Biocontrol of yeast spoilage in selected food and beverages by yeast mycocin

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ABSTRACT

Yeasts constitute a highly heterogeneous group of unicellular organisms and have been most intimately associated with man from the dawn of his existence. Fruit juices contain various concentrations of sucrose, which constitutes a very important component of the medium for the growth of fungi. Dairy products are especially favorable environment for growth of yeasts due to the acidic reaction of the medium. Yeasts and molds can have both positive and negative effects on fermented food and beverages. Yeasts are used as the starter culture in various food and beverages products, but they can also initiate spoilage. Wild yeast contamination in the wine, beer processing or bread proving can retard fermentation and lead to loss of yield in fermentation, foaming and scum formation. Many of these fermentative processes use non-pasteurized medium, which can allow the predominance of wild yeast strains coming from the raw material outnumbering the starter yeast. This contamination can bring about the sluggish fermentation process, increased acidity and fusel oil production with a subsequent decrease in productivity. These problems challenge the industries to look forward and contribute to the management of wild yeast contamination. Understanding the bioload of various yeast genera and their killer toxin effects could be utilized in food and beverage industry in order to maintain food quality and food security.

Keywords: Fermentation, wild yeast strains, mycocin, food quality.

INTRODUCTION

From models of a bakery and brewery excavated at Thebes as early as 2000 BC, there is ample evidence about the use of yeasts by ancient civilization for enrichment of food quality. The concept of yeast and awareness about its fermentation capabilities may be considered to have its formal origin with the descriptions and drawings of yeast cells sent to the Royal Society in London in 1680 by Antonie van Leeuwenhoek. He observed these 'tiny animalcules' in a droplet of fermenting beer by use of tiny, hand ground and polished lenses which he made as a leisure pursuit. The origin of the word 'yeast' in many languages relates primarily to its ability to ferment. The English 'yeast' and the Dutch "gist" are derived from the Greek term 'zestos' which means 'boiled', a reference to the bubbling foam caused by the evolution of carbon dioxide. The German 'hefe' and the French 'levure' both have their origins in verbs meaning 'to raise' again referring to the bubbling foam. Yeast and molds can have both positive and deleterious effects on fermented products.

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Yeasts constitute a highly heterogeneous group of unicellular organisms and have been most intimately associated with man from the dawn of his existence [1]. Fruit juices contain various concentrations of sucrose, which constitutes a very important component of the medium for the growth of fungi [2]. Citrus juices are acidic beverages (pH 3 to 4) with high sugar content. Under these conditions, acidolactic bacteria, molds and yeasts comprise the representative microbiota [3]. Dairy products are especially favorable environment for growth of yeasts due to the acidic reaction of the medium. Another important condition for their development is their ability to grow at low temperatures and also lactose dissimilation [4]. The microbial composition of the sourdough is a consequence of ecological factors and therefore, every sourdough has a specific and unique range of microorganisms [5]. Killer yeasts are known to secrete a killer protein (mycocin) that is lethal to specific yeasts, but to which they are themselves immune.

A detailed investigation was undertaken to assess the bioload of various yeast genera and to understand the yeast with broad spectrum killer activity towards indigenous wild yeast killer toxin effects which has a potential to be utilized in food industry owing to their broad-spectrum activity against spoilage yeasts and food preservation.

MATERIALS AND METHODS

Isolation and bioload estimation of yeast strains in food samples

1 ml each of olive brine, honey, fermented fruit juices, beer, wine, soya sauce, curd along with surface rinses of grapes, saffron with ringers solution and 1g sour dough emulsifed 2% sodium citrate were seperately subjected to selective enrichment in 5ml Yeast Extract Peptone Dextrose (YEPD) broth media with anitbacterial antibiotics. Following which semi quantitative techniques for bio load estimation was carried out by planting a specified amount onto Sabourauds dextrose agar (SDA) plates by spread plate method. All steps carried out in triplicates and culture dishes incubated at 37ºC and 28ºC for 1-3 days till isolated colonies appeared. The colonies were purified and identified using standard mycological methods.

Screening for mycocin production by yeast strains isolated from food samples

Methylene blue agar plates were employed to diametrically streak the killer strain and the test strains isolated from food sample to look for inhibition. Three replicates of each strain were incubated both at room temperature and 37ºC each for 48 hours to check if there was any inhibition on the growth of the test strains.

RESULTS AND DISCUSSION

Sour dough, fermented juices, beer, wine, curd, olive brine, lemon juice, honey, saffron, grapes, soya sauce etc. were used in the study for the determination of killer yeast occurrence. All the isolates had an average growth rate of 24 to 72hrs and were tested negative for germ tube production. Killer toxins were detected in *Saccharomyces*, *Pichia* and *Candida* in the study conducted. Maximum killer activity was between pH 4.2 and 4.8 and inactivated at temperature above 40ºC. Wild yeast contamination in the wine, beer fermentation system or bread can retard fermentation and lead to serious problems including loss of yield in fermentation, foaming and scum formation [6]. Many of these fermentative processes use non-pasteurized medium, which can allow the predominance of wild yeast strains coming from the raw material outnumbering the starter yeast. This contamination can bring about the sluggish fermentation process, increased acidity and fusel

Table 1. Morphological characteristics of the various yeast isolates from the food samples.

Food isolate	Shape of Blastoconidia	Appearance in CMA	Colony morphology	Other features	I	II	III	IV	V
Saccharomyces cereviseae	Elliptical	Ellipsoidal yeast cells with multilateral budding	Dull white colonies	Eliptical ascospores	Yes	No	No	Yes	Yes
Candida pintolopesii	Oval	Large oval blastoconidia with budding	Smooth cream coloured colonies	Pseudohyphal forms along with budding	Yes	No	No	Yes	No
Candida parapsilosis	Oval	Short, thin cells with pronounced curves; Blastoconidia developed singly and in clusters	Cream colored colonies with lacy appearance	Old colonies became wrinkled	Yes	No	No	Yes	No
Candida tropicalis	Oval	Long branching pseudohyphae with blastoconidia	Cream colored colonies	Peripheral fringe was submerged in the agar	Yes	No	No	Yes	No
Pichia anomala	Elliptical	Yeast cells with multilateral budding	Cream moist colonies	Elliptical blastoconidia with multilateral budding	No	No	No	***	Yes
Brettanomyces lambicus	Elongated	Large pseudohyphal forms	Round smooth cream colored colonies	Budding in clusters, hyphae breaking into arthrospores	Yes	Yes	Yes	Yes	No
Geotrichum candidum	Barrel	Hyphae fragmented into arthroconidia	Spreading white color colonies	Germinating arthroconidia showed hockey stick appearance	No	Yes	Yes	No	No
Trichosporon asahii	Oval	Blastoconidia on pseudohyphae	Creamy moist colonies	True hyphae breaking into arthroconidia	Yes	Yes	Yes	Yes	No

I: Pseudohyphae on CMA; II: Arthroconidia on CMA; III: True hyphae on CMA; IV: Growth at 37ºC; V: Ascospore production; *** - Variable.

oil production with a subsequent decrease in productivity. These problems challenge the industries to look forward and contribute to the management of wild yeast contamination [7].

Yeasts are used as starter cultures in wine, beer, cheese, breads and other fermented alcoholic beverages. Yeast can also initiate spoilage in food such as yoghurt, fruit juices, mayonnaise and salads. *Saccharomyces cervisiae* with an impressive history in the fermentation industry is referred to as the 'true' brewer's yeast and it is only about 1 in 1000 berries. But, the berries posses many wild yeast strains, molds and bacteria. During the initial stage of the natural fermentation of the must, wild yeast strains from the grapes or any additives dominate the 'true' wine yeast till the alcohol level reaches up to 3 to 4 %. Since the initial fermentation is activated by the wild yeast's uncontrolled fermentation, it may alter the quality of the wine or ruin the wine. Only reliable and rapid identification of yeast species during process and quality control enables enologists to assess the role of yeasts as a main protagonist of alcohol fermentation or as a contaminant (Table 1).

The wild yeast contamination in wine and beer may lead to premature cessation of alcoholic fermentation, high volatile acidity, hydrogen sulfide production and off flavor caused by fusel oil, acetaldehyde and lactic acid. In order to maintain the product quality, the wild yeast strains need to be discarded or deactivated from the initial fermentation process, and fermentation process should be brought under the control of the *Saccharomyces cervisiae* [8,9]. The yeast killer toxin (mycocin) may be one of the approaches to combat the incumbent effects caused by undesirable yeast fermentation process. Wild yeast can either be of the *Saccharomyces* or non-*Saccharomyces* genus. *Saccharomyces diastaticus* breaks down the dextrin which is not being used by *S. cerevisiae*. This action of the wild yeast results in over attenuated beers. Non-*Saccharomyces* strains like *Brettanomyces* spp. produces strong mousey flavors and *Candida* form films on wine and beer. All these factors can result in serious financial losses to the brewery industry. *Pichia membranifaciens* and *Pichia anomala* secretes a killer toxin that is inhibitory to a variety of spoilage yeast and fungi present in fermented food stuffs [10,11]. *Williopsis saturnus* could be an effective bio representative for cheese spoilage control [12,13]. The killer characteristics can be transferred into starter yeasts in order to control wild strains during the production of wine, beer and bread [14]. Killer strains generate competitive advantages to the starter ethanol making yeast.

In conclusion, the analyzed strains in this study produced different amounts of active killer toxins and they possessed industrially significant killer properties. With more controlled and scale up experiments they could be recommended for fermentation of particular type of beer, wine or bread. We suggest that killer toxins isolated from food and beverage ingredients may be effective in suppressing wild yeast strains during fermentation.

REFERENCES

[1] Reed G. Prescott and Dunn's Industrial Microbiology, Globe Bookservices, London, 1983, 15-18.
[2] Marquina D, Santos A, Peinado J. Int. Microbiol. 2002, 5:65-71.
[3] Salo S, Wirtanen G. Food and Bioprocessing 2005, 83:288-296.
[4] Antonini SRC, Sanino A, Araujo JC, Tosta CD. Brazilian Journal of Food Technology 2005, 5:40-46.
[5] Peiia P, Barros F, Gascon S, et al. J. Biol. Chem. 1901, 256:10420-10425.
[6] Suzzi, G, Romano P, Ponti I, et al. J. Appl. Bacteriol. 1995, 78:304-308.
[7] Vaughan A, O'Sullivan T, Van Sinderen D. J. Inst. Brewing 2005, 111:355-371.
[8] Izgu F, Altinbay D, Yucelis A. Food Microbiol. 1997, 14:125-131.
[9] Rosini G. Canadian Journal of Microbiology 1983, 29:1462-1464.
[10] Björnberg A, Schnurer J. Canadian Journal of Microbiology 1993, 39:623-628.
[11] Petersson S, Schnurer J. Canadian Journal of Microbiology 1998, 44:471-476.
[12] Kimura T, Kitamoto N, Ohta Y, et al. J. Ferment. Bioeng. 1995, 80:85-87.
[13] Suzzi G, Romano P, Ponti I, et al. J. Appl. Bacteriol. 1995, 78:304-308.
[14] Lacey J. J. Appl. Bacteriol. 1989, 67:11-25.