Isolation and Evaluation of Indigenous Yeast Strains for Improving Sugarcane Molasses Fermentation Efficiency in Sri Lankan Alcohol Distilleries

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ABSTRACT

A study was conducted to isolate yeast strains from the natural environment and to screen them for efficient ethanol production from sugarcane molasses aiming to introduce a more efficient yeast strain for commercial level fermentation of sugarcane molasses into ethanol. Fourty nine indigenous yeast strains were isolated from sugar-containing materials collected from diverse sources. They were named from Y1 to Y49, characterised morphologically, and their performance in ethanol production under laboratory conditions were evaluated. The majority of the isolated yeast strains produced alcohol in molasses medium during a 72-hour laboratory fermentation. Out of the 49 strains evaluated, seven strains, Y5, Y8, Y10, Y11, Y21, Y22 and Y39 were found to be superior to bakers' yeast in terms of molasses fermentation. The isolate Y39 produced the highest ethanol concentration (7.5% v/v) compared to bakers' yeast (6.5% v/v).

Keywords: Alcohol distillery, Bakers' yeast, Fermentation, Molasses, Sri Lanka, Sugarcane

INTRODUCTION

Yeasts are easily grown unicellular eukaryotic fungi, which naturally live as either saprophytes or parasites. They are found in many diverse environments; in plants, flowers, fruits, tree exudates, tanning liquors, necrotic tissues of plants, mushrooms, animals (occasionally as pathogens), and in soil and aquatic environments. They are also found in insects (e.g., in bark beetles, *Ambrosia* beetles and other wood-boring insects and in *Drosophila*), crustaceans and other aquatic animals (Phaff and Starmer,1987; Chandrasena *et al.*, 2006).

Yeasts are of great economic importance as they are used in agricultural and industrial purposes. Many saprophytic yeasts along with bacteria decompose dead organic matter, and thereby, they help in returning the nutrients (derived from the organic matters) to the soil in a form available to green plants. Many types of yeasts are used to produce various foods. They include; bakers' yeast in bread production, brewers' yeast in beer fermentation, yeast in wine fermentation and for xylitol production (Chatterjee *et al.*, 2011). Most of the yeasts produce enzymes, alkaloids and various other organic compounds of great economic importance (Wickerham and Burton, 1952; Yamada,1999; Kurtzman and Fell, 1999). The important metabolic products produced by yeasts are the antibiotics and organic acids such as citric acid. Some yeast strains are used in industrial single-cell protein production from lignocelluloses materials, methanol, n-alkanes, starch, oils and other cheap carbon sources. The pigmented yeasts are used as feed and food colourants, and some of them as single-cell oil (Chatterjee *et al.*, 2011).

Ethanol production is one of the major economic important uses of yeasts. In view of the rising fossil fuel prices and its adverse environmental impacts, worldwide interest in the utilisation of bio-ethanol as a renewable energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production (Sheela *et al.*, 2008; Chatterjee *et al.*, 2011). Yeast cells carryout alcoholic fermentation using a range of enzymes by converting sugar into carbon dioxide gas (CO_2) and ethyl alcohol. Ethyl alcohol is manufactured by the fermentation of potatoes, cereals, molasses, etc., but in most countries, sugarcane molasses is used as the carbohydrate substrate for the production of ethanol.

Ethyl alcohol production is a major sugarcane-based industry in Sri Lanka. The distillery integrated to each sugar factory produces ethyl alcohol, a green energy source, from the co-product, molasses of sugar production. This directly influences the economic viability and environmental sustainability of the Sri Lankan sugar industry. However, the overall efficiency of the existing processes is low, compared to similar industries elsewhere in the world resulting in high production costs and loss of potential revenue. The overall processing efficiency is generally below 80%, and the alcohol yield is about 290 l/t of molasses due to the use of contaminated water for dilution of molasses, use of inferior yeast (bakers' yeast) for fermentation at Sevanagala distillery, poor control of temperature and pH, inadequate yeast nutrition and unskilled handling of the fermentation process. Therefore, rectification of these shortcomings is essential to increase the fermentation efficiencies of Sri Lankan distilleries (Chandrasena *et al.*, 2006).

The 'ideal' ethanol-producing yeast strains should possess fermentation and growth properties such as fast fermentation rates, high ethanol yields, high tolerance to high ethanol concentrations and low pH levels and high temperature tolerance during fermentation. The use of efficient yeast strains with higher ethanol tolerance to improve ethanol yields in the fermented wash would reduce distillation costs, and hence, increase the profitability of the overall process (Chen and Chen,1985; Patrascu *et al.*,2009).

This research aims at isolation of yeast strains with economically important properties for more efficient fermentation of molasses to increase ethyl alcohol yield in Sri Lankan distilleries compare to bakers' yeast.

MATERIALS AND METHODS

Collection of samples

Samples of waste molasses, waste sugarcane juice, baggasse, filter-mud, distillery effluent and other sugar-containing plant materials from various locations in Sri Lanka were collected in to sterilised containers and transported to the laboratory of the Sugarcane Research Institute, Uda Walawe where experiments were carried out.

Isolation of yeasts

Yeasts were isolated using suitably-diluted samples by streak plating onto MYPG agar (yeast extract 0.3%, malt extract 0.5%, peptone 0.3%, glucose 1% and agar 1.5%) with pH adjusted to 4.8. All isolates were named as Y1, Y2, and so on.

Purification of yeasts

A loopfull of colonies from the agar plates were streaked on MYPG agar medium (0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 1% glucose, 1.5% agar at 4.8 pH) and incubated at 30°C for 3 days. The isolation and streaking were repeated on MYPG agar medium at pH 4.8 until pure cultures were obtained. Colony characters of the pure yeast isolates were examined.

Morphological characterisation

The yeast colonies grown on MYGP agar for 24 hours at 30°C were characterised

morphologically in terms of size, shape, colour and margin.

Fermentation of sugarcane molasses by yeast isolates under laboratory conditions

The samples of sugarcane molasses obtained from the Sevanagala sugar factory were transported to the Sugarcane Research Institute, Uda Walawe for laboratory studies.

The seed culture medium was prepared by diluting molasses to obtain 10% total sugars and adding 0.6% ammonium sulphate and 0.15% potassium dihydrogen phosphate. The medium pH was adjusted to 4.5 and was pasteurised at 80° C for 15 minutes. The yeast isolates sub-cultured on MYPG agar plates were used as seed cultures for molasses fermentation.

The fermentation medium was prepared by diluting molasses to 16% fermentable sugar and adding 0.6% ammonium sulphate and 0.15% potassium dihydrogen orthophosphate.

The initial pH was adjusted to 4.5 with sulphuric acid and pasteurised at 80°C for 15 minutes. The seed culture was added in shaking flasks which contained fermentation medium. The flasks were incubated in a rotary water bath at 30°C with mild shaking (at 100 rpm) (Chandrasena *et al.*, 2006).

The samples were taken from the water bath incubator at 6-hour intervals to determine the alcohol concentration. Ebiliometer was used to measure the alcohol concentration.

Data analysis

All experiments were carried out in triplicate to analyse the significance of the differences in alcohol production ability of the locally-isolated yeast by one-way analysis of variance (ANOVA). The mean differences of ethanol concentration among the isolates were tested using Tukey's test at the 5% level of probability. MINITAB 16 was used for the statistical analysis.

RESULTS AND DISCUSSION

Morphological features

Fourty nine yeast strains were isolated from various sugar-rich sources in natural environments. The yeast isolates were identified based on their colony morphology (Table 1). Most of the isolated colonies exhibited smooth surfaces with circular margins. The colour of the colonies showed a wide variation of creamy white and white. The cells were found with various shapes such as round oval and ellipsoidal.

Molasses fermentation and ethanol production capability

The yeast strains isolated from various sources were tested for their alcohol- producing capabilities in molasses medium. Table 2 indicates that most of the isolates produced high levels of alcohol from molasses fermentation compared to the bakers' yeast after 72 hours.

The results of ANOVA indicated that there was a significant variation in alcohol yields obtained from fermentation of molasses by 49 yeast strains for 72 hours ($p = 0.000$). Seven strains, namely, Y 5, Y 8, Y 10, Y11, Y21, Y 22 and Y 39 showed the best performance in molasses medium than bakers' yeast. The highest alcohol yield (7.5%v/v) was produced by the yeast isolate Y 39. It was isolated from the molasses collected from the Pelwatte distillery premises. According to the results, the maximum ethanol concentration was achieved around 48 hours after fermentation and amounted to 7.5% by Y39 yeast strain.

The yeast isolates which performed well in the fermentation of molasses than bakers' yeast were further evaluated to test their alcohol production capabilities in molasses medium. The results of the evaluation of seven selected superior yeast isolates and bakers' yeast are presented in Figure 1.

Table 1 Morphological features of the yeast isolates

Strain	Surface	Colour	Cell Shape	Margin
Y 1	Smooth	Off White	Oval	Irregular
Y 2	Smooth	Off White	Oval	Irregular
Y 3	Smooth	White	Oval	Circular
Y 4	Smooth	White	Oval	Circular
Y 5	Rough	Off White	Oval	Irregular
Y 6	Rough	White	Rounded	Circular
Y 7	Smooth	White	Oval	Circular
Y 8	Smooth	White	Oval	Circular
Y 9	Smooth	White	Oval	Circular
Y 10	Smooth	Red	Oval	Irregular
Y 11	Smooth	White	Oval	Circular
Y 12	Smooth	White	Rounded	Circular
Y 13	Smooth	Off White	Oval	Irregular
Y 14	Rough	White	Oval	Irregular
Y 15	Rough	Off White	Rounded/ Oval	Circular
Y 16	Smooth	White	Rounded/ Oval	Circular
Y 17	Smooth	White	Rounded/ Oval	Circular
Y 18	Smooth	White	Rounded	Circular
Y 19	Smooth	White	Rounded	Irregular
Y 20	Smooth	White	Ellipsoidal	Circular
Y 21	Smooth	White	Oval	Circular
Y 22	Smooth	White	Oval	Circular
Y 23	Smooth	Off White	Rounded	Circular
Y 24	Smooth	White	Oval	Circular

Y 25	Smooth	Off White	Oval	Irregular
Y 26	Smooth	Off White	Oval	Irregular
Y 27	Smooth	Off White	Oval	Irregular
Y 28	Smooth	Off White	Cylindrical	Irregular
Y 29	Smooth	White	Rounded	Irregular
Y 30	Smooth	Off White	Oval	Irregular
Y 31	Rough	White	Oval	Irregular
Y 32	Smooth	Off White	Oval	Irregular
Y 33	Smooth	White	Oval	Circular
Y 34	Smooth	Off white	Rounded	Circular
Y 35	Smooth	White	Oval	Circular
Y 36	Smooth	Off White	Oval	Irregular
Y 37	Smooth	Off White	Oval	circular
Y 38	Smooth	Off White	Oval/rounded	Circular
Y 39	Rough	White	Oval	Irregular
Y 40	Smooth	Off White	Oval	Circular
Y 41	Smooth	Off White	Oval	Irregular
Y 42	Rough	White	Oval	Irregular
Y 43	Smooth	Off White	Oval	Irregular
Y 44	Smooth	Off White	Oval	Irregular
Y 45	Smooth	Off White	Oval	Irregular
Y 46	Smooth	White	Oval	Circular
Y 47	Smooth	Off White	Oval/rounded	Irregular
Y 48	Smooth	Off White	Oval	Irregular
Y 49	Smooth	White	Oval	Circular
Bakers' yeast	Smooth	White	Oval/ Rounded	Circular

Table 2 Alcohol yields of the yeast isolates

Isolate	Source	Alcohol %
Y 1	1st mill juice	6.1IJ
Y 2	2nd mill juice	2.4W
Y 3	Bagasse	6.2IJ
Y 4	Sugar cane residues from Sevanagala	5.8L
Y 5	Rotten Cashew	6.6DE
Y 6	Rotten banana	6.4FGH
Y 7	Bagasse after 1stmilling	3.9R
Y 8	Bagasse after 2ndmilling	6.7CD
Y 9	Spent wash	6.1JK
Y 10	Spent wash (Pelwatte)	6.7CD
Y 11	Old molasses	6.9B
Y 12	Waste trickle	6.2IJ
Y 13	Jaggary juice	2.4VW
Y 14	Sugarcane base	3.1S
Y 15	Rotten tomato	2.6UV
Y 16	Rotten potato	2.6U
Y 17	Rotten orange	2.6U
Y 18	*Kithul* toddy (Kuruwita)	6.2IJ
Y 19	Rotten carrot	5.5M
Y 20	Filter mud	2.6U
Y 21	Factory waste water	6.8BC
Y 22	Spent wash	6.8BC
Y 23	Waste molasses	2.8T
Y 24	Waste molasses	6.2HIJ
Y 25	Waste molasses	2.1X
Y 26	Killinochchi coconut toddy	6.3 GHI
Y 27	Poonagarpalmyra toddy	5.8L

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