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# SCREENING OF KILLER-SENSITIVE PATTERN (KSP) FOR BIOTYPING YEAST STRAINS ISOLATED FROM DAIRY PRODUCTS

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**Abstract**

Killer-Sensitive Pattern (KSP) was screened by cross reactions in 50 yeast species belonging to 20 genera which were previously isolated from different dairy products. The Killer-Sensitive Pattern (KSP) appeared as strain character rather than species level. Among all yeasts, strain designated as YF19-*Lipomyces starkeyi* appeared as the most killer *i.e.* showed 46.93% killing activity and strains appeared as most sensitive were YF45-*Bullera pyricola* (77.55%), YF42-*Pichia heimii* (77.50%), YF87-*Bullera pseudoalba* (51.02%) and Y90-*Williopsis californica* (42.86%).

## Introduction

Certain yeasts produce killer toxins (mycocins), which are lethal to closely related strains but the killer yeast itself has a killer resistant phenotype (Bevan & Makower, 1963; Woods & Bevan, 1968; Bussey, 1972; Pfeiffer & Radler, 1982; Spencer & Spencer, 1997). The killer phenomenon provides an excellent model system to study host-virus interactions in eukaryotic cells (Wickner, 1979, 1989) 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 & 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).

Killer toxins are protein in nature and active at low pH (Young & Yagiu, 1978; Pfeiffer & Radler, 1982; Radler *et al*., 1985). They are secreted in an inactive glycosylated form that once secreted to the cell plasma membrane, becomes cleaved (Zhu *et al*., 1993). 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. However 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 KSP profiles may have taxonomic relevance. The theoretical rationale supporting this conclusion is related to the resistance mechanism. In a previous study we screened Killer-Sensitive-Pattern (KSP) among yeast species previously isolated from slime fluxes of different trees and flowers' nectar (Mushtaq *et al*., 2010). In the present study Killer-Sensitive-Pattern (KSP) has been screened by cross reactions in yeast species isolated from different dairy products (Mushtaq *et al*., 2007; Mushtaq *et al*., 2006).

## Materials and Methods

A modified method of Abranches *et al*., (1997) was used to screen Killer-Sensitive Pattern (killer, sensitive and neutral phenotypes) in 23 yeasts species belonging to 13 genera previously isolated from slime fluxes of trees and 57 yeast species belonging to 23 genera from flowers' nectar, on yeast extract-malt extract agar supplemented with 0.003% methylene blue (YM-MB Agar). Twenty-four h old yeast culture grown on YM agar (Kreger-van Rij, 1984) was diluted in double distilled sterile 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 killer if a blue colored killing zone appeared on streak and sensitive if killing zone appeared around the streak on lawn. Intensity of the killer activity was recorded as $K^{+1}$ (very light blue killing zone), $K^{+2}$ (blue killing zone), $K^{+3}$ (dark blue killing zone) and $K^{+4}$ (intense dark blue killing zone) reaction. The sensitivity of the yeast was also recorded in the same manner as $S^{+1}$, $S^{+2}$, $S^{+3}$ and $S^{+4}$. A negative reaction indicated by (-) when yeasts did not show any reaction. Percentages of killing activity and sensitivity of yeast species were calculated. Strains that showed >40% killing activity or sensitivity were considered as super killers and super sensitive.

## Results

Killer-Sensitive Pattern (KSP) (killer, sensitive & neutral phenotypes) was screened by cross reactions in 50 yeast species belonging to 20 genera previously isolated from different dairy products. Spectrum of killing activity and sensitivity (species vise) is presented respectively in table 1 and their percentages in table 2. One of the strain of *Lipomyces starkeyi* designated as YF19 showed 46.93% killing activity and appeared as the most killer strain, on the other hand, yeast strains designated as YF45-*Bullera pyricola* (77.55%), YF42-*Pichia heimii* (77.50%), YF87-*B. pseudoalba* (51.02%) and Y90-*Williopsis californica* (42.86%) appeared as the most sensitive yeast strains.

**Table 1. Cross reaction screening of killer, sensitive and neutral phenotypes in yeast species isolated from dairy products.**

| | | Streak strains | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| No. | Seeded strains | *Arxula adeninovorans* | *Bensingtonia intermedia* | *B. naganoensis* | *Bullera pseudoalba* | *B. pyricola* | *C. diddensiae* | *C. etchellsii* | *C. friedrichii* | *C. haemulonii* | *C. membranifaciens* | *C. pseudointermedia* | *C. shehatae* | *C. succiphila* | *C. valdiviana* | *C. xestobii* | *Clavispora lusitaniae* | *Cryptococcus albidus* |
| 1. | *Arxula adeninovorans* | X | - | $S^{+1}$ | - | $K^{+1}$ | - | - | - | $S^{+1}$ | - | - | - | - | - | - | - | - |
| 2. | *Bensingtonia intermedia* | - | X | - | $K^{+2}$ | $K^{+2}$ | $S^{+3}$ | - | - | - | - | - | - | - | - | - | - | - |
| 3. | *B. naganoensis* | $K^{+1}$ | - | X | - | $K^{+1}$ | - | - | - | - | - | $S^{+2}$ | - | - | - | - | - | - |
| 4. | *Bullera pseudoalba* | - | $S^{+2}$ | - | X | $K^{+1}S^{+2}$ | $S^{+2}$ | $S^{+1}$ | - | $S^{+2}$ | $S^{+1}$ | - | $S^{+3}$ | - | $K^{+2}$ | - | $S^{+4}$ | $S^{+1}$ |
| 5. | *B. pyricola* | $S^{+1}$ | $S^{+2}$ | $S^{+1}$ | $K^{+2}S^{+1}$ | X | $S^{+1}$ | - | $S^{+1}$ | - | $K^{+2}S^{+1}$ | - | $S^{+1}$ | $S^{+2}$ | $S^{+1}$ | $K^{+2}$ | $S^{+1}$ | $K^{+2}S^{+1}$ |
| 6. | *Candida diddensiae* | - | $K^{+3}$ | - | $K^{+2}$ | $K^{+2}$ | X | - | - | - | - | - | - | - | - | $K^{+2}$ | $K^{+1}$ | $K^{+2}$ |
| 7. | *C. etchellsii* | $K^{+1}$ | - | - | $K^{+1}$ | - | - | X | $K^{+1}$ | - | - | - | - | - | $K^{+1}$ | - | - | - |
| 8. | *C. friedrichii* | - | $K^{+3}$ | - | $K^{+1}$ | $K^{+2}$ | - | - | X | - | $K^{+1}$ | - | - | - | $K^{+2}$ | - | - | - |
| 9. | *C. haemulonii* | - | - | - | $K^{+2}$ | - | - | - | - | X | - | - | - | - | - | - | - | $K^{+1}$ |
| 10. | *C. membranifaciens* | - | - | - | $K^{+1}$ | $K^{+1}S^{+2}$ | - | - | - | - | X | - | $S^{+3}$ | - | - | - | $S^{+1}$ | - |
| 11. | *C. pseudointermedia* | - | - | $K^{+2}$ | - | - | - | - | - | - | - | X | - | - | - | - | - | - |
| 12. | *C. shehatae* | - | - | - | $K^{+3}$ | $K^{+2}$ | - | - | - | - | $K^{+3}$ | - | X | - | $K^{+3}$ | $S^{+1}$ | $K^{+3}$ | $K^{+3}$ |
| 13. | *C. succiphila* | - | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | X | - | - | - | - |
| 14. | *C. valdiviana* | - | - | - | $S^{+2}$ | $K^{+1}$ | - | $S^{+1}$ | - | - | - | - | $S^{+3}$ | - | X | $K^{+1}$ | - | - |
| 15. | *C. xestobii* | - | - | - | - | $S^{+2}$ | $K^{+2}$ | - | - | - | - | - | $S^{+1}$ | - | $S^{+1}$ | X | - | - |
| 16. | *Clavispora lusitaniae* | - | - | - | $K^{+2}$ | $K^{+1}$ | $S^{+2}$ | - | - | - | $K^{+1}$ | - | $S^{+3}$ | - | - | - | X | $K^{+1}$ |
| 17. | *Cryptococcus albidus* | - | - | - | $K^{+1}$ | $K^{+1}S^{+2}$ | $S^{+2}$ | - | - | $S^{+1}$ | - | - | $S^{+3}$ | - | - | - | $S^{+1}$ | X |
| 18. | *C. gastricus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 19. | *Debaryomyces castellii* | - | $S^{+1}$ | - | $K^{+2}$ | $K^{+1}$ | - | - | - | - | - | - | - | - | - | $K^{+1}$ | - | - |
| 20. | *D. hansenii* | $S^{+1}$ | $K^{+1}$ | - | $S^{+1}$ | $K^{+1}$ | - | - | - | - | - | $S^{+1}$ | - | - | - | $S^{+1}$ | - | - |
| 21. | *D. nepalensis* | - | - | - | - | $K^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 22. | *D. vanrijii* | - | - | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23. | *D. yamadae* | - | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 24. | *Fibulobasidium inconspicuum* | - | - | $K^{+2}S^{+2}$ | $K^{+1}$ | - | $S^{+1}$ | - | - | - | - | $S^{+1}$ | - | - | $K^{+1}$ | - | - | - |
| 25. | *Filobasidiella neoformans* | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26. | *Filobasidium uniguttulatum* | - | - | - | $K^{+1}$ | $K^{+2}$ | - | - | - | - | - | - | - | - | $K^{+1}$ | - | - | - |
| 27. | *Kluyveromyces polysporus* | $S^{+1}$ | - | $S^{+1}$ | - | $K^{+1}$ | $S^{+1}$ | - | - | - | - | $S^{+1}$ | $S^{+3}$ | - | - | - | - | - |
| 28. | *Lipomyces lipofer* | $S^{+2}$ | - | - | - | $K^{+2}$ | - | - | - | - | $K^{+3}$ | - | - | - | $K^{+1}$ | - | $K^{+2}$ | $K^{+1}$ |
| 29. | *L. starkeyi* | - | $K^{+1}$ | $K^{+3}$ | $K^{+3}$ | $K^{+3}$ | $K^{+2}$ | - | $K^{+2}$ | $K^{+2}$ | $K^{+2}$ | - | $K^{+2}$ | - | $K^{+1}$ | - | $K^{+2}$ | $K^{+2}$ |
| 30. | *Phaffia rhodozyma* | - | - | - | - | $K^{+3}$ | - | - | $K^{+1}$ | - | - | - | - | - | $K^{+1}$ | - | - | - |
| 31. | *Pichia angusta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32. | *P. anomala* | $S^{+1}$ | $S^{+1}$ | - | - | $K^{+2}$ | $S^{+3}$ | $K^{+1}$ | - | $K^{+1}$ | - | $S^{+1}$ | $S^{+3}$ | - | - | $K^{+1}$ | - | - |
| 33. | *P. euphorbiiphila* | - | - | - | $K^{+1}$ | $K^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 34. | *P. guilliermondii* | - | - | - | $K^{+2}$ | $K^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 35. | *P. heimii* | $S^{+2}$ | $S^{+4}$ | $S^{+3}$ | $K^{+4}S^{+2}$ | $K^{+3}S^{+2}$ | $S^{+3}$ | - | - | $S^{+1}$ | $S^{+3}$ | $S^{+2}$ | $K^{+2}S^{+2}$ | - | $K^{+3}S^{+2}$ | $K^{+3}$ | $K^{+2}S^{+2}$ | $K^{+3}S^{+2}$ |
| 36. | *P. jadinii* | - | - | - | - | $K^{+1}$ | - | - | - | - | - | - | $S^{+1}$ | - | - | - | - | - |
| 37. | *P. lynferdii* | $K^{+2}$ | - | $K^{+2}$ | $K^{+2}$ | $K^{+3}$ | - | - | - | - | $K^{+2}$ | $K^{+2}$ | - | - | - | - | - | $K^{+2}$ |
| 38. | *P. methanolica* | - | - | - | $K^{+2}S^{+2}$ | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 39. | *P. mexicana* | $S^{+1}$ | $S^{+2}$ | - | - | $K^{+1}$ | - | - | - | - | - | - | $S^{+3}$ | - | - | - | - | - |
| 40. | *P. ofunaensis* | - | - | - | $K^{+3}$ | - | - | - | - | - | $K^{+1}S^{+1}$ | $S^{+2}$ | - | - | - | - | - | $K^{+2}S^{+1}$ |
| 41. | *P. ohmeri* | - | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 42. | *P. strasburgensis* | - | - | - | $K^{+2}$ | - | $S^{+1}$ | - | - | - | - | - | - | - | - | - | - | - |
| 43. | *P. sydowiorum* | - | - | $K^{+1}$ | - | - | - | - | - | - | - | $S^{+3}$ | - | - | - | - | - | - |
| 44. | *Saccharomycodes ludwigii* | - | - | - | $K^{+2}$ | $K^{+1}$ | - | $K^{+3}$ | - | - | - | - | - | $S^{+1}$ | - | - | - | - |
| 45. | *Sporidiobolus ruineniae* | - | - | $K^{+2}$ | $K^{+2}$ | - | - | - | - | - | - | $S^{+2}$ | - | - | - | - | $K^{+2}$ | - |
| 46. | *S. salmonicolor* | - | - | $K^{+2}$ | $K^{+4}$ | $K^{+2}$ | - | - | $K^{+3}$ | $S^{+1}$ | - | $S^{+1}$ | $S^{+1}$ | $S^{+1}$ | $K^{+1}$ | - | - | $S^{+2}$ |
| 47. | *Sporobolomyces tsugae* | $K^{+1}$ | $S^{+3}$ | $K^{+2}$ | $K^{+2}$ | $K^{+2}$ | - | $S^{+2}$ | - | $S^{+2}$ | - | $K^{+1}S^{+1}$ | - | - | $K^{+2}$ | $K^{+3}$ | - | - |
| 48. | *Stephanoascus ciferrii* | - | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| 49. | *Tremella encephala* | - | - | - | - | $K^{+1}$ | $S^{+1}$ | - | - | - | - | - | $K^{+1}$ | - | - | - | $S^{+1}$ | - |
| 50. | *Williopsis californica* | - | $S^{+2}$ | $K^{+1}S^{+2}$ | $S^{+1}$ | $K^{+1}$ | $S^{+2}$ | - | $S^{+3}$ | - | - | $S^{+2}$ | - | - | $K^{+1}$ | $K^{+2}$ | - | - |

**Table 1. (Cont'd.).**

| No. | Seeded strains | Streak strains | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
| | | *C. gastricus* | *Debaryomyces castellii* | *D. hansenii* | *D. nepalensis* | *D. vanrijii* | *D. yamadae* | *Fibulobasidium inconspicuum* | *Filobasidiella neoformans* | *Filobasidium uniguttulatum* | *Kluyveromyces polysporus* | *Lipomyces lipofer* | *L. starkeyi* | *Phaffia rhodozyma* | *Pichia angusta* | *P. anomala* | *P. euphorbiiphila* | *P. guilliermondii* |
| 1. | *Arxula adeninovorans* | - | - | $K^{+1}$ | - | - | - | - | - | - | $K^{+1}$ | - | - | - | - | - | - | - |
| 2. | *Bensingtonia intermedia* | - | $K^{+1}$ | $S^{+1}$ | - | - | - | - | - | - | - | - | $S^{+1}$ | - | - | $K^{+1}$ | - | - |
| 3. | *B. naganoensis* | - | - | - | - | - | - | - | - | - | $K^{+1}$ | - | - | - | - | - | - | - |
| 4. | *Bullera pseudoalba* | - | $S^{+2}$ | $K^{+1}$ | - | - | - | $S^{+1}$ | $S^{+2}$ | $S^{+1}$ | - | - | $S^{+3}$ | - | - | - | $S^{+1}$ | $S^{+2}$ |
| 5. | *B. pyricola* | - | $S^{+1}$ | $S^{+1}$ | $S^{+1}$ | $S^{+2}$ | $K^{+2}$ | $K^{+2}S^{+2}$ | - | $S^{+2}$ | $S^{+1}$ | $S^{+1}$ | $S^{+3}$ | $S^{+2}$ | - | $S^{+1}$ | $S^{+1}$ | $S^{+1}$ |
| 6. | *Candida diddensiae* | - | - | - | - | - | - | $K^{+1}$ | - | - | $K^{+1}$ | - | $S^{+3}$ | - | - | $K^{+4}$ | - | - |
| 7. | *C. etchellsii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | - | - |
| 8. | *C. friedrichii* | - | - | $K^{+3}$ | - | $K^{+2}$ | - | - | - | - | - | - | $K^{+1}$ | $S^{+1}$ | - | - | - | - |
| 9. | *C. haemulonii* | - | - | - | - | - | - | - | - | - | - | - | $S^{+2}$ | - | - | $K^{+1}$ | - | - |
| 10. | *C. membranifaciens* | - | - | - | - | - | - | - | - | - | - | $K^{+3}$ | $S^{+2}$ | - | - | - | - | - |
| 11. | *C. pseudointermedia* | - | - | $K^{+1}$ | - | - | - | $K^{+1}$ | - | - | $K^{+1}$ | - | - | - | - | $K^{+1}$ | - | - |
| 12. | *C. shehatae* | - | - | - | - | - | - | - | - | - | $K^{+3}$ | - | $S^{+1}$ | - | - | $K^{+4}$ | - | - |
| 13. | *C. succiphila* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. | *C. valdiviana* | - | - | - | - | - | - | $S^{+1}$ | - | $S^{+1}$ | - | - | $S^{+1}$ | $S^{+1}$ | - | - | - | - |
| 15. | *C. xestobii* | - | $S^{+1}$ | $K^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | - | - |
| 16. | *Clavispora lusitaniae* | - | - | - | - | - | - | - | - | - | - | $S^{+2}$ | - | - | - | - | - | - |
| 17. | *Cryptococcus albidus* | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | $S^{+2}$ | - | - | - | - | - |
| 18. | *C. gastricus* | X | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | - | - | $K^{+4}$ | - | - |
| 19. | *Debaryomyces castellii* | - | X | - | - | - | - | - | $S^{+1}$ | - | - | - | $S^{+2}$ | - | - | - | - | - |
| 20. | *D. hansenii* | - | - | X | - | - | - | $K^{+3}$ | - | - | - | - | $S^{+2}$ | - | - | - | $S^{+1}$ | - |
| 21. | *D. nepalensis* | - | - | - | X | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22. | *D. vanrijii* | - | - | - | - | X | - | - | - | - | - | - | - | - | - | - | - | - |
| 23. | *D. yamadae* | - | - | - | - | - | X | - | - | - | - | - | - | - | - | - | - | - |
| 24. | *Fibulobasidium inconspicuum* | - | - | $S^{+3}$ | - | - | - | X | - | - | - | - | - | - | - | $K^{+1}$ | - | - |
| 25. | *Filobasidiella neoformans* | - | $K^{+1}$ | - | - | - | - | - | X | - | - | - | - | - | - | - | - | - |
| 26. | *Filobasidium uniguttulatum* | - | - | - | - | - | - | - | - | X | - | $S^{+3}$ | - | - | - | - | - | - |
| 27. | *Kluyveromyces polysporus* | - | - | - | - | - | - | - | - | - | X | $S^{+2}$ | - | - | - | - | - | - |
| 28. | *Lipomyces lipofer* | - | - | - | - | - | - | - | - | $K^{+3}$ | $K^{+2}$ | X | $S^{+1}$ | - | - | $K^{+4}$ | - | - |
| 29. | *L. starkeyi* | $K^{+2}$ | $K^{+2}$ | $K^{+2}$ | - | - | - | - | - | - | - | $K^{+2}$ | X | - | - | $K^{+2}$ | - | - |
| 30. | *Phaffia rhodozyma* | - | - | - | - | - | - | - | - | - | - | - | - | X | - | - | - | - |
| 31. | *Pichia angusta* | - | - | - | - | - | - | - | - | - | - | - | - | - | X | - | - | - |
| 32. | *P. anomala* | $S^{+3}$ | - | - | - | - | - | $S^{+1}$ | - | - | - | $S^{+3}$ | $S^{+1}$ | $S^{+1}$ | - | X | $S^{+1}$ | - |
| 33. | *P. euphorbiiphila* | - | - | $K^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | $K^{+1}$ | X | - |
| 34. | *P. guilliermondii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| 35. | *P. heimii* | $S^{+1}$ | $S^{+2}$ | $S^{+3}$ | $S^{+2}$ | - | $K^{+2}$ | $K^{+1}S^{+3}$ | $S^{+4}$ | $S^{+3}$ | $K^{+2}S^{+3}$ | $S^{+3}$ | - | $S^{+3}$ | - | $K^{+3}S^{+2}$ | $S^{+3}$ | $S^{+3}$ |
| 36. | *P. jadinii* | - | - | - | - | - | - | - | - | - | $K^{+1}$ | $S^{+1}$ | - | - | - | - | - | - |
| 37. | *P. lynferdii* | - | - | - | - | - | - | $S^{+1}$ | - | $S^{+1}$ | $S^{+1}$ | - | - | - | - | - | $K^{+2}$ | - |
| 38. | *P. methanolica* | - | $K^{+1}$ | - | - | - | - | - | - | - | $K^{+2}$ | - | - | - | - | $K^{+1}$ | - | - |
| 39. | *P. mexicana* | - | - | - | - | - | - | $S^{+1}$ | $S^{+1}$ | - | $S^{+1}$ | - | $S^{+1}$ | $K^{+2}S^{+1}$ | - | - | - | - |
| 40. | *P. ofunaensis* | - | - | - | - | - | - | $S^{+1}$ | - | $K^{+2}S^{+1}$ | $S^{+3}$ | - | $S^{+3}$ | - | - | - | - | - |
| 41. | *P. ohmeri* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 42. | *P. strasburgensis* | - | $K^{+1}$ | $S^{+1}$ | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | $K^{+1}$ | - | - |
| 43. | *P. sydowiorum* | - | - | $K^{+1}S^{+1}$ | - | - | - | $K^{+1}$ | - | - | $K^{+1}$ | $S^{+1}$ | - | - | $K^{+1}$ | - | - | - |
| 44. | *Saccharomycodes ludwigii* | - | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | - | - | - | - | - |
| 45. | *Sporidiobolus ruineniae* | - | - | - | - | - | - | $S^{+1}$ | - | $K^{+2}S^{+1}$ | $S^{+3}$ | $S^{+2}$ | $S^{+2}$ | - | - | - | - | - |
| 46. | *S. salmonicolor* | - | - | $K^{+2}$ | - | - | - | $K^{+1}$ | - | $K^{+1}$ | $K^{+1}$ | $S^{+1}$ | $S^{+1}$ | - | - | - | - | - |
| 47. | *Sporobolomyces tsugae* | $S^{+2}$ | $S^{+3}$ | - | - | - | - | $K^{+1}$ | $S^{+3}$ | $K^{+1}$ | $K^{+2}$ | - | $S^{+2}$ | $S^{+3}$ | - | - | - | $K^{+3}S^{+3}$ |
| 48. | *Stephanoascus ciferrii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 49. | *Tremella encephala* | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | $S^{+2}$ | - | - | - | - | - |
| 50. | *Williopsis californica* | - | $S^{+2}$ | $S^{+3}$ | $S^{+1}$ | - | - | $K^{+1}S^{+2}$ | $S^{+2}$ | | | | | | | | | |

**Table 1. (Cont'd.).**

| | | Streak strains | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
| No. | Seeded strains | *P. heimii* | *P. jadinii* | *P. lynferdii* | *P. methanolica* | *P. mexicana* | *P. ofunaensis* | *P. ohmeri* | *P. strasburgensis* | *P. sydowiorum* | *Saccharomycodes ludwigii* | *Sporidiobolus ruineniae* | *S. salmonicolor* | *Sporobolomyces tsugae* | *Stephanoascus ciferrii* | *Tremella encephala* | *Williopsis californica* |
| 1. | *Arxula adeninovorans* | $K^{+2}$ | - | $S^{+1}$ | - | - | $S^{+1}$ | - | - | - | - | - | - | $S^{+1}$ | - | - | $K^{+2}$ |
| 2. | *Bensingtonia intermedia* | $K^{+4}$ | - | - | - | $K^{+2}$ | - | - | - | - | - | - | - | $K^{+3}$ | - | - | $K^{+2}$ |
| 3. | *B. naganoensis* | $K^{+3}$ | - | $S^{+1}$ | - | - | - | - | - | $S^{+1}$ | - | $S^{+2}$ | $S^{+2}$ | $S^{+2}$ | - | - | $K^{+2}S^{+1}$ |
| 4. | *Bullera pseudoalba* | $K^{+2}S^{+4}$ | - | $S^{+2}$ | $K^{+2}S^{+2}$ | - | $S^{+3}$ | - | $S^{+2}$ | - | $S^{+2}$ | $S^{+2}$ | $S^{+4}$ | $S^{+2}$ | - | - | $K^{+1}$ |
| 5. | *B. pyricola* | $S^{+3}$ | $S^{+1}$ | $S^{+3}$ | $S^{+2}$ | $S^{+1}$ | - | $S^{+1}$ | - | - | $S^{+1}$ | $S^{+1}$ | $S^{+1}$ | $K^{+2}S^{+2}$ | $S^{+2}$ | $S^{+1}$ | $S^{+1}$ |
| 6. | *Candida diddensiae* | $K^{+3}$ | - | - | - | - | - | - | $K^{+1}$ | - | - | - | - | - | - | $K^{+1}$ | $K^{+2}S^{+1}$ |
| 7. | *C. etchellsii* | - | - | - | - | - | - | - | - | - | $S^{+2}$ | - | - | $K^{+3}$ | - | - | - |
| 8. | *C. friedrichii* | - | - | - | - | - | - | $S^{+2}$ | - | - | - | - | $K^{+1}S^{+1}$ | - | $K^{+2}$ | - | $S^{+1}$ |
| 9. | *C. haemulonii* | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | $K^{+1}$ | $K^{+3}$ | - | - | - |
| 10. | *C. membranifaciens* | $K^{+3}$ | - | $S^{+2}$ | - | - | $K^{+1}S^{+1}$ | - | - | - | - | - | - | $K^{+1}$ | - | - | - |
| 11. | *C. pseudointermedia* | $K^{+2}$ | - | $S^{+2}$ | - | - | $K^{+2}$ | - | - | $K^{+3}$ | - | $S^{+1}$ | $K^{+1}$ | $K^{+1}S^{+1}$ | - | - | $K^{+2}$ |
| 12. | *C. shehatae* | $K^{+2}S^{+2}$ | $K^{+1}$ | - | - | $K^{+3}$ | - | - | - | - | $K^{+1}$ | - | $K^{+1}$ | - | - | $S^{+1}$ | - |
| 13. | *C. succiphila* | - | - | - | - | - | - | - | - | - | $K^{+1}$ | - | $K^{+2}$ | - | - | - | - |
| 14. | *C. valdiviana* | $K^{+3}S^{+3}$ | - | - | - | - | - | - | - | - | - | - | $S^{+1}$ | $S^{+2}$ | - | - | $S^{+2}$ |
| 15. | *C. xestobii* | $S^{+3}$ | - | - | - | - | - | - | - | - | - | - | - | $S^{+3}$ | - | - | $S^{+2}$ |
| 16. | *Clavispora lusitaniae* | $K^{+2}S^{+2}$ | - | - | - | - | - | - | - | - | $K^{+1}$ | $S^{+2}$ | - | - | - | $K^{+1}$ | - |
| 17. | *Cryptococcus albidus* | $K^{+2}S^{+3}$ | - | $S^{+2}$ | - | - | $K^{+1}S^{+2}$ | - | - | - | - | - | $K^{+2}$ | - | - | - | - |
| 18. | *C. gastricus* | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | $K^{+3}$ | - | - | - |
| 19. | *Debaryomyces castellii* | $K^{+2}$ | - | - | $S^{+1}$ | - | - | - | $S^{+1}$ | - | - | - | - | $K^{+3}$ | - | - | $K^{+2}$ |
| 20. | *D. hansenii* | $K^{+3}$ | - | - | - | - | - | - | $K^{+1}$ | $K^{+1}S^{+1}$ | - | - | $S^{+2}$ | - | - | - | $K^{+3}$ |
| 21. | *D. nepalensis* | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22. | *D. vanrijii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23. | *D. yamadae* | $S^{+1}$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24. | *Fibulobasidium inconspicuum* | $K^{+3}S^{+1}$ | - | $K^{+1}$ | - | $K^{+1}$ | $K^{+1}$ | - | $S^{+2}$ | $S^{+1}$ | - | - | $S^{+1}$ | $K^{+1}S^{+1}$ | - | - | $K^{+2}S^{+1}$ |
| 25. | *Filobasidiella neoformans* | $K^{+4}$ | - | - | - | - | - | - | - | - | - | - | - | $K^{+3}$ | - | - | $K^{+2}$ |
| 26. | *Filobasidium uniguttulatum* | $K^{+3}$ | - | $K^{+1}$ | - | $K^{+1}$ | $K^{+1}S^{+2}$ | - | - | - | - | - | $S^{+1}$ | $S^{+1}$ | - | - | $K^{+2}$ |
| 27. | *Kluyveromyces polysporus* | $K^{+3}S^{+2}$ | $S^{+1}$ | $K^{+1}$ | $S^{+1}$ | $K^{+1}$ | $K^{+3}$ | - | - | $S^{+1}$ | - | $S^{+2}$ | $K^{+1}$ | $S^{+2}$ | - | - | $K^{+2}S^{+1}$ |
| 28. | *Lipomyces lipofer* | $K^{+3}$ | $K^{+1}$ | - | - | $K^{+1}$ | - | - | - | $K^{+1}$ | - | $K^{+3}$ | $K^{+1}$ | - | - | $K^{+1}$ | - |
| 29. | *L. starkeyi* | - | - | - | - | $K^{+1}$ | $K^{+3}$ | - | - | - | $K^{+2}$ | $K^{+3}$ | $K^{+2}$ | $K^{+2}$ | - | $S^{+2}$ | - |
| 30. | *Phaffia rhodozyma* | $K^{+2}$ | - | - | - | $K^{+1}S^{+2}$ | - | - | .- | - | - | - | - | $K^{+1}$ | - | - | $K^{+2}$ |
| 31. | *Pichia angusta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32. | *P. anomala* | $K^{+4}$ | - | - | $S^{+1}$ | - | - | - | $S^{+1}$ | $S^{+1}$ | - | - | - | - | - | - | - |
| 33. | *P. euphorbiaphila* | $K^{+3}$ | - | $S^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | - | $K^{+2}$ |
| 34. | *P. guilliermondii* | $K^{+2}$ | - | - | - | - | - | - | - | - | - | - | - | $K^{+3}S^{+3}$ | - | - | - |
| 35. | *P. heimii* | X | $S^{+2}$ | $S^{+2}$ | $S^{+4}$ | $K^{+2}S^{+1}$ | - | $S^{+3}$ | $S^{+4}$ | $K^{+2}S^{+4}$ | $S^{+1}$ | $S^{+1}$ | $S^{+3}$ | $K^{+3}S^{+2}$ | - | $S^{+3}$ | - |
| 36. | *P. jadinii* | $K^{+2}$ | X | - | - | $K^{+1}$ | $K^{+3}$ | - | - | - | - | $S^{+2}$ | $S^{+2}$ | $K^{+2}$ | - | - | $K^{+2}$ |
| 37. | *P. lynferdii* | $K^{+3}$ | - | X | - | - | - | - | - | $K^{+2}$ | - | - | - | $K^{+3}$ | - | $K^{+2}$ | $K^{+2}S^{+3}$ |
| 38. | *P. methanolica* | $K^{+4}$ | - | - | X | $K^{+2}$ | - | - | - | - | - | - | - | $K^{+3}$ | - | - | $K^{+2}$ |
| 39. | *P. mexicana* | $K^{+1}S^{+3}$ | $S^{+1}$ | - | $S^{+2}$ | X | - | - | $S^{+1}$ | - | - | - | $S^{+1}$ | - | - | - | - |
| 40. | *P. ofunaensis* | - | $S^{+3}$ | - | - | - | X | - | - | $K^{+2}$ | $K^{+1}$ | - | - | - | - | $S^{+2}$ | - |
| 41. | *P. ohmeri* | $K^{+4}$ | - | - | - | - | - | X | - | - | - | $S^{+1}$ | $K^{+2}$ | $K^{+3}$ | - | - | - |
| 42. | *P. strasburgensis* | $K^{+4}$ | - | - | - | $K^{+1}$ | - | - | X | - | - | - | - | $K^{+3}$ | - | - | $K^{+2}$ |
| 43. | *P. sydowiorum* | $K^{+4}S^{+2}$ | - | $S^{+2}$ | - | - | $K^{+2}$ | - | - | X | - | - | $K^{+1}$ | $S^{+1}$ | - | - | $K^{+2}$ |
| 44. | *Saccharomycodes ludwigii* | $K^{+3}$ | - | - | - | - | $S^{+2}$ | - | - | - | X | $S^{+2}$ | $S^{+2}$ | $K^{+3}$ | - | - | - |
| 45. | *Sporidiobolus ruineniae* | $K^{+2}$ | $S^{+3}$ | - | - | - | - | $K^{+1}$ | - | $K^{+2}$ | $K^{+1}$ | X | $K^{+3}$ | $K^{+2}$ | - | - | - |
| 46. | *S. salmonicolor* | $K^{+3}$ | - | - | - | $K^{+1}$ | - | $S^{+1}$ | - | $S^{+1}$ | $K^{+3}$ | $S^{+2}$ | X | $K^{+2}$ | - | $K^{+2}$ | $K^{+2}$ |
| 47. | *Sporobolomyces tsugae* | $K^{+3}S^{+2}$ | $S^{+2}$ | - | $S^{+3}$ | - | - | $S^{+2}$ | $S^{+3}$ | $K^{+1}$ | $S^{+2}$ | $S^{+2}$ | $S^{+2}$ | X | - | - | - |
| 48. | *Stephanoascus ciferrii* | - | - | - | - | - | - | - | - | - | - | - | - | - | X | - | - |
| 49. | *Tremella encephala* | $K^{+3}$ | - | $S^{+2}$ | - | - | $K^{+2}$ | - | - | - | - | - | $S^{+2}$ | - | | X | - |
| 50. | *Williopsis californica* | $K^{+3}S^{+2}$ | $S^{+2}$ | - | $S^{+2}$ | - | - | - | $S^{+3}$ | $S^{+2}$ | - | - | $S^{+2}$ | - | - | - | X |

**Table 2. Percentages of killer, sensitive and neutral phenotypes in yeast species isolated from dairy products.**

| S.No. | Yeast species | Phenotypes of seeded yeast strains (%) | | |
|---|---|---|---|---|
| | | Killer | Sensitive | Neutral |
| 1. | *Arxula adeninovorans* | 10.2 | 10.20 | 79.59 |
| 2. | *Bensingtonia intermedia* | 16.32 | 6.12 | 77.55 |
| 3. | *B. naganoensis* | 10.2 | 14.29 | 7.55 |
| 4. | *Bullera pseudoalba* | 12.25 | 51.02 | 42.86 |
| 5. | *B. pyricola* | 14.29 | 77.55 | 18.37 |
| 6. | *Candida diddensiae* | 26.53 | 2.04 | 73.47 |
| 7. | *C. etchellsii* | 10.2 | 4.08 | 85.71 |
| 8. | *C. friedrichii* | 20.40 | 8.16 | 73.47 |
| 9. | *C. haemulonii* | 12.25 | 2.04 | 85.71 |
| 10. | *C. membranifaciens* | 10.2 | 12.25 | 79.59 |
| 11. | *C. pseudointermedia* | 22.45 | 6.12 | 73.47 |
| 12. | *C. shehatae* | 26.53 | 8.16 | 67.35 |
| 13. | *C. succiphila* | 6.12 | 0.00 | 93.88 |
| 14. | *C. valdiviana* | 6.12 | 22.45 | 73.47 |
| 15. | *C. xestobii* | 4.08 | 16.33 | 79.59 |
| 16. | *Clavispora lusitaniae* | 14.29 | 10.2 | 77.55 |
| 17. | *Cryptococcus albidus* | 10.2 | 20.4 | 75.51 |
| 18. | *C. gastricus* | 10.2 | 2.04 | 71.43 |
| 19. | *Debaryomyces castellii* | 12.24 | 10.2 | 77.55 |
| 20. | *D. hansenii* | 14.29 | 16.33 | 71.43 |
| 21. | *D. nepalensis* | 4.08 | 0.00 | 95.92 |
| 22. | *D. vanrijii* | 2.04 | 0.00 | 97.96 |
| 23. | *D. yamadae* | 2.04 | 2.04 | 95.92 |
| 24. | *Fibulobasidium inconspicuum* | 20.4 | 20.4 | 67.35 |
| 25. | *Filobasidiella neoformans* | 10.20 | 0.00 | 89.80 |
| 26. | *Filobasidium uniguttulatum* | 16.32 | 8.16 | 77.5 |
| 27. | *Kluyveromyces polysporus* | 14.29 | 26.53 | 63.26 |
| 28. | *Lipomyces lipofer* | 30.61 | 4.08 | 65.3 |
| 29. | *L. starkeyi* | 46.93 | 2.04 | 51.02 |
| 30. | *Phaffia rhodozyma* | 14.29 | 2.04 | 85.71 |
| 31. | *Pichia angusta* | 0.00 | 0.00 | 100.00 |
| 32. | *P. anomala* | 10.2 | 28.52 | 61.22 |
| 33. | *P. euphorbiiphila* | 12.25 | 2.04 | 85.71 |
| 34. | *P. guilliermondii* | 8.16 | 2.04 | 91.84 |
| 35. | *P. heimii* | 28.57 | 77.50 | 18.37 |
| 36. | *P. jadinii* | 14.29 | 8.16 | 77.55 |
| 37. | *P. lynferdii* | 26.53 | 8.16 | 67.35 |
| 38. | *P. methanolica* | 18.37 | 2.04 | 81.63 |
| 39. | *P. mexicana* | 6.12 | 26.53 | 71.43 |
| 40. | *P. ofunaensis* | 12.25 | 18.37 | 75.51 |
| 41. | *P. ohmeri* | 8.16 | 2.04 | 89.8 |
| 42. | *P. strasburgensis* | 16.32 | 4.08 | 79.59 |
| 43. | *P. sydowiorum* | 18.37 | 12.25 | 73.47 |
| 44. | *Saccharomycodes ludwigii* | 10.20 | 10.20 | 79.59 |
| 45. | *Sporidiobolus ruineniae* | 20.4 | 14.29 | 67.35 |
| 46. | *S. salmonicolor* | 30.61 | 20.4 | 48.98 |
| 47. | *Sporobolomyces tsugae* | 26.53 | 36.37 | 42.86 |
| 48. | *Stephanoascus ciferrii* | 2.04 | 0.00 | 97.96 |
| 49. | *Tremella encephala* | 8.16 | 12.25 | 79.59 |
| 50. | *Williopsis californica* | 14.29 | 42.86 | 50.02 |

Whereas, other yeasts that showed lesser killing activity were *Candida diddensiae* (26.53%), *C. friedrichii* (20.40%), *C. pseudointermedia* (22.45%), *C. shehatae* (26.53%), *Fibulobasidium inconspicuum* (20.40%), *Lipomyces lipofer* (30.61%), *Pichia heimii* (26.53%), *P. lynferdii* (26.53%), *Sporidiobolus ruineniae* (20.40%), *S. salmonicolor* (30.61%) and *Sporobolomyces tsugae* (26.53%). Several strong ($K^{+3}$) and very strong ($K^{+4}$) killing zones were produced by *Lipomyces starkeyi*, and other killer strains against sensitive strains.

A number of yeasts which, neither showed killing nor sensitive reactions even against the supper sensitive and killer strains are considered as neutral or resistant strains. The phenomenon of insensitivity towards killer yeasts generally occurs at the cell wall level. It is known that resistant (neutral) 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). Taxonomically, different cell wall chemical compositions are used for classification of organisms, hence resistance causing insensitivity towards killer yeasts could be a taxon-related property as well (Golubev, 1998, 2006). In some studies Golubev (Golubev, 1992; Golubev *et al*., 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). However, in the present studies, we observed a mixed effectiveness of killer yeasts against the neutral (insensitive) yeasts (Table 1). In this context, we emphasize (which Golubev (2006) also emphasized in his studies) that the use of killer toxins as a taxonomic tool should be preceded by a careful study of their KSP. Broad-spectrum killer toxins should 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)].

Similarly, a number of yeast strains showed strong killing activity against sensitive yeasts during cross reactions. In nature certain strains of killer yeasts dominate only in particular niches (Zorg *et al*., 1988). Killer activity is one of the mechanisms of antagonism among yeasts during spontaneous fermentations and because of this mechanism killer strains may be used to avoid contamination by sensitive spoilage yeasts (Starmer *et al*., 1987; Bussey *et al*., 1988; Jacobs *et al*., 1988; Longo *et al*., 1990; Vagnoli *et al*., 1993; Hidalgo & Flores, 1994).

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