Scientific Bulletin. Series F. Biotechnologies, Vol. XXII, 2018
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

SCREENING OF KILLER ACTIVITY IN YEAST STRAINS ISOLATED FROM FERMENTED AND NON-FERMENTED FOODS AGAINST SPOILING FOOD YEASTS

Arzu KART, Hüseyin ÖZTÜRK

Süleyman Demirel University, Faculty of Engineering and Department of Food Engineering, West Campus, 32260, Isparta, Turkey

Corresponding author email: arzukart@sdu.edu.tr

Abstract

Killer activity is one of the mechanisms of antagonism among yeasts during fermentations. A variety of yeast species secrete a compound which is protein produced outside the cell and is called the killer toxin. Killer toxins are protein in nature and active at low pH. These toxins have a lethal effect against yeasts, bacteria and the moulds, except for their own species. Killer yeasts can be used to prevent the contamination of many spoilage yeasts which are sensitive to killer toxins in food processing via this mechanism during the food fermentations. In this way, it is possible to control of the food spoilage microorganisms and prolongation of the spoilage processes in foods by using of killer yeasts or direct using of killer toxins. The shelf life of foods can be extended by using killer features of yeast, so food resources can be better assessed and economic losses can be prevented. Therefore, it is important to determine the killer yeasts which are effective against spoilage food yeast. For these reasons, it is aimed to determine the effective killer yeast strains against spoiling food yeasts in this study.

In the study, yeast isolation was performed to obtain spoilage yeasts from various spoiled fermented foods. At the same time, in this study, the yeasts, which were isolated from different products and obtained from Süleyman Demirel University Food Engineering Department laboratory, were evaluated to determine the killer activities against these spoilage yeasts isolated from various spoilage fermented products.

It has been determined that those yeasts, which are effective against sensitive spoilage food yeasts, were investigated by using the agar diffusion method in the solid medium and have a low, medium and high spectrum activities as a result of quantitative experiments.

The study was carried out under the 30°C incubation temperature and pH 4 acidic conditions to the determine the killer activity. The results that were obtained in the study were calculated and evaluated over the zone radius. It was determined that the yeasts obtained from laboratory, showed 50% killer activity against spoilage yeasts as a results of quantitative analyses in solid medium. These yeast isolates were classified according to their effect degrees. It was determined that the yeasts obtained from Süleyman Demirel University Department of Food Engineering, showed killer activity against spoilage yeasts isolated from spoilaged food products as a results of in vitro experiments.

Key words*: spoilage yeast, antagonism, killer yeasts, food fermentation, biocontrol.*

INTRODUCTION

The yeasts are commonly found in nature as one of the groups of microorganisms. Yeast cells are widely obtained from the natural environments such as soil, water, fruit, fermented foods, animal and human biologic systems. However, it is important that the yeasts strains are used in different food products (Van Vuuren and Jacobs, 1991). In recently, the killer properties of the yeast strains have attracted more attention. Many strains of yeasts secrete toxins called as killer toxins (Bevan and Makower, 1963).

Many yeasts strains are evaluated as killer yeast because of their abilities to produce killer toxin. It was well-known that killer toxins produced by some yeast strains are low molecular mass proteins or glycoproteins. These proteins cause the death of sensitive cells of the same or related yeast genera and of the fungi and bacteria cells. The killer yeast strains are resistant to their own toxin and can be sensitive to the toxins produced by other killer yeasts (Bevan and Makower, 1963; Marquina et al., 2002).

Many studies indicate that the killer phenomenon is especially widespread among yeasts. Also, it can be found the killer character in natural yeast isolates and laboratory yeast strain collections (Florentina and Gageanu, 2011).

In many studies have been determinated that killer yeasts have been identified in many genera, such as: *Williopsis, Kluyveromyces,*

Debaryomyces, Saccharomyces, Torulopsis, Hanseniaspora, Metschnikowia, Pichia, Hansenula, Cryptococcus, Zygosaccharomyces and Ustilago. Several research articles have shown that killer yeasts can be applied to control growth of undesirable yeasts in food productions (Bevan and Makover, 1963; Woods et al., 1968; Woods et al., 1974; İzgü et al., 1996; İzgü et al., 2004; Santos et al., 2004; Hatoum et al., 2012).

The determination of killer yeast strains can also provide important information for combating different food deteriorate processes caused by certain spoiling strains of the yeasts (Van Vuuren and Jacobs, 1991; Garcia-Garibay et al., 2009; Ullivarri et al., 2011; Hatoum et al., 2012).

The main aims of the present study are to determine killer activity of laboratory yeasts isolated from different foods in previous studies against spoiling yeast strains. Yeast strains obtained from Microbiology Laboratory at Süleyman Demirel University in Turkey were screened for their killer activity against yeast strains which could cause spoiling in vinegar, olive and pickle food products.

In this study, killer characteristic was screened by *in vitro* tests in twenty-five strains which were previously isolated from different food products.

Killer activity of 25 isolates previously isolated from different food sources against nineteen yeast isolates which have potential spoilage character was analyzed.

For this reason, these nineteen yeast strains were isolated from spoiled fermented foods in this study.

The aim of our trial was to develop an effective biological and natural tool by using killer strains as biocontrol material against undesirable yeast strains.

MATERIALS AND METHODS

Materials

Yeast strains

Twenty-six yeast strains used in present study as a killer toxin producers were isolated from different fermented or non- fermented food products in previous studies in our laboratory. These strains were conserved in the Department of Food Engineering, Suleyman Demirel University in Turkey. Laboratory strain codes and isolation sources of yeasts were demonstrated in Table 1.

Table 1. Laboratory strain codes and isolation sources of yeasts

Laboratory strain codes	Isolation sources of yeasts
LM-1	bread dough
LM-3	Milk
LM-4	Milk
LM-5	Milk
LM-6	Milk
LM-7	Yoghurt
LM-8	Yoghurt
LM-9	Cheese
LM-10	Cheese
LM-11	home-made vinegar
LM-12	Pickle
LM-13	Kefir
LM-14	Kefir
LM-15	tarhana (traditional product)
LM-16	tarhana (traditional product)
LM-17	Pickle
LM-18	turnip (traditional product)
LM-19	goruk (traditional product)
LM-20	goruk (traditional product)
LM-21	goruk (traditional product)
LM-22	Kefir
LM-23	fermented olive
LM-24	fermented olive
LM-25	Bousa
LM-26	Bousa

LM: Laboratory yeast strain

Nineteen yeast strains were also used in present study as a spoilage yeast strains. Spoilage strain codes and isolation sources of yeasts were demonstrated in Table 2.

Table 2. Spoilage strain codes and isolation sources of yeasts

Spoilage strain codes	Isolation sources of yeasts
BM-41	fermented olive
BM-42	fermented olive
BM-43	fermented olive
BM-44	fermented olive
BM-45	fermented olive
BM-46	fermented olive
BM-47a	fermented olive
BM-47b	fermented olive
BM-48	pickle
BM-49	pickle
BM-50	pickle
BM-51	pickle
BM-52	pickle
BM-53	pickle
BM-54	pickle
BM-55	pickle
BM-56	pickle
BM-57	home-made vinegar
BM-58	home-made vinegar

BM: Potential spoilage yeast strain

Culture media

Growth medium was YEPD broth medium containing 1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) dextrose. The medium was buffered to pH 4.0 with 0.1 M citrate-phosphate buffer, and YEPD-MB agar (medium containing 0.003% (w/v) methylene blue and 2% (w/v) agar) was used in assays for the killer activity (Ullivarri et al., 2014).

Malt extract broth (MEB), yeast peptone dextrose broth (YPDB), potatoes dextrose agar (PDA), dichloran rose bengal chloramphenicol agar (DRBC), malt extract agar (MEA), yeast peptone dextrose agar (YPDA) mediums were also used to activate and cultivate all yeast isolates.

Methods

Isolation of yeast strains

Sampling. Spoilage yeast strains were isolated from spoiled food samples. Different spoiled samples were collected during experimental studies. These foods (cucumber, cabbage, pepper pickles, home- made vinegar, fermented olive samples) were purchased from local market place in various regions in Isparta-Turkey.

Isolation of spoilage yeast strains

The spoilage yeast strains were isolated by routine methods. Ten grams or milliliters of the spoiled food products were homogenized with ninety milliliters of sterile 0.85% NaCl solution and then were diluted. After suitable dilution of the cell cultures, the dilute was plated on appropriate solid medium and the plates were incubated at 28-30^{0}C for 5 days. Different colonies were selected according to colony properties of yeast strains from the plates and were transferred to the solid medium slants, respectively. Yeasts cells were also counted in appropriate solid medium.

Screening of yeasts strains for killer activity

The killer activity was investigated by method described below. Spoilage yeast strains were grown in YEPD broth medium at 28-30 ^{0}C for 24-48 h and the cells from the culture were suspended in sterile 0.85% NaCl solution and cell density was adjusted to 10^5-10^6 cells/ml. Adjusted and standardized cell densities of yeast strains were used. Each yeast culture was mixed with YEPD-MB containing 2 % (w/v) agar, and the mixture was poured in a sterile Petri dish containing the assay medium. The plates were incubated for 1-2 h until the agar hardened. For determination of the killing activity, the potential killer yeast strain cells (obtained from Department of Food Engineering laboratory) were inoculated onto the YEPD-MB containing 2 % (w/v) agar assay medium which cells of the spoilage yeast strain were mixed. The Petri dishes were incubated at 28-30^{0}C for 2-5 days, and checked daily (Gulbiniene et al., 2004).

Measurement of killer toxin activity

We assayed killer toxin activity with an agar diffusion test that was mentioned above. Finally, in the end of the incubation time, the diameter of the inhibition zone was used as a measure of the yeast killer activity. A killer effect was considered when the clear killing zone of inhibition around the tested isolates appeared on the Petri cups. Also, yeast strains which were surrounded by bluish colored cells which to be potential sensitive strains were considered as killer strains. Killer activity was measured by subtracting diameter of the yeast colony from diameter of the inhibition zone.

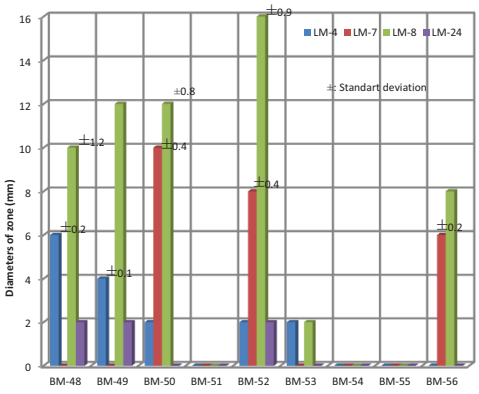
RESULTS AND DISCUSSIONS

The results of the obtained killer activity tests were evaluated according to three different product groups (pickle, table olive and vinegar). In each product group, it was tried to detected the killer yeast strains separately. In addition, potential best killer strains to be used in fermentation processes against spoilage yeasts were detected.

Killer activity experiments between potential killer strains and spoilage yeasts isolated from spoiled pickles

There was great variation in killer effect percentages against yeasts that have spoilage potential. There were killer effect percentage values between 11-67 %, except LM-11, LM-12, LM-19 and LM-23 strains, towards spoilage yeast strains isolated from different pickle products. Most killer effect percentage value was 66.7 %. LM-11, LM-12, LM-19 and

LM-23 yeast strains were not able to kill any of potential spoilage strains used in the study.
The highest killer activity was observed in LM-8 and LM-17 strains. The highest killer activity was also observed in LM-4 and LM-13 yeast strains. Therefore, these strains were considered to be superior killer yeast strains as suitable yeasts to be used as starter cultures in pickle fermentation.
According to results of diameter rate of clear zone as shown in Figure 1, the highest killer activity was determined in LM-8, LM-13, LM-3 and LM-7, respectively. Concurrently, it was revealed that LM-8 yeast ensured similar diameter rate of clear zone when compared with the results were obtain from tests by reference yeast strain.
Also it was observed that BM-49 spoilage yeast strain isolated from spoiled pickle products was the most sensitive strain from the tested laboratory strains.



BM: Spoilage Yeast ; LM: laboratory Yeast, Diameters of zone (mm), ±: Standart deviation

Figure 1. The diameter of clear zones of killer toxin on potential spoilage yeast strains isolated from spoiled fermented pickle products

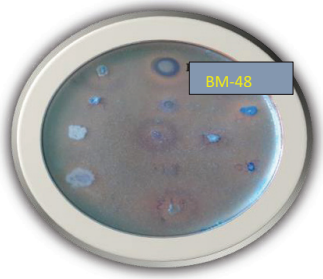


Figure 2. The image of inhibition zones of killer toxins secreted by laboratory and reference strains on a potential spoilage yeast strain BM-48 at pH 4.0

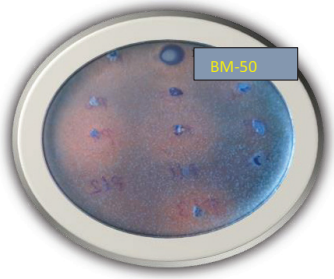


Figure 3. The image of inhibition zones of killer toxins secreted by laboratory and reference strains on a potential spoilage yeast strain BM-50 at pH 4.0

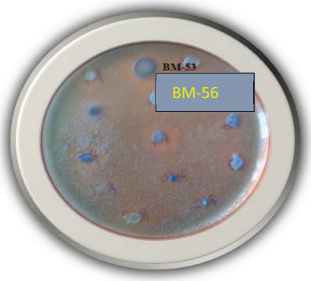


Figure 4. The image of inhibition zones of killer toxins secreted by laboratory and reference strains on a potential spoilage yeast strain BM-56 at pH 4

Killer activity experiments between potential killer strains and spoilage yeasts isolated from spoiled table yeasts

Similarly, according to the results of analyses carried on potential spoilage yeast strain isolated from spoiled fermented olives, it was determined that there were great variations in killer effect percentages against spoilage yeasts.
There were killer effect percentage values between 14-72 %, except LM-23, LM-25 and LM-26 strains, against spoilage yeast strains isolated from spoiled table olives. At the end of the study, it was detected that LM-5, LM-6 and LM-8 strains were the most active against the spoilage strains isolated form spoiled table olives.
Most killer effect percentage value was 71.5 % and LM-8 yeast strain shown the highest killer effect. LM-23, LM-25 and LM-26 strains were not able to kill any of potential spoilage strains that were used in this stage of the study.
The highest killer activity was also observed in LM-5, LM-15 and LM-21 yeast strains. For this reason, these strains and LM-8 yeast strain were considered to be superior killer yeast

strains as suitable yeasts to be used as starter cultures in pickle fermentation.
It was observed in this part of the study that LM-23, LM-25 and LM-26 yeast strains were not able to kill any of potential spoilage strains that were used in the study.
It was also appreciated that the highest killer activity was in LM-9 and LM-10, according to the diameter ratio of the clear zone.
Also, killer activity was observed in reference killer strain. Reference killer strain shown inhibition effect against only 6 strains of all to be spoilage yeasts isolated from table olives. It was measured killer activity values of reference strain according to the diameter ratio of the clear zone and it was found between 6-12 mm.
According to mentioned inhibition zone diameter range, yeast strains were appreciated as suitable potential killer strains in this study and were considered to have similar activity to reference strain also. In addition, it was observed that BM-43, BM-47, BM-45 and BM-41 spoilage yeast strains isolated from table olive products were most sensitive strains to the tested laboratory strains.

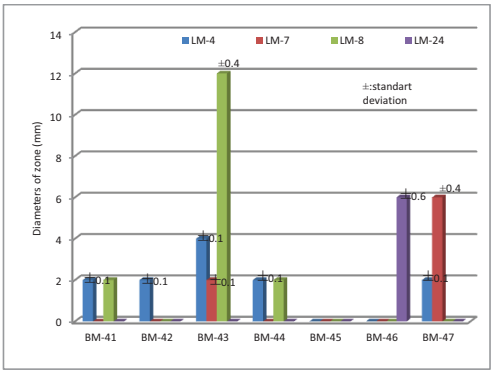


Figure 5. The diameter of clear zones of killer toxin on potential spoilage yeast strains isolated from spoiled table olive products

Killer activity experiments between potential killer strains and spoilage yeasts isolated from spoiled vinegar

Killer activity assay was also performed against two different spoilage yeast strains (BM-57 and BM-58) isolated from the home- made vinegar samples. Tested 25 laboratory yeast strains and reference strain were evaluated to killer effects towards two spoilage yeast strains.

Among yeasts isolates that were tested to determine killer strains, lowest killer activity was detected in this product group.
It was determined that there was killer effect percentage value at 50 % in some test yeast strains.
These strains, LM-4, LM-5, LM-7, LM-8, LM-14, and LM-24, were able to kill one of the potential spoilage strains used in the study.
It was also observed that other remaining tested strains were not killer against any spoilage strains.
It was detected that BM-58 spoilage yeast isolate was most resistance strain from the tested laboratory strains.
As a result, there was no observed killer activity of 25 yeast against BM-58 strain. However, it was affected by reference strain.

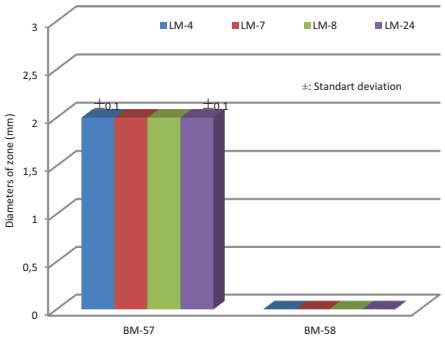


Figure 6. The diameter of clear zones of killer toxin on potential spoilage yeast strains isolated from spoiled vinegar products

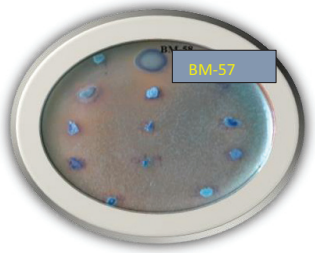


Figure 7. The image of inhibition zones of killer toxins secreted by laboratory and reference strains on a potential spoilage yeast strain BM-57 at pH 4.0

CONCLUSIONS

Killer yeast strains or their toxins have been recommended to control spoilage yeasts and

other undesirable microorganisms in food industry. In our study, it was determined that potential killer strains can be used in food industry by pre -screening killer activity of laboratory yeast isolates. Some of tested yeast strains were considered as new industrially killer strains according to results of our study. Among the killer yeasts isolated from different food products a wide range of killer activity was observed. These laboratory yeast strains could be recommended to biological control of undesirable yeasts in especially pickles, table olives and vinegar fermentations.

ACKNOWLEDGEMENTS

This research work was carried out at Süleyman Demirel University.

REFERENCES

Bevan, E.A., Makower, M., 1963. The physiological basis of the killer character in yeast. Proceeding of the 11th international conference on Genetics, 1: 203.

Florentina, M., Gageanu, A., 2011. Killer profile of wine yeast strains isolated in Dealurile Bujorului vineyard. Romanian Biotechnological Letters, 16(6): 144-147.

Garcia-Garibay, M., Gomez-Ruiz, L., Cruz-Guerrero, A.E., Lappe, P., Ulloa, M., 2009. Killer yeasts and alcoholic beverages. 13. Congreso Nacional de Biotecnologia Bioingeniera, 2009, Mexico.

Gulbiniene, G., Kondratiene, L., Jokantaite, T., Serviene, E., Melvydas, V., Petkuniene, G., 2004. Occurrence of Killer Yeast Strains in Fruit and Berry Wine Yeast Populations., Food Technologies Biotechnology, 42(3):159-163.

Hatoum, R., Labrie, S., Fliss, I., 2012. Antimicrobial and probiotic properties of yeasts: from fundemental to novel applications. Frontiers in Microbiology, 421(3): 1-12.

Izgu, F., Altınbay, D., Yüceliş, A., 1996. Identification and killer activity of a yeast contaminating starter cultures of *Saccharomyces cerevisiae* strains used in the Turkish baking industry. Food Microbiology, 14: 125-131.

Izgu, F., Altınbay, D., Derinel, Y., 2004. Immunization of the industrial fermentation starter culture strain of *Saccharomyces cerevisiae* to a contaminating killer toxin producing *Candida tropicalis*. Food Microbiology, 21: 635-640.

Marquina, D., Santos, A., Peinado, J.M., 2002. Biology of killer yeasts. International Microbiology, 5, 65-71.

Santos, A., Marquina, D., 2004. Killer toxin of *Pichia membranafaciens* and its possible use as a biocontrol agent against grey mould disease of grape wine. Microbiology, 150: 2527-2534.

Ullivarri, M.F.D, Mendoza, L.M, Raya, R.R, Farias, M.E., 2011. Killer phenotype of indigenous yeasts isolated from Argentinian wine cellars and their potential starter cultures for winemaking. Biotechnol Letters, 33: 2177-2183.

Ullivarri, M.F., Mendozaa, L.M., Raya, R.R., 2014. Killer yeasts as biocontrol agents of spoilage yeasts and bacteria isolated from wine. BIO Web of Conferences 3, 02001: p1-p4.

Van Vuuren, H.J.J., Jacobs, C.J., 1992. Killer yeasts in the wine industry. American Journal of Enology and Viticulture, 43, 119-128.

Waema, S., Maneesri, J., Masniyom, P., 2009. Isolation and identification of killer yeast from fermentedvegetables. Asian Journal of Food and Agro-Industry. 2(04): 126-134.

Woods, D.R., Bevan, E.A., 1968. Studies on nature of killer factor produced by *Saccharomyces cerevisiae*. Journal of General Microbiology, 51: 115-126.

Woods, D.R., Ross, J.W., Hendry, D.A., 1974. A New Killer Factor Produced by a Killer/Sensitive Yeast Strain. Journal of General Microbiology, 81: 285-289.