

Isolation of Indigenous Yeast Strains and Screening for Economically Important Properties

S. M. T. A.1. Maralanda, G. Chandrasena
Uva Wellassa University, Badulla., Sri Lanka

and

A. P. Keerthipala
Sugarcane Research Institute, Uda-Walawe

Introduction

The yeast is used to ferment carbohydrate substrate present in sugarcane molasses for the production of ethanol. Molasses-based alcohol production is one of the major sugarcane base industries in Sri Lanka. Distillery of each sugar factory consumes all the molasses produced as co-products of sugar production and for ethyl alcohol production. This directly influences the economic viability of the Sri Lankan sugar industry. However, overall efficiency of the existing processes is low, compared with similar industries elsewhere in the world resulting in high production costs and loss of potential revenue.

The use of inferior yeast cultures is one of the major reasons for low fermentation efficiencies in Sri Lankan alcohol distilleries that use sugarcane molasses. The 'ideal' ethanol producing yeast should possess fermentation and growth properties such as fast fermentation rates, high ethanol yields, high ethanol tolerance, low pH, and high temperature tolerance for fermentation. Use of efficient yeast strains improve ethanol yields in the fermented wash would reduce distillation costs and hence the profitability of the overall process. (Chen, M. and Chen, J.C.P., 1985).

Methodology

Samples of sugar-containing plant materials were collected in sterile sampling containers and kept at 4°C. These samples were used for the isolation of the yeast strains.

Yeasts were isolated using suitably diluted samples by streak plating in MYPG agar (yeast extract 0.3%, malt extract 0.3%, peptone 0.3%, glucose 1% and agar 1.5%) with pH adjusted to 4.8. All isolates were named as UWU M 100 series and isolation and streaking were repeated on MYPG agar medium at pH 4.8 until pure cultures were obtained.

The yeast colonies grown on MYPG agar for 24 h at 30°C were characterized morphologically in terms of size, shape, color, and margin.

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 pasteurized at 80°C for 15 minutes. Yeast isolates sub cultured on MYPG agar plates were used as seed cultures for molasses fermentations.

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 pasteurized at 80°C for 15 min. Seed culture was added in shake-flasks which contain fermentation medium. The flasks were incubated in a rotary water bath at 30°C with mild shaking (100 rpm). (Chandrasena *et al.*, 2006)

Samples were taken from the water bath incubator at 6 h intervals to determine the alcohol concentration. Ebulliometer was used to measure the alcohol concentration.

Results and Discussion

In an attempt to isolate naturally occurring yeasts superior in fermentation performance to baker's yeast, 26 yeast strains were isolated from various sugar-rich sources. The yeast isolates were identified based on their colony morphology. 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.

Yeast strains isolated from various sources were subjected to ethanol fermentation for test their alcohol producing capabilities in molasses medium. Table I indicates that most of the isolates produced high levels of alcohol from molasses fermentations. ANOVA (analysis of variance) results indicated that there was a significant variation in alcohol yields obtained from fermentation of molasses for 72 h ($p = 0.000$). The highest alcohol yield (6.98%) was produced by the yeast isolate UWU M 111. It was isolated from the old molasses collected from the Sugar cane research institution distillery premises.

All superior yeast isolates produced maximum levels of ethanol after 48 h of fermentation under laboratory conditions. The results indicate that there is a significant variation in alcohol yield produced by the six selected yeast strains compared to the baker's yeast. UWU M 111 showed the best performance in terms of ethanol production within shortest fermentation time, producing nearly 6.9% (v/v) ethanol in 48h.

Table 1: Alcohol production from Yeast

Isolates	Source	Alcohol % after 72hr
UWU M 100	1st mill juice	6.1
UWU M 101	2nd mill juice	2.4
UWU M 102	Bagasses	6.2
UWU M 103	Sugar cane residues from Sewanagala	5.8
UWU M 104	Rotten Cassue	6.6
UWU M 105	Rotten banana	6.4
UWU M 106	Bagasses after 1stmilling	3.9
UWU M 107	Bagasses after 2ndmilling	6.7
UWU M 108	Spent wash	6.1
UWU M 109	Spent wash (Pelwatta)	6.7
UWU M 110	Baker's Yeast	6.5
UWU M 111	Old molasses	6.9
UWU M 112	Waste trickle	6.2
UWU M 113	Jaggary juice	2.4
UWU M 114	Sugar cane base	3.1
UWU M 115	Rotten tomato	2.6
UWU M 116	Rotten potato	2.6
UWU M 117	Rotten orange	2.6
UWU M 118	Kithul toddy(Kuruwita)	6.2
UWU M 119	Rotten carrot	5.5
UWU M 120	Filter mud	2.6
UWU M 121	Factory waste water	6.8
UWU M 122	Spent wash	6.8
UWU M 123	Waste molasses	2.8
UWU M 124	Waste molasses	6.2
UWU M 125	Waste molasses	2.1

Conclusion

Yeast strains with superior molasses fermentation features were isolated from sugar containing materials. Out of the 26 isolates evaluated, six strains were found to be superior to baker's yeast in terms of sucrose fermentation. Six strains, namely UWU M 104, UWU M 107, UWU M 109, UWU M 111, UWU M 121, and UWU M 122 show the best performance in molasses medium. The present study revealed that the yeast strain UWU M 111 has the potential to produce high alcohol yields than other strains isolated from sugary materials.

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