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Article / Book Information

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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Increasing awareness regarding environmental problems related to the consumption of petroleum-based materials had inspired many studies aiming at the establishment of eco-friendly systems as the alternatives to the existing infrastructure. One of the proposed alternatives is bio-industry; however, it is still associated with high cost, which is chiefly attributed to cost of feedstock and purification. One of the compounds which is still mainly synthesized from petroleum derivatives is the short-chain alcohol isopropanol, even though it can be produced by microorganisms. Isopropanol is extensively used as a solvent in various industries, and to a lesser extent as disinfectant. The potential future applications of isopropanol include biofuel and precursor of propylene, which is the monomer of widely used polyester polypropylene. This study investigateed the production of isopropanol in facultative chemolitoautotrophic bacterium *Ralstonia eutropha*, which has been extensively studied especially in its capability as a polyhydroxyalkanoates (PHAs) producer, a family of biopolymer sharing many characteristics with petroleum-based plastics. *R. eutropha* had been shown as an attractive platform for the production of various bioproducts, such as alcohols and fatty acids, in addition to PHAs. Moreover, it has been shown to reutilize CO2 released during heterotrophic growth via Calvin-Benson-Bassham (CBB) cycle, which is a promising characteristic for improvement in yield of bio-products.

An isopropanol-producing strain of *R. eutropha* was constructed by introducing codon-optimized clostridial genes adh_{Cb} (encoding NADPH-dependent primary-secondary alcohol dehydrogenase (ADH) from *Clostridium beijerinckii*) and adc_{Ca} (encoding acetoacetate decarboxylase (ADC) from *Clostridium acetobutylicum*) in tandem on a plasmid into the glucose-utilizing, PHA-negative *R. eutropha* strain H16G*ΔphaC*. Although the initial strain showed only low level production of isopropanol, the block of acetoacetyl-CoA reduction pathways by deletions of *paaH1* and *had* (encoding NAD+-dependent 3-hydroxyacyl-CoA dehydrogenases) in addition to deletion of *phaB1* and *phaB3* (encoding NADPH-dependent acetoacetyl-CoA reductases) greatly increased isopropanol production from glucose. However, significant by-production of acetone was observed by the constructed strain IP-007/pBPP-adh-adc, which was likely attributed to low activity of ADH in the established strains. ADH activity was improved by replacement of P_{phaP1} for expression of adh_{Cb} and adc_{Ca} on the plasmid with the strong synthetic promoter $P_{j5[c2]}$. The resulting strain IP-007/pBj5c2-adh-adc showed higher ADH and ADC activity, and consequently high isopropanol production of 3.74 g/L (corresponding to 56% of the maximum theoretical yield) with minimal acetone by-production, which was further improved to 4.13 g/L (corresponding to 60% of maximum theoretical yield) using two-stage cultivation allowing isopropanol synthesis not associated the cell growth.

Meanwhile, chromosomal insertion of the adh_{Cb}-adc_{Ca} tandem within *pha* locus was investigated and found to be not suitable for isopropanol production. Instead, adh_{Cb}-adc_{Ca} tandem was inserted replacing *phaP1* gene and the P_{phaP1} transcription regulator gene *phaR* was deleted. The resulting strain IP-015 also produced higher amount of isopropanol relative to the strain having the adh_{Cb}-adc_{Ca} tandem within *pha* locus, but lower than IP-007/pBj5c2-adh-adc without any by-products. Two-stage cultivation of IP-015 resulted in 3.59 g/L of isopropanol, corresponding to up to 60% of maximum theoretical yield, comparable to strain IP-007/pBj5c2-adh-adc.

Next, fixation of CO_2 during heterotrophic isopropanol production was investigated and discussed. The ^{13}C abundance in isopropanol produced by IP-007/pBj5c2-adh-adc from [1-$^{13}C_1$]-glucose was determined to be 4.61%, which was much higher than 1.15% on natural glucose. This indicated actual reutilization of CO_2 emitted through sugar degradation into isopropanol, most likely by CBB cycle partially de-repressed under heterotrophic conditions in *R. eutropha*. Unfortunately, the contribution of CBB cycle to isopropanol production could not be evaluated due to severe impacts on growth and solvent production by deletion of two sets of Rubisco genes.

Taken together, the author demonstrated isopropanol production from glucose with high yields by engineered

R. eutropha strains. The quadruple deletions of *phaB1/phaB3/paaH1/had*, which is a novel modification strategy, increased production of isopropanol synthesized form acetyl-CoA precursor. The author also suggested that one of great advantages of *R. eutropha* is the ability to reutilize CO_2 emitted through sugar degradation, leading to high yield of not only P(3HB) but also other target compounds. Further engineering and optimization of culture conditions will be expected to improve the productivity.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

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