International Food Research Journal 20(2): 975-979 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Screening of yeast strains for vinification of fruits from cold desert regions of North West India

Negi, B., Sharma, P., Kashyap, S., Seth, S. and *Dey, G.

Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, HP, India 173234

Article history

Received: 7 May 2012
Received in revised form: 7 August 2012
Accepted: 9 August 2012

Keywords

Ethanol-tolerance
osmo-tolerance
Saccharomyces cerevisiae
thermo-tolerance
wine

Abstract

The selection of yeast strains is important in the wine industries because yeasts contribute to the microbial ecology of wine production. The appropriate oenological process involves the screening of large numbers of natural yeast isolates in order to select desirable variants within a population of yeast strains. In this context, the 14 yeast strains isolated from different parts of Himachal Pradesh, India and identified as genus *Saccharomyces cerevisiae* were screened for their ethanol-, osmo- and thermo-tolerance. Among 14 strains, only one strain 'N' showed ethanol-tolerance (upto 12% (v/v)), osmo-tolerance (upto 30% (w/v) dextrose) and thermo-tolerance (upto 40°C) and it was found to be on par with other two industrial strains, 'I-1' and 'I-2'. The strain 'N' was used for vinification as an alternative post harvest technique for lesser utilized fruits from the cold desert regions of Himachal Pradesh, such as sea buckthorn.

Introduction

The term wine is most commonly used to refer to the fermented product of grape juice. Similar products can be obtained from different fruit juices with appropriate processing and additions. There are numerous reports available on wine production from other fruits such as apple, pear, bananas, jackfruit, mango, plum, litchi and strawberry (Sandhu and Joshi, 1995; Zeng *et al*., 2008; Singh and Kaur, 2009). There is an emerging interest in these fruit wines because of their phenolic compounds which act as antioxidants. The cold desert regions of Himachal Pradesh are also known for fruits like sea buckthorn (*Hippophae rhamnoides* L.), wild apricot (*Armeniaca vulgaris*), apricot (*Prunus armeniaca*), wild almond (*Brabejum stellatifolium*). Due to lack of appropriate post harvest technologies, these fruits are left unutilized. An attractive post-harvest preservation technique would be making wines from these fruits, as they are also rich source of antioxidant phenolics and vitamins. However for the vinification process an appropriate yeast strain is mandatory which should cope with stress conditions. More so because throughout alcoholic fermentation yeast cells are exposed simultaneously and sequentially to several stress conditions like increase in temperature, variations in osmotic conditions, high concentration of ethanol and the presence of competing organisms (Attfield, 1997). Yeasts should be able to detect and respond to these stress conditions without viability loss (Bauer and Pretorius, 2000). Hence, the selection and screening of yeast strains is an essential part in the wine industries. This often involves the screening of large numbers of natural isolated yeast strains within the genus *Saccharomyces*, in order to select desirable variants within a population of yeast strains, or alternatively, the evaluation of variants of established yeasts that have been optimized for specific properties (Pretorius, 2000; Rainieri and Pretorius, 2000). The present study reports the screening of yeast strains for vinification on the basis of ethanol-, osmo- and thermo-tolerance.

Materials and Methods

Yeast strains

Yeast strains were collected from various sites around Himachal Pradesh, India, and identified as genus *Saccharomyces cerevisiae* by Department of Microbiology, CSK HP Agricultural University, Palampur, Himachal Pradesh, India. The two industrial strains (*Saccharomyces cerevisiae*), 'I-1' and 'I-2', were generously provided by breweries, Vintage and Minchy's Pvt. Ltd., Solan, India and taken as control strains for the study. All yeast strains were maintained individually as frozen stocks in 40% glycerol and stored at -80°C.

Chemicals used in this study were yeast extract,

*Corresponding author.
Email: *drgargi.dey@gmail.com*

malt extract, peptone, dextrose, sodium hydroxide (NaOH), ethanol, potassium-sodium tartarate, phenolphthalein, potassium dichromate, sulfuric acid (H_2SO_4) and 3, 5-dinitrosalicylic acid (DNS). All chemicals used for the study were of analytical grade and were purchased from Sigma–Aldrich.

Estimation of ethanol tolerance

Yeast strains were revived in YMPD (Yeast extract, Malt extract, Peptone and Dextrose) broth overnight at 30ºC. Ethanol tolerance was estimated by the method of Benitez *et al*. (1983). Yeasts were inoculated in 10ml of YMPD broth and incubated for 12 hrs in 121 rpm at 30ºC. An aliquot of 200µl of the above inoculum was used for inoculation in 10ml of YMPD broth containing different percentage of ethanol (2-20%, v/v). The experiment was carried out with all the 16 strains of *Saccharomyces cerevisiae* (14 lab isolated and 2 industrial strains) for 24 hrs. The results were expressed in percent survival.

Estimation of osmo-tolerance

Yeast strains were inoculated in 10ml of YMPD medium and incubated for 12hrs in 121 rpm at 30ºC. An aliquot of 200µl of the above inoculum was used for inoculation in 10ml of YMPD medium containing 15% (w/v) and 30% (w/v) of dextrose concentration. The experiment was performed with all the 16 strains of *S. cerevisiae* (14 lab isolated and 2 industrial strains) for 24 hrs. The results were expressed in percent survival.

Estimation of thermo-tolerance

For testing the thermo-tolerance, an aliquot of 200µl of overnight grown culture of individual yeast strain was inoculated in the autoclaved YMPD medium and incubated at three different temperatures (30ºC, 40ºC and 50ºC) for 24 hrs. The results were expressed in percent survival.

Application of selected strain for vinification

Sea buckthorn berries were collected and identified by Ecosphere, a Non Government Enterprise in Spiti, Himachal Pradesh. Juice extraction from the berries was carried out using pressing technique according to Bump (1989). Extracted juice was thermally treated at 85ºC for 30 min and stored at 4ºC for further use. The wine production for sea buckthorn was carried out by the method described by Dey and Negi (2012).

Residual sugar was estimated by the method of Miller (1959). The ethanol content and the total acidity were estimated by the methods described by Joshi (1997).

Statistical analysis

All data were presented as the average of triplicate experiments with standard deviation. Results were statistically interpreted with one-way analysis of variance (ANOVA) followed by post hoc analysis (Tukey's test) to locate the significant differences indicated with ANOVA. The data for ANOVA were analyzed at different significance level using statistical package MSTAT/Minitab (Minitab Inc. USA, Version 13, 2004 for Windows®).

Results and Discussion

Selection of yeast strain during wine production is a crucial step because it can have a great influence on the volatile and non-volatile components of the end product (Fundira *et al*., 2002). In this context, the present study aimed to screen the ethanol-tolerant, osmo-tolerant and thermo-tolerant yeast strain for wine production.

It has been well established that at the commencement of fermentation, yeast is subjected to high sugar concentration and as the ethanol is produced, both the sugar and ethanol causes stress to the yeast strain (Guyot *et al*., 2005). It is also known that tolerance to ethanol is variable from one yeast strain to another. Thus, selection for yeast strains with a high resistance to ethanol stress is of immense importance for understanding the evolution of the organism and their economic value for traditional brewing. Figure 1(A-F) depicts the effect of increasing concentrations of ethanol on the different yeast strains as compared to the two industrial strains. To determine the high ethanol-tolerant strain, we tentatively divided percent survival of these strains into three categories on the basis of three independent experiments: highly ethanol-tolerant (50 to 100% survival), moderately ethanol-tolerant (25-50% survival) and slightly ethanol- tolerant (<25% survival). Based on 50-100% survival, the ethanol-tolerance of yeast strains could be arranged in the following order: strain 'N' (12% v/v) > 'I-2' (11% v/v) >'I-1', strain 'G', 'J', 'K', 'L' and 'M' (10% v/v) > strain 'C' and 'H' (9% v/v) > strain 'B', 'D' and 'F' (8% v/v) > strain 'I' (6% v/v) > strain 'A' and 'E' (5% v/v). Furthermore, the strain 'N' showed 25% survivability in presence of 13% (v/v) of ethanol concentration in comparison to industrial strains, 'I-1' (8% survival) and 'I-2' (20% survival). The difference in percent survival of strain 'N' appeared to be statistically significant ($P \leq 0.001$) in comparison to industrial strains ('I-1' and 'I-2'). The ethanol-tolerance influences the efficiency of the fermentation process because optimal conversion of sugar to ethanol requires yeast strains that are

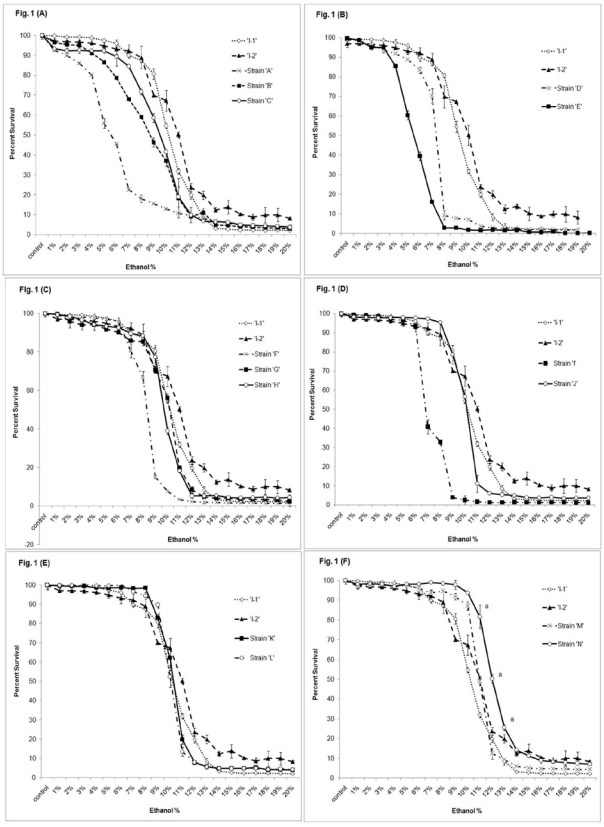

Figure 1 (A - F). Screening of *Saccharomyces cerevisiae* strains for high ethanol-tolerance. The data represents the average of three replicates with standard deviation. All the 16 strains were analyzed by means of one-way analysis of variance (ANOVA) followed by post hoc (Tukey's test) analysis ($P \leq 0.001$). The small letter 'a' depicts the significant difference ($P \leq 0.001$) of strain 'N' in comparison to industrial strains ('I-1' and 'I-2')

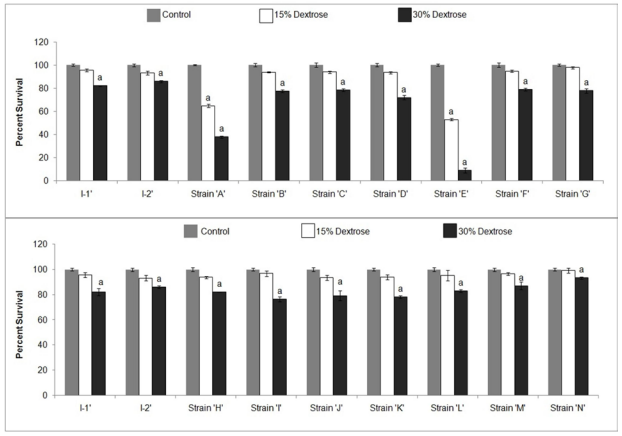

Figure 2. Screening of *Saccharomyces cerevisiae* strains for osmo-tolerance at different sugar concentrations (15% and 30% (w/v) Dextrose). The data represents the average of three replicates with standard deviation. aThe values are statistically significant ($P \leq 0.001$) based on one-way ANOVA followed by post hoc (Tukey's test) analysis

tolerant to high concentrations of ethanol at relatively ambient temperatures. From the present results, the strain 'N' was screened for vinification based on its high ethanol tolerance. The reduced ethanol tolerance exhibited by the other strains could be due to the toxic effect of ethanol (Alexandre and Charpentier, 1998). The rising ethanol level during fermentation

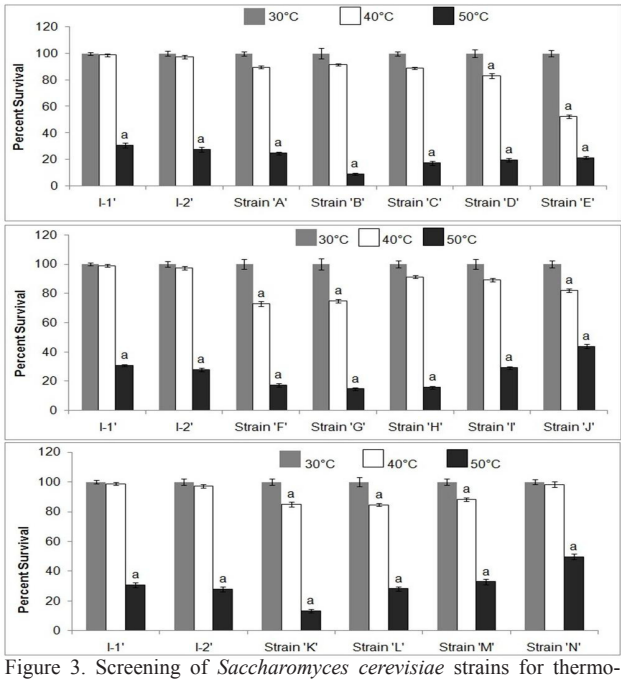

Figure 3. Screening of *Saccharomyces cerevisiae* strains for thermo-tolerance at different temperatures (30°C, 40°C and 50°C). The data represents the average of three replicates with standard deviation. aThe values are statistically significant ($P \leq 0.001$) based on one-way ANOVA followed by post hoc (Tukey's test) analysis.

on high concentration of sugar acts to reduce cell viability because ethanol has been reported to be non-competitive inhibitor (Piper, 1995; Boulton *et al*., 1996).

Osmo-tolerance by yeast is an essential property for wine production (Jimenez-Marti *et al*., 2011). As a result, the capability of the yeast strains to survive on medium with high sugar concentrations by tolerating high osmotic pressure was examined in YMPD (Yeast extract, Malt extract, Peptone and Dextrose) medium containing 15% (w/v) and 30% (w/v) dextrose. All the yeast strains showed more than 93% survivability in YMPD medium containing 15% (w/v) dextrose except strain 'A' (65% survival) & 'E' (53% survival), (Figure 2). The percent survival of yeast strains in YMPD medium containing 15% (w/v) dextrose could be arranged in the following order: strain 'N' (99%) > strain 'G' (98%) > strain 'I' and 'M' (97%) > 'I-1' (96%) > strain 'F' and 'L' (95%), strain 'B', 'C', 'D', 'H' and 'K' (94%) > 'I-2' and strain 'J' (93%) > strain 'A' (65%) > strain 'E' (53%), (Figure 2). For the purpose of vinification, the strain 'N' showed the required high osmo-tolerance (93% survival) in YMPD medium containing 30% (w/v) dextrose, which is equivalent to approximately 24° Brix of sugar for wine production (Lee *et al*., 2011). There was only 6% decrease in the percent survival of strain 'N' in the presence of 30% (w/v) dextrose. Whereas, the industrial strains, 'I-1' and 'I-2' showed 14% and 7% decrease in the presence

of 30% (w/v), respectively. The strain 'N' showed both high ethanol- and osmo-tolerance, similarly strain 'A' and 'E' showed poor ethanol- and osmo-tolerance. Our results corroborate previous study which reported that ethanol tolerant yeast strains are likely to be sugar-tolerant (Osho, 2005).

The temperature tends to increase during fermentation process as heat is liberated due to exothermic reactions i.e. around 42°C in case of industrial scale fermentation (Attfield *et al*., 1992). Therefore, the capability of the yeast strains to grow on medium at different temperatures (30°C, 40°C and 50°C) for 24 hrs was examined further by culturing them in YMPD medium. As illustrated in Figure 3, the percent survival of yeast strains at temperature 40oC could be arranged in the following order: 'I-1' (99%) > strain 'N' (98%) > 'I-2' (97%) > strain 'B' (92%) > strain 'H' (91%) > strain 'A' (90%) > strain 'C' and 'I' (89%) > strain 'M' (88%) > strain 'K' and 'L' (85%) > strain 'D' (83%) strain 'J' (82%) > strain 'G' (75%) > strain 'F' (73%) > strain 'E' (52%). Some strains showed reduction in cell growth at 40°C and were not able to retain their cell growth at 50°C (Figure 3). However, the yeast strain 'N' was able to retain 50% survivability at temperature 50°C in comparison to industrial strains ('I-1' and 'I-2'), (Figure 3).

Wine quality is significantly influenced by the fermentation technique and the yeast strain which can influence the flavour, appearance, aroma and texture of the end product (Fleet, 2003; Romano *et al*., 2003). Based on the present study, we were able to select one strain 'N' with high ethanol-, osmo- and thermo-tolerance which could be compared to the industrial strains ('I-1' and 'I-2'). This strain was further used for wine production with the fruits from cold regions of Himachal Pradesh in North West India. One of the wines from sea buckthorn (*Hippophae rhamnoides* L.) berries made with the above strain 'N' had ethanol content of 8.6% (v/v), pH of 3.89, residual sugar of 800mg/100ml and total acidity of 230mg/100ml. The wine made could be classified as table wine, because these usually contain 11-14% (v/v) alcohol and may have as low as 7% (v/v), (Joshi, 1997). Thus, the strain 'N' performed well in this study; however, this strain deserves further evaluation for its robustness in large scale fermentation set up.

Acknowledgement

The authors are grateful to Prof. S.S. Kanwar (Department of Microbiology, CSK HP Agricultural University, Palampur, Himachal Pradesh, India) for providing the yeast strains for this study.

References

Alexandre, H. and Charpentier, C. 1998. Biochemical aspects of stuck and sluggish fermentation in grape must. Journal of Industrial Microbiology and Biotechnology 20: 20-27.

Attfield, P. V., Raman, A. and Northcott, C. J. 1992. Construction of *Saccharomyces cerevisiae* strains that accumulate relatively low concentrations of trehalose, and their application in testing the contribution of the disaccharide to stress tolerance. FEMS Microbiology Letters 94: 271-276.

Attfield, P. V. 1997. Stress tolerance: the key to effective strains of industrial baker's yeast. Nature Biotechnology 15: 1351-1357.

Bauer, F. F. and Pretorius, I. S. 2000. Yeast stress response and fermentation efficiency: how to survive the making of wine- a review. South African Journal of Enology and Viticulture 21: 27-51.

Benitez, T., Castillo, L. D., Aguilera, A., Conde, J. and Cerdaolmedo E. 1983. Selection of wine yeasts for growth and fermentation in the presence of ethanol and sucrose. Applied and Environmental Microbiology 45: 1429-1436.

Boulton, B., Singleton, V. L., Bisson, L. F. and Kunkee, R. E. 1996. Yeast and biochemistry of ethanol fermentation. In Boulton, B., Singleton, V. L., Bisson, L. F. and Kunkee, R. E. (Eds). Principles and Practices of Winemaking, p. 139-172. New York: Chapman and Hall.

Bump, V. L. 1989. Apple pressing and juice extraction. In Downing, D. L. (Ed.). Processed apple products, p. 53-82. New York: Avi, Van Nostrand Reinhold.

Dey, G. and Negi, B. (Publication Date (U/S11A) 10/02/2012). Novel method for production of sea buckthorn wine and wine thereof. Indian Patent, filed March 5, 2010 (Application no. 499/DEL/2010).

Fleet, G. H. 2003. Yeast interactions and wine flavour. International Journal of Food Microbiology 86: 11-22.

Fundira, M., Blom, M., Pretorius, I. S. and Rensburg, P. V. 2002. Selection of yeast starter culture strains for the production of marula fruit wines and distillates. Journal of Agricultural and Food Chemistry 50: 1535-1542.

Guyot, S., Ferret, E. and Gervais, P. 2005. Responses of *Saccharomyces cerevisiae* to thermal stress. Biotechnology and Bioengineering 92: 403-409.

Jimenez-Marti, E., Gomar-Alba, M., Palacios, A., Ortiz-Julien, A. and del Olmo, M. L. 2011. Towards an understanding of the adaptation of wine yeasts to must: relevance of the osmotic stress response. Applied Microbiology and Biotechnology 89:1551-1556.

Joshi, V. K. 1997. Fruit Wine. In Joshi, V. K. (Ed). India: Directorate of Extension Education, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP).

Lee, Y. J., Choi, Y. R., Lee, S. Y., Park, J. T., Shim, J. H., Park, K. H. and Kim, J. W. 2011. Screening wild yeast

strains for alcohol fermentation from various fruits. Mycobiology 39: 33-39.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426-428.

Osho, A. 2005. Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. African Journal of Biotechnology 4: 660-662.

Piper, P. W. 1995. The heat-shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiology Letters 134: 121-127.

Pretorius, I. S. 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16: 675-729.

Rainieri, S. and Pretorius, I. S. 2000. Selection and improvement of wine yeasts. Annals of Microbiology 50: 15-31.

Romano, P., Fiore, C., Caruso, P. M. and Capece, A. 2003. Function of yeast species and strains in wine flavor. International Journal of Food Microbiology 86: 169-180.

Sandhu, D. K. and Joshi, V. K. 1995. Technology, quality and scope of fruit wines especially apple beverages. Indian Food Industry 11: 24-34.

Singh, R. S. and Kaur, P. 2009. Evaluation of litchi juice concentrate for the production of wine. Natural Product Radiance 8: 386-391.

Zeng, X. A, Chen, X. D., Qin, F. G. F. and Zhang. L. 2008. Composition analysis of litchi juice and litchi wine, International Journal of Food Engineering 4: 1-16.