

SCREENING KILLER ACTIVITY OF SOME YEAST STRAINS ISOLATED FROM SLIME FLUXES OF TREES

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ABSTRACT

Killer activity of yeast strains viz., Y21-*Bullera pseudoalba*, Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae* (previously isolated from slime fluxes) was determined against 555 isolates of 89 yeast species belonging to 29 genera previously isolated from dairy products, flower's nectar, slime fluxes and soil. Y21-*Bullera pseudoalba* appeared as the most killer strain.

Key-words: Killer activity of yeast, *Bullera pseudoalba*, Y16-*Pichia anomala*, Y20-*Sporidiobolus ruineniae*.

INTRODUCTION

Certain yeast strains produce killer toxins that have ability to kill closely related yeast strains and the killer yeast itself has killer resistant phenotype (Bevan and Makower, 1963; Woods and Bevan, 1968; Bussey, 1972; Pfeiffer and Radler, 1982; Rosini, 1983; Spencer and Spencer, 1997). The killer toxins are proteins, active at low pH and are secreted in an inactive glycosylated form that once secreted to the cell plasma membrane, becomes cleaved (Zhu *et al.*, 1993; Young and Yagiu, 1978; Pfeiffer and Radler, 1982; Radler *et al.*, 1985). A portion of the toxin with the glycosylated site remains associated with the membrane and conveys immunity to the cell. The cleaved mature toxin is available to bind at the sites located on the cell wall and the plasma membrane of sensitive yeasts. The phenomenon of insensitivity towards killer toxins generally occurs at the cell wall level. Resistant yeasts lack receptors necessary for the formation of the link and thus for the action of the killer toxin (Marquina *et al.*, 2002; Golubev, 2006). The killer phenomenon was originally observed in certain strains of *Saccharomyces* (Bevan and Makower, 1963) and subsequently found in other genera such as *Candida*, *Cryptococcus*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Torulopsis*, *Trichosporon*, *Williopsis* etc. (Philliskirk and Young, 1975; Stumm *et al.*, 1977; Asheda *et al.*, 1983; Hodgson *et al.*, 1994; Vadkertiova and Slavikova, 1995; Nout *et al.*, 1996; Golubev, 1999).

Killer strains are known to occur in a variety of sources including grape skins, must fermentations and wine cellar equipment (Jacobs *et al.*, 1988; van Vuuren and Jacobs, 1992). In nature certain strains of killer yeasts dominate only in particular niches (Zorg *et al.*, 1988). Bussey *et al.* (1988) showed that the K1 and K2 killer strains of *Saccharomyces cerevisiae* had different pH and temperature optima in grape juice cultures. Possible uses of killer phenomenon, which aroused great interest, include the differentiation of pathogenic strains (Morace *et al.*, 1984) and their possible role in ecosystems mainly in natural fermentation processes (Starmer *et al.*, 1987; Vagnoli *et al.*, 1993; Hidalgo and Flores, 1994). The killer phenomenon provides an excellent model system to study host-virus interactions in eukaryotic cells (Wickner, 1979) and to investigate the mechanisms of protein processing and secretion (Douglas *et al.*, 1988).

Killer activity is one of the mechanisms of antagonism among yeasts during spontaneous fermentations and because of this mechanism killer strains could dominate at the end of the wine fermentation (Bussey *et al.*, 1988; Longo *et al.*, 1990; Jacobs and van Vuuren, 1991).

In the present study killer activity has been screened by cross reactions in yeast species previously isolated from slime fluxes of different trees (Mushtaq *et al.*, 2008b; Mushtaq *et al.*, 2006b). Furthermore, activity of most killer

strains was also detected against yeast species previously isolated from dairy products (Mushtaq *et al.*, 2007a; Mushtaq *et al.*, 2006a), flower's nectar (Mushtaq *et al.*, 2008a; Mushtaq *et al.*, 2007b; Mushtaq *et al.*, 2006b) and soil (Mushtaq *et al.*, 2004).

MATERIALS AND METHODS

Three (3) yeast strains viz., Y21-*Bullera pseudoalba*, Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae* which were originally isolated from slime fluxes of trees (Mushtaq *et al.*, 2008b) and appeared as super killer strains during cross reactions (Mushtaq *et al.*, 2010) were selected for screening their killer activity against 555 isolates of 89 yeast species belonging to 29 genera previously isolated from dairy products, flower's nectar, slime fluxes and soil. A modified method of Abranches *et al.* (1997) as adopted earlier (Mushtaq *et al.*, 2010) was used. Twenty four hours old yeast culture grown on YM agar was diluted in double distilled sterile water to obtain a suspension of ca. 4×10^5 cells/ml and spread with a sterile cotton swab as seeded (lawn) cultures on the surface of yeast extract-malt extract agar supplemented with 0.003% methylene blue (YM-MB Agar) in Petri plates and dried. Fresh cultures of the yeasts to be tested were grown on YM agar for 24 h and each inoculated in a single streak on plates seeded with the yeast culture and incubated at $25 \pm 1^\circ\text{C}$ for 10 days and observed daily. The seeded yeast was considered as killer if a blue colored killing zone appeared on streak and sensitive if killing zone appeared around the streak on lawn. Killing activity and sensitivity of yeast species were calculated in percent.

RESULTS

Screening of Killer-Sensitive Pattern Using Killer Strains

Killer activity of strains Y21-*Bullera pseudoalba*, Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae* was tested against a total of 555 isolates of 89 yeast species belonging to 31 genera previously isolated from dairy products, slime fluxes, flowers' nectar and soil.

Y21-*Bullera pseudoalba* showed maximum killing activity when tested against 228 isolates of 50 yeast species belonging to 20 genera, previously isolated from dairy products against *Bullera pyricola*, *Candida succiphila*, *C. clavigiana*, *Debaryomyces castellii*, *D. hansenii*, *D. vanrijii*, *Pichia angusta*, *P. anomala*, *P. lynferdii*, *P. mexicana*, *P. strasburgensis*, *Sporidiobolus salmonicolor* and *Stephanoascus ciferrii* (Table 1). This killer strain also showed maximum killer activity as compared to Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae* when tested against 41 isolates of 23 yeast species belonging to 14 genera previously isolated from slime fluxes of trees (Table 2). 26 isolates of 16 different yeast species belonging to 11 genera were found to be killed by this strain. Yeasts from slime fluxes that showed sensitivity against this killer strains were *Candida succiphila*, *C. valdiviana*, *Cryptococcus albidus*, *Debaryomyces hansenii*, *Fibulobasidium inconspicuum*, *Mrakia frigida*, *Pichia angusta*, *Pichia anomala*, *Pichia strasburgensis*, *Saitoella complicata*, *Sporidiobolus ruineniae* and *Williopsis californica* (Table 2).

As compared to other two killer strains, Y21-*Bullera pseudoalba* also showed maximum killing activity against yeast species from flowers' nectar (Fig. 1). This strain killed 139 isolates of 38 yeast species belonging to 18 genera, when tested against a total of 263 isolates of 56 yeast species belonging to 21 genera previously isolated from flowers' nectar (Table 3). Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae* also showed remarkable killing activity against yeast species of nectar (Fig. 1). The most killer strain i.e., Y21-*Bullera pseudoalba*, on the other hand also showed certain amount of sensitivity against yeast species of flowers' nectar (Fig. 1). Whereas, Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae*, showed least sensitivity than Y21-*Bullera pseudoalba*. Yeast strains from flowers' nectar that showed sensitivity towards killer strains were *Bullera pyricola*, *Candida succiphila*, *Cryptococcus albidus*, *C. curvatus*, *C. laurentii*, *Debaryomyces castellii*, *D. hansenii*, *Fibulobasidium inconspicuum*, *Pichia angusta*, *P. lynferdii*, *Sporidiobolus ruineniae* and *Zygoascus helinicus* (Table 3).

Like those of slime fluxes, dairy products and flowers' nectar, Y21-*Bullera pseudoalba*, also showed maximum killing activity as compared to Y16-*Pichia anomala* and Y20-*Sporidiobolus ruineniae*, when tested against 23 isolates of 19 species belonging to 14 genera, previously isolated from soil yeast species of soil (Fig. 1). *Debaryomyces hansenii*, *Pichia euphorbiae* and *Sporidiobolus ruineniae* from soil particularly showed sensitivity against the tested killer strains (Table 4).

Table 1. Activity of most killer yeast strains against different yeast species isolated from dairy products.

No.	Streak Yeast species	No. of isolates tested	Y21	Y16	Y20
			K/S/N	K/S/N	K/S/N
1	<i>Arxula adeninovorans</i>	1	1/0/0	0/0/1	0/0/1
2	<i>Bensingtonia intermedia</i>	1	1/1/0	0/0/1	0/0/1
3	<i>B. naganoensis</i>	1	1/0/0	0/0/1	0/0/1
4	<i>Bullea pyricola</i>	29	6/0/23	3/2/24	0/0/29
5	<i>B. pseudoalba</i>	6	0/0/6	1/0/0	0/0/6
6	<i>Candida diddensiae</i>	1	0/0/1	0/0/1	0/0/1
7	<i>C. etchellsii</i>	1	0/0/1	0/0/1	0/0/1
8	<i>C. haemulonii</i>	1	1/0/0	0/0/1	1/0/0
9	<i>C. membranaefaciens</i>	1	0/0/1	0/0/1	0/0/1
10	<i>C. pseudointermedia</i>	1	1/0/0	0/0/1	0/0/1
11	<i>C. shehatae</i>	1	0/0/1	0/0/1	0/0/1
12	<i>C. succiphila</i>	15	3/0/12	2/1/12	0/0/15
13	<i>C. valdiviana</i>	5	3/0/2	1/1/3	0/0/5
14	<i>C. xestobii</i>	1	0/0/1	0/0/1	0/0/1
15	<i>Clavispora lusitanae</i>	4	0/0/4	0/0/4	0/0/4
16	<i>Cryptococcus albidus</i>	2	0/0/2	0/0/2	0/0/2
17	<i>C. gastricus</i>	1	0/0/1	0/0/1	0/0/1
18	<i>Debaryomyces castellii</i>	29	11/2/16	2/3/24	4/0/25
19	<i>D. hansenii</i>	15	2/0/13	3/2/10	4/0/11
20	<i>D. nepalensis</i>	2	2/1/0	0/0/2	0/0/2
21	<i>D. vanrijii</i>	8	4/1/3	2/0/6	0/0/8
22	<i>D. yamadae</i>	1	0/0/1	0/1/0	0/0/1
23	<i>Fibulobasidium inconspicuum</i>	5	1/0/4	0/0/5	0/0/5
24	<i>Filobasidiella neoformans</i>	1	0/1/0	0/0/1	0/0/1
25	<i>Filobasidium uniguttulatum</i>	1	0/0/1	0/0/1	0/0/1
26	<i>Kluyveromyces polysporus</i>	1	0/0/1	0/0/1	0/0/1
27	<i>Lipomyces lipofer</i>	1	0/0/1	0/0/1	0/0/1
28	<i>L. starkeyi</i>	2	1/0/1	0/0/2	0/0/2
29	<i>Phaffia rhodozyma</i>	1	1/0/0	0/0/1	0/0/1
30	<i>Pichia angusta</i>	12	4/1/7	1/2/12	1/0/11
31	<i>P. anomala</i>	10	2/1/7	1/0/9	2/0/8
32	<i>P. euphorbiaphila</i>	1	1/0/0	0/0/1	0/0/1
33	<i>P. guilliermondii</i>	2	2/1/0	0/0/2	0/0/2
34	<i>P. heimii</i>	2	0/0/1	0/0/2	0/0/2
35	<i>P. jadinii</i>	2	2/1/0	0/0/2	0/0/2
36	<i>P. lynferdii</i>	20	5/0/15	2/3/15	2/0/18
37	<i>P. methanolica</i>	1	0/0/1	0/0/1	0/0/1
38	<i>P. mexicana</i>	2	2/0/0	1/0/1	1/1/0
39	<i>P. mississippiensis</i>	1	0/1/0	1/0/0	1/0/0
40	<i>P. ofunaensis</i>	3	2/0/1	0/0/3	0/0/3
41	<i>P. ohmeri</i>	5	3/0/2	1/0/4	1/2/2
42	<i>P. strasburgensis</i>	3	3/0/0	0/0/3	0/0/3
43	<i>P. sydowiorum</i>	1	1/0/0	0/0/1	0/0/1
44	<i>Saccharomycodes ludwigii</i>	1	1/0/0	0/0/1	1/0/0
45	<i>Sporidiobolus ruineniae</i>	5	1/0/4	1/0/4	0/1/4
46	<i>S. salmonicolor</i>	2	2/0/0	0/0/2	1/0/1
47	<i>Sporobolomyces tsugae</i>	4	1/0/3	1/0/3	0/0/4
48	<i>Stephanoascus ciferii</i>	8	4/1/3	2/1/5	2/2/4
49	<i>Tremella encephala</i>	2	0/0/2	0/0/2	0/0/2
50	<i>Wiliopsis californica</i>	2	0/0/2	0/0/2	0/0/2
	Total isolates	228	75/12/144	25/16/185	21/6/201

* Values given in the table (K/S/N) are the behavior of super killer strains in numbers against tested yeasts from dairy products (K = killer, S = sensitive or N=neutral)

Table 2. Activity of most killer yeast strains against yeast species isolated from slime fluxes of trees *.					
			Y21	Y16	Y20
No.	Streak Yeast species	No. of isolates tested	K/S/N	K/S/N	K/S/N
1	<i>Bullera pseudoalba</i>	1	0/0/1	0/0/1	0/0/1
2	<i>Candida lyxosophila</i>	1	0/0/1	0/0/1	0/0/1
3	<i>C. succiphila</i>	1	1/0/0	1/0/0	1/1/0
4	<i>C. valdiviana</i>	4	3/1/0	0/0/4	0/0/4
5	<i>Cryptococcus albidus</i>	3	2/0/1	3/0/0	0/0/3
6	<i>C. gastricus</i>	1	1/0/0	1/0/0	0/0/1
7	<i>Debaryomyces castellii</i>	1	1/0/0	1/0/0	1/0/0
8	<i>D. hansenii</i>	1	1/0/0	1/0/0	1/0/0
9	<i>D. yamadae</i>	1	0/0/1	1/0/0	0/0/1
10	<i>Fibulobasidium inconspicuum</i>	2	2/0/0	2/0/0	2/0/0
11	<i>Mrakia frigida</i>	1	1/0/0	1/0/0	0/0/1
12	<i>Phaffia rhodozyma</i>	1	1/0/0	1/0/0	1/0/0
13	<i>Pichia angusta</i>	1	1/1/0	1/0/0	1/0/0
14	<i>P. anomala</i>	8	3/1/4	3/2/3	2/5/1
15	<i>P. lynferdii</i>	2	0/0/2	1/0/1	1/1/0
16	<i>P. methanolica</i>	1	1/1/0	0/0/1	0/0/1
17	<i>P. rabaulensis</i>	1	0/0/1	1/0/0	1/0/0
18	<i>P. strasburgensis</i>	2	2/0/0	2/0/0	0/1/1
19	<i>Rhodospiridium toruloides</i>	1	1/0/0	1/0/0	1/0/0
20	<i>Rhodotorula bacarum</i>	1	0/0/1	0/0/1	0/0/1
21	<i>Saitoella complicata</i>	1	1/0/0	0/0/1	1/0/0
22	<i>Sporidiobolus ruineniae</i>	3	3/0/0	2/0/1	1/1/0
23	<i>Wiliopsis californica</i>	2	2/0/0	0/0/2	2/0/0
	Total isolates (41)	41	26/4/12	23/2/17	16/9/16
* Values given in the table are the positive lineages of K (= seeded strain killer) and S (= seeded strain sensitive)					

Table 3. Activity of most killer yeast strains against species of yeast isolated from flowers' nectar*.

No.	Streak Yeast species	No. of isolates tested	Y21 K/S/N	Y16 K/S/N	Y20 K/S/N
1	<i>Bensingtonia miscanthi</i>	1	1/0/0	1/0/0	0/0/1
2	<i>Bullera megalospora</i>	2	1/1/0	1/0/1	2/0/0
3	<i>B. pyricola</i>	15	10/4/1	6/1/8	0/0/15
4	<i>B. pseudoalba</i>	3	1/0/2	1/0/2	0/0/3
5	<i>Candida friedrichii</i>	1	1/0/0	1/0/1	0/0/1
6	<i>C. gropengiesseri</i>	1	0/0/1	0/0/1	0/0/1
7	<i>C. magnoliae</i>	1	1/0/0	1/0/0	1/0/0
8	<i>C. membranaefaciens</i>	1	1/0/0	1/0/0	1/0/0
9	<i>C. rhagii</i>	4	2/2/0	0/0/4	0/0/4
10	<i>C. sake</i>	1	0/0/1	1/0/0	0/0/1
11	<i>C. succiphila</i>	7	4/2/1	3/0/4	1/0/6
12	<i>C. valdiviana</i>	4	4/0/0	1/0/3	0/0/4
13	<i>C. versatilis</i>	1	1/0/0	1/0/0	0/0/1
14	<i>C. xestobii</i>	1	0/0/1	0/0/1	0/0/1
15	<i>Cryptococcus albidus</i>	24	15/2/7	14/0/10	1/0/23
16	<i>C. curvatus</i>	3	2/1/0	1/0/2	1/0/2
17	<i>C. flavus</i>	2	1/0/1	1/0/1	0/0/2
18	<i>C. heveanesis</i>	2	0/0/2	1/0/1	1/1/0
19	<i>C. humicolus</i>	1	0/0/1	1/0/0	0/0/1
20	<i>C. hungaricus</i>	3	0/0/3	1/0/2	0/0/3
21	<i>C. laurentii</i>	27	14/3/10	6/0/21	4/0/23
22	<i>C. macerans</i>	5	0/3/2	4/0/1	0/0/5
23	<i>Cystofilobasidium bisporidii</i>	2	1/0/1	1/0/1	1/0/1
24	<i>Debaryomyces castellii</i>	16	11/2/3	4/0/12	4/1/11
25	<i>D. hansenii</i>	8	5/1/2	4/1/3	2/0/6
26	<i>D. vanrijii</i>	2	1/0/1	0/0/2	0/0/2
27	<i>Exophila salmonis</i>	11	0/2/9	0/0/11	0/0/11
28	<i>Fibulobasidium inconspicuum</i>	10	7/1/2	2/1/7	1/0/9
29	<i>Filobasidiella neoformans</i>	2	2/0/0	0/0/2	1/0/2
30	<i>Issatchenkia occidentalis</i>	1	0/0/1	1/0/0	1/0/0
31	<i>Lipomyces starkeyi</i>	1	0/0/1	0/0/1	0/0/1
32	<i>Mrakia frigida</i>	5	3/0/2	2/0/3	2/0/3
33	<i>Phaffia rhodozyma</i>	5	2/1/2	2/1/2	0/0/5
34	<i>Pichia angusta</i>	13	9/0/4	5/0/8	2/0/11
35	<i>P. castillae</i>	1	0/0/1	0/0/1	0/1/0
36	<i>P. dryadoides</i>	1	0/0/1	0/0/1	0/0/1
37	<i>P. fabianii</i>	1	1/0/0	1/0/0	0/0/1
38	<i>P. guilliermondii</i>	2	0/0/2	2/0/0	1/0/1
39	<i>P. jadinii</i>	3	3/0/0	1/0/2	0/0/3
40	<i>P. lynferdii</i>	23	19/4/0	7/1/15	1/0/22
41	<i>P. methanolica</i>	3	2/1/0	1/0/2	0/0/3
42	<i>P. mississippiensis</i>	2	1/1/0	0/0/2	0/0/2
43	<i>P. ofunaensis</i>	2	0/1/1	0/0/2	0/0/2
44	<i>P. ohmeri</i>	3	2/0/1	0/0/3	0/0/3
45	<i>Pseudozyma Antarctica</i>	2	1/0/1	0/0/2	0/0/2
46	<i>P. fusiformata</i>	4	0/2/2	2/0/2	0/0/4
47	<i>Rhodospiridium toruloides</i>	3	1/0/2	1/0/2	0/0/3
48	<i>Rhodotorula fragaria</i>	2	1/1/0	0/0/2	0/0/2
49	<i>R. hinnulea</i>	4	2/0/2	1/0/3	0/0/4
50	<i>Saccharomyces kluyveri</i>	1	0/0/1	1/0/0	0/0/1
51	<i>Sporidiobolus ruineniae</i>	8	3/1/4	1/0/7	2/0/6
52	<i>Stephanoascus ciferii</i>	1	0/0/1	0/0/1	0/0/1
53	<i>Tremella aurentia</i>	1	1/0/0	0/0/1	0/0/1
54	<i>Wiliopsis californica</i>	5	0/1/4	2/1/2	0/0/5
55	<i>W. pratensis</i>	1	1/0/0	0/0/1	0/0/1
56	<i>Zygoascus helinicus</i>	4	1/0/3	0/0/4	1/0/3
Total isolates (263)		263	139/37/	88/6/170	31/3/229

* Values given in the table are the positive lineages of K (= seeded strain killer) and S (= seeded strain sensitive)

Table 4. Activity of most killer yeast strains against species of yeast isolated from soil*.					
No.	Streak Yeast species	No. of isolates tested	Y21	Y16	Y20
			K/S/N	K/S/N	K/S/N
1	<i>Bensingtonia phyllada</i>	1	1/0/0	0/0/1	1/0/0
2	<i>Bullera pyricola</i>	1	1/0/0	1/0/0	1/0/0
3	<i>B. pseudoalba</i>	1	0/0/1	1/0/0	1/0/0
4	<i>Candida succiphila</i>	1	0/0/1	1/0/0	1/0/0
5	<i>C. valdiviana</i>	1	1/0/0	1/0/0	0/0/1
6	<i>Cryptococcus albidus</i>	3	3/0/0	1/0/2	0/0/3
7	<i>Debaryomyces castellii</i>	1	1/0/0	1/0/0	0/1/0
8	<i>D. hansenii</i>	2	2/0/0	2/0/0	1/2/0
9	<i>Fibulobasidium inconspicuum</i>	1	0/0/1	1/0/0	1/0/0
10	<i>Filobasidiella neoformans</i>	1	1/0/0	1/0/0	0/1/0
11	<i>Filobasidium uniguttulatum</i>	1	1/0/0	1/0/0	1/0/0
12	<i>Phaffia rhodozyma</i>	1	1/0/0	0/0/1	1/0/0
13	<i>Pichia euphorbiae</i>	1	1/0/0	1/0/0	0/0/0
14	<i>P. jadinii</i>	1	0/0/1	0/0/1	0/0/1
15	<i>P. lynferdii</i>	1	1/0/0	1/0/0	0/0/1
16	<i>Rhodospiridium toruloides</i>	1	0/0/1	1/0/0	1/0/0
17	<i>Rhodotorula pilati</i>	1	1/0/0	0/0/1	0/0/1
18	<i>Sporidiobolus ruineniae</i>	2	2/0/0	2/0/0	2/0/0
19	<i>Zygosaccharomyces bailii</i>	1	0/0/1	0/0/1	0/0/1
	Total isolates	23	17/0/6	16/0/7	11/5/8
* Values given in the table are the positive lineages of K (= seeded strain killer) and S (= seeded strain sensitive)					

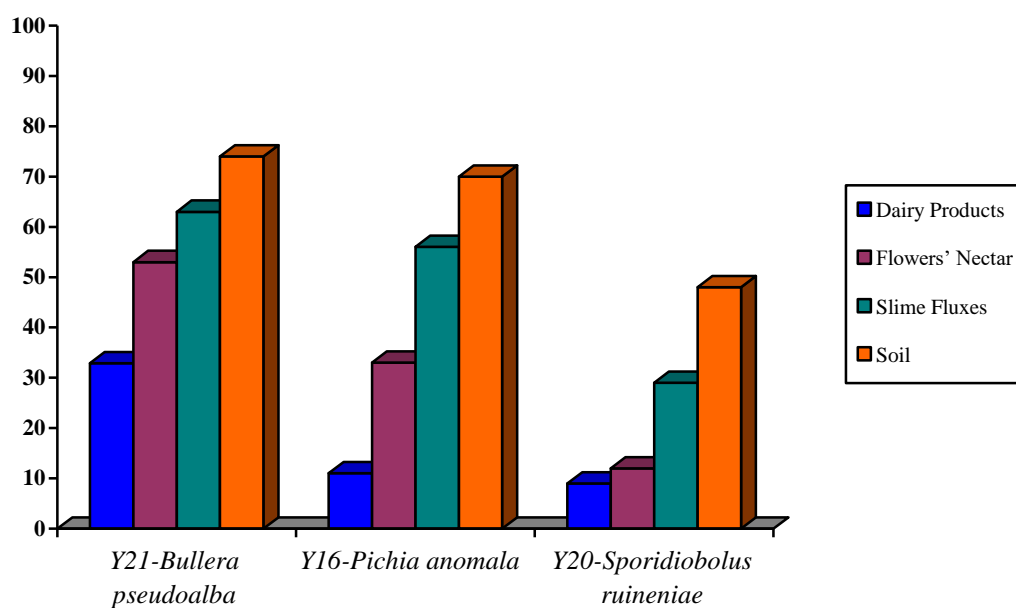


Fig. 1. Killing activity of different super killer yeast strains (in percentage) against total isolates of yeast species from different sources.

DISCUSSION

Figure 1 clearly showed the predominant killing activity of all three (3) super killer yeasts strains especially Y21-*Bullera pseudoalba*. It may be mentioned here that these super killer strains were originally isolated from slime fluxes of trees but they showed highest killing activity against yeasts isolated from soil and subsequently against yeast species from slime fluxes, flowers' nectar and dairy products. Moreover, it is also evident from the results that a single super killer strain showed different behavior i.e. killer, sensitive or neutral against several isolates of same species. This clearly indicates that the phenomenon of killer activity is a strain character rather than species specific. These interactions occur because of the sensitivity of toxins of killer strain that kills the test strain, but at the same time it shows sensitivity against the toxins produced by the test yeast isolate. It is known that the killer activity phenomenon plays an important role during antagonistic mechanism not only in artificial habitats but also in nature (Zorg *et al.*, 1988). Moreover, they also have great potential to minimize contaminating spoilage yeasts if used in fermentation process as starter culture (Starmer *et al.*, 1987; Bussey *et al.*, 1988; Jacobs *et al.*, 1988; Longo *et al.*, 1990; Vagnoli *et al.*, 1993; Hidalgo and Flores, 1994).

On the other hand the phenomenon of insensitivity of certain strains of same species is defined as lack of killer toxin receptors in their cell walls necessary for activity of killer toxin (Marquina *et al.*, 2002; Golubev, 2006). It is interesting to note that ascomycetous yeasts are usually insensitive to toxins produced by basidiomycetous species and vice versa (Golubev, 1992; Golubev *et al.*, 1997). In the present study we also observed that several strains of ascomycetous yeasts such as *Debaryomyces*, *Pichia*, *Stephanosascus*, or their anamorphs such as *Candida* showed sensitivity against tested basidiomycetous yeasts super killer strains Y21-*Bullera pseudoalba* and Y20-*Sporidiobolus ruineniae* (Table 1-4). Hence, based on killer toxins' receptors, different cell wall chemical compositions may be used for their classification (Golubev, 1998, 2006). Similarly, Y16-*Pichia anomala* showed killing activity against several isolates of ascomycetous yeasts such as *Bullera*, *Cryptococcus*, *Cystofilobasidium*, *Fibulobasidium*, *Filobasidiella*, *Mrakia*, *Rhodotorula*, *Sporidiobolus*, *Tremella* etc. There are studies where killer yeasts have been screened from alcoholic fermentations processes for not only beverage productions such as brewing, sake, cachaca, wine (Maule and Thomas, 1973; Imamura *et al.*, 1974; Morais *et al.*, 1997; Sulo and Michalcáková, 1992; Carrau *et al.*, 1993) but also from ethanol producing units (Sato *et al.*, 1993; Ceccato-Antonini *et al.*, 1999, 2004; Soares and Sato, 1999, 2000). Antonini *et al.* (2005) reported that a non-*Saccharomyces* yeast strain (CCA 510) which has slightly lower ethanol yield as compared to baker's yeast has great potential for commercial use as it showed 92% killing activity against 102/110 industrial strains. In the present study, we reported up to 74% killing activity of tested yeast strains, indicating that they can be used for fermentations process as well.

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