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Outline of Research Result

Genetic analysis of the ethanol fermentation ability of yeast using a next-generation DNA sequencer

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Research aims

Sake yeast is a diploid yeast classified as *Saccharomyces cerevisiae*. In sake brewing, it is well known to produce sake with good aroma and flavor as well as a high concentration of ethanol. Identification of the genes involved in these sake brewing characteristics is important from the viewpoints of scientific research and for application in yeast breeding. Quantitative trait locus (QTL) analysis is a method used to identify genetic loci linked to a specific phenotype on the basis of phenotyping and genotyping of many meiotic segregants of a hybrid, and has been applied to many organisms with sexual life cycles. The authors previously isolated haploid segregants from the hybrid diploid of a sake yeast haploid and a laboratory yeast, and reported QTL analysis of their sake brewing characteristics using microsatellite DNA[1]. However, the resolving power of this study was not high because of the low density of the DNA markers on the whole genome. In this study, the genotypes of a pool of superior (or inferior) haploid segregants with ethanol fermentation ability were analyzed at a single nucleotide polymorphism (SNP) level, using a next-generation sequencing method, in order to analyze more precise QTLs.

Methods

The best 10 (18.5–17.3%) and worst 10 (10.2–5.8%) haploid segregants in terms of ethanol concentration in sake brewing were selected among 100 haploid segregants derived from the hybrid diploid of a sake yeast Kyokai no. 7 (K7) haploid strain (K7H868) and a laboratory yeast haploid strain (X2180-1B)[1]. Each strain of the best 10 haploid segregants was cultured separately and an equivalent quantity of each strain was pooled, following which genome DNA preparation was carried out (the best 10 pool). Genome DNA was also prepared from a pool of the worst 10 segregants and from the parental strains (K7H868 and X2180-1B). DNA sequences of the samples were analyzed using Illumina HiSeq 2500 with the paired-end method. The reads obtained were mapped on the *S. cerevisiae* S288C genome (a laboratory strain) with the BWA software, after removing low-quality reads. Extraction of SNPs from the mapped reads was performed with the GATK toolkit, whereas annotation of the SNPs was done with the SnpEff toolbox.

Results

The total of high-quality sequencing reads obtained from the best 10 pool and the worst 10 pool was 2,092,574,200 bases and 1,682,096,400 bases, with sequencing redundancies of 138 and 114, respectively. After mapping on the laboratory yeast genome, 71,618 SNPs that were different between K7H868 and X2180-1B were extracted. For these SNPs, whether each read obtained from the best 10 pool and the worst 10 pool was originated from the sake yeast (K7H868) genome or the laboratory yeast (X2180-1B) genome was determined[2]. The SNP frequency was defined as 1 when all SNPs were originated from the sake yeast genome, and as 0 when all SNPs were originated from the laboratory yeast genome (Fig. 1). A high SNP frequency at a given SNP position in the best 10 pool indicates that the sake yeast-type SNP is associated with higher ethanol fermentation ability, whereas a low SNP frequency indicates that the laboratory yeast-type SNP is associated with higher ethanol fermentation ability. In the worst 10 pool, the reverse association would be seen between SNP frequency and ethanol fermentation ability.

One of the regions with high SNP frequency in the best 10 pool and low SNP frequency in the worst 10 pool was at the position of ~70 kb on chromosome VI (Fig. 1). This region contains *RIM15*, the mutation of which has been shown by individual genetic analysis to be involved in ethanol fermentation ability. *RIM15* is required for the acquisition of stress tolerance after entering the stationary phase. Sake yeast K7 has, however, a loss-of-function mutation in *RIM15*. This *rim15* mutation is thought to be one of the reasons for the high ethanol fermentation ability of K7 and related sake yeast strains, since the laboratory yeast strain having the *rim15* mutation showed higher ethanol fermentation ability than the parental *RIM15* strain[3].

On the contrary, a region with very low SNP frequency (0) in the best 10 pool and high SNP frequency in the worst 10 pool was observed at the position of ~350 kb on chromosome XIII. This suggests that sake yeast K7 contains a mutation with a negative effect on ethanol fermentation ability.

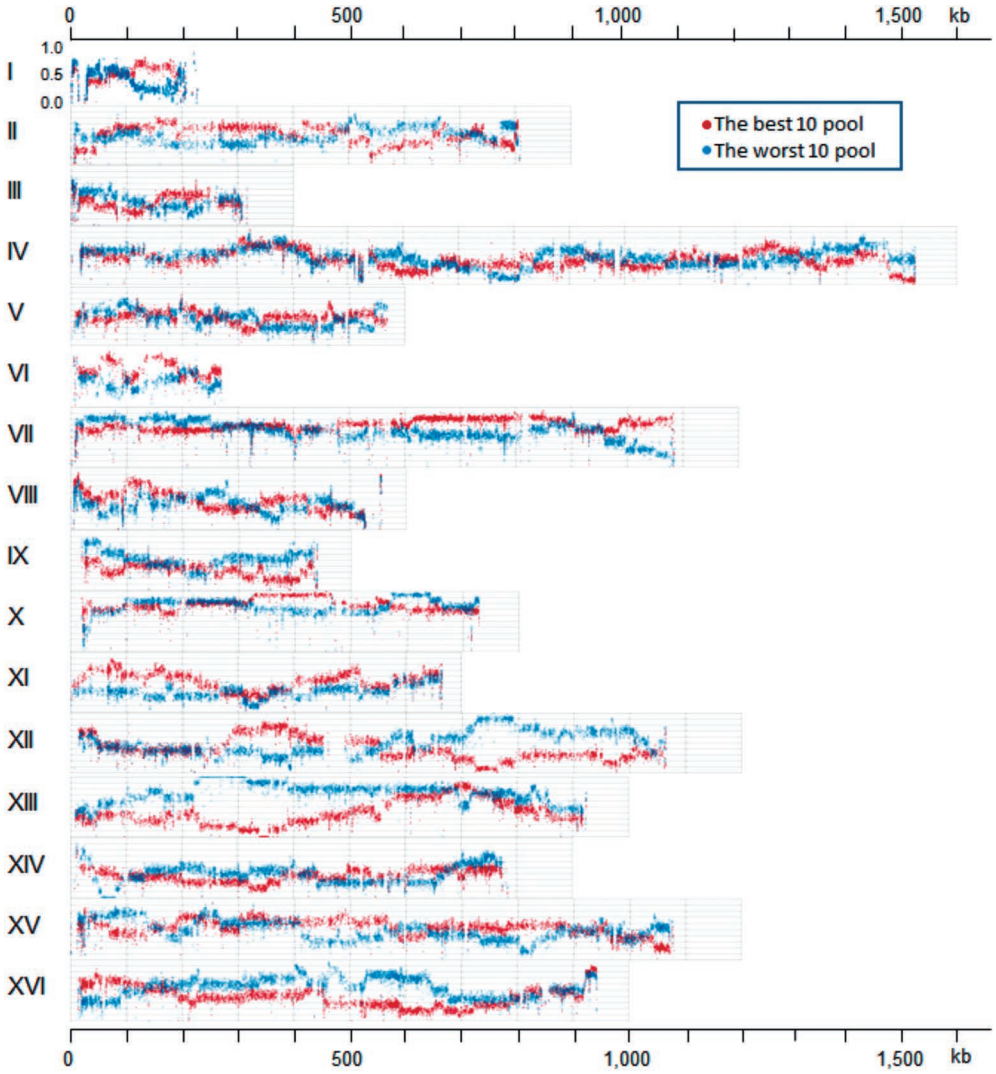

Fig. 1. Genome-wide SNP frequency of the pooled segregants.
SNP frequency of each pool was plotted against the chromosome coordinate. Red points indicate SNP frequency of the best 10 pool and blue points indicate SNP frequency of the worst 10 pool. When this value is 1, all SNP of this position are derived from the sake yeast. When this value is 0, all SNP of this position are derived from the laboratory yeast.

Conclusion

QTL analysis of the ethanol fermentation ability of sake yeast was carried out using next-generation sequencing of genome DNA of the pooled segregants derived from the hybrid between the sake yeast and laboratory yeast strains. This method is very effective for QTL analysis of industrial yeast strains since SNP frequencies at a large number of SNP positions can be simultaneously determined by using only one sequencing run.

References

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2) Pais, T. M., Foulquié-Moreno, M. R., and Thevelein, J. M. (2014) QTL mapping by pooled-segregant whole-genome sequencing in yeast. *Methods Mol. Biol.* **1152**: 251–266.
3) Watanabe, D., Araki, Y., Zhou, Y., Maeya, N., Akao, T., and Shimoi, H. (2012) A loss-of-function mutation in the PAS kinase Rim15p is related to defective quiescence entry and high fermentation rates in *Saccharomyces cerevisiae* sake yeast strains. *Appl. Environ. Microbiol.* **78**: 4008–4016.