Isolation and Characterisation of Sri Lankan Yeast Germplasm and Its Evaluation for Alcohol Production

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ABSTRACT

J. Inst. Brew. 112(4), 302–307, 2006

Use of inferior yeast cultures represents one of the reasons for low fermentation efficiencies in Sri Lankan alcohol distilleries that use sugarcane molasses. The present study isolated and characterised yeast strains found in natural environments in Sri Lanka and evaluated their performance under laboratory conditions in an effort to select superior strains for industrial fermentations. Yeasts were characterised based on morphological and physiological features such as sugar fermentation and nitrate assimilation. Ethanol production, alcohol tolerance and growth rate of the most promising strains were monitored following laboratory fermentations of molasses. Over a thousand yeast cultures were collected and screened for fermentative activity and a total of 83 yeast isolates were characterised as higher ethanol producers. Most of these belonged to the genus *Saccharomyces*. Certain strains produced over 10% (v/v) alcohol in molasses media during 72 h laboratory fermentations. Only two strains, SL-SRI-C-102 and 111, showed an appreciable fermentation efficiency of about 90%. The latter strain produced the highest level of ethanol, 11% (v/v) within a 48 h fermentation and exhibited improved alcohol tolerance when compared with the baker's yeast strains currently used in Sri Lankan alcohol distilleries. This study highlights the benefits of exploiting indigenous yeasts for industrial fermentation processes.

Key words: Alcohol fermentation, indigenous yeast, molasses, Sri Lanka.

INTRODUCTION

Alcohol fermentation represents one of the oldest industrial uses of molasses and has been reported in this context as far back as the 17th century in the West Indies and in Europe[2]. Sugar refining and molasses-based distilleries are major industries in Sri Lanka. Fermentation consumes all the molasses produced as co-products of sugar production and the resultant ethyl alcohol directly influences the economic viability of the Sri Lankan sugar industry[11]. However, overall efficiency of existing processes is low compared with similar industries elsewhere in the world resulting in high production costs and loss of potential revenue. Such efficiencies are generally below 80% and this is attributable to factors including use of contaminated water for dilution of molasses, use of inferior yeast (baker's yeast) for fermentation, poor control of temperature and pH, inadequate yeast nutrition and unskilled handling of the fermentation process[4]. Therefore, rectification of these shortcomings is essential to increase the fermentation efficiencies of Sri Lankan distilleries. Use of efficient yeast strains with higher ethanol tolerance to improve ethanol yields in the fermented wash would reduce distillation costs and hence the profitability of the overall process[4]. According to Chinjen and Gran[3], isolation and development of indigenous yeasts is one of the most appropriate options available to overcome problems with respect to yeast strains used to ferment sugarcane molasses under local conditions. Yeasts 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. Yeast species have highly specialised natural habitats and, therefore, it is possible to isolate specific strains from appropriate substrates. The community composition of yeast in various habitats is determined by many factors, such as nutrient composition and inhibitory compounds and the present study focused on naturally occurring yeasts that can be grown on sugary media for alcoholic fermentations.

We therefore attempted to isolate and characterise yeast strains from various natural sugary sources available in Sri Lanka and to evaluate molasses fermentation capabilities of those yeast strains.

EXPERIMENTAL METHODS

Isolation of yeasts

Samples of waste molasses, waste sugarcane juice, sugar factory and distillery effluents, top soils in milling areas of sugar factories, cane residues from harvested fields, bagasse, filter mud, and other sugar-containing plant materials from various locations in Sri Lanka were collected in sterile sampling containers and transported to the laboratory. After preliminary microscopic investigations of the samples, yeasts were isolated using suitably diluted samples by spread plating onto MYPG agar (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1% and

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Publication no. G-2006-1303-481

agar 1.5%). The isolated yeasts were subcultured on molasses agar medium consisting of (w/v) 16% molasses, 0.1% ammonium sulphate, 0.05% potassium dihydrogen orthophosphate, with pH adjusted to 4.8. All isolates were named as Sri Lanka-Sugarcane Research Institute-Chandrasena (SL-SRI-C) series and stored for further evaluation.

Purification of yeast isolates

A loopful of colonies from the agar plates were streaked on Wickerham medium (0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 1% glucose, 1.5% agar and 0.05% ferric ammonium citrate at 4.5 pH) and incubated at 30°C for 3 days. Isolation and streaking were repeated on malt extract and Rose Bengal streptomycin agar at pH 3.5 until pure cultures were obtained. Colony characters of the pure yeast isolates were examined and cultures were stored in MYPG agar medium under sterile mineral oil at 4°C.

Characterisation of yeast isolates

New yeast isolates may be characterized by their morphology, physiology and biochemistry and by using more advanced DNA-based methods. In the present study, the simplified identification methods developed by Beech et al.[1] and by Lodder[9] were used and these were based mainly on the morphological and physiological characteristics of the isolates.

Characterisation experiments were conducted both in the laboratory of the Sugarcane Research Institute (SRI), Sri Lanka and by the Yeast Research Group of the University of Abertay Dundee, UK. All purified yeast isolates were tested for their morphological and physiological characteristics such as fermentation and assimilation of different sugars (glucose, sucrose, maltose, galactose, raffinose, melibiose and lactose) and assimilation of nitrate compounds[1,9].

Study of morphological characters

Morphological characters were studied following the methods adopted by Beech et al.[1] and Lodder[9]. Pellicle formation and pseudomycelium formation were studied as described below.

Pellicle formation

The morphology broth medium (Difco Morphology agar containing 0.05% ferric ammonium citrate and 2 µg biotin/L) was prepared and 15 mL aliquots were poured into 25 mL McCartney bottles which were then inoculated with yeast and incubated for 5 days at 30°C to observe pellicles.

Pseudomycelium formation

Agar plates were prepared using 1.7% Difco Corn Meal agar and allowed to dry for 2 days at room temperature. Loopfuls of actively growing yeast cultures were then streaked on agar plates, covered with sterile cover slips and incubated for 4 days to observe for pseudomycelium formation.

Fermentation studies

A base medium with 0.45% Difco Yeast Extract and 0.75% Difco Bacto Peptone was prepared and bromothymol blue indicator added to give the medium an intense blue colour after adjusting the medium to pH 6.4. A 4 mL aliquot of this medium was then transferred to 150 × 15 mm test tubes, each with an inverted 50 × 10 mm Durham tube. The medium was sterilised at 121°C for 15 min. Filter sterilised solutions of glucose, galactose, sucrose, maltose (all 6%), lactose and melibiose (8%) and raffinose (12%) were then added prior to inoculation with actively growing yeast cultures. The Durham tubes were observed for gas formation every day for 7 days.

Assimilation of nitrates

A culture medium of 0.5 mL of Difco Carbon Base (11.7%) plus 0.78% potassium nitrate was added to test tubes containing 4.5 mL sterile deionised water and inoculated with 0.2 mL of washed yeast cells. Cultures were incubated for 7 days and observed for growth. A second set of tubes with similar culture media were then inoculated with 0.1 mL inocula taken from the first set of tubes and observed for growth after 7 days of incubation.

Assimilation of sugars

Glucose, galactose, lactose, maltose and sucrose were used for this test. Filter sterilised solutions, 5% (w/v) of each sugar, were added to 5 mL of 6.7% filter sterilised solution of Difco Yeast Nitrogen Base and inoculated with yeast cultures to be tested prior to 4 days of incubation at 30°C. Sugar assimilation was estimated by observing turbidity at weekly intervals for a period of one month[1].

Fermentation of sugarcane molasses by yeast isolates under laboratory conditions. Based on the results of the preliminary evaluation of yeast isolates, the promising isolates producing alcohol yields above 10% (v/v) were selected for further evaluation of their molasses fermentation capabilities.

Samples of sugarcane molasses obtained from the Hingurana sugar factory located in the Eastern province of Sri Lanka were transported to the Sugarcane Research Institute, for laboratory and pilot plant studies. This molasses contained 55% fermentable sugar, 3% unfermentable sugar, 9.3% total ash, 0.03% phosphate and 0.26% total nitrogen. Total solid content of the molasses was 78%. The original pH of the molasses was 5.4.

The seed culture medium was prepared by diluting molasses to obtain 8% 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. Yeast isolates subcultured on MYPG agar plates were used as seed cultures for molasses fermentations. The initial inoculum was multiplied up to 800 mL by serial transfers after 6 h of growth. Cultures were incubated at 30°C in a rotary water bath.

Fermentation medium was prepared by diluting molasses to 20% 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 min. Yeast inoculum, 20% (v/v) was added in shake-flask fermenters containing airlocks[8]. The flasks were incubated in a rotary water bath at 30°C with mild shaking (100 rpm)[5].

Determination of ethanol concentration. Samples were taken aseptically from the fermentors at 12 h intervals to determine the alcohol concentration. The samples

were micro centrifuged and supernatants analysed using gas chromatography (Hewlett Packard 5710A) with isopropanol, 5% (v/v), as an internal standard.

Studies on ethanol tolerance of superior yeast isolates. Studies were conducted to examine the effect of added ethanol on cell growth and glucose consumption by the superior yeast isolates compared with baker's yeast under laboratory conditions. The yeast isolates were grown in media with 1.25% (w/v) glucose and varying quantities of ethanol (97.7% neutral spirit from BDH) added aseptically at concentrations of 0% (control), 4%, 6%, 8%, 10% and 12% (w/v). Yeast inocula were prepared as described previously and inoculated at an initial cell density of 2×10^6 cells/mL prior to incubation at 30°C for 48 h in a shaking water bath at 50 rpm[8]. Cells were then filtered through 0.45 µm Gelman membrane filters. Residual glucose concentrations in supernatants were determined by the Dubois phenol sulphuric acid method[7]. In addition, yeast growth was determined by measuring cell numbers with a haemocytometer after 0, 4, 8, 16, 24, 32, 40 and 48 h incubation.

RESULTS AND DISCUSSION

Selection and characterisation of indigenous Sri Lankan yeast strains

In an attempt to isolate naturally-occurring yeasts superior in fermentation performance to baker's yeast, over 1000 isolates were collected from various sugar-rich

Table I. Sri Lankan yeast isolates: their characteristics and fermentation performance in molasses.

Isolate SL-SRI-C	SF[1]	SA[2]	NA[3]	MOR[4]	Species	Source	% Alcohol	FE[5]
100	GMA	4	0	1	*Saccharomyces cerevisiae*	Waste molasses	5.9	48.3
101	GMA	4	0	1	*S. cerevisiae*	Millyard soil	5.2	42.6
102	GMA	4	0	1	*S. cerevisiae*	Waste molasses	10.9	89.3
103	GMA	4	0	FF	*Schizosaccharomyces pombe*	Sugarcane base	6.8	55.7
104	GMA	1SM	0	FF	*Schizo. pombe*	Sugarcane base	6.5	53.2
105	MA	1SM	N	2	*Hansenula anomala*	Cane tops	5.8	48.3
106	S	5	0	1	*Debaryomyces hansenii*	Sugar	6.1	50.0
107	MA	1SM	0	2B	*S. bayanus*	Waste bagasse	5.3	41.8
108	MA	1SM	N	2	*H. anomala*	Filter mud	4.8	39.3
109	MA	4	0	FF	*Schizo. pombe*	Filter mud	5.8	48.3
110	GB	4	0	1	*S. microellipsoides*	Mixed sugar cane juice	6.7	54.9
111	GMA	4	0	1	*S. cerevisiae*	Water molasses	11.1	90.9
112	M	2M	0	1	*S. rouxii*	Factory waste water	6.4	64.7
114	GMA	4	0	2	*S. cerevisiae*	Sugarcane base	8.7	71.3
115	GMA	1SM	0	FF	*Schizo. pombe*	Distillery waste (Kantale)	8.7	71.3
116	GA	4	0	2	*S. chevalieri*	Mango	7.4	60.6
117	GMA	4	0	2	*S. ellipsoides*	Sugarcane base (Hingurana)	6.2	59.0
118	M	2	0	1	*S. rouxii*	Soil (distillery site)	8.0	80.5
119	GMA	4	0	1	*S. cerevisiae*	Filter mud (Higurana)	7.2	59.0
120	GMA	4	0	1	*S. cerevisiae*	Factory waste water	9.5	80.3
121	GMA	4	0	1	*S. cerevisiae*	1st mill juice	9.7	80.3
122	GMA	4	0	2	*S. cerevisiae*	2nd mill juice	7.4	60.6
123	GMA	4	0	2	*S. cerevisiae*	Sugarcane base	7.0	57.3
124	GMB	4	0	2	*S. uvarum*	Decaying papaw	8.9	72.3
125	GAL	3L	0	2B	*Kluyveromyces marxianus*	Decaying pineapple	5.8	48.3
126	A	1S	0	BB	*Saccharomycodes ludwigii*	Decaying avocado	3.0	24.5
127	A	1S	0	BB	*Saccharomycodes ludwigii*	Coconut toddy	7.0	57.3
128	GMA	4	0	2	*S. cerevisiae*	Kitul toddy	7.4	61.4
129	GMA	4	0	2	*S. cerevisiae*	Palmyrah sap	6.0	49.1
130	MA	1SM	0	2B	*S. bayanus*	Sugarcane syrup	6.5	53.2
131	MA	1M	0	FF	*Schizo. octosporus*	Kelanitissa Flowers	3.2	26.2
132	M	2M	0	1	*S. rouxii*	Jaggery (Passara)	6.9	55.7
133	S	1S	0	BB	*Kloeckera lafarii*	Cane juice (Passara)	6.8	56.5
134	MA	1SM	0	1	*S. fermentatii*	Coconut toddy (Passara)	6.9	56.5
135	MA	4	0	1	*S. fructuum*	Sugarcane syrup (Passara)	7.5	61.4
136	MA	4	0	2	*S. cerevisiae*	Sugar (Nuwara Eliya)	7.7	63.1
137	MA	4	0	2B	*S. cerevisiae*	Coconut toddy (Kegalle)	7.9	64.7
138	GMA	4	0	FF	*Schizo. pombe*	Sugar (Kurunegala)	6.4	60.5
139	GMA	4	0	2	*Trichosporon behrendii*	Coconut toddy (Kurunegala)	7.5	61.4
140	GM	4	0	2B	*Candida albicans*	Sugar (Kantale)	6.5	53.2
141	GMA	4	0	FF	*Schizo. pombe*	Coconut toddy (Matara)	6.6	54.9
142	GA	4	0	FF	*Pichia ohmeri*	Sugar (Matara)	6.6	54.0
143	GM	4	0	1	*S. italicus*	Sugar (Colombo)	5.6	45.9
144	GA	4	0	2B	*S. fructuum*	Jack fruit (Kegalle)	5.7	47.5
145	MA	1SM	0	1	*S. ovifirmis*	Grapes	6.5	53.2
146	GA	4	0	2B	*S. fructuum*	Apple	6.3	51.6
147	D	1	0	2B	*S. acidifaciens*	Tomato	4.2	35.2
148	GMS	4	0	2B	*C. tropicalis*	Jack fruit	3.5	20.4
149	DUI	1	0	2B	*S. bayanus*	Orange	4.4	36.8
150	GMA	4	0	1	*S. bayanus*	Mango	8.0	65.5

(*continued on next page*)

sources in Sri Lanka. A total of 83 yeast strains with alcoholic fermentation capabilities at least over 2% (v/v) ethanol were subsequently selected for further study. Information on the yeasts' morphology and physiology is presented in Table I.

The majority of isolates belonged to morphological group 1 which included yeasts with multilateral budding, without pseudomycelia and pellicle formation, and group 2 with multilateral budding, with pseudomycelia and pellicle formation[1]. Most of the strains with higher ethanol producing capability belonged to the genus *Saccharomyces.* Other genera were *Hansenula, Debaryomyces, Schizosaccharomyces, Trichosporon, Pichia, Candida* and *Torula.* None of the non-*Saccharomyces* spp. were very good alcohol producers. The results of the sugar fermentation experiments showed that all isolates studied fermented at least one type of sugar used, but none of the isolates fermented all the sugars. However, a majority of the isolates which fermented glucose, galactose, maltose, sucrose and raffinose belonged to the genus *Saccharomyces.* Isolates Sl-SRI-C169 and 175 only fermented glucose.

The sugar assimilation pattern of the isolates was also highly variable. Over 50% of the isolates assimilated glucose, galactose, sucrose and maltose. Very few isolates (e.g. SL-SRI-C-106 and 166) assimilated all the sugars tested except raffinose. There was only one isolate with lactose assimilation capability. None of the isolates belonging to the genus *Saccharomyces* utilised potassium nitrate as a sole nitrogen source. Isolates SL-SRI-C-105, 108, 153 and 172 which belonged to the genera *Hansenula, Candida* and *Torula,* respectively, assimilated nitrate compounds.

Table I. (*continued*)

Isolate SL-SRI-C	SF[1]	SA[2]	NA[3]	MOR[4]	Species	Source	% Alcohol	FE[5]
151	MA	1SM	0	2B	*S. fructuum*	Mango	7.9	64.7
152	GA	4	0	2B	*S. cerevisiae*	Mango	2.8	22.9
153	A	1SM	N	1	*C. utilis*	Mango	9.1	74.5
154	GMA	1SM	0	FF	*Schizo. pombe*	Cocoa	2.1	17.2
155	GMA	4	0	1	*S. cerevisiae*	Toddy (Warakapola)	7.5	61.4
156	GA	4	0	2B	*S. fructuum*	Bee honey	6.4	53.2
157	GA	3	0	2	*S. chevalieri*	Banana	5.5	45.0
158	GMA	4	0	2	*S. cerevisiae*	Coconut toddy (Negambo)	6.9	56.5
159	GA	4	0	2	*Pichia omari*	Dates	3.1	25.4
160	GB	4	0	2	*C. melibiosica*	Brown sugar	4.4	37.7
161	S	1S	0	BB	*K. lafarii*	Cane	7.6	62.2
162	GB	3	0	1	*S. microellipsoides*	Banana flowers	4.1	33.6
163	MA	4	0	2B	*S. uvarum*	Coconut toddy (Kegalle)	7.4	60.6
164	GA	3	0	2	*S. chevalieri*	Coconut toddy (Kandy)	7.0	58.1
165	GMA	4	0	2	*C. robustus*	Coconut toddy (Weeraketiya)	7.1	59.8
166	A	5	0	2	*P. polymorpha*	Coconut toddy (Kurunegala)	7.5	64.1
167	MA	4	0	1	*P. robertsiae*	Sugar (Kegalle)	5.0	45.9
168*								
169	D	1	0	2	*Pichia/Candida*	Wood apple	4.5	36.8
170	A	1S	0	1	*Debaryomyces/Saccharomyces/ Torulopsis*	Beet root (decaying)	5.8	48.3
171	GMS	4	0	2A	*Torula sake*	Toddy (Colombo)	7.0	57.3
172	GMS	4	N	1	*T. versatilis*	Kitul toddy (Kegalle)	7.5	61.4
173	GMA	1SM	0	FF	*Schizo. pombe*	Mango (Kegalle)	8.1	67.2
174	MA	1SM	N	2	*H. anomala*	Shoe flower	5.5	45.9
175	D	1M	0	BB	*K. magna*	Aricanut sap	2.2	18.0
176	GB	3	0	1	*S. microellipsoides*	Sugar (Anuradhapura)	6.9	56.5
177	GA	3	0	1	*S. exiguus*	Guava	5.7	46.7
178	GMA	4	0	2	*C. robustus*	Kitul toddy (Peradeniya)	7.8	63.9
179	MA	1SM	0	2/2B	*S. oviformis*	Coconut toddy (Kantale)	7.3	59.8
180	MB	1SM	0	FF	*Schizo. versalitis*	Coconut toddy (Hingurana)	7.8	63.9
181	GA	4	0	2	*P. ohmeri*	Kitul toddy (Nuwara Eliya)	7.4	60.6
182	GMA	4	0	2	*S. cerevisiae*	Kitul toddy (Badulla)	7.6	62.2
183	GMA	4	0	2	*S. cerevisiae*	Baker's yeast (Saflaora)	7.0	56.5

[1] SF (Sugar fermentation pattern): O – Glucose not fermented. D – Glucose only fermented. G – Glucose and galactose fermented. M – Glucose and maltose fermented. S – Glucose and sucrose fermented. MS – Glucose, maltose and sucrose fermented. MA – Glucose, maltose and sucrose and raffinose fermented. MB – Glucose, maltose, sucrose raffinose and melibiose fermented. B – Glucose, sucrose, raffinose and melibiose fermented. GM – Glucose, galactose and maltose fermented. GS – Glucose, galactose and sucrose fermented. GA – Glucose, galactose, sucrose and raffinose fermented. GAL – Glucose, galactose, sucrose, and lactose fermented. GB – Glucose, galactose, sucrose, raffinose and melibiose fermented. GMS – Glucose, galactose, maltose and sucrose fermented. GMA – Glucose, galactose, maltose, sucrose, and raffinose fermented. GMB – Glucose, galactose, maltose, raffinose and melibiose fermented.

[2] SA (Sugar assimilation pattern): 1 – Glucose assimilated only. 1M – Glucose and maltose assimilated. 2M – Glucose, galactose and maltose assimilated. 1S – Glucose and sucrose assimilated. 1SM – Glucose, sucrose and maltose assimilated. 3L – Glucose, galactose, sucrose and lactose assimilated. 2 – Glucose and galactose assimilated. 4 – Glucose, galactose, sucrose, and maltose assimilated. 5 – All sugars assimilated except raffinose.

[3] NA (Nitrate assimilation): N – Assimilate potassium nitrate as sole source of nitrogen. 0 – Cannot utilize potassium nitrate.

[4] MOR (Morphology): FF – Fission yeast. BB – Bipolar budding. 1 – Multilateral budding, no psudomycelium, no pellicle. 2 – Multilateral budding, with pseudomycelium, with pellicle. 2A – Multilateral budding, no pseudomycelium, with pellicle. 2B – Multilateral budding, with pseudomycelium, no pellicle.

[5] FE (Fermentation efficiency), % = actual alcohol yield/theoretical (Gay-Lussac) alcohol yield × 100.

*Unidentified.

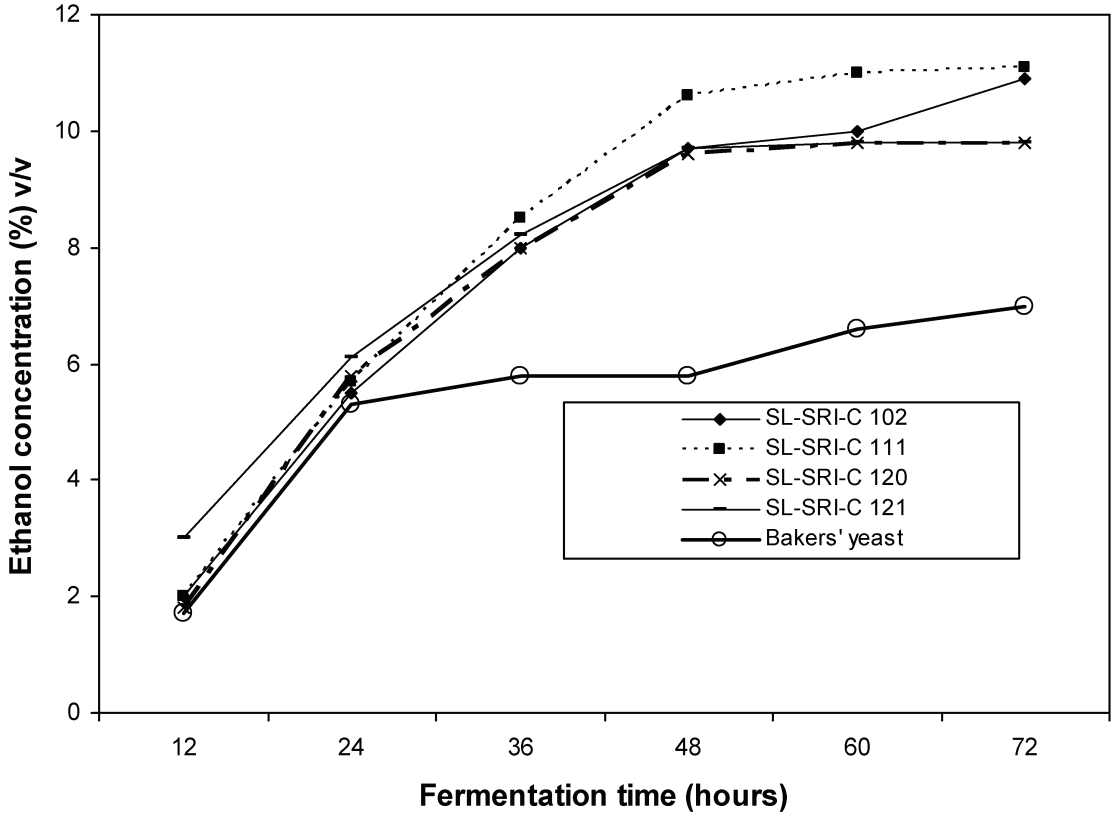

Fig. 1. Kinetics of alcohol production from molasses by selected Sri Lankan yeast isolates at 30°C.

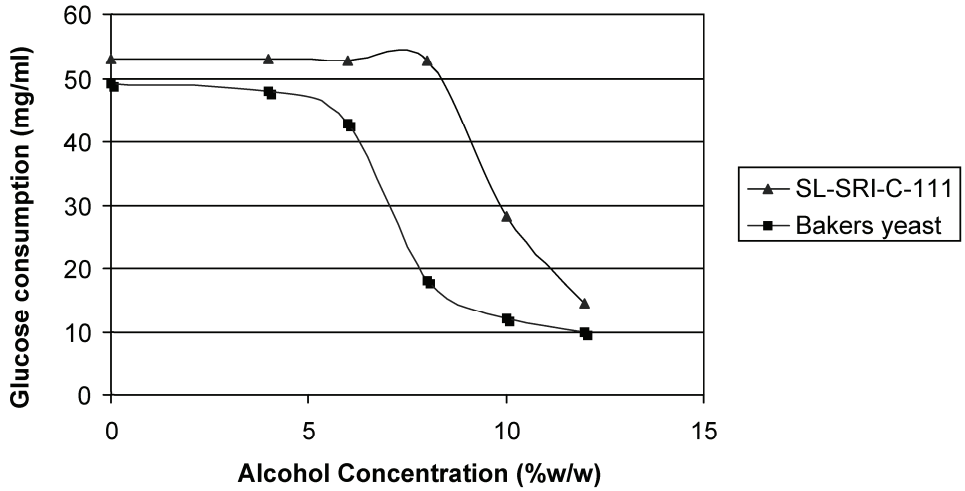

Fig. 2. Effect of added ethanol on glucose utilization by yeast isolates.

Yeasts which performed well in the fermentation of sugars were further evaluated to test their alcohol producing capabilities in molasses medium (sucrose fermentation) under laboratory conditions. 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 ($p = 0.001$). The highest alcohol yield of over 10% (v/v) was produced by the yeast isolates SL-SRI-C-102, 111, 120 and 121. However, only the strains SL-SRI-C-102 and 111 showed appreciable fermentation efficiencies of around 90%. *Saccharomyces* strains SL-SRI-C-102, 111 and 121 were isolated from waste molasses collected from distillery premises. Indigenous *Saccharomyces* yeasts with very high ethanol producing capabilities in the natural environment are thought to be very rare[10]. It is possible that the *S. cerevisiae* yeasts isolated from Sri Lankan distilleries may be contaminants of the baker's yeast used in the distillery for the fermentation process.

Table II. Effect of ethanol on growth of a selected Sri Lankan yeast compared with baker's yeast.

Yeast isolate	Ethanol % (v/v)	Cell no. (10^6/mL) Hours after inoculation 0	4	8	16	24	32	40
SL-SRI-C-111	0	1	5	15	35	48	55	59
	4	1	5	14	33	47	56	59
	6	1	4	10	14	32	40	50
	8	1	4	9	12	30	37	45
	10	1	2	3	10	22	24	24
	12	1	2	2	4	5	9	9
Baker's yeast	0	1	4	9	16	32	46	50
	4	1	4	8	15	30	39	44
	6	1	3	7	10	24	29	30
	8	1	3	7	10	14	15	15
	10	1	2	2	2	4	5	5
	12	1	1	1	2	4	5	5

Performances of yeast isolates under laboratory conditions

The results of further evaluation of these selected superior yeast isolates are presented in Fig. 1. All yeast isolates produced maximum levels of ethanol after 48 h of fermentation under laboratory conditions with significant variation in alcohol yield produced by the four selected yeast strains compared to the baker's yeast ($p = 0.001$). SL-SRI-C-111 showed the best performance in terms of maximum ethanol production within shortest fermentation time, producing nearly 11% (v/v) ethanol in 48 h.

The possibility that strain SL-SRI-C 111 performed much better in test fermentations compared with baker's yeast due to its greater alcohol tolerance was evaluated. For both yeasts, glucose consumption decreased with increasing exogenous ethanol concentrations. However, yeast strain Sl-SRI-C 111 utilised higher levels of glucose than baker's yeast at the same levels of ethanol, indicating that this natural yeast isolate exhibits higher ethanol tolerance than baker's yeast (Fig. 2). Similarly, the new yeast strain SL-SRI-C 111 had a higher rate of multiplication in the presence of ethanol than the baker's yeast (Table II).

CONCLUSION

Yeast strains with superior molasses fermentation features were isolated from natural sugary habitats in Sri Lanka. Out of the 83 isolates evaluated, 11 strains were found to be superior to baker's yeast in terms of sucrose fermentation. Four strains, namely SL-SRI-C 102, 111, 120 and 121 showed the highest fermentation efficiency in molasses medium under laboratory conditions. Of these four strains, SL-SRI-C 111 was found to be the best strain for molasses fermentation. Further evaluations of this strain under scaled-up conditions are planned to evaluate its suitability for commercial use in alcohol distilleries.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the distillery staff of the Hingurana Sugar Industries Ltd. for supplying molasses and providing labotratory facilities, colleagues who provided samples to isolate yeast, laboratory staff of the Yeast Research Group at University of Abertay Dundee, UK for their assistance in fermentation experiments, staff of the microbiology laboratory of Sugarcane Research Institute, Sri Lanka, for their assistance and the Sugarcane Research Institute, Sri Lanka for financing this study.

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(Manuscript accepted for publication December 2006)