Development of DNA markers for identification of good sake yeast

DNA markers, which are characteristic sequences or structures of DNA to a species or strain, are widely used for identification of species and strains of plants, animals and microbes. However, some phenotypic markers have been used for differentiation of sake yeast strains with good brewing properties from other yeast strains, and no DNA markers were known for this purpose.

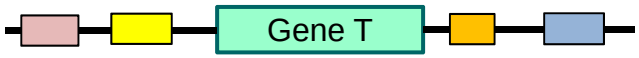
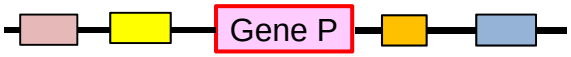
Recently, NRIB analyzed various yeast genomes and identified three DNA polymorphisms specific to widely used good sake yeast strains such as Kyokai no. 6 (K6), K7, K9, K10 and their derivative strains. These polymorphic sequences were common and specific to these sake yeast strains and were not found in the other yeast including wild sake yeast. Thus, these sequences are useful for DNA markers for differentiation of good sake yeast from the others. In addition, a DNA marker specific to the strain K7 was also found. We are now working on finding new markers that are specific to other respective strains by comparing the various yeast genome sequences.

We expect that these identification/differentiation methods using DNA markers will become powerful tools for maintenance of strain libraries, protection of patented strains, screening of yeast belonging to novel or known lineages, *etc*.

Latest methods for identification of good sake yeasts

Useful DNA markers for identification of good sake yeast strains were developed using genome information.

1) Differentiation of good sake yeast strains from other strains by three DNA markers

	DNA marker A	DNA marker B	DNA marker C
Type 1 Good sake yeast strains	ACA **A** GCC	CAA **T** AAC	
Type 2 Other yeast strains	ACA – GCC	CAA C AAC	
Types of Difference in DNA sequence or structure	Frameshift mutation in Type 1	Nonsense mutation in Type 1	Gene conversion (Gene P is lost in Type 1.)

Unknown strains → DNA marker typing → Type 1 in all markers → Strains in the lineage of sake yeasts with good brewing properties

→ Type 2 in all markers → Strains with unknown or diverse brewing properties

◆ Strains with a combination of Type 1 and 2 have not been found yet.

2) Identification of Kyokai no. 7 lineage by a specific marker

	DNA marker D
K7 lineage	TTC A/G TAT or TTC A/A TAC
Other yeast strains	TTC G/G TAT

<u>Application of the identification/differentiation method</u>

- ✓ Detection of mislabeling of strains
- ✓ Detection of contamination of unfavorable wild yeast
- ✓ Protection of patented strains
- ✓ Application for screening of novel yeast strains

◆ More DNA markers are needed for identification of an individual good sake yeast strain.

➢ Good sake yeast：Kyokai no. 6 (K6), K7, K9, K10 and their derivative strains. These strains are closely related to one another. Most of sake yeast strains in commercial use are in this group.