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# 学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

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| 学位論文題目<br>(Dissertation Title) | Metabolic flow at high temperatures and laboratory adaptation in thermotolerant yeast *Kluyveromyces marxianus* DMKU 3-1042<br><br>(耐熱性酵母 *Kluyveromyces marxianus* DMKU 3-1042 の高温代謝フローと実験室適応) |
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Nowadays, bioethanol from cellulosic biomass is widely recognized as an alternative to fossil fuels, but the conversion is still costly for industrial application. High-temperature fermentation (HTF) is a new technology that has several benefits such as reduction of cooling cost, reduction of contamination risk and efficient saccharification and fermentation. It became trendy in ethanol fermentation especially in tropical countries. To achieve efficient ethanol production at high temperatures, yeast strain that is thermotolerant is essential. *Kluyveromyces marxianus* is a thermotolerant yeast that can grow well at high temperatures, and it has beneficial properties for industrial ethanol fermentation such as high ethanol production at high temperatures, high growth rate, short doubling times, weak glucose repression and assimilation ability of various sugars. This strain may have an intrinsic mechanism to survive at high temperatures.

In general, during the fermentation process, yeast cells are exposed to various stresses that have negative effects on cell viability and ethanol production. In addition, fermentation with lignocellulosic biomass also exposed cells to various by-products. Stresses including by-products provide negative effects on the cell growth and fermentation. There are different strategies developed such as sexual breeding, genetic engineering, mutagenesis and adaptation, to improve yeast strains.

*K. marxianus* DMKU 3-1042 that is one of the most thermotolerant strains isolated from Thailand was used for this study. It is widely utilized after complete genome sequencing. However, the mechanism of survival at high temperatures in this strain is unclear. In addition, it produces less amount of ethanol and weaker tolerance to inhibitors than *Saccharomyces cerevisiae* that is widely used in industry. For HTF, the improvement of *K. marxianus* DMKU 3-1042 is thus needed.

In this study, two research topics were performed: 1) distinct metabolic flow in response to temperature and 2) evolutionary adaptation by repetitive long-term cultivation with gradual increase of temperature for acquiring multi-stress tolerance and high ethanol productivity. To understand the mechanism of thermotolerant yeast for survival at high temperatures, several experiments were performed. At high temperatures, acetate accumulation and levels of reactive oxygen species (ROS)

increased that caused to reduce cell viability. NADPH that is a major product of the pentose phosphate pathway (PPP) is required for ROS scavenging via regeneration of glutathione. Transcriptome analysis revealed that the metabolic flow was changed from glycolysis to PPP at high temperatures. This finding suggests that this strain survives at a high temperatures by scavenging ROS via the metabolic change.

To improve yeast strain for high ethanol production and stress resistance, *K. marxianus* DMKU 3-1042 was adapted by repeated long-term cultivations at gradual increase of temperatures (RLCGT). As a result, four adapted strains were obtained. They achieved higher ethanol concentrations and exhibited stronger tolerance to multiple stresses than the parental strain at high temperatures. Interestingly, three ACT mutants showed similar phenotypes such as strong resistance to acetic acid, formic acid and low pH and increased ethanol production. In addition, to understand the mechanism of stress tolerance, the transcriptome analysis of ACT001was performed. Three genes *ICL1*, *CIT3* and *ADY2* may be related to acetic acid tolerance by maintaining a low level of intracellular acetate. Therefore, these results suggest that RLCGT is an effective evolutionary adaptation procedure for industrial ethanol fermentation.

(様式9号)

# 学位論文審査の結果及び最終試験の結果報告書

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【論文審査の結果及び最終試験の結果】

Pattanakittivorakul Sornsiri さんによる学位論文「Metabolic flow at high temperatures and laboratory adaptation in thermotolerant yeast *Kluyveromyces marxianus* DMKU 3-1042 (耐熱性酵母 *Kluyveromyces marxianus* DMKU 3-1042 の高温代謝フローと実験室適応)」について，その論文審査と口頭発表による最終試験を行った。本学位論文では，第1章で耐熱性酵母である *K. marxianus* DMKU 3-1042 の耐熱性機構を理解するため、生理学的、生化学的そして転写情報解析をすすめ、高温での細胞内の ROS の蓄積に対して代謝を改変して対応しており、それに伴って酢酸の蓄積やミトコンドリアの形態変化が起こることを示した。第2章では、*K. marxianus* DMKU 3-1042 のストレス耐性の向上を目指し、比較的長期の培養と継代を組み合わせた。得られた適応変異株は、エタノール生産性の向上が見られただけでなく、酢酸やフルフラールといった工業的に生産されるストレス原因物質に対して耐性を示した。そして適応株は、酢酸耐性に関わる遺伝子の転写向上が見られた。

本学位論文について審査委員による審査が行われ，研究内容の説明が口頭により行われた。その後，審査委員ならびに出席者からの質問を受け，それらに対して的確に回答した。これらの結果から，本論文が高度な内容を有していること，また本人が十分に本研究内容を理解して主体的に本研究を推進したことが明らかになった。また，本研究にはいくつかの独創的な内容が含まれており，しかも，それらの多くは，本人の主体的な発想と研究によって産みだされたものと判断された。

以上のことより，Pattanakittivorakul Sornsiri さんによる本研究は十分に博士号を与えるにふさわしい内容を有するものと判定された。