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

学位論文題目 (Dissertation Title)	Development of xylose-fermenting microbes for high-temperature fermentation of ethanol and 2,3-butanediol (エタノール及び 2,3 ブタンジオール高温発酵のためのキシロース発酵微生物の開発)
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In recent years, the approach of using lignocellulosic biomass as a renewable source has become a concern for biotechnology, because of reduced petroleum resources, greenhouse gas emission risks, and fluctuations in crude oil market prices. Ethanol and 2,3-butanediol (2,3-BD) are a valuable bulk chemical specially used in industries such as chemicals, cosmetics, agriculture and pharmaceuticals. Both can be produced via the fermentation process by microbes. Lignocellulose is composed of three major carbohydrate polymers, cellulose, hemicelluloses and lignin. However, lignocellulose hydrolysates contain various sugars, including xylose and glucose as an abundant sugar. Therefore, the microbial conversion of lignocellulosic biomass requires microbes with highly efficient utilization ability of both glucose and xylose.

Furthermore, during the fermentation process, microbes are simultaneously and continuously exposed to various stresses, such as high concentrations of sugars, final products including ethanol and elevated temperature in addition to lignocellulose-derived toxic compounds, which cause decrease in end-products, decrease in sugar consumption or accumulation of intermediates like xylitol in the case of xylose as a substrate.

In this study, two research objectives were conducted: 1) adaptive laboratory evolution of thermotolerant and xylose-fermenting *Candida tropicalis* for improvement of tolerance to multistress including high glucose concentrations. To improve the sensitiveness of the strain to high concentrations of glucose, repetitive long-term

cultivation with gradual increase of temperature (RLCGT) was performed in the presence of a high concentration of glucose. RLCGT is an efficient laboratory adaptation procedure for the development of adapted strains because it allows cells to be exposed to various stresses, such as accumulated metabolites including ethanol and organic acids, toxic compounds formed by chemical reactions, nutrient starvation, high temperatures and oxidative stress. Compared to previously reported laboratory adaptation procedures that were performed in the presence of main stress factors like high concentrations of substrate or high temperatures, RLCGT containing these factors at the same time may have advantages for acquiring adapted strains. As expected, an adapted strain isolated by RLCGT in the presence of 200 $g \cdot L^{-1}$ glucose was found to be resistant not only to high concentrations of glucose but also other stresses. This was the first trial of RLCGT performed in the presence of high levels of glucose, and its success may encourage us to further challenge cell capacity improvement to achieve a cost-effective fermentation for lignocellulosic bioethanol production. 2) highly efficient production of 2, 3-butanediol from xylose or glucose by newly isolated thermotolerant *Cronobacter sakazakii*. In order to obtain strains that quickly utilize xylose but hardly accumulate xylitol for efficient ethanol-producing microorganisms in xylose medium, thermotolerant xylose-fermenting microbes isolated in Lao PDR were screened for a low accumulation of xylitol in xylose medium. Isolated strains were subjected to identification and fermentation tests at high temperatures in glucose and xylose media. One strain was found to produce 2,3-butandiol (2,3-BD) at high productivity from xylose as well as glucose as a carbon source at high temperatures compared to other microbes. Therefore, it is likely that the strain has a capacity for industrial applications.

(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】

令和4年8月1日（月）、Phommachan Koudkeoさんによる学位論文「Development of xylose-fermenting microbes for high-temperature fermentation of ethanol and 2,3-butanediol (エタノール及び2,3ブタンジオール高温発酵のためのキシロース発酵微生物の開発)」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，再生可能な物質の生産を効率的に行える微生物の単離と育種を目指した。まず、グルコースやキシロースからエタノールを生産可能な酵母 *Candida tropicalis* を単離し、その株のグルコース耐性の向上のために適応育種を試みた。得られた変異株はグルコース耐性が向上しただけでなく多くの生育阻害物質に対する耐性を獲得していた。また、転写量が増加した遺伝子に膜蛋白が含まれていることが明らかとなった。一方、キシロースを資化し物質生産が可能な菌株を得ることを目的に細菌 *Cronobacter sakazakii* を単離し、この株が高温でキシロースから2,3ブタンジオールを生産すること、そしてその生産速度が優れていること示した。

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，Phommachan Koudkeo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。