested for their killer activity against four yeast species – *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*.

Table I
Reference strains for testing killer activity

Species	CCY number	Origin
Candida albicans	29-3-19	bronchomycosis
	29-3-32	vaginal mycosis
	29-3-100	Hasenclever 207 II-8, serotype A
	29-3-101	Hasenclever B 311 II-15, serotype A
	29-3-102	Hasenclever 526 II-15, serotype B
	29-3-103	Hasenclever 192 II-8, serotype B
	29-3-108	infected nail
	29-3-159	Novak, Hungary *erg-, nysR, ade- = erg-*
	29-3-161	sediments of fresh-water lake
	29-31-2	CBS
	29-31-3	pulmonary mycosis
	29-64-1	Tsuchiya, Japan, serotype B
	29-64-3	Hasenclever c.s.2864 II-8, serotype B
	29-64-4	Hasenclever Y-244 II-8, serotype B
Candida krusei	29-9-13	vaginal mycosis
	29-9-34	Danube river water
	29-9-38	sputum
Candida parapsilosis	29-20-6	skin mycosis
	29-20-13	case of sprue
	29-20-18	sputum of bat
	29-20-20	soil, Brazil
Candida tropicalis	29-7-10	vaginal mycosis
	29-7-48	activated sludge
	29-7-58	Morava river water

Table II
The origin and killer activity of individual strains

Species	Strain No	Origin	% of sensitive strains*
Candida lambica	CCY 29-97-7	fresh-water lake	4
Candida maltosa	1_1	tilled soil	8
Cryptococcus albidus	32’	tilled soil	60
	19	tree leaves	52
	29_2	tree leaves	80
Cryptococcus laurentii	CCY 17-3-26	grassy soil	8
	2_3	tilled soil	32
	21a	tree leaves	40
	29	tree leaves	52
Cystofilobasidium capitatum	38	tilled soil	40
Debaryomyces castellii	CCY 41-9-8	forest soil	0
Debaryomyces hansenii	CCY 41-6-14	grassy soil	0
Hanseniaspora guilliermondii	CCY 25-9-4	forest soil	0
Hanseniaspora uvarum	CCY 46-3-15	sediments of fresh-water lake	24
Metschnikowia pulcherrima	12_1	tilled soil	48
	1c	tree leaves	56
	36c	tree leaves	72
Pichia americana	CCY 38-19-4	forest soil	8
Pichia anomala	CCY 38-1-21	fresh-water lake	0
	CCY 38-1-30	river water	0
	4b	tree leaves	84
	6a	tree leaves	76
	16d	tree leaves	68
	28a	tree leaves	52
Pichia membranifaciens	21_1	tilled soil	4
Pseudozyma aphidis	28b	tree leaves	40
Pseudozyma floculosa	40_2	tree leaves	20
Rhodotorula glutinis	CCY 20-2-1	soil	100
	CCY 20-2-8	plants	100
	CCY 20-2-24	sediments of fresh-water lake	100
	3	tree leaves	100
	16b	tree leaves	96
	38_1	tree leaves	96
	40a	tree leaves	92
Rhodotorula graminis	CCY 20-9-6	forest soil	100
	CCY 20-7-9	plant material	92
Rhodotorula mucilaginosa	CCY 20-7-27	sediments of fresh-water lake	100
	6	tree leaves	96
Sporobolomyces salmonicolor	CCY 19-4-16	forest soil	40
	1_5’	tilled soil	40
	50_3	tilled soil	28
Sporobolomyces roseus	11_2	tree leaves	4
	11_6	tree leaves	32
Williopsis californica	CCY 38-6-9	river water	0

* The number says how many % of the strains from the panel of tested strains were sensitive to the killer activity of an individual strain

The best killer activity was observed among *Rhodotorula* species, which were active against almost all tested strains. Two strains isolated from sediments of fresh-water lake *R. glutinis* CCY 20-2-24 and *R. mucilaginosa* CCY 20-7-27 gave the positive killer reaction against all tested strains.

Table III
The killer activity of ascomycetous and basidiomycetous yeast species

Killer species	Sensitive species			
	C. albicans (14)	*C. krusei* (3)	*C. tropicalis* (3)	*C. parapsilosis* (5)
C. maltosa (1)	#1→1	1→1		
C. albidus (3)	3→11	2→2	1→1	2→4
C. laurentii (4)	3→10	2→1		3→4
C. capitatum (1)	w 1→7	1→1		w 1→2
H. uvarum (1)	w 1→3	1→2		
M. pulcherrima (3)	3→12	2→1		3→3
P. anomala (6)	4→12	3→2	3→2	4→5
P. aphidis (1)	1→8	1→1		1→2
P. floculosa (1)	1→2	1→2		
R. glutinis (7)	6→13	7→2	7→3	7→5
R. graminis (1)	1→13	1→3	1→3	w 1→5
R. mucilaginosa (3)	3→13	2→3	3→2	2→3
S. salmonicolor (3)	w 2→5	3→2	w 2→1	w 3→3
S. roseus (2)	1→8	1→3		

Values in the table are the ratio of the number of killer strains to the number of sensitive strains;
The number of tested strains is given in parentheses; Blank – no killer reaction;
w – weak reaction of tested strains

The other carotenoid producing species *C. capitatum*, *S. salmonicolor*, and *S. roseus* showed the less activity than *Rhodotorula* strains. They were active only against about 40 % of the tested strains and demonstrated weak activity (Table II).

Among *Cryptococcus* species three strains of both *C. laurentii* and *C. albidus*, isolated from grassy and tilled soil, were active against the strain *C. krusei* isolated from the river Danube. The only *C. albidus* strain, isolated from tree leaves, showed activity against the strain *C. tropicalis* originating from vaginal mycosis. All strains of *C. tropicalis* were resistant to the *C. laurentii* strains.

The strains of species *C. tropicalis* were also resistant to the activity of *Pseudozyma* species (Table III).

The broadest killer activity among ascomycetous yeasts was shown by the strains *P. anomala* isolated from tree leaves. They were active against all tested species. The strains of *M. pulcherrima* were also active against the majority of tested strains (Tables II and III).

The strain *H. uvarum* showed the killer activity against strains of *C. krusei* isolated from sputum and Danube river water respectively, but no activity to the strain originating from vaginal mycosis.

The highest sensitivity was demonstrated by the strain *C. krusei* CCY 29-9-34, isolated from river water. On the other hand *C. tropicalis* was the most resistant species among all the tested species (Tables II, III).

The species C. lambica, *D. castellii*, *D. hansenii*, *H. guilliermondii*, *P. americana*, *P. membranifaciens*, and *W. californica* did not show any killer activity.

Discussion

Among 44 yeast strains of natural origin tested for their killer activity, only 7 strains belonging to 6 species did not have it (Table II).

The broadest killer spectrum was shown by the strains of *Rhodotorula* species. They demonstrated killer activity against almost all pathogenic strains. Some exceptions were found among the strains of *R. mucilaginosa*; three strains were not active against two strains *C. albicans* and one strain of *C. parapsilosis*. This is in agreement with our previous work, in which the strains of the genus *Rhodotorula* were active against the majority of the tested strains originating from fresh-water lakes (Vadkertiová and Sláviková 1995). No activity patterns of basidiomycetous species were found by Polonelli *et al.* (1986) and only weak inhibition activities were reported by Buzzini and Martini (2000).

In contrast to Golubev and Kuznetsova (1989) and our previous report (Vadkertiová and Sláviková, 1995), the strains of *C. laurentii* showed killer activity against some *Candida* strains. This is in agreement with Buzzini and Martini (2000) who found the killer activity among strains of *C. laurentii* isolated from Brazilian rain forest, directed against *C. tropicalis* and *C. parapsilosis*. We observed the activity against *C. krusei*, *C. parapsilosis*, and *C. albicans* (Tables II and III).

Two strains of the basidiomycetous genus *Pseudozyma*, isolated from tree leaves, showed only narrow

action spectra against *Candida* species (Table III), which is in agreement with Buzzini and Martini (2000).

The killer potential of *P.* (formerly *Hansenula*) *anomala* against *C. albicans* has been studied for a long period. Polonelli *et al.* (1983) reported that the strains of *H. anomala* were able to kill more than 97% of tested *C. albicans* strains. Abranches *et al.* (1998) isolated from fecal pellets of small mammals *P. anomala* strains that killed 58% of species and they were active against *C. albicans* of the same origin. Buzzini and Martini (2000) found killer activity in strains of *P. anomala* isolated in a Brazilian rain forest. We found no killer activity of strains *P. anomala* isolated from the water environment, but broad action spectra of strains isolated from tree leaves.

Similarly to *H. anomala*, *M. pulcherrima* had the broad killing patterns, too (Table III). Nguyen and Panon (1998) also reported a large spectrum of activity for *M. pulcherrima*. Its inhibitory effects extend to many unrelated species, including *C. albicans*. The "killer effect" of *M. pulcherrima* is not a classical one. The inhibition of yeast cells involves the production of pulcherrimic acid, which complexes iron.

The results of this study have shown that the majority of yeasts isolated from soil, water and leafy environment demonstrated killer activity against pathogenic yeasts. We have found that the best killer activity was shown by strains isolated from leafy material. Up to 80 % of these strains showed killer activity against more than 50 % of studied strains. On the other hand, the lowest activity pattern was found among strains originating from the soil environment (Table II).

The broadest spectrum activity was found among *Rhodotorula* strains – they were active against more than 90 % of the tested strains regardless of their origin. Therefore, further investigations related to killer activity against pathogenic yeasts could be focused on the yeasts of *Rhodotorula* species.

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