学 位 論 文 要 旨

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題　目	Functional analysis of *THI3* and development of a novel gene disruption method using shochu yeast （焼酎酵母を用いた *THI3* 機能解析および新規対立遺伝子破壊法の開発）

The aroma compounds produced by yeast are crucial in alcoholic beverages. Among these aroma compounds, higher alcohols play a key role as essential precursors to acetic acid esters that contribute to the fruity fragrance in alcoholic beverages. Although much breeding and research has been conducted to understand higher alcohol biosynthesis in yeast, the role of *THI3* remains unclear among the many identified related genes. Furthermore, a limitation of the genetic engineering in these studies is that diploid yeast target genes cannot be fully eliminated in a single transformation, posing a challenge for application to industrial strains like shochu and sake yeasts.

This study aimed to clarify role of *THI3* in higher alcohol production and develop a novel gene disruption method for future gene functional analysis using industrial strains.

A *THI3* disrupted strain (Δ*thi3*) was engineered from the parent strain C4, shochu yeast strain with high production of higher alcohol. Culture tests were conducted to assess isoamyl alcohol production, primarily associated with *THI3* among higher alcohols. The results revealed that the isoamyl alcohol production by Δ*thi3* was influenced by the concentration of thiamine in the growth environment. This dependence arises because Δ*thi3* cannot synthesize thiamine intracellularly and relies on external uptake. The findings demonstrate that the intracellular concentration of thiamine diphosphate (TPP), a cofactor essential for α-ketoisocaproic acid decarboxylation in isoamyl alcohol biosynthesis, is influenced by extracellular thiamine content.

Homozygous gene disruption method applicable to diploid industrial strains was developed using the parental strain K2, a shochu yeast widely employed for shochu production. A disruption cassette, composed of functional *LYS5* flanked by non-functional *ura3* and given repetitive sequences inside and outside, allowed for gradual removal from the chromosome through loop-out recombination. This process involved sequential elimination: first, functional *LYS5*, followed by reversion of functional *URA3*, and finally complete elimination of *URA3*. A single transformation and loss of heterozygosity resulted in complete disruption of the target gene. Loop-out recombination and counter-selection allowed for the complete removal of the cassette after disrupting the target gene. As all the genes introduced by this method are derived from K2 genomic DNA and no foreign genes are used, the resulting mutant strain can be regarded as an industrially available self-cloning strain.

This study revealed that the role of *THI3* in higher alcohol production involves maintaining thiamine biosynthesis, ensuring a stable supply of the cofactor TPP. Furthermore, a novel homozygous gene disruption disruption method was developed that completely disrupts target genes in diploid shochu yeast in a single transformation, avoiding the use of foreign genes. These findings contribute to a precise understanding of the mechanism underlying higher alcohol biosynthesis in yeast and to future yeast breeding and genetic analyses employing industrial strains.