Mutation breeding of sake yeast using heavy-ion-beam irradiation

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In recent years, the total sake consumption has been decreasing, although the consumption of fruity sake variants such as Ginjo-shu is increasing. Taste of fruity sake results from malic acid.[1] Malic acid is produced by yeast during main fermentation (moromi).[2] The main objective of this study is to isolate a high-malic-acid-producing yeast strain through the irradiation of heavy-ion beams to develop high-value-added products.

The parent strain is an in-house yeast strain, T-66. It is isolated from Kyokai-kobo K-6 and tends to produce more organic acid more than K-6. T-66 was precultured in 5 mL of a koji extract medium (a Baumé degree of 6.0) at 30°C for 2 days. The cultured yeast cells were spread on YPD agar medium plates [20 g/L (w/v) glucose, 20 g/L (w/v) polypeptone, 10 g/L (w/v) yeast extract, and 18 g/L (w/v) agar]. We irradiated the plates with 500 Gy of Fe-ion beams (790 keV/μm) at the RIKEN Nishina center in Jury 2018. After incubation at 30°C for 5 days, 88 mutant yeast strains were isolated from the plates.

As primary screening, the mutagenized yeast strains was precultured in 5 mL of *koji* extract medium. 3 mL of yeast culture was added to 20 mL of *koji* extract medium supplemented with 7 g alcohol-dehydrated *koji* and incubated at 20°C for 10 days.[3] Filtrated mediums were measured using an alcohol densitometer (Alcomate AL-2, Woodson Riken Keiki, Tokyo), and titratable acidity was measured using the official analysis method of the National Tax Agency. We selected 8 mutant yeast strains that produced more organic acids than the parent strain without producing any off-flavor. Next, the amount of organic acid (succinic acid, lactic acid, citric acid, malic acid, and acetic acid) was measured using an F-kit (J. K. International, Tokyo). Table 1 lists the results of primary screening. The malic-acid production amounts of P1-8, P3-10, and P7-6 strains were higher than those of the parent strains.

Table 1. Analysis of mutant yeast strains.

Sample name	Alcohol content [%]	Titratable acidity [-]	Malic acid [mg/L]
Parent strain	19.7	4.5	639.2
P1-8	19.5	5.2	908.3
P2-11	18.9	4.8	624.8
P3-10	18.2	4.9	975.6
P4-3	16.6	4.8	797.8
P6-12	20.2	4.9	778.5
P6-9	20.0	4.8	725.7
P7-13	19.7	5.0	884.3
P7-6	20.1	4.8	1100.5

Table 2. Analysis of small-scale sake brewing.

Sample name	Sake meter value [-]	Alcohol content [%]	Titratable acidity [-]	Amino acid content [-]	Sensory evaluation
Parent strain	+4.6	17.1	2.9	0.8	3
P1-8	+4.1	16.8	3.6	0.8	2
P3-10	+1.8	16.6	3.6	0.8	1
P7-6	+9.1	17.3	3.4	0.7	2

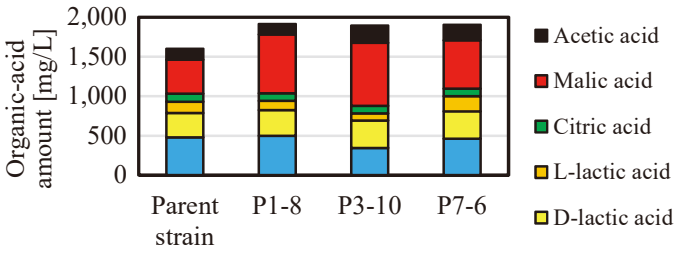

Fig. 1. Organic-acid production of isolated mutant yeast strains.

As secondary screening, the parent strain, T-66, and 3 selected mutant yeast strains were examined by small-scale sake brewing. After the filtration of fermentation mash, the sake meter value (SMV), an indicator of sweetness of sake; alcohol content; titratable acidity; and amino-acid content were measured using the official analysis method of the National Tax Agency. Additionally, we performed sensory evaluation (3-step rating: 1 is good and 3 is bad). Table 2 lists the results. The three selected mutant yeast strains produced more organic acid than the parent strain. While the SMVs are different, the three mutants show almost the same level of alcohol production as T-66. This result shows that the three mutant yeasts have good brewing ability. P3-10 scored the highest value in sensory evaluation. To verify the reason for this result, the amount of organic acid was measured using an F-kit (Fig. 1). P3-10 showed a higher malic-acid content and lower succinic-acid content than the other mutants, indicating that the sake brewed with this strain will have a light taste that matches the preference of recent consumers. It was shown that the P3-10 strain produces a lighter-tasting sake than the parent strain, T-66.

We isolated the high-malic-acid-producing yeast strain P3-10 derived from T-66. P3-10 produces not only more malic acid but also more acetic acid more than T-66. Because acetic acid produces an off-flavor for sake, we plan to improve on the P3-10 strain.

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References

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