（学位第３号様式）

学　位　論　文　要　旨

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題　目	Breeding of sake yeasts and evaluation of the brewing characteristics （清酒酵母の育種と醸造特性評価）

Sake yeast, classified as *Saccharomyces cerevisiae*, is a yeast with high ethanol productivity under low temperature, and is an essential brewing microorganism for sake brewing. In conventional sake brewing, foaming sake yeasts, which form a layer of foam at *moromi*, has been used, but breeding of non-foaming mutants has been actively performed to ensure stable sake production and improve the efficiency of the production. The characteristics of sake yeasts also are a major factor in determining the flavor and taste of sake because they produce many metabolites such as aroma compounds and organic acids. Therefore, it is important to develop new sake yeasts with different metabolite compositions. The purpose of this study is isolating and breeding sake yeasts and evaluating their brewing characteristics, and the following five researches were performed. The following is a detailed description of the researches 3 to 5.

1 Obtaining the non-foaming mutant of sake yeast strain Y52.

2 Breeding of sake yeast with high productivity of aroma compounds by synchrotron light irradiation.

3 Isolation of sake yeast from the mud flats of Ariake Sea and evaluation of their brewing characteristics.

4 Breeding of malic acid high producing sake yeast mutants.

5 Identification of the mutation genes introduced into malic acid high producing mutant strains and elucidating the mechanism of getting malic acid high productivity.

Saccharomyces cerevisiae H3-1 strain with sake brewing aptitude were isolated from mud flats in the Ariake Sea, and breeding of strain H3-1 was performed to obtain malic acid high producing mutants. Of 6000 mutants induced by EMS, 236 dimethyl succinate-sensitive strains (DMSS) were obtained, and fermentation test and small-scale brewing tests were carried out. The mutant strain DMSS233 showed approximately 6 times higher malic acid productivity than that of strain H3-1.

Next, comparative genomic analysis between H3-1 and DMSS233 was performed, and 13 nonsense mutations, 2 frameshift mutations and 325 missense mutations were identified. In addition, about five malate biosynthesis genes, *MDH1*, *MDH2*, *MDH3*, *MLS1* and *DAL7*, malic acid productivity of constructed gene disrupt strains were analyzed. The production of malic acid in *MDH3* disrupt strain of DMSS233 was reduced to about half of that in the wild type, but the production of malic acid in *MDH3* disrupt strain of H3-1 was not different from that in the wild type. These results suggest that Mdh3p is involved in malic acid high productivity of DMSS233 strain. Therefore, localization analysis was performed. Microscopic observations of the constructed H3-1 GFP-MDH3 and DMSS233 GFP-MDH3 showed that Mdh3p was transported normally into peroxisomes in both strains at 8-24 hours of incubation. However, at 48-72 hours of incubation, Mdh3p was localized in the cytoplasm of DMSS233 GFP-MDH3 strain, whereas it was localized in the vacuole of H3-1 GFP-MDH3 strain. This suggests that DMSS233 strain accumulated Mdh3p in the cytoplasm without being degraded, resulting in obtaining malic acid high productivity.