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Killer yeast isolated from some foods and its biological activity

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Abstract

Seventy eight yeasts were isolated from different foodstuffs. Out of the seventy eight isolates four yeast species namely *C. parapsilosis* Q3, *C. solani* F8, *C. versatilis* J3 and *K. jensenii* H1 were selected to study their biological activity. The four strains termed as killer yeast by observing its activity against microorganisms. Killer yeasts secrete proteinaceous killer toxins lethal effect against some of Gram positive and negative bacteria, molds and yeasts. The antagonistic effect of the four killer yeast strains on the growth of different microorganisms recorded as zone of inhibition (mm), demonstrated that *C. versatilis* J3 was an active stains against majority test microorganisms. Consumed sugar determined for the four strains and showed that *C. versatilis* J3 and *C. parapsilosis* Q3 reached to its maximum on the 12 hours of incubation both yeast consumed 97.5 and 95% of initial sugar (sugar utilization efficiency) while both of *C. solani* F8 and *K. jensenii* H1 sugar utilization efficiency was recorded on the 24 hours being 99.65 and 99.30%, respectively.

Keywords: yeasts; killer strains; consumed sugar; glucose affinity.

Practical Application: Killer yeast activity against different microorganisms.

1 Introduction

The term called yeast is originated from the old Dutch word gist and the German word gischt, which refers to fermentation. There are approximately 100 genera and 800 detected species of yeasts (Kurtzman & Fell, 1998). Yeasts are distributed in different food known to contaminate and spoilage of foods and dairy products (Mushtaq et al., 2006). Yeasts are of wide distributions in the environment, and may be found as a part of the normal flora of a food product, on inadequately sanitized equipment, or as air borne contaminants. Their habitats may include not only the upper layers of the soil but also many forms of organic matter, especially of plants origin, where carbohydrates are common occurrence. Yeasts may be isolated particularly from the soil of vineyards and orchards; from the surface of grapes, apples, and most sweet fruits and from the leaves and other parts of plants (Prescott & Dunn, 1959). Mushtaq et al., (2006) have been used yeasts in the food industry principally for the production of ethanol and carbon dioxide, which are important to the brewing, wine distilling and baking industries, Yeasts are rich of proteins, lipids and vitamins (Kutty & Philip 2008). Over the years, morphological, biochemical and physiological characteristics have been used to identify yeasts (Barnett et al., 1990. This conventional methodology requires the evaluation of some 60 to 90 tests, resulting in a complex (Arias et al., 2002). From the biological activity of yeasts found a Killer yeasts which produce antimycotic compounds and form immune (Magliani et al., 1997). Yeast killer toxins are proteinaceous compounds which are active against members of the same species or closely related species, and the activities of these toxins are similar to the activities of bacteriocins in bacterial species. Some authors (Lowes et al., 2000) prefer to call yeast killer toxins mycocins and killer strains mycogenic in order to emphasize the general nature of the antagonistic interactions (Golubev, 1998). Mycocins were first found in brewing strains of *Saccharomyces cerevisiae* (Bevan & Makower, 1963) and since then have been shown to occur in a large number of yeast species of agronomic, environmental, industrial, and clinical interest, including *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Torulopsis*, and *Williopsis* species (Golubev, 1998; Philliskirk & Young, 1975; Young, 1987; Young & Yagiu, 1978). Certain mycotoxins have also been shown to have inhibitory effects on some pathogenic gram-positive bacteria, including *Staphylococcus aureus* (Izgü & Altinbay, 1997). The aim of our study is to determine the common yeast isolates in some foods and select the biologically active strain (killer strain) against some other microorganisms. Also to determine the affinity of yeast strains for glucose at different concentrations (m mole/L) and the sugar consumption for the four killer yeasts as follows (Equation 1 and 2):

$$Consumed\ sugar\ g^{-1} = \left(Initial\ sugar - residual\ sugar\right) \quad (1)$$

$$Sugar\ utilization\ efficient\ \left(SUE\%\right) = Initial\ sugar - residual\ sugar\ /\ Initial\ sugar \times 100 \quad (2)$$

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2 Materials and methods

2.1 Samples

Samples included fresh fruits (dates, grapes, figs and strawberries), juices (carrot, orange, sugar cane and tomato), high test molasses syrup, pickles (black and green olive, carrot, lemon and cucumber), dairy products (milk, butter, ice cream, old white cheese and yoghurt), salted fish and sausage. These materials were collected from the local markets of Cairo, Egypt, and directly transferred to the laboratory for microbiological analysis.

2.2 Media used for culturing yeasts

The following media were used throughout this work, malt extract agar (Lodder & Kreger-van-Rij, 1967), it was used or isolation and of yeasts from different foodstuffs. It has the following composition; malt extract 20, agar 20 tap water 1000 and pH was adjusted to 5.5. also the malt extract broth prepared without addition of agar. YEPD medium (Seki et al., 1985), was used for the production of high titre of killer solution. It was consisted of g/1 L: glucose (20), yeast extract (10) , peptone (20) and pH was adjusted to 4.7. Nutrient glucose agar medium (Difco Laboratories, 1988), was used for seeded bacterial strains and its composition g/1L: glucose (10), beef extract (3), peptone (5), agar (20) and pH was adjusted to 7-7.2. The medium were autoclaved at 110 or 120 °C for 20 minutes, this depends on the composition of the media and pH.

2.3 Antagonistic Effect

The Technique described by Woods & Bevan (1968) was The used throughout this investigation

2.4 Yeast yeast interaction

YEPD agar medium was used to produce the killer substances. For obtaining stable high titer of killer solution, conical flasks (250 mL in volume)containing 100 mL of YEPD broth medium was inoculated with tested yeast and incubated on an orbital shaker (150 rpm) at 30 °C for 3 days. Discs (5 mm in diameter) or sterilized filter paper were saturated with 100 µL of yeast cultures under aspect conditions. Thereafter, they were placed on the surface of 10 mL of seeded malt agar(inoculated with one mL tested yeast containing 10^3 cells, which kept for 2 hours at room temperature before incubation). The plates were incubated at 30 °C for 1- 3 days and zone of inhibition was detected. *Candida albicans* (R12) was also used in seeded agar plates as a sensitive yeast.

2.5 Yeast- bacteria interaction

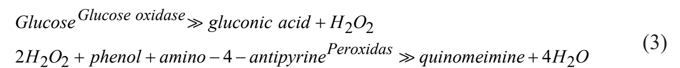
Nutrient glucose agar medium was used as seeded agar plates. bacteria used in seeded agar (10^3 cell/mL) were *Staphylococcus aureus* (A3), *Proteus vulgaris* (H9), *Escherishia coli* (S17), and *Bacillus cereus* (K11). The plates were incubated at 37 °C for 1-2 days and examined for inhibition zone..

2.6 Yeast fungi interaction

Malt extract agar medium was used as seeded agar plates. fungi used as seede plates (10^3 spores/mL) were *Aspergillus flavus* (F5), *A. niger (*N70) and *Penicillium notatum* (H3). The plates were incubated at 30 °C for 3-5 days. The yeasts, bacteria and fungi tested strains were obtained from Dep. of Microbiology, Faculty of agriculture, Ain Shams University, cairo, Egypt.

2.7 Glucose determination

Glucose was determined according to the method of Trinder (1969) using special kits. The principal of the method could be explained as follow (Equation 3):



$$Glucose \xrightarrow{Glucose\ oxidase} gluconic\ acid + H_2O_2$$
$$2H_2O_2 + phenol + amino-4-antipyrine \xrightarrow{Peroxidas} quinomeimine + 4H_2O \quad (3)$$

The procedure was carried out by micropiptting 10 µL of the tested sample (or standard solution 2.0 gl^{-1} glucose) in a test tube (5 mL capacity), then one mL of enzymatic reagent solution (reagent 3.18.7) was added, mixed gently and incubated at 37 °C for 10 minutes. The developed color (stable for 30 minutes) was measured colurmetrically at 505 nm.

2.8 Saturation constant of glucose

Glucose is considered to be the best carbon source for propagation of yeasts. All yeasts utilize this substrate aerobically (fermented yeast). The specific growth rate of yeasts highly affected by glucose concentration. This effect is only when substrate levels become very low that the growth rate begins to be severely affected. In order to evaluate this effect, maximum specific growth rate (µmax) and saturation constant (Ks) which is inversely proportional to the affinity of yeasts for the particular substrate (glucose) were determined.

In this experiment, conical flasks (250 mL in volume) containing 100 mL fermentation broth medium. Without carbon source were autoclaved at 121 °C for 15 minutes. Different concentrations of glucose (sterilized by filtration using Millipore filter) being 0, 1, 2, 4, 8, 16, 32, 64 mmole were added. The flasks containing different concentrations of glucose were inoculated with one mL standard inoculum of tested yeasts (4 selective strains were used) and were incubated at 30 °C for 7 days using an orbital shaker (150 rpm). Yeast growth (optical density at 570 nm) was determined periodically (6-12 hours). Growth is numerically equal to the concentration of growth- limiting substrate (glucose) at half of the maximum rate (µmax/2)

Hence, a plot of 1/max against 1/s. i.e. a lineweaver-Burk plot, will gave straight line with an intercept abscissa at -1/Ks. and an intercept on the ordinate 1/µmax. S = the substrate (glucose) concentration.

3 Results and discussion

Table 1 show the number of percentage of different yeast genera isolated from different foodstuffs. Results sin Table 1 revealed that seventy eight yeast cultures were isolated from different foodstuffs. The morphological and physiological proportion of

these isolates were studied according to Lodder & Kreger-van-Rij (1967), Barnett et al. (1983) and Kreger-van-Rij (1984). All yeast isolates belonged to seven genera being Candida, Geotrichum, Hansenula, Klockera, Rhodotorula, Schizoblastosporion, and Trichosporon, represented the most dominant genera being (74.36% (58 isolates out of 78 yeast isolates). This yeasts belonged to genera represented of 74.4, 3.8, 2.6, 6.4, 8.8, 2.6 and 1.3%. Seven isolates (8.92) were found to be Rhodotorula. Other yeast genera showed the lowest percentage among the yeasts ranged from 1.28% to 6.41%. it is also interesting to notice that the yeast isolates belonging to candida were observed in all tested foodstuffs except yoghurt and milk i.e. nineteen foods out of twenty one foodstuffs. Kloeckera and Rhodotorula isolates were only recorded in five and four foodstuffs respectively. On the contrary Hansenula and Schizosaccharomyces yeasts were isolated from lemon pickles brine and sausage respectively. Active species (killer yeast) belonged to the genus of candida were *C. parapsilosis* Q3, *C. solani* F8 and *C. versatilis* J3, while the active specie belonged to Klocecker genus was *K. jensenii* H1. These four strains were used for further study throughout the following work as its activity against other microorganisms through the antagonistic effect, consumed sugar and affinity to glucose.

3.1 Antagonistic effect of isolates

It has been known for many years that antagonisms can exist between microorganisms growing in a common environment. Some organisms may produce metabolic products or specific toxic substrate which inhibit or kill other microorganisms. This phenomenon is widely studied in Table: Inhibition of microbial growth by yeast strains. is widely studied in bacteria and fungi. Certain yeast strains termed killer yeasts produce an extracellular toxin which is lethal to another yeasts (sensitive yeasts). This killer interaction is restricted between strains of species within one genus but reactions and different genera have been reported (Bevan & Makower, 1963). In this work, the interaction between seventy eight yeast isolated from some foodstuffs were studied. The antagonistic effect of these yeasts against certain bacteria and fungi was elucidated. Results in Table 2 showed that (3.5%) *C. solani* F8, *C. parapsilosi* Q3 snd *K. jensenii* H1 out of 78 yeast isolates showed inhibitory effect against *Schizoblastosporon strakeii* P2, *Sch. starkeii* P3 and *C. parapsilosis* A3, respectively. The area of inhibition 6, 3, and 1 mm respectively. Regarding to the interaction between yaeast isolates and other microorganisms, the results showed that four yeast strains had a deleterious effect on some microorganisms associated with foods. Additionally, the inhibitory effect was highly varied from one yeast to another. Besides, *C. versatilis* J3 was capable to retard the growth of all tested microorganisms except *Aspergillus flavus* (F5) and *penicillium notatum (*H3). Moreover, the highest inhibition zone was recorded in the case of *Staphylococcus aureus* (A3) being 8 mm whereas the the lowest value was shown in the case of *E. coli* (1 mm, however, *K. jensenii* H1 did not inhibit the growth of C. albicans (R12) and *Penicillium notatum* (H3), while highly inhibited *Proteus vulgaris* (H9), (8 mm inhibition

Table 1. Different yeast genera isolated from foodstuffs.

Yeast genera	No., of isolates	Percentage (%)	Source of isolation	No., of foodstuffs	Active strains (killer yeast)
Candida sp.	58	74.36	All tested foodstuffs except cheese and yoghurt	19	*C. parapsilosis* Q3, *C. solani* F8, *C. versatilis* J3
Geotrichum sp.	3	3.85	Old white cheese and yoghurt	2	
Hansenula sp.	2	2.56	Lemon pickles	1	
Kloeckera	5	6.41	Black olive pickles, dates, green olive pickles, lemon and grapes.	5	*K. jensenii* H1
Rhodotorula sp.	7	8.97	Dates, milk, sausage and sugar cane juice	4	
Schizosaccharomyces sp.	2	2.56	sausage	1	
Tricosporon sp.	1	1.28	Milk and sausage	2	
Total	**78**	**100**	-----------------------------	**21**	

Table 2. Inhibition of microbial growth by yeast strains.

Yeast Strain Tested microorganisms	Zone of inhibition (mm)			
	***C. solani* F8**	***K. jensenii* H1**	***C. versatilis* J3**	***C. parapsilosis* Q3**
***Staph. aureus* A3**	5	4	8	5
***P. vulgaris* H9**	5	8	4	0
***E. coli* S17**	5	6	1	0
***C. albicans* R12**	3	0	5	0
***A. flavus* F5**	0	2	0	0
A. niger N70	0	2	2	3
***P. notatum* H3**	0	0	0	0
***B. cereus* K11**	3	3	5	0

Table 0 = no effect

zone) furthermore *Aspergillus flavus* (F5), *A. niger* (N70) and *P. notatum* (H3) not affected by *C. solani* F8.on the contrary, only two tested organisms (*Staph. aureus* (A3) and *A. niger* (N70))] were sensitive to *C. parapsilosis* Q3.

On the other hand, all tested yeasts had antagonistic effect against *Staph. aureus* (A3), while *A. flavus* (F5) was inhibited by only one yeast strain (*K. jensenii*). It could be concluded that, the occurrence of these yeasts in foods may play a role in retardation of some undesirable microorganisms associated with food. On the other hand, the the inhibitory effect of these yeasts may be due to some metabolic products which was excreted from the cells to the media. These results are in line with those observed by Yokomori et al. (1988) and Palpacelli et al., 1991. Killer activity was recorded in some yeast strains such as *Hansenula saturn* (Bussey & Sherman, 1973), *Candida galabrata* (Bussey & Skipper, 1975), *Saccharomyces cerevisiae* (Palfree and bussey, 1979), *Klyveromyces lactis* (Sugisaki et al., 1984), H. markeii (Ashida et al., 1983), Candida sp. (Yokomori et al., 1988) and *Metscenikowia pulcherima* (Farris et al., 1991). On the other hand, Wilson & Chalutz (1989) explained the role of antagonistic yeast to biocontrol of Penicillium rots of citrus. McLaughlin et al. (1990) studied the effect of inoculum concentration and salt solutions on the biological control of postharvest disease of apple with Candida sp. and *Debaromyces hansenii* (ascosporogenous yeast) was also used to biocontrol of green and blue mold and sour rot of citrus by Chalutz & Wilson (1990). Izgü & Altinbay (1997), mentioned that certain mycocins have been shown to have inhibitory effects on some pathogenic gram-positive bacteria, including *Staphylococcus aureus* and these results were agreed with us. There is evidence that interactions between mycogenic yeasts and sensitive yeasts are widespread in natural habitats and are probably ecologically significant (Stumm et al., 1977). The researches of mycogenic yeasts based on the activity assays in vitro focused on the molecular aspects of production, properties of the mycocins, and the mechanisms of action. Little attention has received about the role of killer yeasts in ecological community structure and it is assumed that these organisms have an advantage over sensitive competitors. When mycogenic yeasts are present in natural communities, a single killer strain usually predominates (Starmer et al., 1987). The action between killer yeast and sensitive yeast in mixed culture lead to predominant the killer and can be detrermined through kinetic studies (Petering et al., 1991; Ramon-Portugal et al., 1998). It is essential to develop an understanding of the interactions of killer strains in communities if mycocins are to be used as biological control agents. Starmer et al., (1987) found that mycogenic yeasts are widespread in natural populations of fruit and decaying vegetable matter and concluded that these organisms have an important effect on the development and composition of the yeast flora. Generally it could be concluded that our yeast strain termed killer strains and may delay some pathogenic bacteria,, yaest and fungi and these all enhancement it role in food preservation from contaminant microorganisms

3.2 Consumed sugar

Table 3 declared the amount of consumed sugar by the tested yeasts in a 100 mL medium containing 2.0% glucose. During the first ,12 hours of incubation, consumed glucose by *C. versatilis* J3 and *C. parapsilosis* Q3 was increased rapidly reaching to 19.5 and 19.0 g/L on the 12 th hours respectively, which means that both yeast consumed 97.5 and 95% of initial sugar (sugar utilization efficiency) respectively, during this period (12 hours). Besides, the corresponding figures of specific sugar consumption rate were 0.248 and 0.447 h^{-1}. In contrast, *C. solani* F8 and *K. jensenii* H1 did not exhibit the same trend where the highest sugar utilization efficiency (SUE) was recorded on the 24 hours being 99.65 and 99.30%, respectively. These yeasts also showed the lowest specific sugar consumption rate (0.0484 and 0.114 h^{-1}) as compared with other two strains. There are direct proportional between the consumed sugar and the percentage of fermentation. This could be due to the utilization of sugar for the formation of other products and depends on the strain efficiency in utilized sugar and other factor intrinsic and extrinsic factor as the type of sugar and its concentration, pH, temperature, nitrogen source agitation rate.

3.3 Affinity of yeast strains for glucose

Glucose is considered to be the best carbon source for propagation of yeasts. All yeasts utilize this substrate aerobically (fermented yeast). The specific growth rate of yeasts highly affected by glucose concentration. In this study *C. versatilis* J3, *C. parapsilosis* Q3, *C. solani* F8 and *K. jensenii H1* were grown at different glucose concentration (1, 2, 4, 8, 16 and 64 mmol/L) to determine saturation constant (Ks) for each strain. Tables 4, 5, 6, 7 showed gradual increase of yeast of yeast growth, during the exponential growth pahse (24 hors) where the growth increased from 0.43 to 3.8 O.D for *C. solani* F8, from 1.0 to 3.6 O.D for *C. parapsilosis* Q3 and from 0.47 to 2.8 O.D for *C. versatilis* J3 and from 1.0 to 3.3 O.D for *K. jensenii* H1 where

Table 3. Consumed sugar, sugar utilization efficiency and specific consumption rate of sugar during propagation of yeast strains in medium containing 2% glucose at 30 C using shake flasks as a batch culture.

Yeast species	Time in hours												specific consumption rate of sugar
	3		6		12		24		48		72		
	Consumed sugar gl^{-1}	SUE%	Consumed sugar gl^{-1}	SUE%	Consumed sugar gl^{-1}	SUE%	Consumed sugar gl^{-1}	SUE%	Consumed sugar gl^{-1}	SUE%	Consumed sugar gl^{-1}	SUE%	h^{-1}
C. versatilis J3	2.1	10.5	5.0	25	19.5	97.5	19.6	98	19.5	98	19.5	98	0.248
C. parapsilosis Q3	0	0	1.3	6.5	19.0	95	19.99	99.95	19.99	99.95	19.99	99.95	0.447
C. solani F8	3.4	17	4.3	21.5	6.2	31	19.93	99.65	19.93	99.96	19.93	99.65	0.084
K. jensenii H1	1.8	9	2.2	11	4.0	20	19.86	99.30	19.94	99.7	19.94	99.7	0.114

Consumed sugar g^{-1} = (Initial sugar – residual sugar); Sugar utilization efficient (SUE%) = Initial sugar – residual sugar/ Initial sugar × 100.

glucose was increased from 1 mmole to 64 mmole. According to the specific rate as influenced by the highest concentration Figures 1, 2, 3, 4 showed that the highest value of this parameters was recorded at 64 mmole of glucose being 0.1839, 0.1875, 0.1897 h^{-1} for

Table 4. Growth (O.D) of *C. solani* F8 at different concentrations of glucose (m mole/L) using a shake flasks as a batch culture at 30 °C.

Glucose concentration (m mole/L)	*C. solani* F8				
	Time in hours				
	24	48	72	96	120
1	0.43	0.45	0.49	0.52	0.52
2	0.52	0.52	0.52	0.53	0.54
4	1.0	1.5	2.1	2.1	2.1
8	1.3	1.8	2.6	2.6	2.4
16	1.5	2.1	2.7	2.7	2.5
32	1.8	2.2	3.0	4.1	4.0
64	3.8	3.9	4.0	4.0	4.2

Table 5. Growth (O.D) of *K. jensenii* H1 at different concentrations of glucose (m mole/L) using a shake flasks as a batch culture at 30 °C.

Glucose concentration (m mole/L)	*K. jensenii* H1				
	Time in hours				
	24	48	72	96	120
1	1.0	1.7	2.0	2.4	2.4
2	2.0	2.2	2.2	2.6	2.7
4	2.2	2.5	2.5	2.7	2.9
8	2.5	2.7	2.9	3.3	3.1
16	2.7	4.2	4.1	4.1	4.0
32	3.1	5.0	5.2	5.5	4.6
64	3.3	5.2	5.4	5.6	6.2

Table 6. Growth (O.D) of *C. versatilis* J3at different concentrations of glucose (m mole/L) using a shake flasks as a batch culture at 30 °C.

Glucose concentration (m mole/L)	*C. versatilis* J3				
	Time in hours				
	24	48	72	96	120
1	0.47	0.86	1.8	2.2	2.0
2	0.5	0.98	1.9	2.3	2.2
4	0.75	1.3	2.0	2.4	2.3
8	1.6	2.1	2.2	2.5	2.4
16	2.2	2.2	2.4	2.6	2.6
32	2.8	2.9	3.3	3.7	3.7
64	2.8	3.3	3.4	3.7	4.0

Table 7. Growth (O.D) of *C. parapsilosis* Q3 at different concentrations of glucose (m mole/L) using a shake flasks as a batch culture at 30 °C.

Glucose concentration (m mole/L)	*C. parapsilosis* Q3				
	Time in hours				
	24	48	72	96	120
1	1.0	2.1	2.7	3.1	3.0
2	1.3	2.2	2.9	3.3	3.2
4	1.5	2.3	3.0	3.4	3.3
8	1.8	2.8	3.6	4.1	3.9
16	2.0	2.9	3.7	4.2	4.2
32	3.0	4.7	5.1	5.5	5.5
64	3.6	6.0	8.4	8.5	8.5

K. jensenii H1, *C. parapsilosis* Q3, *C. solani* F8, *C. versatilis* J3, respectively. The plotting of reciprocal number of μ (1/μ) against the reciprocal number of substrate S (1/s) gave straight line (lineweaver-Burk plot) with an intercept on the abscissa at 1/KS (reciprocal number of saturation constant) and an intercept on the ordinate at 1/μmax (reciprocal of maximum specific growth rate in Figures 1, 2, 3, 4 as well as, saturation constant Ks for each yeast strain was calculated. Results clearly showed that *C. solani* F8 recorded the highest affinity to utilize glucose than other yeast strains showing the lowest value of saturation constant Ks being 0.7 * 10 $^{-4}$ mole glucose. It means that this yest

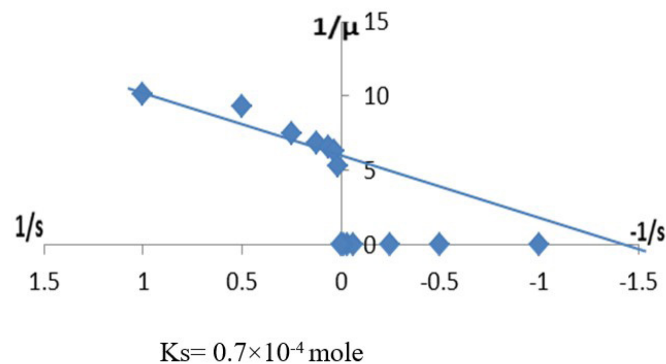


Figure 1. The straight line of reciprocal number of specific growth rate (1/μ) against reciprocal number of glucose concentration (1/s) for the strain of *C.solani* F8.

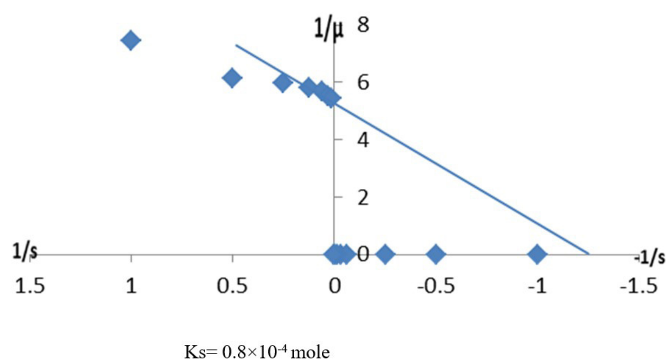


Figure 2. The straight line of reciprocal number of specific growth rate (1/μ) against reciprocal number of glucose concentration (1/s) for the strain of *K.jensenii* H1.

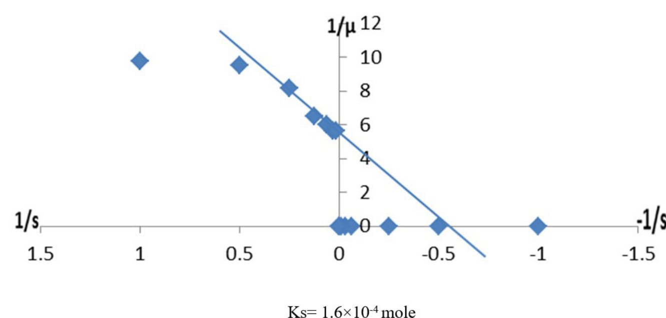
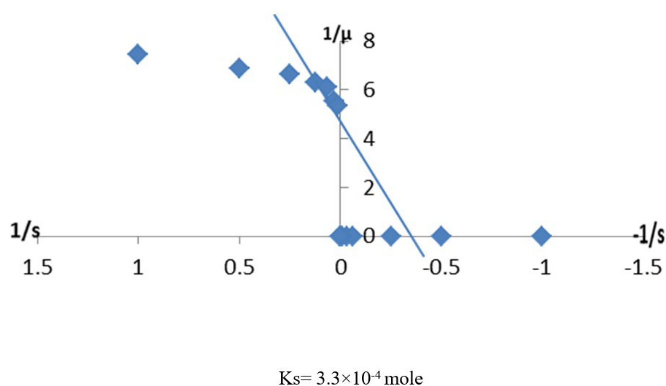


Figure 3. The straight line of reciprocal number of specific growth rate (1/μ) against reciprocal number of glucose concentration (1/s) for the strain of *C. versatilis* J3.



Ks= 3.3×10^{-4} mole

Figure 4. The straight line of reciprocal number of specific growth rate (1/μ) against reciprocal number of glucose concentration (1/s) for the strain of *C. parapsiolsis* Q3.

utilized the lowest amount of sugar to produce a unit of growth. On the contrary *C. parapsilosis* Q3 showed the higest value of Ks being 3.3 * 10^{-4} mole, i.e. it utilize higest amount of sugar per a unit of sugar per unit of growth. Generally, these yeasts had different saturation constant, i.e. their effecieny to utilze glucose varied from one strain to another, where the highest efficiency was recorded in the case of *C. solani* F8. Higgins et al., (1985) reported that Ks values for carbon energy substrat are usually 10^{-5} mole. Rose & Harrison (1970) also reported thaty the μmax and Ks for some yeasts were found to be 0.37 ± 0.03 h^{-1} and 3.6 ± 0.5 * 10^{-4} mole at 30 °C and pH value 4.0.

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References

Arias, C. R., Burns, J. K., Friedrich, L. M., Goodrich, R. M., & Parish, M. E. (2002). Yeast species associated with orange juice: evaluation of different identification methods. *Applied and Environmental Microbiology*, 68(4), 1955-1961. http://dx.doi.org/10.1128/AEM.68.4.1955-1961.2002. PMid:11916718.

Ashida, S., Shimazaki, T., Kitano, K., & Hara, S. (1983). New killer toxin of Hansenula mrakii. *Agricultural and Biological Chemistry*, 47(12), 2953-2955.

Barnett, J. A., Payne, R. W., & Yarrow, D. (1983). *Yeast – characteristics and identification*. Cambridge, United Kingdom: Cambridge University Press.

Barnett, J. A., Payne, R. W., & Yarrow, D. (1990). *Yeasts: characteristics and identification* (2nd ed.). Cambridge, United Kingdom: Cambridge University Press.

Bevan, E. A., & Makower, M. (1963). The physiological basis of the killer character in yeast. In S. J. Geerts (Ed.), *Proceedings of the 11th International Congress on Genetics* (Vol. 1, pp. 202–203). Oxford: Pergamon Press.

Bussey, H., & Sherman, D. (1973). Yeast killer factor: ATP leakage and coordinate inhibition of macromolecular synthesis insensitive cells. *Biochimica et Biophysica Acta*, 298(4), 868-875. http://dx.doi.org/10.1016/0005-2736(73)90391-X. PMid:4580980.

Bussey, H., & Skipper, N. (1975). Membrane-mediated killin g of Saccharomyces cere visiae glycoprote in s from Torulopsis glabrata. *Journal of Bacteriology*, 124(1), 476-483. http://dx.doi.org/10.1128/jb.124.1.476-483.1975. PMid:240809.

Chalutz, E., & Wilson, C. L. (1990). Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*. *Plant Disease*, 74(2), 134-137. http://dx.doi.org/10.1094/PD-74-0134.

Difco Laboratories. (1988). *Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures*. New York: Scholar's Choice.

Farris, G. A., Mannazzu, I., & Budroni, M. (1991). Identification of killer factor in the yeast genus Metschnikowia. *Biotechnology Letters*, 13(4), 297-298. http://dx.doi.org/10.1007/BF01041488.

Golubev, W. I. (1998). Mycocins (killer toxins). In C. P. Kurtzman & J. W. Fell (Eds.), *The yeasts: a taxonomic study* (pp. 55–62). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-044481312-1/50011-3.

Higgins, I. J., Best, D. J., & Jones, J. (1985). *Biotechnology principles and application*. London: Blackwell.

Izgü, F., & Altinbay, D. (1997). Killer toxins of certain yeast strains have potential growth inhibitory activity on Gram-positive pathogenic bacteria. *Microbios*, 89(358), 15-22. PMid:9218351.

Kreger–van-Rij, N. J. W. (1984). *The yeast, a taxonomic study* (3rd ed.). Amsterdam: Elsevier Biomedical Press.

Kurtzman, C. P., & Fell, J. W. (1998). *The yeasts: a taxonomic study* (4th ed.). Amsterdam: Elsevier.

Kutty, S. N., & Philip, R. (2008). Marine yeasts: a review. *Yeast*, 25(7), 465-483. http://dx.doi.org/10.1002/yea.1599. PMid:18615863.

Lodder, J., & Kreger-van-Rij. N. J. W. (1967). *The yeasts: a taxonomic study*. Amsterdam: North Holland Publishing.

Lowes, K. F., Shearman, C. A., Payne, J., MacKenzie, D., Archer, D. B., Merry, R. J., & Gasson, M. J. (2000). Prevention of yeast spoilage in feed and food by the yeast mycocin HMK. *Applied and Environmental Microbiology*, 66(3), 1066-1076. http://dx.doi.org/10.1128/AEM.66.3.1066-1076.2000. PMid:10698773.

Magliani, W., Conti, S., Gerloni, M., Bertolotti, D., & Polonelli, L. (1997). Yeast killer systems. *Clinical Microbiology Reviews*, 10(3), 369-400. http://dx.doi.org/10.1128/CMR.10.3.369. PMid:9227858.

McLaughlin, R. J., Wisniewski, M. E., Wilson, C. L., & Chalutz, E. (1990). Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. *Phytopathology*, 80(5), 456-461. http://dx.doi.org/10.1094/Phyto-80-456.

Mushtaq, M., Iftikhar, F., & Nahar, S. (2006). Detection of yeast micro-flora from milk and yogurt in Pakistan. *Pakistan Journal of Botany*, 38(3), 859-868.

Palpacelli, V., Ciani, M., & Rosini, G. (1991). Activity of different killer yeasts on strains of yeast species undesirable in the food industry. *FEMS Microbiology Letters*, 68(1), 75-78. http://dx.doi.org/10.1111/j.1574-6968.1991.tb04572.x. PMid:1769559.

Petering, J. E., Symons, M. R., Langridge, P., & Henschke, P. A. (1991). Determination of killer yeast activity in fermenting grape juice by using a marked *Saccharomyces* wine yeast strain. *Applied and Environmental Microbiology*, 57(11), 3232-3236. http://dx.doi.org/10.1128/aem.57.11.3232-3236.1991. PMid:1781684.

Philliskirk, G., & Young, T. W. (1975). The occurrence of killer character in yeasts of various genera. *Antonie van Leeuwenhoek*, 41(2), 147-151. http://dx.doi.org/10.1007/BF02565046. PMid:239627.

Prescott, S. C., & Dunn, C. G. (1959). *Industrial microbiology*. New York: McGraw-Hill.

Ramon-Portugal, F., Delia, M. L., Strehaiano, P., & Riba, J. P. (1998). Mixed culture of killer and sensitive *Saccharomyces cerevisiae* strains in batch and continuous fermentations. *World Journal of Microbiology & Biotechnology*, 14(1), 83-87. http://dx.doi.org/10.1023/A:1008880618359.

Rose, A. H., & Harrison, J. S. (1970). The yeast. (Vol. 3). London: Academic press.

Seki, T., Choi, E., & Ryu, D (1985). Construction of killer wine yeast. *Applied and Environmental Microbiology*, 49(5), 1211-1215. http://dx.doi.org/10.1128/aem.49.5.1211-1215.1985. PMid:16346794.

Starmer, W. T., Ganter, P. F., Aberdeen, V., Lachance, M. A., & Phaff, H. J. (1987). The ecological role of killer yeasts in natural communities of yeasts. *Canadian Journal of Microbiology*, 33(9), 783-796. http://dx.doi.org/10.1139/m87-134. PMid:3690423.

Stumm, C., Hermans, J. M. H., Middelbeek, E. J., Croes, A. F., & Vries, G. J. M. L. (1977). Killer-sensitive relationships in yeasts from natural habitats. *Antonie van Leeuwenhoek*, 43(2), 125-128. http://dx.doi.org/10.1007/BF00395667. PMid:596861.

Sugisaki, Y., Gunge, H., Sakaguchi, K., Yamasaki, M., & Tamura, G. (1984). Characterization of a novel killer toxin encoded by a double -stran ded linear DNA plasmid of Kluyveromyces lactis. *European Journal of Biochemistry*, 141(2), 241-245. http://dx.doi.org/10.1111/j.1432-1033.1984.tb08183.x. PMid:6734597.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*, 6(1), 24-27. http://dx.doi.org/10.1177/000456326900600108.

Wilson, C. L., & Chalutz, E. (1989). Postharvest biological control of *Penicillium* rots of citrus with antagonistic yeasts and bacteria. *Scientia Horticulturae*, 40(2), 105-112. http://dx.doi.org/10.1016/0304-4238(89)90092-7.

Woods, D. R., & Bevan, E. A. (1968). Studies on the nature of the killer factor produced by Saccharomyces cerevisiae. *Journal of General Microbiology*, 51(1), 115-126. http://dx.doi.org/10.1099/00221287-51-1-115. PMid:5653223.

Yokomori, Y., Akiyama, H., & Shimizu, K. (1988). Toxin of wild Candida killer yeast with a novel killer property. *Agricultural and Biological Chemistry*, 25(11), 2791-2796. http://dx.doi.org/10.1271/bbb1961.52.2797.

Young, T. W. (1987). Killer yeasts. In A. H. Rose & J. S. Harrison (Eds.), *The yeasts* (pp. 131–164). London: Academic Press.

Young, T. W., & Yagiu, M. (1978). A comparison of the killer character in different yeasts and its classification. *Antonie van Leeuwenhoek*, 44(1), 59-77. http://dx.doi.org/10.1007/BF00400077. PMid:655699.