

INTERSPECIFIC HYBRID BETWEEN *Saccharomyces mikatae* AND *Saccharomyces cerevisiae* AS AN ALTERNATIVE STRAIN FOR BREAD MAKING

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ABSTRACT

Hybrids of the homothallic diploid *Saccharomyces mikatae* and heterothallic haploid *Saccharomyces cerevisiae* isolated from local fruits in Hokkaido and a baking strain, respectively, were constructed by the spore to cell mating method. Interspecific hybridization of the selected strain was confirmed by polymerase chain reaction using species-specific primers, fermentation profiles, and spore formation. The leavening ability of the dough and enzyme activities of the hybrid exhibited intermediate traits of their parental strains. A baking test by the sponge-dough method with a standard formulation of white bread showed that breads produced using the hybrid differed in aroma and taste from those made using a conventional baking strain. Thus, the hybrid may be used to produce baked goods with diverse quality, which may be more acceptable to specific consumers.

Keywords: Bread making, Hybrid, *Saccharomyces mikatae*, *Saccharomyces cerevisiae*

INTRODUCTION

Saccharomyces is a teleomorphic ascomycetous genus that generally exhibits vigorous fermentation and includes at least eight species (Alsammar et al., 2019). *Saccharomyces cerevisiae* is the most widely used species in fermentation processes, including bread making. Yeasts used for bread making leaven dough by producing carbon dioxide through glycolysis and impart desirable qualities to bread based on their fermentation by-products, including alcohols, organic acids, and esters. Domestication of wild yeasts through selection based on rapid dough leavening has resulted in the development of species similar to yeasts used in alcoholic beverage production (Carbonetto et al., 2018). While these selected yeasts may have acquired properties specific to their respective industries, their diversity has declined (Gallone et al., 2018).

Flavor is a complex parameter that determines the quality of baked products (Heitmann et al., 2018). Improvements in bread flavor using different techniques were reported (Martinez-Anaya, 1996, Salim-ur-Rehman et al., 2006), but it is unclear if these methods fully respond to the various and complex demands of consumers. As social structures change including a declining low birth rate and increasing aged population in Japan (Nomura & Koizumi, 2016), manufacturers in the food industry such as in baking have used differentiated products focused on specific consumers because developing products acceptable to the entire commercial market is difficult (Thakur et al., 2020). One approach for responding to these challenges is to prepare a wider diversity of baking yeast strains.

Saccharomyces yeasts, other than *S. cerevisiae*, are used in certain fermentation processes; *S. pastorianus*, the natural hybrid of *S. eubayanus* and *S. cerevisiae*, is a representative yeast used to produce lager beer (Gallone et al., 2018). *Saccharomyces bayanus*, *S. kudriavzevii*, *S. uvarum*, and their hybrids with *S. cerevisiae* are sometimes employed during vinification to improve fermentation characteristics or product quality (Pérez-Torrado et al., 2018). Industrial use of *Saccharomyces mikatae*, a wild yeast found exclusively in the natural environment in Japan (Naumov et al., 2000), has not been reported. Interspecific hybrids between *S. mikatae* and *S. cerevisiae* derived from a commercial starter culture altered the metabolite profiles of wine, with respect to the parental wine yeast (Bellon et al., 2013). Another hybrid between *S. mikatae* and an *S. cerevisiae* ale strain efficiently fermented wort at 12°C under lager brewing conditions, and produced more alcohols and esters compared to the parental strains (Nikulin et al., 2018). However, interspecific hybrids between *Saccharomyces* species for bread making have not been reported.

During a survey of local genetic resources, we found a fermentative yeast strain, identified as *S. mikatae*, which was unsuitable for usual bread making as it exhibited a defect in maltose fermentation. In the present study, this strain of *S. mikatae* was hybridized with haploid *S. cerevisiae* to construct an alternative baking strain for preparing bread with diverse quality, which may be acceptable to specific consumers.

MATERIAL AND METHODS

Yeast strains

In this study, the following yeast strains were used (Table 1). The hybrid was constructed by the spore to cell mating method (Garcia Sanchez et al., 2012). Twenty-four spores, dissected from the asci of *S. mikatae* AK40, formed on SPO agar and were individually paired with single cells of *S. cerevisiae* H24U1M using a micromanipulator on YPD agar. After two days, all colonies were transferred onto SD-Glu agar and further incubated for two days. Colonies that appeared on the plate were inoculated onto SD-Mal agar and grown under anaerobic conditions for one day. One well-growing colony was selected from among 18 colonies and isolated as strain DHM15.

Strain AK40 was isolated from a sample of blueberry-like fruits with some leaves attached, which was collected from the Tokachi District of Hokkaido, Japan, in July 2007 after enrichment culture, and identified as *S. mikatae* based on sequences of the 18S-26S rDNA spacer region (Oda et al., 2010). Strains DHM15, AK40, and H24U1M were deposited as NITE P-02672, NITE P-02673, and NITE P-02508, respectively, in the National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depository (Kisarazu-shi, Chiba, Japan).

Culture conditions

Media used for hybridization and related experiments were YPD, SPO (Oda & Ouchi, 1990), and SD (0.67% yeast nitrogen base without amino acids, Difco, Detroit, MI, USA) supplemented with either 2% glucose (SD-Glu) or 2% maltose (SD-Mal), and thereafter solidified with 2% agar when necessary. Fermentation of sugar was tested as described previously (Kurtzman et al., 2011). All cultivations were performed at 30°C.

Table 1 Yeast strains used in the present study

Strain	Species	Origin
DHM15	<i>S. mikatae</i> × <i>S. cerevisiae</i>	Hybrid between strains AK40 and H24U1M
AK40	<i>S. mikatae</i>	Homothallic diploid (wild strain)
H24	<i>S. cerevisiae</i>	Haploid (<i>MATa</i>) derived from a baking strain HP203
H24U1M	<i>S. cerevisiae</i>	Uracil-auxotrophic mutant of strain H24
HP467	<i>S. cerevisiae</i>	Conventional baking strain as the reference

Leavening ability in dough

Cells grown in YPS medium containing 2% NaCl (Oda *et al.*, 2010) were collected and washed with water to prepare a yeast cake including 33% (w/w) cells as dry matter. The dough components, which were 10 g of bread flour, 5.5 mL of water or the solution containing 0.5 g of sucrose plus 0.2 g of NaCl, and 1.0 mL of a yeast suspension containing 0.2 g of the yeast cake, were kept at 30°C and mixed quickly by hand for 1 min after adding the yeast suspension. CO₂ evolved from the mixed dough was collected in graduated cylinder over the saturated saline solution and its volume was measured at 30°C for 2 h to determine the leavening ability in dough.

Enzyme assays

For the α -glucosidase assay, 0.05 mL of the permeabilized cell suspension prepared by vortexing in the presence of chloroform and sodium dodecyl sulfate was added to 1.0 mL of 50 mM phosphate buffer (pH 6.8) containing 5 mM *p*-nitrophenyl α -D-glucopyranoside and incubated at 30°C for 30 min. The reaction was stopped by adding 1.0 mL of 3% sodium carbonate and released *p*-nitrophenol was determined.

For the invertase assay, a mixture containing 100 mM acetate buffer (pH 4.5), 150 mM sucrose, and the cell suspension in 0.25 mL was incubated at 30°C for 3 min. The reaction was terminated by mixing 0.25 mL of 3,5-dinitrosalicylic acid reagent to measure the reducing sugar produced from sucrose.

The specific activities of both enzymes were expressed as nanomoles of products per minute per milligram of cells as dry matter.

Baking test

Breads were made using the sponge-dough method with a standard formulation of white bread (Oda *et al.*, 2010). These breads were subjected to preliminary sensory evaluation of the rating method by 11 panels made up of students. The panels were trained and frequently perform sensory evaluation of bread. The scales of crumb grain and crumb color are from 0 (poor) to 100 (excellent), that of crumb texture from 0 (hard) to 100 (soft), and those of aroma and taste from 50 (same) to 100 (different). The scores were compared to that of the reference setting at 50. Student's *t*-tests were conducted to detect significant differences between the two baked products.

RESULTS AND DISCUSSION

Characterization of the hybrid

Polymerase chain reaction (PCR) was performed to investigate the hybrid DHM15 strain (Figure 1). Specific amplicons corresponding to *S. mikatae* genomic loci (508 base pairs (bp)) were amplified in the DHM15 (lane b) and AK40 (lane d) strains; 1,710-bp amplicons corresponding to *S. cerevisiae* genomic loci were produced in DHM15 (lane b) and H24 (lane e). Similar patterns were observed when the cells of strain DHM15 were grown in YPD medium 10 times (lane c), suggesting that the genome of strain DHM15 can be stably maintained even under non-selective conditions.

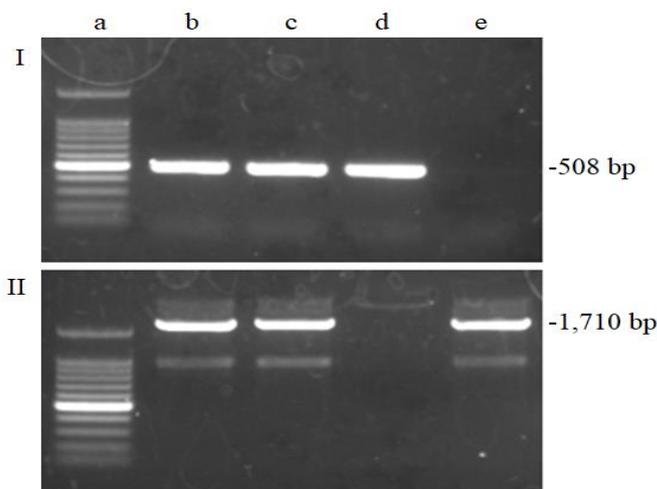


Figure 1 PCR with species-specific primer sets for *S. mikatae* (I) and *S. cerevisiae* (II)

The primer sets used were Smik-F1 (5'-ACAAGCAATTGATTTGAGGAAAAG-3') and Smik-R1 (5'-CCAGTCTTCTTTGTCACGTTG-3') for *S. mikatae* (Pengelly & Wheals, 2013) and YC1f (5'-CTTATGCTTGGAACCTCAAGACA-3') and YC2r (5'-AGAAGCAACAACAGCAACAACCCAA-3') for *S. cerevisiae* (Torriani *et al.*, 2004).

Lanes: a, 100-bp DNA ladder; b, DHM15; c, DHM15 after growth (10 times) in YPD medium; d, AK40; e, H24.

Strains DHM15, AK40, and H24 fermented glucose, galactose, sucrose, and raffinose but not lactose. Maltose fermentation was lacking in strain AK40 but observed in strains DHM15 and H24. In contrast, melibiose fermentation by strain AK40, a typical trait of *S. mikatae* (Naumov *et al.*, 2000), was inherited by strain DHM15 but was not observed in strain H24. Melibiose fermentation ability may be an advantage for a baking strain to completely consume sugars when beet molasses, including raffinose composed of a melibiose moiety, is used as a raw material in commercial propagation (Oda & Tonomura, 1996). Trehalose was fermented by strains DHM15 and H24 but not by strain AK40.

When cultured on SPO agar for six days, most cells of strain AK40 were converted to asci containing four spores, while those of the haploid strain H24 were not (Figure 2). Strain DHM15 produced spores but much less efficiently than strain AK40. *Saccharomyces mikatae* has been proposed for use in producing genetically isolated populations of the *Saccharomyces sensu stricto* complex in Japan, as hybrids between strains NBRC 1815 and NBRC 1816 and four other species do not produce viable spores (Naumov *et al.*, 1995). In the present study, *S. mikatae* was successfully hybridized with *S. cerevisiae* by the spore to cell mating method, whereas hybrid DHM15 may produce no progeny.

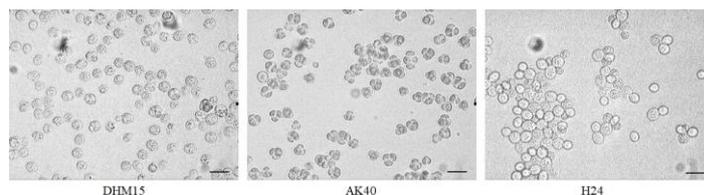


Figure 2 Spore formation of the hybrid and parental strains

Cells of each strain were grown on SPO agar for 6 days at 30°C. The bar indicates 10 μ m.

Some properties required for baker's yeast

In a comparison of strain DHB15 with its parental strains, AK40 and H24, the cell yield and leavening ability of strain AK40 in two doughs were increased by hybridization (Table 2). The basal activity of α -glucosidase produced by strain AK40 showed the lowest leavening ability in the dough without added sugar, which was explained by a defect in the fermentation of maltose, the principal fermentable sugar derived from starch via the action of amylases. In contrast, strain AK40 synthesized enough invertase to hydrolyze sucrose. Hybrid DHM15 showed intermediate properties of the parental strains to reach sufficient levels compared to those of the baking strain HP467. These results suggest that the hybrid can be applied in bread making by the sponge-dough method.

Table 2 Properties required for baker's yeast

Strain	Cell yield (g as dry matter /60 mL of medium)	Leavening ability in dough (mL of CO ₂ /2 h/10 g flour)		Enzyme activity (nmol/min/mg cells as dry matter)	
		Without sugar	+5% sucrose +2% NaCl	α -Glucosidase	Invertase
DHM15	0.749 \pm 0.017	48.5 \pm 3.8	48.0 \pm 4.1	635 \pm 161	1,280 \pm 40
AK40	0.631 \pm 0.013	17.1 \pm 0.9	41.1 \pm 3.4	13 \pm 4	3,620 \pm 80
H24	0.561 \pm 0.002	55.6 \pm 1.1	51.7 \pm 4.1	1,330 \pm 230	126 \pm 3
HP467	0.711 \pm 0.018	32.0 \pm 2.6	48.8 \pm 3.3	84 \pm 31	400 \pm 23

These experiments were performed three times independently and the obtained data are shown as the average values \pm standard deviations.

Baking performance

The specific volume of bread produced using hybrid DHM15 was lower than that of the bread produced using strain HP467. A value higher than 5.0 mL/g is acceptable in the market. In the sponge-dough method, the DHM15 hybrid rapidly fermented the sponge in 4 h, and may not have evolved CO₂ rapidly after remixing with the remaining materials, including sugar and other ingredients, as determined from the leavening ability of dough with 5% sucrose and 2% NaCl

(Table 2). Preliminary sensory evaluation of these breads revealed slight differences in the quality of the crumb grain, texture, and crumb color (Table 3). The scores for the differences in aroma and taste for hybrid DHM15 were higher than those for strain HP467, and a significant difference in taste was found. These results indicate that the bread made using hybrid DHM15 had distinct qualities.

Table 3 Qualities of baked breads by the sponge-dough method

Strain	Specific volume (mL/g) ^a	Crumb evaluation (-) ^b				
		Crumb grain	Texture	Color	Aroma	Taste
DHM15	5.30 \pm 0.18	46.3 \pm 13.4	49.8 \pm 8.2	45.1 \pm 7.6	55.0 \pm 11.5	58.5 \pm 8.3**
HP467	6.01 \pm 0.11**	50.0 \pm 0.0	50.0 \pm 0.0	50.0 \pm 0.0	50.0 \pm 0.0	50.0 \pm 0.0

^an = 4; ^bn = 11, **P < 0.01

CONCLUSION

The hybrid between *S. mikatae* and *S. cerevisiae* may be used to produce baked goods with diverse quality, which may be acceptable to specific consumers. Differences in the quality of fermented products should be investigated by analyzing their soluble and volatile compounds.

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