Study of the High Ethanol Productivity of Sake Yeast by Comprehensive Cellular Morphological Analysis

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Research aims

Sake yeast strains, which belong to the budding yeast species Saccharomyces cerevisiae, produce much more ethanol during sake fermentation than any other type of S. cerevisiae strain. To elucidate the molecular mechanisms responsible for their excellent fermentation properties, we have investigated sake yeast-specific morphological traits using the CalMorph system, which has been developed for high-dimensional and quantitative morphological analysis of yeast cell populations. This analysis led to identification of a novel genetic determinant for ethanol production in S. cerevisiae.

Methods

The sake yeast strains Kyokai no. 6, 7, 9, and 10 and a laboratory strain X2180 were grown to the logarithmic phase in YPD medium and were then fixed with formaldehyde. After triple staining of yeast cell wall mannoproteins, actin, and nuclear DNA, cells were observed under the fluorescent microscope, and the microscopic cellular images were automatically processed using the CalMorph system.

For fermentation tests, yeast cells were inoculated into 20% glucose-containing YPD medium at a final OD660 of 0.1, and were then further incubated at 30°C without shaking. For sake fermentation assays, a sake mash was prepared by mixing 40 g pre-gelatinized rice, 10 g dried koji (rice cultivated with A. oryzae), 20 μl 90% lactic acid, and 80 ml water containing yeast cells at a final OD660 of 1.0, and was then incubated at 15°C without shaking. Fermentation was monitored by measuring the volume of evolved carbon dioxide using Fermograph.

To determine cellular DNA contents, yeast cells were fixed in ethanol, suspended in a propidium iodide solution, and analyzed using a flow cytometer. Gene-specific quantitative real-time PCR (qRT-PCR) was performed to quantify CLN3 mRNA. The actin-encoding gene ACT1 was used as an internal control.

Results

Our high-throughput cytological analysis using CalMorph revealed that sake yeast cells exhibit specific morphological features in the logarithmic phase: Both the ratio-of-no-bud (C119) and long-axis-length-of-mother-cell-on-
Fig. 1. Morphological traits of the sake yeast strains. (A) Three-dimensional PCA mapping of the CalMorph data. Colored dots correspond to data from individual experiments (n>200 cells). Sake yeast strains Kyokai no. 6, 7, 9, and 10 (K6, K7, K9, and K10) and a laboratory strain X2180 were used. (B) Representative cell morphology of a sake and a laboratory yeast. Bar; 3 mm. (C) Morphological parameters that showed significant differences between sake and laboratory yeast.

Fig. 2. Cell cycle-related analysis under a fermentative condition. Phase I: logarithmic growth phase, Phases II–IV: fermentative phases; Phase V: fermentation termination. (A) DNA contents. (B) Analysis of CLN3 mRNA levels using qRT-PCR. Relative expression levels are displayed as fold differences compared to the levels induced in X2180 (phase I), using ACT1 as a reference gene.

Fig. 3. Improvement of the fermentation rate by dysfunction of Rim15p. (A) Fermentation tests in 20% glucose-containing YPD medium. (B) Small-scale sake fermentation tests.
0.18%) than that from wild-type BY4743 (11.19±0.17%). Taken together, these results clearly demonstrated that dysfunction of Rim15p caused by the sake yeast-specific rim155055insA mutation leads to a striking increase of ethanol production.

**Conclusion**

The cellular morphological analysis of sake yeast enabled us to find its novel phenotype related to defective cell cycle control. Furthermore, we revealed that the rim155055insA mutation have played a pivotal influential role in accelerated G1 progression and increased fermentation rate that are both characteristic of sake yeast strains. Identification of multiple genetic determinants for the fermentation properties of sake yeast6–7 will provide significant insights into artificial modification of the yeast metabolic profiles. In future studies, the molecular mechanism on how the Rim15p-mediated cell cycle regulatory system controls fermentation and ethanol production should be intensively investigated.

**References**


