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SCREENING OF KILLER, SENSITIVE AND NEUTRAL PHENOTYPES IN YEASTS USING CERTAIN SENSITIVE *PICHIA* STRAINS

Muhammad Mushtaq[1*], Sharfun-Nahar[2], Muhammad Shahab Alam[3] and M. H. Hashmi[2]

[1*]*Department of Biotechnology & Informatics, Faculty of Life Sciences & Informatics, Baluchistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Takatu Campus, Airport Road, Balili, Quetta, Pakistan; Email: muhammad.mushtaq@buitms.edu.pk mmushtaq72@gmail.com*
[2]*Department of Botany, University of Karachi, Karachi-75270, Pakistan; Email: sharfun_nahar@yahoo.com*
[3]*Department of Chemical Engineering, Dawood University of Engineering and Technology, New MA Jinnah road, Karachi; mshahabalam@gmail.com*

ABSTRACT

Using sensitive *Pichia* strains *viz*., Y246-*P. fabianii*, YF42-*P. heimii*, Y206-*P. jadinii*, Y18-*P. strasburgensis*, Killer and Sensitive Pattern (KSP) was screened in 553 isolates of yeasts belonging to 89 species and 31 genera by cross reactions. Apart from ascomycetous yeasts killer phenomenon was significantly observed in certain basidiomycetous yeast species such as *Bullera pseudoalba*, *B. pyricola*, *Crytococcus albidus*, *C. laurentii*, *C. macerans*, *Fibulobasidium inconspicuum* and *Sporidiobolus ruineniae* against these sensitive *Pichia* strains. Among all the tested sensitive strains, Y18-*Pichia strasburgensis* appeared as the most sensitive strain. Calculation of diversity in percentage clearly showed the occurrence of sensitivity of this strain in all cases. The phenomenon of killer activity appeared as strain character. It is inferred that the use of killer toxins as a taxonomic tool should be preceded by a careful study of their KSP.

Key words: Yeast, *Pichia* strains, killer, sensitive, phenotype.

INTRODUCTION

Certain yeasts have shown killer activity which is readily detectable only when a suitable sensitive strain is tested. There is evidence that killer yeasts secrete different killer toxins with activities specific for different target yeast cells (Wickner, 1996). The Killer toxins (mycocins) are protein in nature, active at low pH (Young and Yagiu, 1978; Pfeiffer and Radler, 1982; Radler *et al*., 1985) and lethal to closely related strains but the killer yeast itself has a killer resistant phenotype (Bevan and Makower, 1963; Woods and Bevan, 1968; Bussey, 1972; Pfeiffer and Radler, 1982; Spencer and Spencer, 1997).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). 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). 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; Jacobs *et al*., 1988; Longo *et al*., 1990).

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). As a result, if different cell wall chemical compositions are taxon-associated; resistance, causing insensitivity could be a taxon-related property as well (Golubev, 1998, 2006). Based on evidence that the chemical composition of yeast cell walls is a taxon related characteristic, Golubev (2006) hypothesized that Killer-Sensitive-Pattern (KSP) profiles may have taxonomic relevance. The theoretical rationale supporting this conclusion is related to the resistance mechanism. In previous studies we screened Killer-Sensitive-Pattern (KSP) by cross reaction among yeast species previously isolated from slime fluxes of different trees, flowers' nectar and dairy products (Mushtaq *et al*., 2010 and 2013). The KSP was also screened using some ascomycetous sensitive strains among yeast species of various ecological niches (Mushtaq *et al*., 2014). The sensitive *Pichia* strains identified in these studies were used in the present study to screen killer, sensitive and neutral phenotypes in yeast species previously isolated from different substrates (Mushtaq *et al*., 2004; 2005; 2006a, 2006b, 2007a, 2007b, 2008a, 2008b).

MATERIALS AND METHODS

A modified method of Abranches *et al*. (1997) was used to screen killer, sensitive and neutral phenotypes (killer-sensitive pattern) in 553 yeast isolates belonging to 89 species and 31 genera previously isolated from dairy products, flowers' nectar, slime fluxes of trees and soil, on yeast extract-malt extract agar supplemented with 0.003% methylene blue (YM-MB Agar). Twenty-four h old sensitive yeast culture grown on YM agar (Kreger-van Rij, 1984) was diluted in double distilled sterilized water to obtain a suspension of 4×10^5 cells/mL and spread with a sterile cotton swab as seeded (lawn) cultures on the surface of 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±1°C for 10 days and observed daily. The seeded yeast was considered as sensitive if a blue colored killing zone appeared around the streak on lawn and it was considered as killer if blue colored zone appeared on streak. A negative reaction indicated that the tested yeast strain is neutral. Percentage of sensitivity of each tested sensitive *Pichia* strain was calculated.

RESULTS AND DISCUSSION

Using 4 sensitive *Pichia* strains, killer, sensitive and neutral phenotypes (killer-sensitive-pattern) was screened in 553 isolates of 89 yeast species belonging to 31 genera previously isolated from different dairy products, flowers' nectar, slime fluxes of trees and soil. The sensitive *Pichia* strains included Y246-*P. fabianii* (from nectar), YF42-*P.heimii* (from dairy product), Y206-*P. jadinii* (from nectar); Y18-*P.strasburgensis* (from slime fluxes). Y206-*P. jadinii* and Y18-*P. strasburgensis* showed highest sensitivity when tested against 224 isolates of 50 yeast species belonging to 20 genera previously isolated from dairy products. *P. mexicana* and *P. mississippiensis* showed killer activity against seeded strains (Table 1). Whereas, *Bullera pyricola*, *Candida succiphila*, *Clavispora lusitaniae*, *Debaryomyces castellii*, *D. hansenii*, *D. vanrijii*, *Fibulobasidium inconspicuum*, *Lipomyces starkeyi*, *Pichia angusta*, *P. anomala*, *P. lynferdii*, *P. ofunaensis*, *P. ohmeri*, *Sporidiobolus ruineniae*, *Sporobolomyces tsugae* and *Stephanoascus ciferrii* showed dual activity i.e., both killer activity and sensitivity against tested sensitive *Pichia* strains. It was also observed that certain isolates of *Candida diddensiae*, *Cryptococcus albidus*, *Pichia heimii*, *Saccharomycodes ludwigii*, *Sporidiobolus salmonicolor*, and *Tremella encephala* showed sensitivity. It was observed that Y246-*Pichia fabianii*, Y206-*P. jadinii* and Y18-*P. strasburgensis* also showed certain killing activity against yeast species isolated from dairy products (Table 1). The calculation of sensitivity percentage also indicating the prevalent sensitivity of Y18-*P. strasburgensis* than other sensitive strains (Fig. 1).

Screening of KSP using the *Pichia* sensitive strains in 260 isolates of 55 yeast species belonging to 23 genera from nectar, revealed that Y206-*P. jadinii* and Y18-*P. strasburgensis* showed more than 20% sensitivity, whereas, YF42-*P. heimii* and Y18-*P. strasburgensis* showed more than 20% killing activity against yeast species from nectar (Table 2). Among the yeast species *Bullera pyricola*, *Candida succiphila*, *Cryptococcus albidus*, *C. laurentii*, *C. macerans*, *Debaryomyces castellii*, *D. hansenii*, *Fibulobasidium inconspicuum*, *Mrakia frigida*, *Pichia angusta*, *P. lynferdii*, *Pseudozyma fusiformata* and *Sporidiobolus ruineniae* mostly showed dual activity, however, *Cryptococcus laurentii*, *P. jadinii* and *P. mississippiensis* showed killing activity against seeded strains (Table 2). The sensitivity of Y18-*P. strasburgensis* is also very clearly indicated in figure 1 when its relative percentage was calculated.

It is interesting to note that sensitive strain Y18-*P. strasburgensis* isolated from slime fluxes, when tested against 44 isolates of 23 yeast species belonging to 13 genera of its own ecological habitat, appeared sensitive against 30 isolates belonging to 19 species and 11 genera (Table 2). As compared to Y18-*P. strasburgensis*, significant sensitivity as well as certain amount of killing activity was also recorded in strain YF42-*P. heimii* which was isolated from dairy products. However, rather than to sensitive Y246-*P. fabianii* showed killing activity, whereas, Y206-*P. jadinii* did not showed any activity against yeast strains isolated from dairy products. Yeast strains from slime fluxes showed killing activity against the above mentioned sensitive strains included *Candida valdiviana*, *Cryptococcus albidus*, *Fibulobasidium inconspicuum* and *P. anomala* (Table 3). The calculated value in percentage of Y18-*P. strasburgensis* was significantly highest than other sensitive strains (Fig. 1).

Similarly, Y18-*P. strasburgensis* also showed significant sensitivity when tested against 25 isolates of 20 yeast species belonging to 13 genera from soil (Table 4). Other tested sensitive yeast strains did not showed any significant activity against the yeasts from soil. The sensitivity of strain Y18-*P. strasburgensis* was also found significantly highest that other sensitive strains when tested against yeast isolates of soil (Fig. 1).

Table 1. Screening of Killer-Sensitive-Neutral (K/S/N) Pattern in yeasts species isolated from dairy products using sensitive *Pichia* strains as seeded culture.

No.	Yeast species	No. of isolates tested	Sensitive *Pichia* Strain used			
			P. fabianii	*P. heimii*	*P. jadinii*	*P. strasburgensis*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Arxula adeninovorans*	1	0/0/1	0/0/1	0/0/1	0/0/1
2	*Bensingtonia intermedia*	1	0/0/1	0/0/1	0/1/0	1/0/0
3	*B. nananoensis*	1	0/0/1	0/0/1	0/1/0	1/0/0
4	*Bullera pseudoalba*	4	0/0/4	0/0/4	0/1/3	0/1/3
5	*B. pyricola*	31	10/5/16	3/3/25	5/11/15	4/7/20
6	*Candida diddensiae*	3	0/0/3	0/0/3	0/0/3	0/0/3
7	*C. etchellsii*	1	0/0/1	0/0/1	0/0/1	0/0/1
8	*C. friedrichii*	1	0/0/1	0/0/1	0/0/1	0/0/1
9	*C. haemulonii*	1	0/0/1	0/0/1	0/0/1	0/0/1
10	*C. membranaefaciens*	1	0/0/1	0/0/1	0/0/1	0/0/1
11	*C. pseudointermedia*	1	0/0/1	0/0/1	0/0/1	0/0/1
12	*C. shehatae*	1	0/0/1	0/0/1	0/0/1	0/0/1
13	*C. succiphila*	15	6/5/4	2/0/13	6/6/6	3/5/7
14	*C. valdiviana*	5	0/1/4	0/0/5	1/0/4	2/3/0
15	*C. xestobii*	1	0/0/1	0/0/1	0/0/1	0/0/1
16	*Clavispora lusitaniae*	4	0/0/4	1/0/3	1/1/2	0/0/4
17	*Cryptococcus albidus*	2	1/0/2	0/0/2	0/0/2	1/1/0
18	*C. gastricus*	1	0/0/1	0/0/1	0/0/1	0/0/1
19	*Debaryomyces castellii*	29	7/2/20	1/2/26	7/7/15	3/8/18
20	*D. hansenii*	15	4/1/10	1/4/10	4/3/8	2/5/8
21	*D. nepalensis*	2	0/0/2	0/0/2	0/0/2	0/0/2
22	*D. vanrijii*	8	1/2/5	0/0/8	0/2/6	1/0/7
23	*D. yamadae*	1	0/0/1	0/0/1	0/0/1	0/0/1
24	*Fibulobasidium inconspicuum*	2	0/0/2	0/0/2	1/1/0	1/1/0
25	*Filobasidiella neoformans*	1	0/0/1	0/0/1	0/0/1	0/0/1
26	*Filobasidium uniguttulatum*	1	0/0/1	0/0/1	0/0/1	0/0/1
27	*Kluyveromyces polysporus*	1	0/0/1	0/0/1	0/0/1	0/0/1
28	*Lipomyces lipofer*	1	0/0/1	0/0/1	0/0/1	0/0/1
29	*L. starkei*	2	0/1/5	1/0/5	0/1/5	1/1/5
30	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/0/1
31	*Pichia angusta*	12	3/0/9	2/1/9	2/1/9	1/4/5
32	*P. anomala*	10	3/0/7	0/1/9	0/2/8	2/0/8
33	*P. euphorbiaphila*	1	0/0/1	0/0/1	0/0/1	0/0/1
34	*P. guilliermondii*	2	0/0/2	0/0/2	0/0/2	0/0/2
35	*P. heimii*	2	1/0/1	0/0/2	0/1/1	0/0/2
36	*P. jadinii*	2	1/0/1	0/0/2	0/0/2	2/0/0
37	*P. lynferdii*	18	5/2/11	1/1/16	5/1/12	2/5/11
38	*P. methanolica*	1	0/0/1	0/0/1	0/0/1	0/0/1
39	*P. Mexicana*	2	0/0/2	0/0/2	1/0/1	2/0/0
40	*P. ofunaensis*	3	0/0/3	0/1/2	0/0/3	0/2/1
41	*P. ohmeri*	5	0/1/4	1/0/4	1/1/4	0/1/4
42	*P. strasbergensis*	3	0/0/3	0/0/3	0/0/3	2/2/3
43	*P. sydowiorum*	1	0/0/1	0/0/1	0/0/1	1/0/0
44	*Saccharomycodes ludwijii*	1	1/0/0	0/0/1	0/1/0	0/0/1
45	*Sporidiobolus ruineniae*	4	1/0/3	0/0/4	0/0/4	1/1/2
46	*S. salmonicolor*	2	1/0/1	1/0/1	0/2/0	1/0/1
47	*Sporobolomyces tsugae*	4	0/1/3	0/0/4	0/0/4	2/2/0
48	*Stephanoascus ciferii*	8	1/0/7	1/0/7	0/1/7	3/2/3
49	*Tremella encephala*	2	0/0/2	0/0/2	0/0/2	0/0/2
50	*Wiliopsis californica*	2	0/0/2	0/0/2	0/0/2	0/0/2
	Total	224	45/21/162	15/14/199	34/45/145	39/51/134

K= *Pichia* strain killed the tested strain; S= *Pichia* strain sensitive to tested strain; N= Neutral

Table 2. Screening of Killer-Sensitive Pattern (KSP) in yeasts species isolated from Flowers' nectar using sensitive *Pichia* strains as seeded culture.

No.	Yeast species	No. of isolates tested	Sensitive *Pichia* Strain used			
			P. fabianii	*P. heimii*	*P. jadinii*	*P. strasburgensis*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Bensingtonia miscanthi*	1	0/0/1	0/0/1	0/0/1	1/0/0
2	*Bullara megalospora*	2	0/0/2	0/0/2	0/0/2	1/0/1
3	*B. pseudoalba*	3	0/0/3	0/0/3	0/0/3	1/0/2
4	*B. pyricola*	15	1/0/14	3/1/11	2/1/12	3/3/9
5	*Candida friedrichii*	1	0/0/1	0/0/1	0/0/1	1/0/0
6	*C. gropengiesseri*	1	0/0/1	0/0/1	0/0/1	0/1/0
7	*C. magnoliae*	1	0/0/1	1/0/0	0/0/1	0/0/1
8	*C. membranaefaciens*	1	0/0/1	0/0/1	0/0/1	1/0/0
9	*C. rhagii*	4	0/0/4	1/0/3	0/0/4	1/2/1
10	*C. sake*	1	0/0/1	0/0/1	0/0/1	1/0/0
11	*C. succiphila*	6	0/0/6	1/0/5	1/1/4	2/3/1
12	*C. valdiviana*	4	0/0/4	0/0/4	0/1/3	3/0/1
13	*C. versatilis*	1	0/0/1	1/0/0	0/0/1	1/0/0
14	*C. xestobii*	1	0/0/1	0/0/1	0/0/1	0/0/1
15	*Cryptococcus albidus*	24	7/2/15	5/0/19	9/2/13	2/4/18
16	*C. curvatus*	3	0/1/2	0/0/3	0/1/2	2/1/0
17	*C. flavus*	2	0/0/2	1/0/1	1/0/1	1/0/1
18	*C. heveanesis*	2	0/0/2	0/0/2	0/0/2	0/0/2
19	*C. humicolus*	1	0/0/1	1/0/0	0/0/1	1/0/0
20	*C. hungaricus*	3	0/0/3	0/0/3	0/0/3	1/0/2
21	*C. laurentii*	27	5/1/21	7/0/20	10/0/17	3/12/12
22	*C. macerans*	5	0/1/4	0/1/4	0/1/4	0/1/4
23	*Cystofilobasidium bisporidii*	2	0/0/2	0/0/2	0/0/2	0/0/2
24	*Debaryomyces castellii*	16	0/1/15	3/1/12	1/1/14	2/5/9
25	*D. hansenii*	9	0/0/8	1/0/7	0/2/6	2/4/2
26	*D. vanrijii*	2	0/0/2	0/0/2	0/0/2	0/0/2
27	*Exophila salmonis*	11	0/0/11	0/0/11	0/0/11	0/2/9
28	*Fibulobasidium inconspicuum*	10	1/0/9	1/0/9	4/0/6	2/3/5
29	*Filobasidiella neoformans*	2	0/0/2	0/0/2	0/0/2	0/0/2
30	*Issatchenkia occidentalis*	1	0/0/1	1/0/0	0/0/1	0/0/1
31	*Lipomyces starkei*	1	0/0/1	0/0/1	0/0/1	0/0/1
32	*Mrakia frigida*	4	0/0/4	1/0/3	0/1/3	1/0/3
33	*Phaffia rhodozyma*	5	0/0/5	0/2/3	0/0/5	1/1/3
34	*Pichia angusta*	13	1/2/10	3/1/9	1/2/10	2/4/7
35	*P. castillae*	1	0/0/1	0/0/1	0/0/1	1/0/0
36	*P. dryadoides*	1	0/0/1	0/0/1	0/0/1	0/1/0
37	*P. fabianii*	1	0/0/1	0/0/1	0/0/1	0/0/1
38	*P. jadinii*	3	0/0/3	1/0/2	0/0/3	1/1/1
39	*P. lynferdii*	23	2/2/19	5/3/15	3/2/18	3/14/6
40	*P. methanolica*	3	0/0/3	1/0/2	0/0/3	2/1/0
41	*P. mississippensis*	2	1/0/1	0/0/2	0/0/2	1/1/0
42	*P. ofunaensis*	2	0/0/2	0/0/2	0/0/2	0/0/2
43	*P. ohmeri*	3	0/0/3	0/0/3	1/0/2	2/1/0
44	*Pseudozyma antarctica*	2	0/0/2	0/0/2	0/0/2	1/0/1
45	*P. fusiformata*	4	0/1/3	0/1/3	1/0/3	0/0/4
46	*Rhodosporidium toruloides*	3	0/0/3	0/0/3	0/1/2	1/2/0
47	*Rhodotorula fragaria*	2	0/0/2	0/0/2	0/0/2	0/0/2
48	*R. hinnulea*	4	0/0/4	1/0/3	0/0/4	2/0/2
49	*Saccharomyces kluyveri*	1	0/0/1	0/0/1	0/0/1	0/0/1
50	*Sporidiobolus ruineniae*	8	1/2/5	0/1/7	1/3/4	1/3/4
51	*Stephanoascus ciferii*	1	0/0/1	0/0/1	0/0/1	0/1/0
52	*Tremella aurentia*	1	0/0/1	0/0/1	0/0/1	0/0/1
53	*Wiliopsis californica*	5	0/0/5	1/0/4	0/0/5	0/2/3
54	*W. pratensis*	1	0/0/1	0/0/1	0/0/1	1/0/0
55	*Zygoascus helinicus*	4	1/0/3	0/0/4	0/0/4	1/0/4
	Total	260	20/13/227	40/11/209	35/19/206	53/73/134

K= *Pichia* strain killed the tested strain; S= *Pichia* strain sensitive to tested strain; N= Neutral

Table 3. Screening of Killer-Sensitive Pattern (KSP) in yeasts species isolated from slime fluxes using sensitive *Pichia* strains as seeded culture.

No.	Yeast species	No. of isolates tested	Sensitive *Pichia* Strain used			
			P. fabianii	*P. heimii*	*P. jadinii*	*P. strasburgensis*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Bullera pseudoalba*	1	0/0/1	0/0/1	0/0/1	0/1/0
2	*Candida lyxosophila*	1	0/0/1	0/0/1	0/0/1	0/1/0
3	*C. succiphila*	1	0/0/1	1/0/0	0/1/0	0/1/0
4	*C. valdiviana*	4	0/0/4	0/0/4	0/0/4	2/2/0
5	*Cryptococcus albidus*	3	1/0/2	3/0/0	0/0/3	0/3/0
6	*C. gastricus*	1	0/0/1	0/1/0	0/0/1	0/1/0
7	*Debaryomyces castellii*	1	0/0/1	0/0/1	0/0/1	0/1/0
8	*D. hansenii*	1	1/0/0	1/0/0	1/0/0	0/0/1
9	*D. yamadae*	1	0/0/1	0/0/1	0/0/1	0/1/0
10	*Fibulobasidium inconspicuum*	5	1/2/2	1/3/1	0/0/5	1/3/1
11	*Mrakia frigida*	1	0/0/1	1/0/0	0/0/1	0/0/1
12	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/1/0
13	*Pichia angusta*	1	1/0/0	0/1/0	0/0/1	0/1/0
14	*P. anomala*	8	1/0/7	3/1/4	0/0/8	1/5/2
15	*P. lynferdii*	2	0/0/2	1/0/1	0/0/2	0/1/1
16	*P. methanolica*	1	0/0/1	0/1/0	0/0/1	0/0/1
17	*P. rabaulensis*	1	0/0/1	0/0/1	0/0/1	0/1/0
18	*P. strasbergensis*	2	0/0/2	0/1/0	0/0/2	0/1/1
19	*Rhodosporidium toruloides*	1	0/0/1	0/0/1	0/0/1	0/1/0
20	*Rhodotorula bacarum*	1	0/0/1	0/0/1	0/0/1	0/0/1
21	*Saitoella complicata*	1	1/0/0	0/0/1	0/0/1	0/1/0
22	*Sporidiobolus ruineniae*	3	0/0/3	2/0/1	0/0/3	0/1/2
23	*Williopsis californica*	2	0/0/2	0/1/1	0/0/2	0/2/0
	Total	44	6/2/36	13/9/22	1/1/42	4/29/11

K= *Pichia* strain killed the tested strain; S= *Pichia* strain sensitive to tested strain; N= Neutral

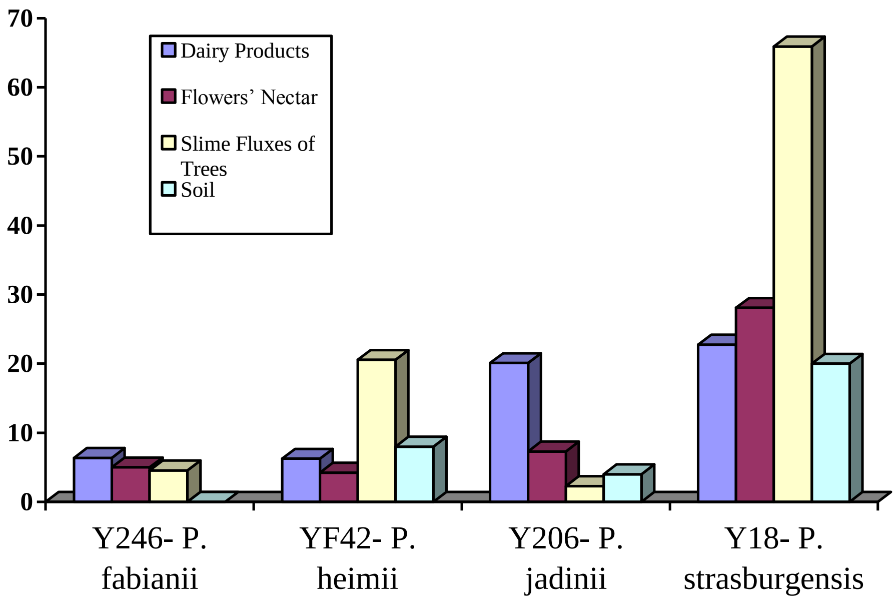

Fig. 1. Comparative analysis of *Pichia* strains sensitivity (in percentage) to yeast species of different sources.

Several yeast strains showed strong killing activity against sensitive *Pichia* strains. The phenomenon of killer activity is one of the mechanisms of antagonism among yeasts in natural as well as artificial habitats. Therefore in nature, this phenomenon leads to the dominance of killer strains in particular ecological niches (Zorg *et al*., 1988). On the other hand, during spontaneous fermentations killer strains may be used to minimize contaminating spoilage yeasts (Starmer *et al*., 1987; Bussey *et al*., 1988; Jacobs *et al*., 1988; Longo *et al*., 1990; Vagnoli *et al*., 1993; Hidalgo and Flores, 1994).

It may be mentioned that certain yeast strains killed sensitive *Pichia* strains but other did not. It is very clear from the results that the strain Y18-*Pichia strasburgensis* showed significant sensitivity against the tested yeast species from all substrates especially against yeast species from slime fluxes and soil (Figure1). The phenomenon of insensitivity of other *Pichia* strains towards killer yeasts could be due to lack of receptors in their cell walls that form the link between killer and sensitive strains for the action of killer toxin (Marquina *et al*. 2002; Golubev 2006). Taxonomically, different cell wall chemical compositions are used for classification of organisms; hence resistance causing insensitivity towards killer yeasts could also be used as a taxon-related property (Golubev 1998, 2006).

Table 4. Screening of Killer-Sensitive Pattern (KSP) in yeasts species isolated from soil using sensitive *Pichia* strains as seeded culture.

No.	Yeast species	No. of isolates tested	Sensitive *Pichia* Strain used			
			P. fabianii	*P. heimii*	*P. jadinii*	*P. strasburgensis*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Bensingtonia phyllada*	1	0/0/1	0/1/0	0/0/1	1/0/0
2	*Bullera pseudoalba*	1	0/0/1	0/0/1	0/0/1	1/0/0
3	*B. pyricola*	1	0/0/1	0/0/1	0/1/0	1/0/0
4	*Candida succiphila*	1	0/0/1	0/0/1	0/0/1	0/0/1
5	*C. valdiviana*	1	0/0/1	1/0/0	0/0/1	1/0/0
6	*Cryptococcus albidus*	3	0/0/3	0/0/3	0/0/3	3/0/0
7	*Debaryomyces castellii*	1	0/0/1	0/0/1	0/0/1	1/0/0
8	*D. hansenii*	3	0/0/2	0/0/2	0/0/2	0/0/2
9	*D. yamadae*	1	0/0/1	0/0/1	0/0/1	1/0/0
10	*Fibulobasidium inconspicuum*	1	0/0/1	0/0/1	0/0/1	0/1/0
11	*Filobasidiella neoformans*	1	0/0/1	0/0/1	0/0/1	1/0/0
12	*Filobasidium uniguttulatum*	1	0/0/1	0/0/1	0/0/1	0/1/0
13	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/1/0
14	*Pichia euphorbiae*	1	0/0/1	0/1/0	0/0/1	0/1/0
15	*P. jadinii*	1	0/0/1	0/0/1	1/0/0	0/0//
16	*P. lynferdii*	1	0/0/1	0/0/1	0/0/1	0/0/1
17	*Rhodosporidium toruloides*	1	0/0/1	1/0/0	0/0/1	1/0/0
18	*R. pilatii*	1	0/0/1	0/0/1	0/0/1	0/0/1
19	*Sporidiobolus ruineniae*	2	0/0/2	0/0/2	1/0/0	2/0/0
20	*Zygosaccharomyces bailii*	1	0/0/1	0/0/1	0/0/0	0/1/0
	Total	25	0/0/25	2/2/21	2/1/22	13/5/7

K= *Pichia* strain killed the tested strain; S= *Pichia* strain sensitive to tested strain; N= Neutral

In some studies Golubev inferred that killer toxin effectiveness is inversely related to phylogenetic affinity e.g. ascomycetous yeasts are usually insensitive to toxins produced by basidiomycetous species and vice versa (Golubev 1992; Golubev *et al*. 1997). However, in the present studies, we observed that *Pichia* strains appeared sensitive against other *Pichia* species as well as ascomycetous and basidiomycetous yeasts species (Tables 1, 2 &3), especially in case of *Bullera pseudoalba*, *B. pyricola*, *Crytococcus albidus*, *C. laurentii*, *C. macerans*, *Fibulobasidium inconspicuum* and *Sporidiobolus ruineniae* (Tables 1-3)

It is, therefore inferred that the use of killer toxins as a taxonomic tool should be preceded by a careful study of their KSP. Broad-spectrum killer toxins may be used for overall phylogenetic evaluation, while those characterized by a narrow range of activity may be used for clarifying relationships between more closely related species, or for grouping phenotypically similar strains before using molecular techniques [e.g. nucleotide composition in the D1/D2 domains and ITS regions of the ribosomal DNA (r-DNA)].

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