Tropical Agricultural Research Vol. 16: 114-120 (2004)

Fermentation of Rice using Yeast Isolated from Coconut and Palmyrah Sap.

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ABSTRACT. *Suitability of twelve yeast isolates from coconut sap and one yeast isolate from palmyrah sap for rice fermentation was studied. All the isolates were identified as Saccharomyces species and categorized into three groups based on the shape and size characteristics of single cells. The ethanol content was calculated during fermentation on 15% glucose medium. The three groups showing, round, oval and ellipsoidal shaped single cells exhibited comparatively fast, medium and slow rates of ethanol production, respectively, on glucose medium. The maximum possible ethanol content produced by the isolates on glucose medium at room temperature (28±2^{0}C) varied from 7.3 to 8.4%. Five yeast isolates selected based on the rate or amount of ethanol production on glucose medium were further assessed for their ability to ferment rice. The maximum possible average ethanol content produced during rice fermentation by the five isolates at room temperature (28±2^{0}C) varied from 10.5 to 13.5%.*

INTRODUCTION

Fermented beverages such as Sake in Japan, Shaoshinchu in China, Tapai in Malaysia and Yakju in Korea are produced using rice (Steinkraus, 1983). In this process, rice starch is broken down into fermentable sugars by fungal enzymes such as alpha amylase and glucoamylase to facilitate fermentation by yeasts, mainly *Saccharomyces* species. The type of *Saccharomyces* strain is one of many factors that contribute to quality and acceptability of the beverage as well as the efficiency of the fermentation process. Palm wine *Saccharomyces* species have been used for bio-ethanol production at industrial level using different sources of fermentable sugars (Agu *et al.*, 1993; Ezeogu and Okolo, 1994). Moreover, these *Saccharomyces* species have shown to possess high level of ethanol and sugar–tolerance (Ezeogu and Emeruwa, 1993), and better sedimentation properties for better product recovery (Ezeogu and Okolo, 1994). In Sri Lanka, there is a good potential for rice fermentation by using locally available palm wine *Saccharomyces* species. As these organisms are well adapted to tropical environmental conditions, a possibility exists to carry out rice fermentation under ambient conditions without using low temperatures as in the Sake production.

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Saccharomyces species which posses high ethanol production capacity has been isolated from coconut palm sap and used successfully, to improve coconut palm wine industry (Atputharajah *et al.*, 1986). However, their use on rice fermentation is not reported in Sri Lanka. This study was undertaken to isolate and study the suitability of palm wine *Saccharomyces* for rice fermentation.

MATERIALS AND METHODS

Yeast isolates

Naturally fermenting coconut and palmyrah saps collected in December 2003 from Panadura in the Kaluthara District and Koppai in the Jaffna District, respectively, were used for isolation of yeasts. The yeasts were isolated and purified on Potato Dextrose Agar (PDA-Oxoid) by streak plate technique, and the purified cultures were maintained on PDA slants at 6^0C.

Starter culture preparation

Starter cultures were prepared in 100 ml of sterile culture medium in 250 ml Erlenmeyer flasks, containing glucose 5 g; peptone (Diplo) 1 g and yeast extract (Diplo) 1 g, buffered at pH 4.5 by 0.1 M KH_2PO_4. A loopfull of cells from the PDA slants maintained at 6^0C was inoculated in the above medium and incubated at room temperature (28 ± 2^0 C) on an orbital shaker (Model SO 1) at 100 rpm to obtain about 10^7 cells ml^{-1}. Cell counts were taken by using a haemocytometer.

Shape and size characteristics

The standard methods described by Lodder (1970) were used to study the shape and size characteristics of single cells of the isolates.

Glucose fermentation

The fermentation medium, pH 4.5 containing glucose 15 g, peptone (Diplo) 1 g and yeast extract (Diplo) 1 g in 0.1 M urea solution (Chaudhari and Chincholkar, 1999), was sterilized at 121^0C for 10 minutes and cooled to room temperature (Atputharajah *et al.*, 1986). Batch fermentation was carried out in triplicate by transferring 1 ml of yeast starter culture containing about 10^7 cells ml^{-1} into 100 ml of the fermentation medium in 250 ml Erlenmeyer flasks. The flasks were incubated at room temperature (28 ± 2^0C) and weighed once daily, after shaking moderately to prevent sedimentation of yeast, until a constant weight was reached. The ethanol content was calculated from the drop in weight due to loss of carbon dioxide by:

$$P = W (M_E/M_C),$$

Where, W is the loss of carbon dioxide (g/100 ml) and M_E and M_C are molecular weights of ethanol and carbon dioxide, respectively (Tabera *et al.*, 1985). The ethanol content was determined after a constant weight was reached by using an ebulliometer (Samarajeewa and Tissera, 1975).

Rice fermentation

Five yeast isolates selected based on the rate or amount of ethanol production were further assessed for their ability to ferment rice. Sake type fermentation was carried out in triplicate. Steamed rice (BG 358), koji or a culture of *Aspergillus oryzae* on rice grains (Japan International Cooperation, 2002), spring water obtained from the Kandy District and the yeast starter were added in five successive batches (Yoshizawa, 1982) every other day as given in Table 1 followed by fermentation at room temperature (28 ± 2^0 C) until a constant weight was reached. At the end of fermentation the fermented liquid was observed under microscope for viability of the yeasts. Ethanol content of the fermented liquid was determined by using an ebulliometer, residual sugar was estimated by the Fehling's test and pH was measured using a pH meter (IM-40S TOA Electronics). The data were subjected to analysis of variance and the means were separated by the LSD mean separation procedure to select the best yeast isolates.

Table 1. Successive addition of ingredients for sake type rice fermentation.

	Starter	1	2	3	4	Total
Total rice (g)	7.00	14.0	26.50	44.5	8.0	100.0
Koji rice (g)	2.25	4.0	6.25	7.5	-	20.0
Residual rice (g)	4.75	10.0	20.25	37.0	8.0	80.0
Spring water (ml)	7.75	12.5	31.75	67.5	8.0	127.5
Yeast starter (ml)	5.00	-	-	-	-	5.0

RESULTS AND DISCUSSION

Single colonies from naturally fermenting coconut and palmyrah sap isolated by using the streak plate technique were further purified by sub-culturing on PDA, and twelve yeast isolates (C1 to C12) from the former and one (P1) from the latter were obtained. All the isolates were identified as *Saccharomyces* species based on the morphological characteristics. These isolates were categorized into three groups based on the size and shape of single cells (Table 2).

Table 2. Shape and size characteristics of palm wine yeast isolates.

Yeast isolate	Shape	Size	
		Length (μm)	Width (μm)
Group 1	Round, oval, lightly pointed ends	8.1-8.3	7.8-8.2
Group 2	Short oval	8.5-10.0	7.5-8.0
Group 3	Ellipsoidal	14.9-15.1	7.5-8.0

Group 1 = C1, C2, C5, C6, C7, C9, C12, P1; Group 2 = C10, C11; Group 3 = C3, C4.

The amount of ethanol produced during fermentation by each yeast isolate on 15% glucose culture medium was calculated by using the equation reported by Tabera

et al. (1985) based on the drop of weight of the culture medium. The rate of alcohol production of the three groups shown similar size and shape characteristics (Table 2) is presented in Fig. 1. A clear relationship between morphological characteristics and the initial rates of fermentation was evident, where the initial rate was the highest in the first group followed by second and third groups (Fig. 1). However, the percentage of ethanol produced by each isolate on 15% glucose medium after reaching the constant weight, varied from 7.3 to 8.4 and the final yield of ethanol was not significantly different among the three morphologically different yeast groups (Table 3). These results were in agreement with the findings of Tabera *et al.* (1985), in which one yeast strain that showed a higher rate of fermentation than another at one stage of fermentation was not always found to be the highest ethanol producer.

Five yeast isolates from all three morphological groups were selected to study their ability to ferment rice, as the initial rate of fermentation among these groups was different. P1, having been an isolate from palmyrah sap, C8, having being the highest producer of the group and C1, having shown the initial rate of ethanol production similar to the other isolates in the same group, were selected from group 1. C10 from group 2, as the two isolates of this group showed similar behavior was also selected.

Table 3. Percentages of ethanol production by yeast isolates on 15% glucose culture medium.

Group 1		Group 2		Group 3	
C1	8.0 ± 0.1	C10	8.3 ± 0.2	C3	7.8 ± 0.3
C2	7.7 ± 0.4	C11	8.1 ± 0.2	C4	7.3 ± 0.0
C5	7.8 ± 0.4				
C6	8.2 ± 0.1				
C7	8.2 ± 0.1				
C8	8.3 ± 0.1				
C9	7.8 ± 0.5				
C12	8.1 ± 0.3				
P1	8.4 ± 0.3				

Each value represents mean ± SD of triplicate.

Table 4. Reducing sugar, ethanol contents and pH values after 7-day batch fermentation of rice at 28±2 °C.

Isolate		Ethanol (%)	Reducing sugar (mg/ 100 mL)	pH
Group 1	C1	10.5 ± 0.6[a]	n.d.	4.4 ± 0.0[c]
	C8	12.2 ± 0.7[a]	n.d.	4.5 ± 0.0[c]
	P1	13.5 ± 0.6[a]	n.d.	4.3 ± 0.0[c]
Group 2	C11	3.6 ± 0.3[b]	0.2±0.0	3.5 ± 0.0[d]
Group 3	C3	13.5 ± 0.6[a]	n.d.	4.4 ± 0.0[c]

Each value represents mean ± SD of triplicate. Means in each column followed by the same letter are not significantly different ($p<0.05$). n.d. = not detectable.

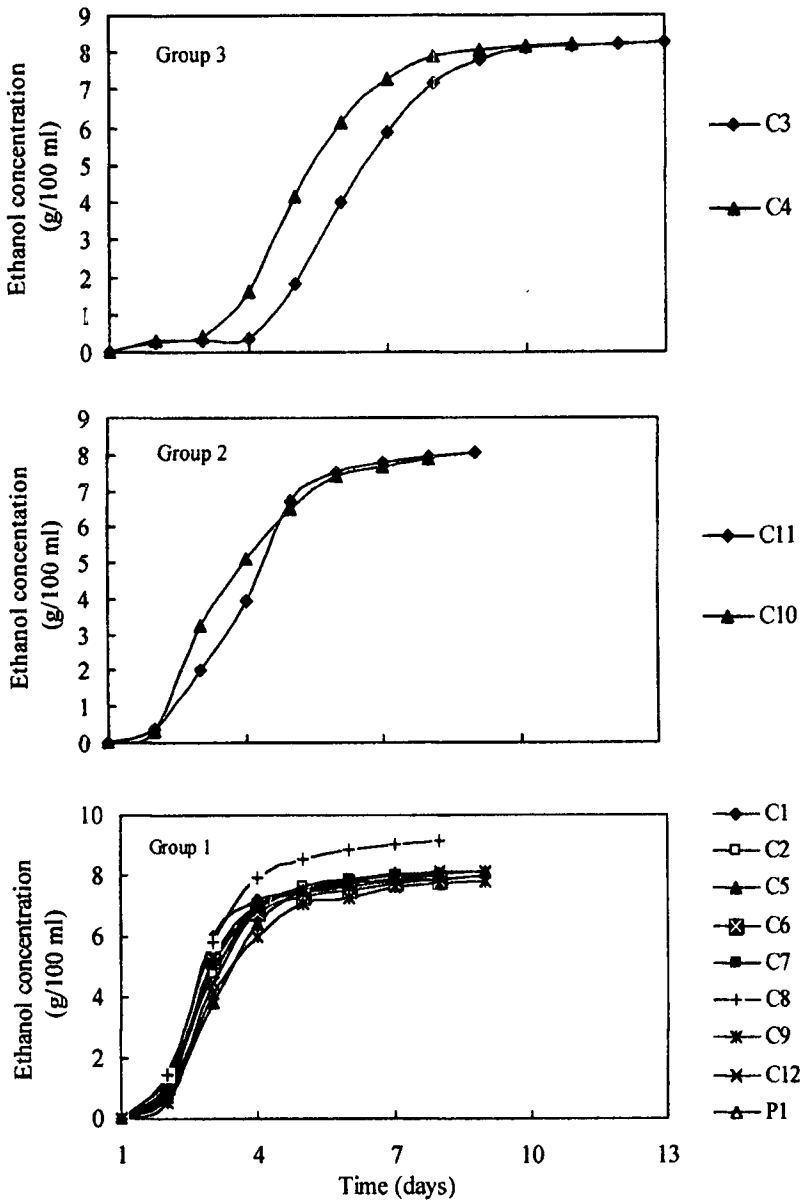

Fig. 1. Rate of ethanol production of yeasts isolated from coconut (C1-C12) and palmyrah sap (P1) on 15% glucose culture medium.

As a slow acting yeast strain might be beneficial for rice fermentation, which is a batch process where different proportion of ingredients are added on a time span, as in Japanese Sake production (Yoshizawa, 1982), C3 from group 3, which showed the slowest rate of ethanol production among the other isolates of the group was also used for further studies.

The average ethanol production during rice fermentation ranged from 10.5 to 13.5% (Table 4). Similar ethanol content of 12.2% was reported for Sake-type fermentation by Nigerian palm wine *Saccharomyces* (Ezeogu and Emeruwa, 1993). However, these ethanol contents were lower than those produced by industrial Sake yeast strains in Japan, where the ethanol content was reported to be 17-19% (Yoshizawa and Kishi, 1994). Difference in the ethanol levels may probably be due to variability in fermentative capacities of yeast strains. Moreover, different alcohol tolerance levels of different strains, as revealed by the absence of viable yeasts in the fermented liquid, may set the maximum possible ethanol content in a fermentation medium.

There is no significant difference in total ethanol production in C1, C8 and P1 yeast isolates of group 1 and C3 of group 3 (Table 4). Non-detectable amounts of reducing sugars present in the fermented liquid indicated that these yeasts had efficiently utilized all the fermentable sugars. However, the C11 isolate of group 2 appears to be unsuitable for rice fermentation as reflected by the low percentage (3.6± 0.6) of ethanol. It would be worthwhile to further study the suitability of all these isolates in terms of their capacity to impart sensory attributes such as flavour, aroma and body as yeast strains also play an important role in improving the sensory quality of fermented beverages (Ayanru, 1989).

CONCLUSIONS

Yeast strains showing, round, oval and ellipsoidal shaped single cells exhibited comparatively fast, medium and slow rates of ethanol production, respectively, on glucose medium. The maximum possible ethanol content produced by the isolates on glucose medium at room temperature (28±2 °C) varied from 7.3 to 8.4%. Five yeast strains isolated from coconut and palmyrah sap produced 10.5 to 13.5% of ethanol during rice fermentation at room temperature (28±2 °C).

ACKNOWLEDGEMENTS

The study was supported by a grant from the Council for Agricultural Research Policy in Sri Lanka (Grant Number: 12/557/423). The authors wish to thank Mr. L. B. Wewegama, Technical Officer, Department of Food Science & Technology, Faculty of Agriculture, University of Peradeniya for his technical assistance.

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