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Note

Baking Properties of *Saccharomyces cerevisiae* Strains Derived from *Brem*, a Traditional Rice Wine from Bali

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Twenty-two strains of *Saccharomyces cerevisiae* isolated from *tape*-mash for the production of *brem*, a traditional rice wine from Bali, Indonesia, were divided into three groups (I, II, and III) based on the sequences of the rDNA spacer region and *SUC2* gene. DNA analyses suggested that the seventeen strains in groups II and III were taxonomically closer to baking strains than the five strains in group I. These differences were reflected in their leavening ability in dough with and without addition of 5% sucrose, and their α-glucosidase activity, although most of the strains did not leaven dough with addition of 30% sucrose. Strain S-10 in group II showed a high rate of CO_2 production from sponge dough without addition of sugar and consumed fermentable sugar within 2.5 h. When baking tests were conducted, strain S-10 and commercial baking strain HP 216 yielded comparable products by the straight-dough and sponge-dough methods.

Keywords: *brem*, *SUC2* gene, leavening, dough, breadmaking

Introduction

The yeast *Saccharomyces cerevisiae* is a microorganism that has been long been used, both knowingly and unknowingly, by humans (Steinkraus, 2004). Various strains are currently used for the production of beer, whisky, sake, wine and breads as pure cultures produced by selection and/or hybridization to possess the characteristics required for their specific purposes (Panchal, 1990). On the other hand, in many Asian countries, indigenous alcoholic beverages using starchy materials are produced using starter cultures containing filamentous fungi and yeasts in rice powder (Tamang and Fleet, 2009). *Brem*, a traditional rice wine from Bali, Indonesia, is one of these products; it contains 8.6% (w/v) ethanol and 27.8% (w/v) glucose and has a sweet taste, with a clear, brownish color (Sujaya *et al.*, 2000). The process of *brem* production includes two fermentation steps (Sujaya *et al.*, 2004). First, *tape*-mash is prepared by mixing a starter culture, *ragi tape*, and steamed rice, followed by incubation for five days at room temperature for liquefaction, saccharification and fermentation. Filtrate obtained by pressing *tape*-mash is collected in a tank and undergoes a second fermentation for about six months.

The microorganisms found in *ragi tape* include the fungi *Rhizopus*, *Mucor* and *Amylomyces*, the yeasts *Saccharomycopsis fibligera* and *Pichia anomala*, and various lactic acid bacteria (Holzapfel, 2002), although the predominant species in *brem* is *Saccharomyces cerevisiae*, which possesses high fermentation activity (Sujaya *et al.*, 2004). The *S. cerevisiae* strains were divided into four types based on the pattern of chromosomal DNA binding, and each type produced *brem* with different organic acids and volatile compounds when used as single strains (Sujaya *et al.*, 2004). The variations in qualities of *brem* suggest that *tape*-mash includes a diverse population of *S. cerevisiae* that can be applied to other fermentation products.

In the present study, we found that several yeast strains isolated from *tape*-mash were taxonomically similar to baking strains on analysis of DNA sequences, and we carried out breadmaking tests using selected strains.

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Materials and Methods

Yeast strains and culture The twenty-two strains used in the present experiments were isolated from *tape*-mash and deposited as *S. cerevisiae* in the Culture Collections of Udayana University (Sjamsuridzal, 2007). Cells were grown at 30°C with shaking (150 rpm), as described elsewhere (Oda and Tonomura, 1993).

Analysis of DNA sequences PCR was conducted using primers pITS1 and pITS4 for the entire internal transcribed spacer (ITS) region spanning ITS1, ITS2 and the intervening 5.8S rDNA, as well as SCS-1F and SCS-12R for the *SUC2* gene (Oda *et al.*, 2010a). The nucleotide sequences of the amplified fragments were determined using a 310 DNA sequencer (Applied Biosystems, Foster City, CA). A phylogenetic tree was constructed using the CLUSTAL W (Thompson *et al.*, 1994) and TreeView programs (Page, 1996) with retrieved sequences (Oda *et al.*, 2010a). Sequences were assigned the DDBJ/EMBL/GenBank Accession Numbers AB602488 and AB602489.

Leavening ability and enzyme activities Gas evolved from dough without addition of sugar and from that containing 5% and 30% sucrose (based on weight of flour) was measured at 30°C for 2 h as the leavening ability. Activities of invertase and α-glucosidase were assayed as described previously (Oda *et al.*, 2010b). Specific activities of both enzymes were expressed as nanomoles of product per minute per milligram of cells as dry matter.

Baking test Breads were baked from 200 g of flour using the straight-dough method and the sponge-dough method (Oda *et al.*, 2010b). A piece (20 g) of sponge dough prepared separately was used to record the rate of CO2 production with a Fermograph II (Atto Corp., Tokyo, Japan) at 30°C for 4 h. The weight and volume of baked goods were measured after cooling at room temperature for 1 h. Volume was determined by displacement of rape seeds. *S. cerevisiae* HP 216 isolated from commercial compressed yeast was used as a reference.

Results

The twenty-two strains of *S. cerevisiae* isolated from *tape*-mash were divided into three groups (I, II, and III) based on sequence analyses of the ITS region and the *SUC2* gene (Table 1). DNA sequences were the same in each group. Five strains in group I possessed an ITS region identical to that of strain NBRC 10516 (EU145764) and an *SUC2* gene (AB602488) with 97.8% identity with that of

Table 1. Properties of *S. cerevisiae* strains isolated from *tape*-mash.

Strain	Group	Cell yield (g as dry matter/ 100 ml of medium)	Leavening ability in dough (ml/2 h/10 g flour)			Enzyme activity (nmol/min/mg cells as dry matter)	
			Without sugar	+5% sucrose	+30% sucrose	α-Glucosidase	Invertase
G-1	I	1.15 ± 0.02	21.2 ± 1.6	36.3 ± 8.1	1.3 ± 0.6	11 ± 0	504 ± 50
G-2	I	0.92 ± 0.04	37.7 ± 3.3	45.0 ± 7.4	0.0 ± 0.0	44 ± 19	18,300 ± 1,700
G-3	I	1.20 ± 0.03	23.7 ± 1.6	40.5 ± 3.5	1.2 ± 1.0	15 ± 3	1,030 ± 100
G-4	I	1.16 ± 0.01	22.0 ± 2.3	35.5 ± 4.4	2.7 ± 1.0	14 ± 1	759 ± 25
G-6	I	1.12 ± 0.01	22.3 ± 1.2	38.7 ± 2.1	2.5 ± 0.5	14 ± 2	770 ± 43
J-1	II	0.78 ± 0.08	40.5 ± 5.7	42.5 ± 5.1	0.0 ± 0.0	316 ± 53	881 ± 202
J-4	II	0.93 ± 0.06	45.7 ± 6.3	46.2 ± 4.5	0.2 ± 0.3	319 ± 60	1,430 ± 530
J-5	II	0.54 ± 0.07	44.7 ± 5.9	44.3 ± 2.3	2.5 ± 2.2	221 ± 57	1,570 ± 530
J-6	III	1.07 ± 0.05	40.3 ± 2.0	46.5 ± 1.8	2.5 ± 2.3	228 ± 51	1,250 ± 530
J-7	III	1.07 ± 0.07	34.4 ± 4.5	45.2 ± 0.8	4.9 ± 3.1	109 ± 26	2,700 ± 320
K-2	II	0.92 ± 0.15	44.2 ± 4.5	45.2 ± 4.0	0.3 ± 0.6	243 ± 103	764 ± 150
S-1	II	0.60 ± 0.12	26.5 ± 8.7	36.0 ± 4.3	0.0 ± 0.0	293 ± 9	882 ± 152
S-2	II	0.36 ± 0.13	22.2 ± 4.1	26.3 ± 6.9	0.0 ± 0.0	180 ± 13	1,800 ± 280
S-3	III	1.06 ± 0.01	38.0 ± 1.7	45.8 ± 0.8	3.3 ± 1.9	107 ± 23	1,590 ± 90
S-4	II	0.56 ± 0.02	32.7 ± 1.6	35.5 ± 3.0	0.0 ± 0.0	87 ± 6	1,170 ± 160
S-6	III	1.07 ± 0.02	41.0 ± 2.6	48.3 ± 1.6	3.7 ± 1.6	75 ± 28	1,590 ± 90
S-7	II	0.91 ± 0.08	44.8 ± 2.5	45.8 ± 0.6	1.2 ± 0.3	219 ± 57	538 ± 17
S-8	II	0.75 ± 0.28	35.7 ± 4.9	40.8 ± 1.0	0.0 ± 0.0	127 ± 61	894 ± 50
S-9	II	0.47 ± 0.17	44.3 ± 2.8	43.0 ± 2.6	0.0 ± 0.0	204 ± 89	785 ± 249
S-10	II	0.88 ± 0.07	45.7 ± 4.5	47.8 ± 3.3	0.2 ± 0.3	365 ± 75	810 ± 16
S-11	II	0.69 ± 0.13	35.0 ± 2.5	37.8 ± 2.8	0.0 ± 0.0	236 ± 42	773 ± 21
S-13	II	1.09 ± 0.28	31.2 ± 8.3	39.3 ± 4.3	0.7 ± 0.3	284 ± 122	517 ± 26
HP 216	–	1.14 ± 0.10	39.5 ± 2.0	50.7 ± 3.3	18.3 ± 0.6	173 ± 84	3,300 ± 946

Data are shown as average values and standard deviation from three independent experiments.

strain NBRC 1953 (AB534211). Thirteen strains in group II and four strains in group III had common ITS sequences with strain HP 216 (AB279753). The same sequence of the *SUC2* gene was observed in the strains of group II and strain HP 203 (AB534208), while the sequence of the *SUC2* gene (AB602489) in group III showed 99.7% identity with that of strain HP 216 (AB534209). A phylogenetic tree based on the sequence of the *SUC2* gene shows that strains in groups II and III are taxonomically closer to baking strains than those in group I (Fig. 1). The relationship among these strains coincided with the previous observation that strains S2, S11, J1 and J4, and strains J6 and S6 could be divided into two distinct groups (Sujaya *et al.*, 2004).

Table 1 also summarizes the properties of the twenty-two strains required for bakery use. The cell yield and leavening abilities in dough without addition of sugar and with 5.0% sucrose varied among the individual strains, but all the strains tested showed much lower leavening ability in dough with 30% sucrose than strain HP 216.

The invertase activities ranged from 500 to 2,000 (nmol/min/mg cells as dry matter) except for strain G-2, which showed much higher activity. Strains in groups II and III expressed more α-glucosidase activity than those in group I.

Based on the results described above, strain S-10 was selected as one of the strains possessing the highest leavening ability, and was compared with strain HP 216 for baking performance using the straight-dough and sponge-dough methods. There were a few differences in the two strains with regard to appearance and specific volume of the baked products (Table 2), which were similarly accepted by the preliminary sensory tests (data not shown), indicating that strain S-10 is applicable to breadmaking with either method.

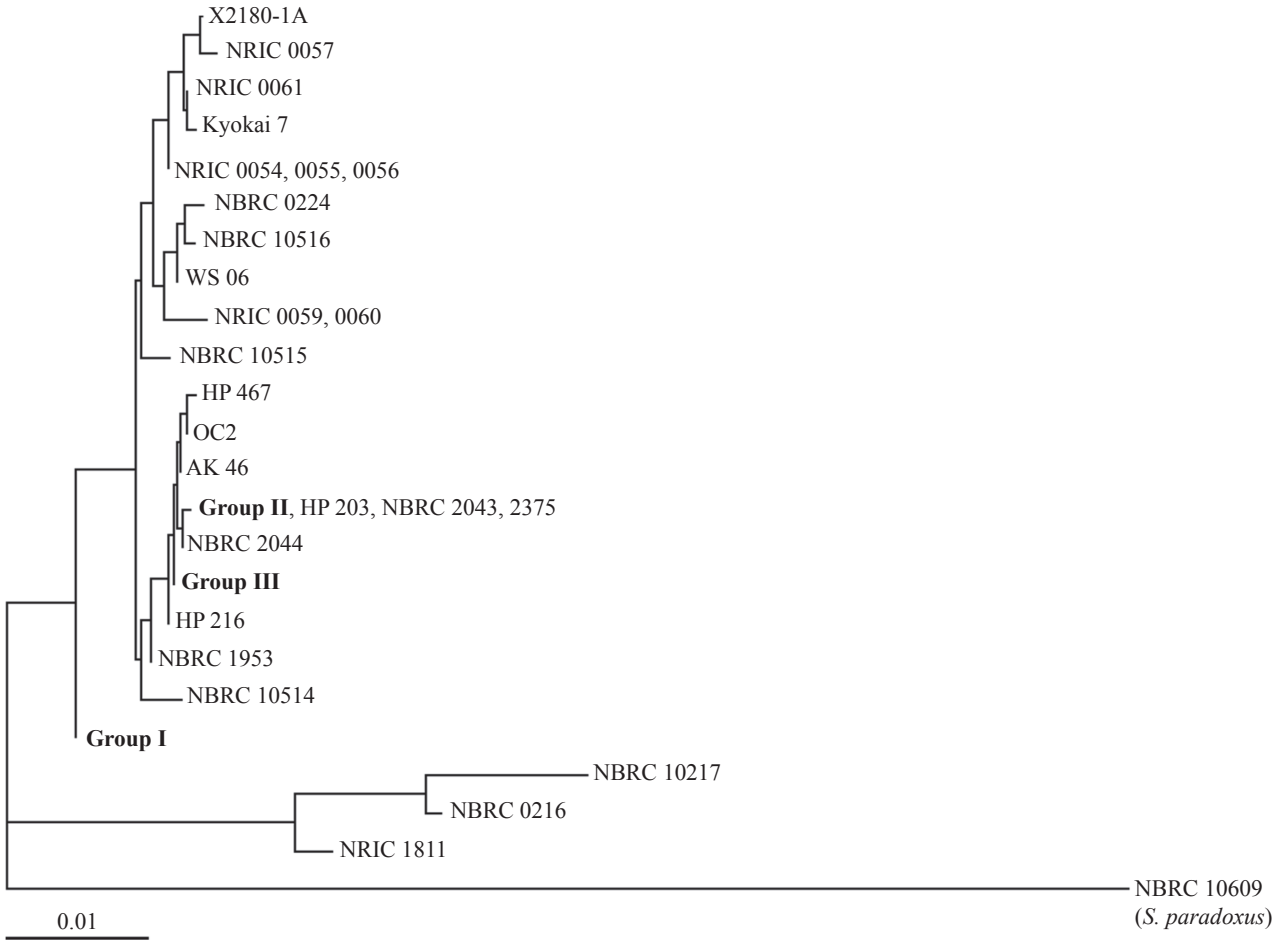

Fig. 1. Phylogenetic tree of *S. cerevisiae* strains based on *SUC2* gene sequences.

Strains for retrieved sequences (WIL162W, AB495285, AB495286, AB534206-AB534231) and their types or origin are as follows: X2180-1A (laboratory); NBRC 2043, NBRC 2044, NBRC 2375 (baking); HP 203, HP 216, HP 467 (baking, retailed outlet); NBRC 1953, NBRC 10217 (brewing); NBRC 0216, NBRC 0224 (ethanol production); NRIC 1811 (Taiwan Sugar Research Institute); NRIC 0057, NRIC 0059, NRIC 0060 (*bubod*, Philippine starter culture); Kyokai 7 (sake making); OC2 (wine making); AK 46 (fermented cherry fruits); WS 06 (Samoan breadfruits); NBRC 10514, NBRC 10515, NBRC 10516 (tree exudates); NRIC 0054, NRIC 0055, NRIC 0056 (apple juice). Retrieved sequence for *S. paradoxus* NBRC 10609 (Oda *et al.*, 2010a) was used as the outgroup for phylogenetic analysis. The bar indicates one estimated substitution per 100 nucleotide positions.

Table 2. Evaluation of bread produced by strains S-10 and HP 216.

Baking Method	Strain	Volume (ml)	Weight (g)	Specific Volume (ml/g)
Straight dough	S-10	438 ± 6	81.0 ± 1.0	5.41 ± 0.09
	HP 216	450 ± 9	80.6 ± 0.3	5.59 ± 0.11
Sponge dough	S-10	483 ± 3	79.5 ± 0.4	6.08 ± 0.06
	HP 216	500 ± 10	78.0 ± 0.2	6.41 ± 0.12

Data are shown as average values and standard deviation from three baked products.

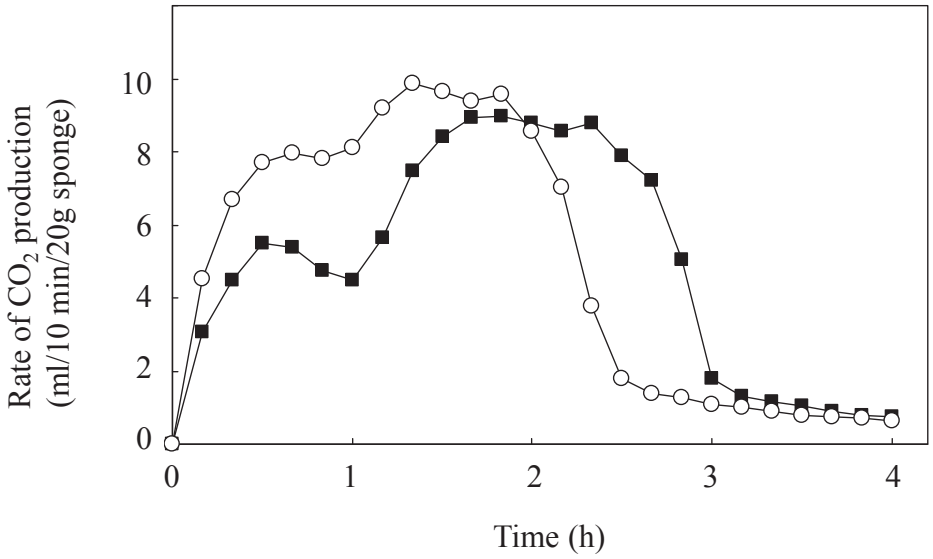

Fig. 2. Change in CO_2 production rate from sponge with strains S-10 (○) and HP 216 (■).

When CO_2 production from the sponge with strains S-10 and HP 216 was observed, the two strains showed a similar curve with two distinct peaks (Fig. 2). The first increase represents the fermentation of sugars present in the flour, and the following large increase corresponds to the fermentation of maltose hydrolyzed from starch by the action of amylases (Oda and Ouchi, 1990). Strain S-10 induced maltose fermentation more rapidly than strain HP 216. These differences may be reflected in the specific volumes of products baked with the sponge-dough method.

Discussion

There are regional differences in the characteristics of commercial baking strains based on accepted dough formulas in individual countries. Japanese baking strains are osmotolerant and suitable for leavening in sweet dough, while those used in North America and Europe rapidly leaven lean dough and possess high maltose fermentation ability (Oda and Ouchi, 1989). Strain S-10 appears to be closer to the latter type and is unsuitable for breadmaking using sweet dough.

Yeast strains isolated from natural habitats often lack or have weak maltose fermentation ability (Bell *et al.*, 2001; Okagbue, 1988), as maltose is a sugar that yeast cells tend to encounter under special conditions. Baking and brewing strains have been selected based on the individual processes, which may require rapid utilization of maltose derived from the substrate, thereby resulting in higher maltose fermentation ability (Jensen, 1998).

In *tape*-mash, starch from rice is hydrolyzed to dextrin, maltose and finally glucose by the action of fungal amylases (Cronk *et al.*, 1977). Maltose fermentation ability is not an essential property for yeast strains in the production of *brem*. The presence of strains capable of fermenting maltose vigorously suggests that maltose is consumed by the yeast cells in immature *tape*-mash. Such starter cultures may therefore be a natural source of microorganisms for industrial use.

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