INT. J. BIOL. BIOTECH., 11 (4): 547-554, 2014.

SCREENING OF KILLER-SENSITIVE PATTERN IN YEASTS USING SENSITIVE ASCOMYCETOUS YEAST STRAINS

Muhammad Mushtaq[1*], Sharfun-Nahar[2], Nazeer Ahmed[3], Muhammad Shahab Alam[4] and M. H. Hashmi[5]

[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 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: nazeer.ahmed@buitms.edu.pk ; nazirdurrani@yahoo.com
[4]Department of Chemical Engineering, Dawood University of Engineering and Technology, New MA Jinnah road, Karachi; mshahabalam@gmail.com
[5]Department of Botany, University of Karachi, Karachi-75270, Pakistan; insramhashmi@ems.att.com

ABSTRACT

Four *ascomycetous* strains *viz.*, Y371-*Candida valdiviana*, Y207-*Saccharomyces kluyveri* and two strains of *Williopsis californica* (Y2- and Y90), which appeared as sensitive in a previous research work (Mushtaq *et al*., 2010 & 2013) were used in this study to screen killer, sensitive and neutral phenotypes (Killer-Sensitive-Pattern, KSP) in 556 isolates of yeasts belonging to 89 species and 31 genera. Y207- *S. kluyveri* and Y371- *C. valdiviana* appeared most suitable strains to screen KSP in yeast species from all substrates as compared to Y90-*W. californica* which did not showed sensitivity against slime flux yeast isolates. Whereas, sensitive strain Y2-*W. californica* appeared sensitive against dairy and flowers' nectar yeast isolates only. The phenomenon of killer activity appeared as strain character, however the ecological habitat was also found to play important role in determining the killer-sensitive pattern. The sensitive ascomycetous yeast strains Y207-*S. kluyveri* and Y2- *W. californica* from flowers' nectar habitat appeared killer as compared to sensitive against yeast isolates from dairy products; whereas, Y90-*W. californica* which was originally isolated from dairy products appeared killer against isolates of different yeast species from flowers' nectar. It is inferred that the killer sensitive pattern should by carefully studied using variety of ascomycetous and basidiomycetous sensitive and killer yeast strains from various ecological habitats for bio-typing yeast strains.

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

INTRODUCTION

Certain yeasts show killer activity by producing killer toxins (mycocins), which 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 phenomenon is readily detectable only when a suitable sensitive strain is tested. The killer phenomenon is used to investigate the mechanisms of protein processing and secretion (Douglas *et al*., 1988) as well as it provides an excellent model system to study host-virus interactions in eukaryotic cells (Wickner, 1979). Other 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). It 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).

In previous studies we screened Killer-Sensitive-Pattern (KSP) by cross reactions among yeast species previously isolated from slime fluxes of different trees, flowers' nectar and dairy products (Mushtaq *et al*., 2010, 2013), and 4 ascomycetous yeast strains were identified as sensitive. In the present study these strains *viz.*, Y371-*Candida valdiviana*, Y207-*Saccharomyces kluyveri* and two strains of *Williopsis californica* (Y2- and Y90), 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

Modified method of Abranches *et al*. (1997) was used to screen killer, sensitive and neutral phenotypes (killer-sensitive pattern) in 556 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 $4x10^5$ 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.

RESULTS AND DISCUSSION

In previous studies (Mushtaq *et al*., 2010, 2013) 4 *ascomycetous* strains *viz*., Y371-*Candida valdiviana*, Y207-*Saccharomyces kluyveri* and Y2- *Williopsis californica* which were initially isolated from flowers' nectar and strain Y90- *W. californica* from dairy products showed prominent sensitivity against yeast species from their own community. Considering their sensitivity, we have selected these yeast strains to screen killer, sensitive and neutral phenotypes in 556 yeasts isolates belonging to 89 species and 31 genera from variety of habitats including flowers' nectar, dairy products, slime fluxes and soil.

The ascomycetous sensitive strain Y371-*C. valdiviana* showed highest sensitivity when tested against 228 isolates of 50 yeast species belonging to 20 genera of dairy products. Among the yeast species from dairy products, *Pichia mexicana* and *P. mississippiensis* showed strong killer activity against seeded sensitive strain, 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 sensitive strain. However, several isolates of *Candida diddensiae*, *Cryptococcus albidus*, *Pichia heimii*, *Saccharomycodes ludwigii*, *Sporidiobolus salmonicolor*, *Tremella encephala* rather than killing activity showed sensitivity to the tested sensitive strains (Table 1).

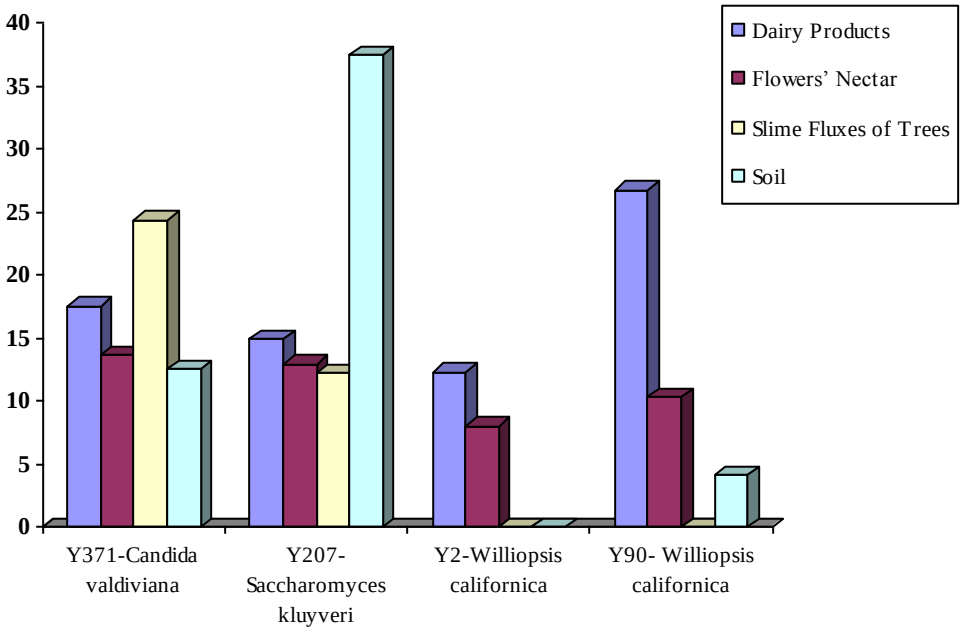

Fig. 1. Comparative analysis (in percentage) of sensitivity of tested ascomycetous yeast strains against yeast species of different habitats.

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

No.	Streak Yeast species	No. of isolates tested	Seeded Sensitive Strains			
			Y371-*Candida valdiviana*	Y207-*Saccharomyces kluyveri*	Y2-*Williopsis californica*	Y90-*Williopsis californica*
			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	0/0/1	0/0/1
3	*B. nananoensis*	1	0/0/1	0/0/1	0/0/1	0/0/1
4	*B. pyricola*	29	3/4/22	8/8/13	9/7/13	1/6/22
5	*B. pseudoalba*	6	0/0/6	0/1/5	1/0/5	1/0/5
6	*Candida diddensiae*	1	0/0/1	0/0/1	0/0/1	0/1/0
7	*C. etchellsii*	1	0/0/1	0/0/1	0/0/1	0/0/1
8	*C. haemulonii*	1	0/0/1	0/0/1	0/0/1	0/0/1
9	*C. membranaefaciens*	1	0/0/1	0/0/1	0/0/1	0/0/1
10	*C. pseudointermedia*	1	0/0/1	0/0/1	0/0/1	0/0/1
11	*C. shehatae*	1	0/0/1	0/0/1	0/0/1	0/0/1
12	*C. succiphila*	15	1/5/9	6/2/7	9/1/5	5/6/4
13	C. *valdiviana*	5	1/0/4	0/1/4	0/0/5	1/1/3
14	*C. xestobii*	1	0/0/1	0/0/1	0/0/1	0/0/1
15	*Clavispora lusitaniae*	4	0/1/3	0/1/3	1/2/1	1/2/1
16	*Cryptococcus albidus*	2	0/1/1	0/1/1	0/1/1	0/0/2
17	*C. gastricus*	1	0/0/1	0/0/1	0/0/1	0/0/1
18	*Debaryomyces castellii*	29	1/8/20	5/5/19	8/4/17	2/12/15
19	*D. hansenii*	15	2/7/6	2/3/10	2/2/11	5/5/5
20	*D. nepalensis*	2	0/0/2	0/0/2	0/0/2	0/0/2
21	*D. vanrijii*	8	1/2/5	2/0/6	2/2/4	0/3/5
22	*D. yamadae*	1	0/0/1	0/0/1	0/0/1	0/0/1
23	*Fibulobasidium inconspicuum*	5	0/1/4	0/1/4	0/0/5	0/0/5
24	*Filobasidiella neoformans*	1	0/0/1	0/0/1	0/0/1	0/0/1
25	*Filobasidium uniguttulatum*	1	0/0/1	0/0/1	0/0/1	0/0/1
26	*Kluyveromyces polysporus*	1	0/0/1	0/0/1	0/0/1	0/0/1
27	*Lipomyces lipofer*	1	0/0/1	0/0/1	0/0/1	0/0/1
28	*L. starkei*	2	0/1/1	1/0/1	0/0/2	1/0/1
29	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/0/1
30	*Pichia angusta*	12	3/0/9	0/2/10	2/2/8	2/5/5
31	*P. anomala*	10	2/0/8	2/1/7	2/0/8	0/5/5
32	*P. euphorbiaphila*	1	0/0/1	0/0/1	0/0/1	0/0/1
33	*P. guilliermondii*	2	0/0/2	0/0/2	0/0/2	0/0/2
34	*P. heimii*	2	0/0/2	0/2/0	0/1/1	0/0/2
35	*P. jadinii*	2	0/0/2	0/0/2	0/0/2	0/1/1
36	*P. lynferdii*	20	2/6/12	5/3/12	6/3/11	3/6/11
37	*P. methanolica*	1	0/0/1	0/0/1	0/0/1	0/0/1
38	*P. Mexicana*	2	1/0/1	0/0/2	0/1/1	1/0/1
39	*P. mississippensis*	1	1/0/0	0/1/0	1/0/0	0/1/0
40	*P. ofunaensis*	3	0/0/3	1/0/2	0/0/3	0/1/2
41	*P. ohmeri*	5	2/1/2	0/0/5	1/0/4	0/1/4
42	*P. strasburgensis*	3	0/0/3	0/0/3	0/1/2	0/1/2
43	*P. sydowiorum*	1	0/0/1	0/0/1	0/0/1	0/0/1
44	*Saccharomycodes ludwijii*	1	0/1/0	1/0/0	1/0/0	0/0/1
45	*Sporidiobolus ruineniae*	5	0/1/4	1/0/4	1/0/4	0/0/5
46	*S. salmonicolor*	2	1/0/1	2/0/0	2/0/0	0/0/2
47	*Sporobolomyces tsugae*	4	0/0/4	0/1/3	0/0/4	1/0/3
48	*Stephanoascus ciferii*	8	1/0/7	1/0/7	1/0/7	0/4/4
49	*Tremella encephala*	2	1/1/0	0/1/1	0/1/1	0/0/2
50	*Wiliopsis californica*	2	0/0/2	0/0/2	0/0/2	0/0/2
	Total isolates	228	23/40/165	37/34/157	49/28/151	24/61/143

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

Table 2. Screening of Killer-Sensitive-Neutral (K/S/N) Pattern in yeasts species isolated from flowers' nectar of different plants using certain ascomycetous sensitive strains as seeded culture*.

No.	Streak Yeast species	No. of isolates tested	Seeded Sensitive Strains			
			Y371- *Candida valdiviana*	Y207- *Saccharomyces kluyveri*	Y2- *Williopsis californica*	Y90- *Williopsis californica*
			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	0/0/1
2	*Bullera megalospora*	2	0/0/2	0/0/2	0/0/2	0/0/2
3	*B. pseudoalba*	3	0/0/3	0/0/3	0/0/3	0/0/3
4	*B. pyricola*	15	6/4/5	2/2/11	1/2/12	6/1/8
5	*Candida friedrichii*	1	0/0/1	0/0/1	0/0/1	0/0/1
6	*C. gropengiesseri*	1	0/0/1	0/0/1	0/0/1	0/0/1
7	*C. magnoliae*	1	0/0/1	0/0/1	0/0/1	0/0/1
8	*C. membranaefaciens*	1	0/0/1	0/0/1	0/0/1	0/0/1
9	*C. rhagii*	4	0/0/4	0/0/4	0/0/4	1/0/3
10	*C. sake*	1	0/0/1	0/0/1	0/0/1	0/0/1
11	*C. succiphila*	7	3/1/3	1/0/6	1/0/6	0/0/7
12	*C. valdiviana*	4	0/1/3	0/1/3	0/1/3	1/0/3
13	*C. versatilis*	1	0/0/1	0/0/1	0/0/1	0/0/1
14	*C. xestobii*	1	0/0/1	0/0/1	0/0/1	0/0/1
15	*Cryptococcus albidus*	24	8/5/11	3/7/14	8/3/13	4/0/20
16	*C. curvatus*	3	0/0/3	0/0/3	0/1/2	0/0/3
17	*C. flavus*	2	0/0/2	0/0/2	1/0/1	0/1/1
18	*C. heveanesis*	2	1/0/1	0/0/2	0/0/2	0/0/2
19	*C. humicolus*	1	0/0/1	0/0/1	0/0/1	0/0/1
20	*C. hungaricus*	3	0/0/3	0/0/3	0/0/3	0/0/3
21	*C. laurentii*	27	2/8/17	7/0/20	5/3/19	5/0/22
22	*C. macerans*	5	1/1/3	0/1/4	1/0/4	2/0/3
23	*Cystofilobasidium bisporidii*	2	0/0/2	0/0/2	0/0/2	0/0/2
24	*Debaryomyces castellii*	16	0/4/12	1/2/13	1/2/13	1/0/15
25	*D. hansenii*	8	0/0/8	0/0/8	0/3/5	2/0/6
26	*D. vanrijii*	2	0/0/2	0/0/2	0/0/2	0/0/2
27	*Exophila salmonis*	11	0/011	0/0/11	0/0/11	0/0/11
28	*Fibulobasidium inconspicuum*	10	1/2/7	2/2/6	4/1/5	2/0/8
29	*Filobasidiella neoformans*	2	0/0/2	0/0/2	0/0/2	0/0/2
30	*Issatchenkia occidentalis*	1	0/0/1	0/0/1	0/0/1	1/0/0
31	*Lipomyces starkei*	1	0/0/1	0/0/1	0/0/1	0/0/1
32	*Mrakia frigida*	5	0/1/4	1/0/4	0/1/4	0/0/5
33	*Phaffia rhodozyma*	5	0/1/4	0/0/5	0/0/5	0/0/5
34	*Pichia angusta*	13	5/0/8	1/2/10	2/1/10	2/0/11
35	*P. castillae*	1	0/0/1	0/0/1	0/0/1	0/0/1
36	*P. dryadoides*	1	0/0/1	0/0/1	0/0/1	0/0/1
37	*P. fabianii*	1	0/0/1	0/0/1	1/0/0	0/1/0
38	*P. guilliermondii*	2	0/0/2	0/0/2	0/0/2	1/1/0
39	*P. jadinii*	3	0/0/3	0/0/3	0/0/3	0/0/3
40	*P. lynferdii*	23	4/2/17	3/2/18	4/3/16	5/0/18
41	*P. methanolica*	3	0/0/3	0/0/3	0/0/3	1/0/2
42	*P. mississippensis*	2	0/0/2	0/0/2	0/0/2	0/0/2
43	*P. ofunaensis*	2	0/0/2	0/0/2	0/0/2	0/0/2
44	*P. olmeri*	3	0/0/3	1/0/2	1/0/2	2/0/1
45	*Pseudozyma antarctica*	2	0/0/2	0/0/2	0/0/2	0/0/2
46	*P. fusiformata*	4	2/1/1	1/0/3	1/0/3	0/1/3
47	*Rhodosporidium toruloides*	3	0/1/2	0/0/3	0/0/3	2/0/1
48	*Rhodotorula fragaria*	2	0/0/2	0/0/2	0/0/2	0/0/2
49	*R. hinnulea*	4	0/1/3	0/0/4	0/0/4	1/0/3
50	*Saccharomyces kluyveri*	1	0/0/1	0/0/1	0/0/1	0/0/1
51	*Sporidiobolus ruineniae*	8	0/3/5	0/2/6	1/3/4	1/0/7
52	*Stephanoascus ciferii*	1	0/0/1	0/0/1	0/0/1	0/0/1
53	*Tremella aurentia*	1	0/0/1	0/0/1	0/0/1	0/0/1
54	*Williopsis californicai*	5	0/0/5	0/0/5	0/0/5	1/0/4
55	*W. pratensis*	1	0/0/1	0/0/1	0/0/1	1/0/0
56	*Zygoascus hellenicus*	4	0/1/3	0/0/4	0/0/4	1/0/3
	Total Isolates	263	33/37/193	23/21/219	32/24/207	43/5/215

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

Table 3. Screening of Killer-Sensitive-Neutral (K/S/N) Pattern in yeasts species isolated from slime fluxes of different trees using certain ascomycetous sensitive strains as seeded culture*.

No.	Streak Yeast species	No. of isolates tested	Seeded Sensitive Strains			
			Y371-*Candida valdiviana*	Y207-*Saccharomyces kluyveri*	Y2-*Williopsis californica*	Y90-*Williopsis californica*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Bullera pseudoalba*	1	0/0/1	1/0/0	0/0/1	0/0/1
2	*Candida lyxosophila*	1	0/0/1	0/0/1	0/0/1	0/0/1
3	*C. succiphila*	1	0/1/0	0/0/1	0/0/1	1/0/0
4	*C. valdiviana*	4	0/0/4	0/0/4	0/0/4	0/0/4
5	*Cryptococcus albidus*	3	0/1/2	0/0/3	0/0/3	0/1/2
6	*C. gastricus*	1	0/0/1	0/0/1	0/0/1	0/0/1
7	*Debaryomyces castellii*	1	0/0/1	0/0/1	0/0/1	0/0/1
8	*D. hansenii*	1	0/0/1	0/0/1	0/0/1	0/0/1
9	*D. yamadae*	1	0/0/1	0/0/1	0/0/1	0/0/1
10	*Fibulobasidium inconspicuum*	2	0/1/1	0/0/2	0/0/2	0/0/2
11	*Mrakia stokesii*	1	0/0/1	0/0/1	0/0/1	0/0/1
12	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/0/1
13	*Pichia angusta*	1	0/1/0	1/0/0	0/1/0	0/1/0
14	*P. anomala*	8	1/2/5	0/0/8	0/0/8	3/0/5
15	*P. lynferdii*	2	0/0/2	0/0/2	0/0/2	0/0/2
16	*P. methanolica*	1	0/0/1	0/0/1	0/0/1	0/0/1
17	*P. rabaulensis*	1	0/0/1	0/0/1	0/0/1	0/0/1
18	*P. strasburgensis*	2	0/0/2	0/0/2	0/0/2	0/1/1
19	*Rhodosporidium toruloides*	1	0/0/1	0/0/1	0/0/1	0/0/1
20	*Rhodotorula bacarum*	1	0/0/1	0/0/1	0/0/1	0/1/0
21	*Saitoella complicata*	1	1/0/0	0/0/1	0/0/1	1/0/0
22	*Sporidiobolus ruineniae*	3	0/0/3	0/0/3	0/0/3	2/0/1
23	*Williopsis californica*	2	0/1/1	0/0/2	0/0/2	0/0/2
	Total isolates	41	2/7/32	2/0/39	0/1/40	7/4/30

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

All four tested ascomycetous yeast strains showed sensitivity when tested to screen KSP in 263 isolates of 58 yeast species belonging to 21 genera from nectar. Among the yeast species from flowers' nectar *Cryptococcus laurentii*, *Pichia jadinii* and *P. mississippiensis* showed strong killing activity against tested ascomycetous sensitive yeast strains. It was also observed that all 4 tested sensitive strains used for screening, showed low or high dual activity especially against different isolates of *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* (Table 2).

On the other hand when these sensitive strains tested against 41 isolates of 23 yeast species belonging to 13 genera from slime fluxes of trees Y371-*C. valdiviana* showed highest sensitivity than Y207-*S. kluyveri*, whereas, both strains of *W. californica i.e.* Y2- and Y90 did not showed any sensitivity but produced some killing activity against yeast species from slime fluxes of trees (Table 3). Yeast species from gum that showed killing activity against sensitive strains included other strains of *Candida valdiviana*, *Cryptococcus albidus*, *Fibulobasidium inconspicuum* and *Pichia anomala* (Table 3). Similarly, when these sensitive strains were tested to screen killer,

sensitive and neutral phenotypes in 23 isolates of 19 yeast species belonging to 14 genera from soil, Y207-*S. kluyveri* showed highest sensitivity than Y371-*C. valdiviana* and Y2- *W. californica*. Whereas, Y90-*W. californica* did not showed any sensitivity against yeast isolates from soil (Table 4).

Table 4. Screening of Killer-Sensitive-Neutral (K/S/N) Pattern in yeasts species isolated from soil using certain ascomycetous sensitive strains as seeded culture*.

No.	Streak Yeast species	No. of isolates tested	Seeded Sensitive Strains			
			Y371-*Candida valdiviana*	Y207-*Saccharomyces kluyveri*	Y2-*Williopsis californica*	Y90-*Williopsis californica*
			K/S/N	K/S/N	K/S/N	K/S/N
1	*Bensingtonia phyllada*	1	0/0/1	0/0/1	0/0/1	0/0/1
2	*B. pseudoalba*	1	0/0/1	0/0/1	0/0/1	0/0/1
3	*Bullera pyricola*	1	0/1/0	0/0/1	0/0/1	0/0/1
4	*C. succiphila*	1	0/0/1	0/0/1	0/0/1	0/0/1
5	*C. valdiviana*	1	0/0/1	0/0/1	0/0/1	0/0/1
6	*Cryptococcus albidus*	3	0/0/3	0/0/3	0/0/3	0/1/2
7	*Debaryomyces castellii*	1	0/0/1	0/0/1	0/0/1	0/0/1
8	*D. hansenii*	3	0/1/2	0/0/3	0/0/3	0/1/2
9	*Fibulobasidium inconspicuum*	1	0/0/1	1/0/0	0/0/1	0/0/1
10	*Filobasidiella neoformans*	1	0/0/1	0/0/1	0/0/1	0/1/0
11	*Filobasidium uniguttulatum*	1	0/0/1	0/0/1	0/0/1	0/1/0
12	*Phaffia rhodozyma*	1	0/0/1	0/0/1	0/0/1	0/0/1
13	*P. euphorbiae*	1	0/0/1	0/0/1	0/0/1	0/1/0
14	*P. jadinii*	1	1/0/0	0/0/1	0/0/1	0/0/1
15	*P. lynferdii*	1	0/0/1	0/0/1	0/0/1	0/0/1
16	*Rhodosporidium toruloides*	1	0/1/0	0/0/1	0/0/1	1/0/0
17	*R. pilatii*	1	0/0/1	0/0/1	0/0/1	0/1/0
18	*Sporidiobolus ruineniae*	2	0/0/2	0/0/2	0/0/2	0/0/2
19	*Zygosaccharomyces bailii*	1	0/0/1	0/0/1	0/0/1	0/0/1
	Total isolates	24	1/3/20	1/0/23	0/0/24	1/6/17

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

In certain findings Golubev (1992, 1997) 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. In the present study, we observed inversely related effectiveness of killer activity in relation to habitat *i.e.*, sensitive ascomycetous yeast strains from flowers' nectar habitat when tested against yeast isolates from dairy products appeared killer as compared to sensitive and vice versa. The results are very clear especially when Y207-*S.kluyveri* and Y2-*W. californica* from flowers' nectar tested against yeast isolates from dairy products (Table1) and Y90-*W. californica* which was originally isolated from dairy products against isolates of different yeast species from flowers' nectar (Table 2). Moreover the results of screening Killer-Sensitive Pattern (KSP) among yeast species of different habitats, it is evident that Y207-*S. kluyveri* and Y371-*C. valdiviana* appeared most suitable strains to screen KSP in yeast species from all substrates as compared to Y90-*W. californica* which did not showed sensitivity against slime flux yeast isolates; whereas sensitive strain Y2-*W. californica* appeared sensitive against dairy and flowers' nectars' yeasts' isolates only. Even though the sensitive strains Y90 and Y2 belonging to same species, they may be different phylogenetically since the killer-sensitive pattern is directly related with the production of killer toxins which are encoded by several different genomes, such as the genome of the double-stranded (ds) RNA virus that persistently resides in its host symbiotically (Bostian *et al*., 1984; Dignard *et al*., 1991;

Schmitt, 1995; Park *et al*., 1994; Tao *et al*., 1990), the chromosomal DNA (Goto *et al*., 1990 a, 1990b; Suzuki and Nikkuni, 1994), and the linear cytoplasmic DNA plasmid (Stark and Boyd, 1986; Bolen *et al*., 1994). There is evidence that killer yeasts secrete different killer toxins with activities specific for different target yeast cells (Wickner, 1996). Moreover these results are indicating the role of environment in the distribution of yeasts in different habitats.

The phenomenon of killer activity is also one of the mechanisms of antagonism among yeasts in natural as well as artificial habitats especially to control phytopathogenic fungi (de Souza *et al*., 2009). Therefore in nature, this phenomenon leads to the dominance of killer strains in particular ecological niches (Zorg *et al*., 1988), especially 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). Apart from sensitivity and killing activity, several tested strains showed neutral activity, probably due to lack of cell wall receptors (Marquina *et al*., 2002; Golubev, 2006) which could be used taxonomically to classify yeast strains. In a recent killer activity has also been recognized in clinical isolates of *Candida glabrata* against a strain of *Saccharomyces cerevisiae* W303 sensitive strain (Arroyo-Helguera *et al*., 2012) where killer activity was found at every pH and temperature tested, however, the role of extrachromosomal genetic elements was not observe associated with killer activity in any of the *C. glabrata* isolates. It is inferred that the screening of killer-sensitive pattern may be observed carefully for overall phylogenetic evaluation and characterization by a narrow range of activity to clarify relationships between more closely related species, or for grouping phenotypically similar strains before using molecular techniques.

REFERENCES

Abranches, J., P.B. Morais, C.A. Rosa, L.C. Mendonca-Hagler and A.N. Hagler (1997). The incidence of killer activity and extracellular proteases in tropical yeast communities. *Can J Microbiol.,* 43: 328-336.

Arroyo-Helguera, O., D.L.P. Alejandro and C. Irene (2012). Occurrence of killer C*andida glabrata* clinical isolates. *Braz J Microbiol*., 43: 880-887.

Bevan, E.A. and M. Mackower (1963). The physiological basis of the killer character in yeast. *Proc Intl Congre Genet*, 1: 202-203.

Bolen, P.L., E.M. Eastman, P.L. Cihak and G.T. Hayman (1994). Isolation and sequence analysis of a gene from the linear DNA plasmid Ppacl-2 of *Pichia acaciae* that shows similarity to a killer toxin gene of *Kluyveromyces lactis. Yeast*, 10: 403–414.

Bostian, K.A., Q. Elliot, H. Bussey, V. Burn, A. Smith and D.J. Tipper (1984). Sequence of the preprotoxin dsRNA gene of type 1 killer yeasts: multiple processing events producing a two component toxin. *Cell*, 36: 741-751.

Bussey, H. (1972). Effects of yeast killer factor in sensitive cells. *Nature New Biol*., 235: 73-75.

Bussey, H., T. Vernet and A.M. Sdicu (1988). Mutual antagonism among killer yeasts: competition between K1 and K2 killers and a novel cDNA-based K1-K2 killer strain of *Saccharomyces cerevisiae*. *Can J Microbiol*., 34: 38 – 44.

de Souza, A., P. Barroso, T. Pinotti, A.N. Hagler, L.C.S. Mendonça-Hagler and A. Macrae (2009). Killer yeasts inhibit the growth of the phytopathogen *Moniliophthora perniciosa*, the causal agent of Witches' Broom disease. *Braz J Microbiol*., 40: 108-110.

Dignard, D., M. Whiteway, D. Germain, D. Tessier and D.Y. Thomas (1991). Expression in yeast of a cDNA copy of the K2 killer toxin gene. *Mol Gen Genet*, 227: 127–136.

Douglas, C.M., S.L. Sturley and K.A. Bostian (1988). Role of protein processing, intracellular trafficking and endocytosis in production and immunity to yeast killer toxin. *Eur J Epidemiol*., 4: 400-408.

Golubev, W.I. (2006). Antagonistic interactions among yeasts. In: *The Yeast Handbook. Biodiversity and Ecophysiology of Yeasts* (Rosa CA & P´eter G, eds), pp. 197–219. Springer, Berlin, Germany.

Golubev, W.I., R. Ikeda, T. Shinoda and T. Nakase (1997). Antifungal activity of *Bullera alba* (Hanna) Derx. *Mycoscience*, 38: 25–29.

Golubev, W.I. (1992). Antibiotic activity and taxonomic position of *Rhodotorula fujisanensis* (Soneda) *Johnson et Phaff. Mikrobiol Zhurnal (Kiev)*. 54: 21–26.

Goto, K., H. Fukuda, K. Kichise, K. Kitano and S. Hara (1990). Cloning and nucleotide sequence of the KHS killer gene of *Saccharomyces cerevisiae. Agricultural Biol Chem*.., 55: 1953–1958.

Goto, K., Y. Iwatuki, K. Kitano, T. Obata and S. Hara (1990). Cloning and nucleotide sequence of the KHR killer gene of *Saccharomyces cerevisiae. Agricultural Biol Chem.*, 54: 979–984.

Hidalgo, P. and M. Flores (1994). Occurrence of the killer character in yeasts associated with wine production. *Food Microbiol.*, 11: 161-167.

Jacobs, C.J., I. Fourie and van H.J.J. Vuuren (1988) Occurrence and detection of killer yeasts on Chenin Blanc grapes and grape skins. *S Afr J Enol Vitic*., 9: 28-31.

Kreger-van Rij N.J.W. (1984). *The Yeasts, A Taxonomic Study*. Elsevier, Amsterdam, The Netherlands.

Longo, E., J.B. Velazquez, J Cansado, P. Calo and T.G. Villa (1990). Role of killer effect in fermentations conducted by mixed cultures of *Saccharomyces cerevisiae*. *FEMS Microbiol Lett.,* 71: 331-336.

Marquina, D., A. Santos and J .M. Peinado (2002). Biology of killer yeasts. *Intl Microbiol*., 5: 65–71.

Morace, G., C. Arehibusacchi, M. Sesito and L. Polonelli (1984). Strain differentiation of pathogenic yeasts by the killer system. *Mycopathol*., 84 (2-3): 81-86.

Mushtaq, M., S. Nahar and M.H. Hashmi (2013). Screening of Killer-Sensitive Pattern (KSP) for biotyping yeast strains isolated from dairy products. *Pak J Bot.,* 45(3): 1039-1044.

Mushtaq, M., S. Nahar and M.H. Hashmi (2010). Screening of Killer-Sensitive Pattern (KSP) for biotyping yeast strains isolated from slime fluxes of trees and flowers' nectar. *Pak J Bot.,* 42(6): 4313-4327.

Mushtaq, M., S. Nahar and M.H. Hashmi (2008). Biodiversity of yeast mycoflora in slime fluxes of some trees. *Pak J Bot*., 40(3): 1291-1299.

Mushtaq, M., J. Ayesha and S. Nahar (2008). Biodiversity of yeast mycoflora in nectar of *Malvaviscus arboreus* and *Pancratium biflorum* flowers. *Pak J Bot.,* 40(2): 877-885.

Mushtaq, M., J. Ayesha and S. Nahar (2007). Biodiversity of yeast mycoflora in nectar of *Hibiscus rosa-sinensis* and *Ixora coccinea* flowers. *Pak J Bot.,* 39(4): 1367-1376.

Mushtaq, M, I. Faiza and S. Nahar (2007). Detection of yeast mycoflora from butter. *Pak J Bot*., 39(3): 887-896.

Mushtaq, M., J. Ayesha and S. Nahar (2006). Biodiversity of yeast mycoflora in nectar of *Bombax cieba* and *Canna indica* flower. *Pak J Bot.,* 38(4): 1279-1288.

Mushtaq, M., I. Faiza and S. Nahar (2006). Detection and identification of yeast mycoflora from milk and yogurt. *Pak J Bot.,* 38(3): 859-868.

Mushtaq, M., S. Nahar and M.H. Hashmi (2005). Yeast mycoflora associated with slime fluxes of trees. *Pak J Bot*., 37(2): 439-450.

Mushtaq, M., S. Nahar and M.H. Hashmi (2004). Isolation and identification of yeast flora from soil of Karachi, Pakistan. *Pak J Bot*., 36(1): 173-180.

Park, C.M., J.A. Bruenn, C. Ganesa, W.F. Flurkey, R.F. Bozarth and Y. Koltin (1994). Structure and heterologous expression of the *Ustilago maydis* viral toxin KP4. *Mol Microbiol.,* 11(1): 155-164.

Pfeiffer, P. and F. Radler (1982). Purification and characterization of extracellular and intercellular killer toxins of *Saccharomyces cerevisiae*- strain 28. *J Gen Microbiol*., 128: 2699-2706.

Radler, F., P. Pfieffer and M. Dennert (1985). Killer toxins in new isolates of the yeasts *Hanseniaspora uvarum* and *Pichia kluyveri*. *FEMS Microbiol Lett.,* 29: 269-272.

Schmitt, M.J. (1995). Cloning and expression of a cDNA copy of the viral K-28 killer toxin gene in yeast. *Mol Gen Genet.,* 246: 236–246.

Spencer, J.F.T. and D. .M. Spencer (1997). *Yeasts in Natural and Artificial Habitats*. Spriner-Verlag Berlin Heidelberg.

Starmer, W.T., P.F. Ganter, V. Aberdeen, M.A. Lachance and H.J. Phaff (1987). The ecological role of killer yeasts in natural communities of yeasts. *Can J Microbiol.,* 33: 783-796.

Suzuki, C. and S. Nikkuni (1994). The primary and subunit structure of a novel type killer toxin produced by a halotolerant yeast, *Pichia farinosa*. *J Biol Chem.,* 269: 3041–3046.

Stark, M.J.R. and A. Boyd (1986). The killer toxin of *Kluyveromyces lactis*: characterization of the toxin subunits and identification of the genes which encode them. *EMBO J*., 5: 1995–2002.

Tao, J., I. Ginsberg, N. Banerjee, W. Held, Y. Koltin and J.A. Bruenn (1990). *Ustilago maydis* KP6 killer toxin: structure, expression in *Saccharomyces cerevisiae*, and relationship to other cellular toxins. *Mol Cell Biol*., 10(4): 1373–1381.

Vagnoli, P., R.A. Musmano, S. Cresti, T. Maggio and G. Croatza (1993). Occurrence of killer yeasts in spontaneous fermentation from the Tuscany region of Italy. *Appl Environ Microbiol.,* 59: 4037-4043.

Wickner, R.B. (1996). Double-Stranded RNA viruses of *Saccharomyces cerevisiae*. *Microbiol Rev.,* 60: 250–265.

Wickner, R.B. (1979). The killer double-stranded RNA plasmids of yeasts. *Plasmid*, 2: 303-322.

Woods, D.R. and E.A. Bevan (1968). Studies on the nature of the killer factor produced by *Saccharomyces cerevisiae*. *J Gen Microbiol*., 51: 115-126.

Young, T.W. and M. Yagiu (1978). A comparison of the killer character in different yeast and its classification. *Anotonie van Leeuwenhoek. J Microbiol Serol*., 44: 59-77.

Zorg, J., S. Kilian and F. Radler (1988). Killer Toxin-Producing Strains of the yeasts *Hanseniaspora uvarum* and *Pichia kluyveri*. *Arch Microbiol.,* 149: 261-267.

(*Accepted for publication May 2014*)