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学位論文名	Molecular mechanism of thermotolerance and efficient bio-ethanol production from cellulosic materials in *Saccharomyces cerevisiae* under high-temperature conditions （酵母 *Saccharomyces cerevisiae* における高温耐性の分子機構と高温条件下でのセルロース系材料からのバイオエタノール生産）
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論文内容の要旨

Global warming, spurt in world population growth, increase in demand for energy coupled with depletion of fossil fuel sources have evoked a global attention on the transitioning from petroleum-based to renewable fuels such as biofuel. Although biofuels offer a diverse range of promising alternatives, bioethanol has received much attention thus far and its use is increasingly widespread worldwide. *Saccharomyces cerevisiae*, is usually the first choice for industrial ethanol production, because of its good fermentative capacity, higher ethanol yield, high tolerance to ethanol, and the capacity to grow rapidly under the anaerobic conditions that are typically established in large-scale fermentation vessels. Although simultaneous saccharification and fermentation (SSF) of cellulosic resources has been recently attracted for fermentation industries, cellulosic ethanol production is not yet in industrial reality due to its poor yield. The possibility of performing fermentations at higher temperatures using thermotolerant yeast strains able to grow at temperatures compatible with optimal cellulase activities would greatly improve the efficiency of SSF processes and thereby make the ethanol production more economically feasible. Thus, breeding of superior *S. cerevisiae* strains that can ferment sugars at high temperatures with a high ethanol yield can be considered as one of the key solutions to reduce its production cost. Although there are several reports about isolation or developing *S. cerevisiae* strains which exhibit high-temperature growth phenotype (Htg^+), our knowledge about the genetic basis of the complex Htg trait is still unclear. The objectives of this study are to improve the efficiency of bioethanol production from cellulosic biomass under high temperature conditions in addition to elucidation of the molecular mechanism of high-temperature (41°C) growth (Htg^+) phenotype in thermotolerant *S.cerevisiae* from scientific point of view.

First generation ethanol made from edible resources such as corn and sugarcane are insufficient to meet the increasing demand for biofuels. In this context, the use of lignocellulosic materials for ethanol production is glimpsed as a promising choice because of their great availability and low cost. However, the large-scale commercial production of fuel bioethanol from lignocellulosic materials has still not been implemented. In this chapter, a Htg^+ ethanol producer, hybrid yeast *S. cerevisiae* strain TJ14 was evaluated for its capability for cellulosic bioethanol production by semi-simultaneous sacharification and fermentation (SSSF) technology under high temperature condition. We found that hybrid TJ14 used all degraded glucose and produced 45g/L ethanol by SSSF of 100 g (w/v)/L cellulose at 39°C. Ethanol concentration and volumetric productivity of TJ14 were higher than that reported in literature. Positive effect of Htg^+ phenotype on SSF ethanol production can be seen when the ethanol yield of TJ14 is compared with those obtained by high ethanol producer strains. The data obtained here using Htg^+ strain TJ14, are highlighting the importance of using this strain in the SSF process at high temperature to decrease production cost by producing ethanol in high level. Thus, this strain could be considered for cost-effective commercial bioethanol production using cellulosic biomass.

SSF process requires the utilization of microorganisms capable of working at high temperatures closer to optimum for commercial cellulases to achieve faster rates of cellulose hydrolysis and shorter SSF times. The breeding of superior *S. cerevisiae* strains that can ferment sugars at high temperatures with a high ethanol yield has become a necessity to increase the efficiency and to reduce the cost of converting biomass to biofuel. We describe an Htg^+ strain that exhibits confluent growth at high temperature (41°C), in addition to an ability to tolerate ethanol, osmotic and oxidative stresses. Genetic study on the thermotolerant *S. cerevisiae* strain C3723 isolated in Thailand suggested that, Htg^+ phenotype of thermotolerant strain was dominant and most plausibly, six genes designated *HTG1* to *HTG6* are responsible for Htg^+ phenotype. *RSP5* encoding E3 ubiquitin ligase was cloned as the *HTG6* gene. *RSP5-C* allele originated from Htg^+ strain likely confers Htg^+ phenotype by increased transcription of the *RSP5* gene. Indeed, transcription level of *RSP5-C* allele was higher than that of designated *RSP5-BY* allele originated from the *htg6* host strain (Htg^-) mainly due to the base changes existed on promoter region of *RSP5-C*. We also revealed that increased ubiquitination of proteins in Htg^+ strain was higher than that in Htg^- strains after exposure to temperature up-shift (41°C). Overexpression of the wild-type *RSP5* allele in Htg^- strain confered thermotolerance at 41°C as in the case of *RSP5-C* allele. Moreover, we found that an Htg^+ strain bearing an over-expressed *RSP5-C* exhibits more ability to tolerate higher temperature (43°C). The data presented here also suggested that overexpression of *RSP5* could be applied to raise the upper limit of thermotolerance in *S. cerevisiae* strain for efficient industrial bioethanol production.

The data presented here revealed that multi-stress tolerant *S. cerevisiae* strain TJ14 has high potential to be used to produce ethanol from cellulosic materials by SSF process under high-temperature condition. The *RSP5* gene encoding ubiquitin ligase was cloned and identified as the *HTG6* gene. Our results revealed that increased transcription level of *RSP5-C* and consequently increased ubiquitination of proteins in Htg^+ strain lead to acquisition

of high temperature growth phenotype. *RSP5-C* allele causes alterations in a diverse cell physiology same as the global regulatory effects of *spt15* and *laeA* in *S. cerevisiae* and *Aspergillus* spp., respectively. We hypothesize that an increase in *RSP5* transcript might increase and facilitate the degradation of damaged proteins or regulate the transcription of some genes and induce the heat stress response through the ubiquitination apparatus and finally allowing the cell to recover after high-temperature stress. This research also illuminated that overexpression of *RSP5* has much potential as a simple technique to develop thermotolerance in *S. cerevisiae* strains which nowadays are used in industrial fermentation.

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. Although simoultaneous saccharification and fermentation (SSF) of cellulosic resources has been recently attracted for fermentation industries, cellulosic ethanol production is not yet in industrial reality due to its poor yield. The possibility of performing fermentations at higher temperatures using thermotolerant yeast strains able to grow at temperatures compatible with optimal cellulase activities would greatly improve the efficiency of SSF processes and thereby make the ethanol production more economically feasible. This study was undertaken to evaluate cellulosic ethanol production in the semi-simultaneous saccharification and fermentation (SSSF) process and understand genetic mechanisms of high-temperature tolerance in superior thermoresistant (Htg^+) *S. cerevisiae* strain. Shahsavarani found that ethanol concentration and volumetric productivity of TJ14 were higher than that reported in prevailing literature. The data obtained in his study using Htg^+ strain TJ14, were highlighted the importance of using this strain in the SSF process at high temperature to decrease production cost by producing ethanol in high level. He then cloned *RSP5*, encoding E3 ubiquitin ligase, as the *HTG6* gene. The *RSP5* allele of the Htg^+ strain, designated *RSP5-C*, possessed five, one and two base changes in the promoter, open reading frame and terminator region, respectively. The base changes in the promoter region of the *RSP5-C* allele were found to be responsible for the thermotolerant phenotype by strongly increasing transcription of the *RSP5* gene and consequently causing a rise in the ubiquitination of cell proteins. The data also revealed that *RSP5-C* allele can protect the yeast cell from DNA damage, ethanol, cell wall, osmotic, oxidative and heat stresses. He also revealed that overexpression of the *RSP5* allele in Htg^- strain conferred thermotolerance at 41℃ as in the case of *RSP5-C* allele. Moreover, he found that an Htg^+ strain bearing an over-expressed *RSP5-C* exhibits more ability to tolerate higher temperature (43°C). Based on these results, Shahsavarani suggests that *RSP5-C* allele confer Htg^+ phenotype through causing alterations in a diverse cell physiology. 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 fermentation industries for developing thermotolerance and the cost-effective commercial bioethanol production using cellulosic biomass using the superior *S. cerevisiae* strain TJ14. Judging from these achievements, this dissertation deserves the degree of Doctor of Engineering.