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| 学位論文名 | Genetic mechanisms of thermotolerance and breeding of a multiple-stress tolerant strain for bioethanol production in Saccharomyces cerevisiae<br>（出芽酵母における高温耐性の遺伝的メカニズムとマルチストレス耐性株の育種） |
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## 論文内容の要旨


Bioethanol, a renewable eco-friendly fuel is now considered to be an alternative to conventional gasoline. *Saccharomyces cerevisiae* is facultative anaerobe and under anaerobic conditions can ferment glucose to ethanol. Improvements in ethanol production by using genetically engineered yeast cells during the fermentation process may lead to a boost in the bioethanol production industry. Among the desirable traits of strains required for efficient bioethanol production, high-temperature growth (Htg), acid tolerance (Acd), and high ethanol production (Hep) are critical for reducing cooling and ethanol recovery costs, and for minimizing the risk of contamination. However, stressful conditions such as high temperatures strongly depress fermentation rate and yeast viability. Although thermotolerant strains have occasionally been isolated, genetic basis and mechanisms underlying the complex hereditary to tolerance to high temperature trait have rarely been reported. The full understanding of genetic mechanisms of thermotolerance could contribute to an increase of the upper-limit temperature for growth of *S. cerevisiae* and, therefore, to breeding robust strains suitable for bioethanol production. In addition, although several S. cerevisiae strains have been bred to achieve the cost-effective bioethanol production, the superior strains which could effectively produce ethanol at even high temperature and low-pH conditions have never been reported.

The molecular mechanisms of thermotolerance in *S. cerevisiae* are not fully understood. To get more insight into the complex hereditary Htg phenotype, I investigated genetically and physiologically a *S. cerevisiae* strain showing a high-temperature (41°C) growth ($Htg^+$) phenotype. The result suggests that the $Htg^+$ phenotype of this $Htg^+$ strain is dominant and under the control of most probably six genes, designated *HTG1* to *HTG6*. As compared with an $Htg^-$ strain, the $Htg^+$ strain exhibited a significantly high content of trehalose when cultured at high temperature and stronger resistance to Congo Red, an agent that interferes with cell wall construction. These results suggest that a strengthened cell wall in combination with increased trehalose accumulation can support growth at high temperature. In addition, the gene *CDC19*, encoding pyruvate kinase, was cloned as the *HTG2* gene. The *CDC19* allele from the $Htg^+$ strain possessed five base changes in its upstream region, and two base changes resulting in silent mutations in its coding region. Interestingly, the latter base changes are responsible for the increased pyruvate kinase activity of the Htg+ strain at a translational but not a transcriptional level. I hypothesized that this increased activity of pyruvate kinase may lead to activation of energy metabolism to maintain cellular homeostasis during heat stress.

To breed a superior strain of *S. cerevisiae* possessing $Htg^+$ $Hep^+$ phenotype suitable for cost-effective bioethanol production, a heterothallic strain showing $Htg^+$ phenotype, a derivative from a strain isolated from nature, was crossed with a homothallic strain displaying high-ethanol productivity ($Hep^+$), a stock culture at the Thailand Institute of Scientific and Technological Research. The resultant hybrid TJ14 displayed superior ability to rapidly utilize glucose, and produced ethanol (46.6 g/l) from 10% glucose fermentation medium at high temperature (41°C). Not only ethanol productivity at 41°C, but also acid tolerance ($Acd^+$) was improved in TJ14 as compared with its parental strains, enabling TJ14 to grow in liquid medium even at pH3. TJ14 maintained high ethanol productivity (46.0 g/l) from 10% glucose when fermentation was done under multiple-stress conditions (41°C and pH3.5). Furthermore, when TJ14 was subjected to a repeated-batch fermentation scheme, the growth and ethanol production of TJ14 were maintained at excellent levels over ten cycles of fermentation. Thus, the multiple-stress ($Htg^+$ $Hep^+$ $Acd^+$) resistant strain TJ14 should be useful for cost-effective bioethanol production under high- temperature and acidic conditions.

In conclusion, the data presented in this study should not only contribute to understanding of the whole picture of the molecular mechanisms responsible for contributing to the $Htg^+$ phenotype but also provide valuable knowledge for application to bioindustries for the cost-effective bioethanol production under multiple-stress conditions by the superior *S. cerevisiae* strains.


## 論文審査の結果の要旨

Bioethanol, a renewable eco-friendly fuel is now considered to be an alternative to conventional gasoline. High-temperature growth ($Htg^+$), acid tolerance ($Acd^+$), and high ethanol production ($Hep^+$) are critical for efficient bioethanol production. Although several *S. cerevisiae* strains have been bred to achieve the cost-effective bioethanol production, the super strains which could effectively produce ethanol at even high temperature and low-pH conditions have never been reported. This study was undertaken to understand genetic mechanisms of thermotolerance and also to breed a super strain of *S. cerevisiae* possessing $Htg^+$ Acd+ $Hep^+$ phenotype. Benjaphokee revealed that the $Htg^+$ phenotype of this $Htg^+$ strain is dominant and under the control of most probably six genes, designated *HTG1* to *HTG6*. $Htg^+$ strain exhibited a significantly high content of trehalose when cultured at high temperature and stronger resistance to Congo Red, an agent that interferes with cell wall construction. These results suggest that a strengthened cell wall in combination with increased trehalose accumulation can support growth at high temperature. He then cloned *CDC19*, encoding pyruvate kinase, as the *HTG2* gene. The *CDC19* allele from the $Htg^+$ strain possessed five base changes in its upstream region, and two base changes resulting in silent mutations in its coding region. Interestingly, the latter was found to be responsible for the increased pyruvate kinase activity of the Htg+ strain at a translational but not a transcriptional level although both mutations are silent mutations. Benjaphokee hypothesized that this increased activity of pyruvate kinase may lead to activation of energy metabolism to maintain cellular homeostasis during heat stress. He then constructed TJ14 by crossing a heterothallic strain showing $Htg^+$ phenotype, a derivative from a strain isolated from nature with a homothallic strain displaying high-ethanol productivity ($Hep^+$), a stock culture at the Thailand Institute of Scientific and Technological Research. The TJ14 maintained high ethanol productivity (46.0 g/l) from 10% glucose when fermentation was done under multiple-stress conditions (41°C and pH3.5). Furthermore, when TJ14 was subjected to a repeated-batch fermentation scheme, the growth and ethanol production of TJ14 were maintained at excellent levels over ten cycles of fermentation. Thus, the multiple-stress ($Htg^+$ $Hep^+$ $Acd^+$) resistant strain TJ14 should be useful for cost-effective bioethanol production under high- temperature and acidic conditions. In conclusion, the data presented in this study should not only contribute to understanding of the whole picture of the molecular mechanisms responsible for contributing to the $Htg^+$ phenotype but also provide valuable knowledge for application to bioindustries for the cost-effective bioethanol production under multiple-stress conditions by the superior *S. cerevisiae* strains. Judging from these achievement, this dissertation deserves the degree of Doctor of Engineering.