学 位 論 文 要 旨

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題　目	Study of metabolic engineering of useful fermentation microorganisms

Sake is produced from rice and water by fermenting with the koji mold *Aspergillus oryzae* and sake yeast *Saccharomyces cerevisiae*.

The primary role of yeast is to convert glucose to ethanol. At the same time, it also produces flavor compounds. This study aimed metabolic engineering of useful fermentation microbes.

(1) Optimization of the method to obtain haploids from brewery yeasts.

The technical barrier for crossbreeding of sake yeasts is the acquisition of haploids. Because sporulation of sake yeasts is generally difficult, we tried to optimize the acquisition method of haploids from brewery yeasts.

As a result, sporulation medium using potassium acetate, cell wall-degrading enzyme Zymolyase 20-T, 2-mercaptethanol and non-ionic surfactant NP-40 were investigated.

(2) Metabolomics-based selection of brewery yeasts and sake yeast haploids generated from diploid sake yeast.

Metabolome analysis of brewery yeasts and sake yeast haploids using GC-FID and GC/MS was performed. As a result, brewery yeasts and sake yeast haploids could be discriminated using metabolome analysis based on GC-FID data. Moreover, using GC/MS analysis, mannitol, glycerol,and inositol were shown to contribute to the discrimination.

(3) Elucidation of pyruvate underproduction mechanism of K7-TCR7

K7-TCR7 is pyruvate-underproducing sake yeast, which was isolated by resistance to ethyl α-transcyanocinnamate.

We analyzed the genomic constitution of K7-TC7. As a result, signals of part of the chromosomes of K7-TC7 were increased. These results suggested that pyruvate-underproducing profile is related with partial increase of the chromosomes.

(4) Correlation of mitochondrial residual activity of sake yeast with unsaturated fatty acid productivity during alcoholic fermentation.

The effect of residual mitochondrial transmembrane potential on the synthesis of unsaturated fatty acids by sake yeast during alcoholic fermentation was investigated.

As a result, inhibition of the residual activity of the mitochondrial electron transport chain increased the synthesis of unsaturated fatty acids.

(5) The effect of koji glucosylceramide on the yeast fermentation profile.

Saccharomyces cerevisiae is incapable of synthesizing glucosylceramide (GlcCer). To the contrary, koji-producing fungus, Aspergillus, synthesizes abundant glucosylceramide. Considering that yeast is exposed to a high concentration of GlcCer in the mash during fermentation, the effect of koji GlcCer on the yeast fermentation profile was investigated. As a result, various GlcCer purified from different species similarly conferred alkali tolerance to yeast. Ceramide also enhanced the alkali tolerance of yeast. To infer the metabolism of GlcCer in the yeast cells, the metabolic fate of GlcCer after addition to yeast was investigated. As a result, no discernible degradation of GlcCer to ceramide in the yeast culture was observed, suggesting that exogenous GlcCer itself exerted the effect. Addition of GlcCer also increased ethanol tolerance of yeast. Furthermore, addition of GlcCer modified the flavor profile of the yeast cells. To understand the mechanism of modification of yeast fermentation profile by GlcCer, membrane properties of yeast added with GlcCer was investigated. As a result, yeast added with GlcCer had a membrane which has a shorter fluorescence lifetime. These results indicate that GlcCer from Aspergillus modifies the physiology of the yeast and demonstrate a new mechanism for cooperation between microbes in food fermentation.