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SELECTION OF THERMOTOLERANT YEAST FOR ALCOHOL FERMENTATION OF RAW CASSAVA STARCH

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Abstract

Alcoholic fermentative activity of isolated thermotolerant yeasts was examined at 40°C using raw cassava starch as substrate and wheat bran koji as saccharifying agent. Thirty of the isolated yeasts were found to produce alcohol, but only one isolate (no. 4) was found to be the most effective in terms of the highest amount of CO_2 evolved and highest concentration of alcohol produced. The alcohol concentration reached 8.45 % (v/v) after 5 days, giving alcohol yield of 81.0 % and residual direct reducing sugar 1.9 % This yeast was then selected for alcoholic fermentation of raw cassava starch at 40°C, using rice bran koji as saccharifying agent. It was found that under optimal condition, an alcohol concentration of 7.95 % (v/v) could be obtained after 5 days, the alcohol yield 76.2 % and residual direct reducing sugar 2.6 %.

Introduction

The energy crisis gave impetus for many countries to search for an alternative source of energy. For agricultural countries, the conversion of alcohol from agricultural products is very attractive. Sugar cane, grains, cassava and sweet potatoes have been used for this purpose. However, because of its availibility and cheapness, cassava has the likelihood of becoming the raw material of choice[1].

In alcoholic fermentation, cassava starch must be digested to fermentable sugar. Sandstedt and Ueda[2] showed that amylase from different sources had different abilities to digest raw starch. Ueda[3] and Ueda and Koba[4] claimed that black-koji amylase is more effective than yellow-koji amylase and malt amylase in the digestion of raw starch, and cereal starches are more easily digested than root starches such as sweet potato and potato. However, raw cassava root starch is as easily digested as raw corn starch. Nunokawa *et al*[5] reported that sugar liberated by the koji amylase can be successively fermented to alcohol by yeast in the same pot. The combination of progressive hydrolysis of starch by koji amylase and alcohol fermentation by yeast are called parallel fermenta-

tion which contributes to the high alcohol production in the mash. From these results, they concluded that raw cassava starch, black-koji amylase and parallel fermentation are the suitable raw material, source of enzyme, and method for the alcohol fermentation respectively.

Many research groups have shown that under the same cultural condition used, various yeast strains have different abilities to produce alcohol in the fermentation broth[6-9]. Chotickai and Ueda[6] studied the production of alcohol at various temperatures using raw cassava starch as substrate, and found that at 40°C although the fermentation time was the shortest, the alcohol conversion efficiency was low. Subsequently, Sivipita-yangoorn and Ueda[8] found yeast strains that were capable of producing alcohol of similar yield either at 37°C and 40°C. Another advantage of producing alcohol at high temperature such as at 40°C is that it reduces the energy consumption for the cooling system during fermentation[9].

Rice is popularly grown in Thailand; consequently, rice bran and husk would be an obvious substitute for wheat bran which are generally used for the preparation of the black koji. After the suitable thermotolerant yeast has been isolated and selected, it was used with black koji, prepared from rice bran and husk, in the alcoholic fermentation of raw cassava starch.

Thus the purpose of this study was to isolate a thermotolerant yeast suitable for raw cassava starch fermentation at 40°C and to examine rice bran enzyme koji as a substitute for wheat bran koji.

Materials and Methods

Microorganisms. The thermotolerant yeasts were isolated from fermenting mash for cottage scale whisky production, mold bran (Look Pang) and fermented fruits by the plate culture method using a yeast extract malt extract agar and incubated at 40°C. These microorganisms were maintained on agar slants of the same medium at 4°C.

Koji preparation. Koji was prepared by inoculating 1 ml spore suspension (approximately 10^8 spores/ml) of *Aspergillus niger* (mold from Kyushu University, Japan) into the 500 ml Erlenmeyer flask containing sterile medium composing of wheat bran or rice bran and husk in various ratios totalling 25 g, urea (1.25 g), potato starch (2.5 g) and tap water (20 ml). The koji preparation was then incubated under stationary condition at 30°C for 4 days and whole bran was used as saccharifying agent.

Fementation broth. The fermentation broth used for the alcohol fermentation study has the following composition : raw cassava starch 20 g, wheat bran koji or rice bran koji 10 g, tap water 100 ml, yeast suspension with a heavy inoculum size, 10^8 cells/ml, 5 ml and pH adjusted to 3.5 with 80 % phosphoric acid.

Different yeast suspensions were prepared from the isolated yeasts. Each yeast

suspension was prepared by inoculating a loopful of 24 hours old stock culture at 40°C into the 9 ml of sterile distilled water which was then vigorously shaken using a rotamixer. The yeast numbers were determined by using the Thoma's haemacytometer.

Selection of alcohol fermenting yeast. The isolated thermotolerant yeasts were examined for their ability to produce alcohol individually. The fermentation broth which used wheat bran koji as saccharifying agent was kept under stationary condition at 40°C. The degree of fermentation was calculated daily from the weight of CO_2 evolved which corresponded to the decrease in weight of the whole culture. Total concentration of alcohol accumulated in fermentation broth was determined by the method indicated by Tax Administration Agency, Tokyo[10]. The alcohol yield was calculated and the residual direct reducing sugar was determined using the method of Kobayashi and Tabuchi[11].

Comparison of rice bran and wheat bran koji. Rice bran koji was prepared as wheat bran koji using various ratios of rice bran and husk instead of wheat bran as saccharifying agent for alcoholic fermentation of the yeast no. 4. The method and efficiency of alcoholic fermentation were determined as described in the selection of alcoholic fermenting yeast. Comparison between the most suitable rice bran and wheat bran koji of this yeast was made.

Results and Discussion

Isolation and selection of yeast strains. Out of 78 thermotolerant yeasts isolated from fermenting mash for cottage scale whisky production, mold bran (Look Pang) and fermented fruits tested for their ability to produce alcohol, thirty were shown to produce alcohol. These are listed in Table 1. Of these thirty isolated veasts. Only three gave significant amount of CO_2 evolved and significant amount of alcohol. The properties of these three yeast strains are compared as shown in Fig. 1, which clearly showed that yeast no. 4 was superior in all respects. It had the highest fermentation rate, produced the highest amount of CO_2 and the highest concentration of alcohol. Fermentation ceased after 5 days and the alcohol concentration reach 8.45 % (v/v) which was equivalent to an alcohol yield of 81.0 % and residual direct reducing sugar was 1.9 % (Table 1 and Fig. 1). The high degree of alcoholic fermentation may be attributed to the high enzyme activity produced by yeast no. 4. This yeast was thus selected for subsequent alcoholic fermentation study. The result of this study is in agreement with those reported by Johnson[12] and others[6-9], i.e., that only certain yeasts were suitable for use in alcohol fermentation and that the fermentation rates and alcohol yield varied with the yeast strains.

Rice bran koji as saccharifying agent. Table 2 shows the alcohol fermentation of yeast no. 4 using rice bran koji which were prepared from rice bran and husk in various ratios. The highest amount of CO_2 evolved and highest concentration of alcohol were obtained from rice bran koji prepared from rice bran : rice husk in the ratio of approxi-

TABLE 1. ALCOHOLIC FERMENTATION OF THE THIRTY ISOLATED THERMOTOLERANT YEASTS, USING WHEAT BRAN KOJI AS SACCHARIFYING AGENT.

Initial yeast concentrations were between 1-2 × 10^8 cells/ml and the total theoretical alcohol concentration possible as 10.436 % (v/v).

Yeast (no.)	Fermentation time[a] (days)	Distilled alcohol (%, v/v)	Alcohol yield (%)	Residual direct reducing sugar (%)
1	3	2.10	20.1	11.9
2	4	4.27	40.9	8.5
3	8	3.54	33.9	9.6
4	5	8.45	81.0	1.9
5	6	4.54	43.5	8.1
6	4	4.78	45.8	7.6
7	7	3.98	38.1	9.0
8	5	6.90	66.1	4.3
9	5	7.32	70.1	3.6
10	4	6.65	63.7	4.8
11	4	4.86	46.6	7.4
12	6	6.40	61.3	5.4
13	4	2.38	22.8	11.6
14	5	5.36	51.4	6.8
15	4	2.48	23.8	11.4
16	5	5.44	52.1	6.8
17	5	7.55	72.3	3.3
18	3	2.30	22.0	11.7
19	4	5.14	49.3	7.1
20	3	4.70	45.0	8.0
21	4	2.05	19.6	12.0
22	4	2.25	21.6	11.8
23	4	2.70	25.9	11.0
24	8	4.16	39.9	8.7
25	4	1.95	18.7	12.2
26	5	4.41	42.3	8.2
27	5	5.86	56.2	6.1
28	4	6.52	62.5	5.2
29	5	3.62	34.7	9.5
30	5	1.48	14.2	13.0

[a] fermentation time means the time during which CO_2 was evolved.

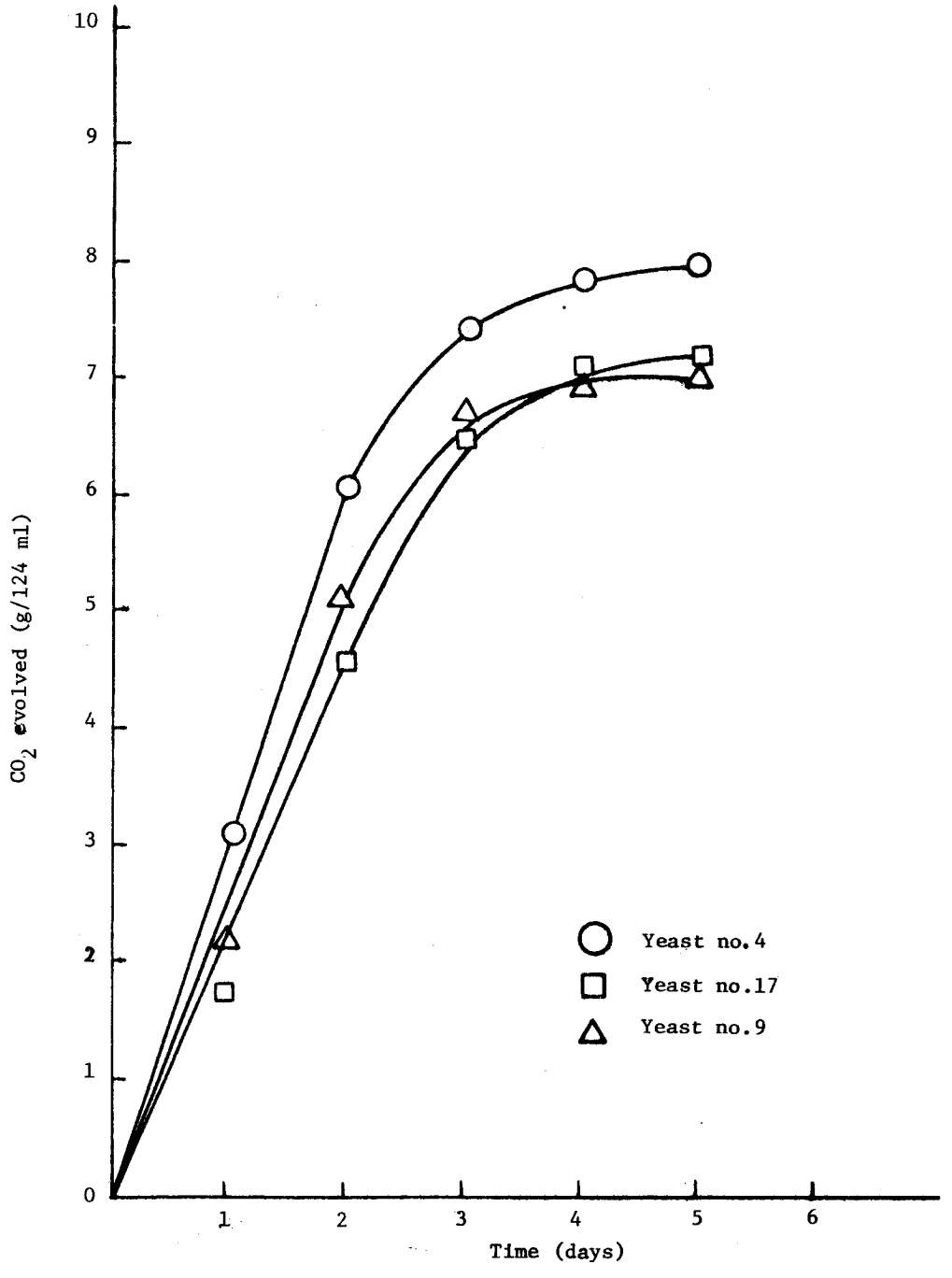

Fig. 1. Comparison of fermentation rates of the three isolated thermotolerant yeasts, when wheat bran koji was used as saccharifying agent.

mately 5 : 1 (w/w). Under these conditions, the fermentation ceased after 5 days and the alcohol concentration reached 7.95 % (v/v) which was equal to an alcohol yield of 76.2 % and the residual direct reducing sugar was 2.6 % (Table 2)

Comparison of the alcohol fermentation rates of the yeast no. 4 using wheat bran koji in one fermentation flask, and rice bran koji which was prepared from rice bran : rice husk in the ratio of approximately 5 : 1 (w/w) in the other flask as saccharifying agents showed that the higher alcoholic fermentation rate and the higher amount of CO_2 evolved were obtained when wheat bran koji was used as saccharifying agent (Fig. 2). This is probably due to the fact that wheat bran koji has a higher concentration of amylase than rice bran koji. Wheat bran koji, therefore, produces larger amount of fermentable sugar and consequently larger amount of alcohol on fermentation[3,4,13,14].

The results of this experiment indicated that yeast no. 4 is a suitable thermotolerant yeast for use in the fermentation of raw cassava starch at 40 °C because it gave a high alcohol concentration of 8.45 % (v/v) (alcohol yield 81,0 %) with wheat bran koji. When used with rice bran koji, prepared from rice bran and husk in the ratio of approximately 5 : 1 (w/w), it gave an alcohol concentration of 7.95 % (v/v) (alcohol yield 76.2 %). Thus, using this optimum of rice bran and husk ratio, the koji prepared is a good substitute for wheat bran koji.

The above result compares favourably with other reports; for instance, Chotickai and Ueda[6] obtained an alcohol concentration of 6.72 % (v/v), and Sivipitayangoorn and Ueda[8] obtained an alcohol concentration of 5.5 % (v/v) from raw cassava starch at 40 °C. Ueda[15], using semi - continuous process at 35°C and *Saccharomyces cerevisiae,* obtained an alcohol concentration of 10 % (v/v). However, all the above works used wheat bran koji as saccharifying agent. Moreover, the latter result was from a semi - continuous process and was conducted at a lower temperature; thus agitation occurred to a certain extent this would promote alcohol production[4,9,15]. Thus we have achieved our initial objective in that a suitable thermotolerant yeast was found and rice bran koji can be used in place of wheat bran koji without significant loss in yield of alcohol. This aspect is particularly appealing because rice and not wheat is grown in Thailand.

Acknowledgement

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TABLE 2. THE ALCOHOLIC FERMENTATION OF THE YEAST NO. 4, WHEN USED RICE BRAN KOJI AS SACCHARIFYING AGENT.
Initial yeast concentrations was 1.26×10^8 cells/ml, fermentation time was 5 days and the total theoretical alcohol concentration possible was 10.436 % (v/v)[a].

Rice bran : rice husk (g)	Distilled alcohol (%, v/v)	Alcohol yield (%)	Residual direct reducing sugar (%)
12.5 : 12.5	3.97	38.0	8.9
16.67 : 8.33	4.32	41.4	8.3
18.75 : 6.25	5.62	53.9	6.3
20.0 : 5.0	6.68	64.0	4.7
20.83 : 4.17	7.95	76.2	2.6
21.43 : 3.57	7.29	69.9	3.6
21.87 : 3.13	7.27	69.7	3.7
22.22 : 2.78	7.08	67.8	4.1
22.50 : 2.50	6.43	61.6	5.0
25 : 0	6.27	60.0	5.2

[a] fermentation time means the time during which CO_2 was evolved.

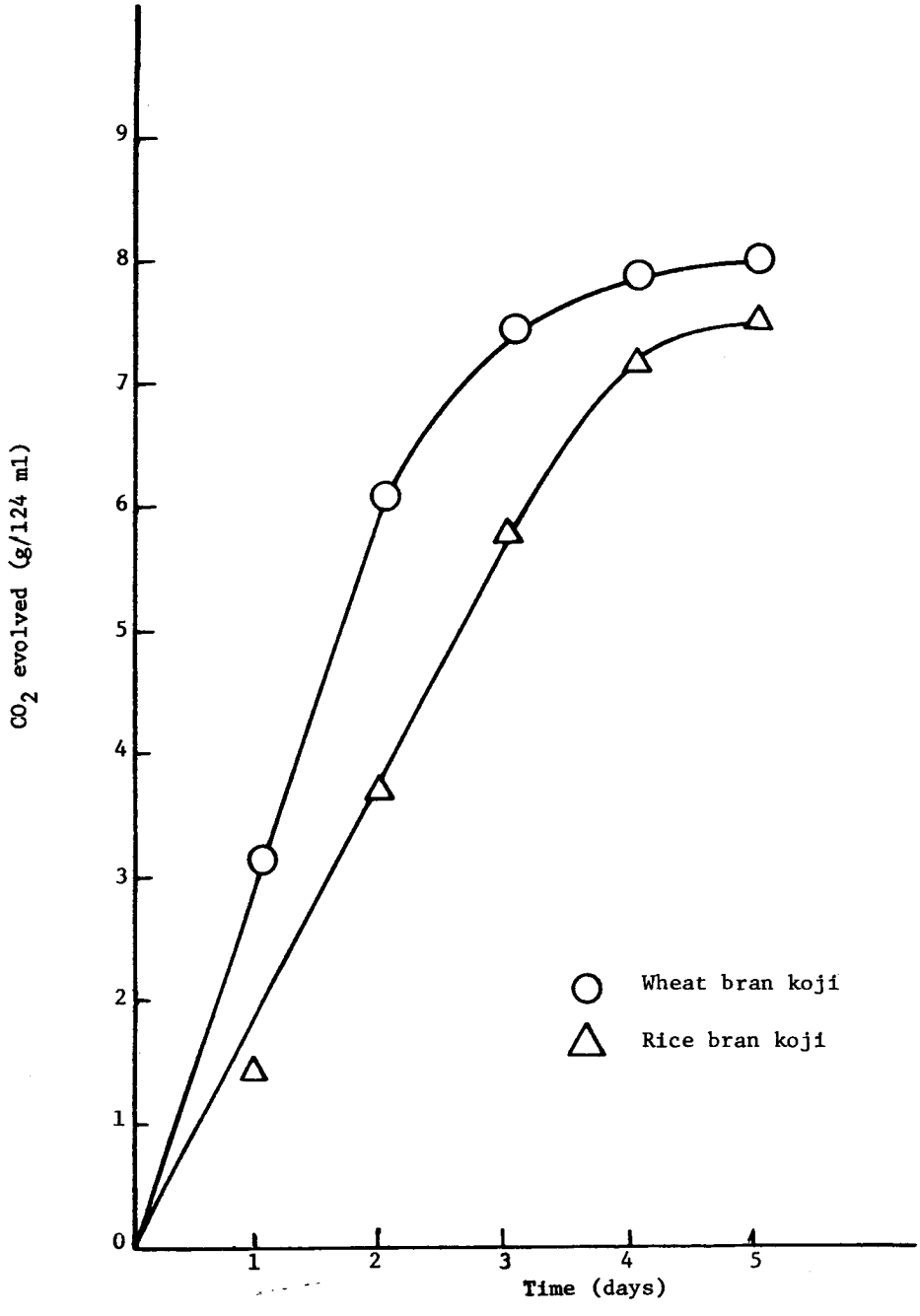

Fig. 2. Comparison of fermentation rates of the yeast no. 4, using rice bran and wheat bran koji as saccharifying agents.

References

1. Mears, L.G. (1979) *Environ* **20,** 17.
2. Sandstedt, R.M. and Ueda, S. (1968) *J Japan Soc. Starch Sci.* **17,** 215.
3. Ueda, S. (1975) *J.Japan Soc. Starch Sci.* **22,** 114.
4. Ueda, S. and Koba, Y. (1980) *J.Ferment. Technol.* **58,** 237.
5. Nunokawa, Y., Sumiya, S. and Iwano, K. (1978) *J.Ferment. Technol.* **56,** 380.
6. Chotickai, P. and Ueda, S. (1980) *J.Ramkhamhaeng Univ.* **3,** 168.
7. Hayashida, S. and Ohta, K. (1981) *J.Inst. Brew.* **87,** 42.
8. Sivipitayangoorn, S. and Ueda, S. (1981) *Ann. Rep. ICME.* **4,** 370.
9. Kamnuanta, J., Punpeng, B., Komagata, K., and Taguchi, H. (1980) *Microbial Utilization of Renewable Resources,* Vol. 1, pp. 136 - 140, ICME, Osaka.
10. Iinkai, T.H. (1973) *Kokuzeityo Shotei Bunsekiho Tyukai Zoteiban,* Nippon Jozo Kyokai, Tokyo.
11. Kobayashi, T. and Tabuchi, T. (1953) *J.Agric.Chem. Soc. Japan.* **28,** 171.
12. Johnson, J.C. (1977) *Yeast for Food and Other Purposes,* Noyes Data Corporation, Park Ridge, New Jersey.
13. Miyoshi, T. and Terui, G. (1971) *J.Ferment.Technol.* **49,** 935.
14. Nagatani, M., Kiso, K. and Nunokawa, Y. (1975) *J.Ferment.Technol.* **53,** 471.
15. Ueda, S. (1982) *Microbial Utilization of Renewable Resources,* vol. 2, pp. 167 - 175, ICME, Osaka.

บทคัดย่อ

นำเทอร์โมโทลีเร้นท์ยีสต์ที่แยกได้จากแหล่งต่าง ๆ มาตรวจสอบความสามารถในการหมักแอลกอฮอล์จากแป้งมันสำปะหลังที่อุณหภูมิ 40 องศาเซลเซียส โดยใช้ขวดหมักและใช้โคจิสีดำซึ่งเตรียมจากรำข้าวสาลีเป็นตัวการเปลี่ยนแป้งมันสำปะหลังไปเป็นน้ำตาล ปรากฏว่าได้เชื้อยีสต์ที่สามารถหมักแอลกอฮอล์ 30 ไอโซเลท และเชื้อยีสต์หมายเลข 4 มีประสิทธิภาพในการหมักแอลกอฮอล์ได้ดีที่สุด คือ ให้ก๊าซคาร์บอนไดออกไซด์สูงสุด หมักแอลกอฮอล์ได้ 8.45% โดยปริมาตร แอลกอฮอล์ยีลด์ 81.0% และมีน้ำตาลรีดิวซ์เหลืออยู่หลังจากการหมักสิ้นสุด 1.9% ในเวลา 5 วัน ดังนั้นจึงคัดเลือกเชื้อยีสต์หมายเลข 4 มาศึกษาการหมักแอลกอฮอล์จากแป้งมันสำปะหลังที่อุณหภูมิ 40 องศาเซลเซียส โดยใช้โคจิสีดำซึ่งเตรียมจากรำข้าวจ้าวและข้าวเปลือกเป็นตัวการเปลี่ยนแป้งมันสำปะหลังไปเป็นน้ำตาล ปรากฏว่า เชื้อยีสต์หมายเลข 4 หมักแอลกอฮอล์ได้สูงสุด 7.95% โดยปริมาตร แอลกอฮอล์ยีลด์ 76.2% และมีน้ำตาลรีดิวซ์เหลืออยู่หลังจากการหมักสิ้นสุด 2.6% ในเวลา 5 วัน