

論文 / 著書情報
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Title(English)	Isopropanol production with CO2 reutilization by engineered <i>Ralstonia eutropha</i>
著者(和文)	ディアチャンドラハップサリ スバギョ
Author(English)	Dyah Candra Hapsari Subagyo
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種別(和文)	要約
Type(English)	Outline

Title: Isopropanol production with CO₂ reutilization by engineered *Ralstonia eutropha*

Thesis: The Gram-negative, facultative chemolithoautotrophic bacterium *Ralstonia eutropha* is a well-characterized producer of biodegradable polyhydroxyalkanoates (PHAs), which share many characteristics with petroleum-based polymers. During lithoautotrophic growth, *R. eutropha* fixes CO₂ via Calvin-Benson-Bassham (CBB) cycle, which was found to be partially active and functional even under heterotrophic growth on sugar. The function of CBB cycle during heterotrophic growth resulted in the reassimilation of CO₂ emitted from various intracellular decarboxylation reactions, leading to higher PHA yield up to 122% in strains with complete CBB cycle genes compared to the CBB gene knock out strains. The effect of CO₂ reutilization in the production of non-native product isopropanol by *R. eutropha* on sugar was investigated.

Chapter 1 General introductions

- 1.1 Microbial production of value-added products from biomass
- 1.2 Hydrogen-oxidizing, polyhydroxyalkanoate-producing *Ralstonia eutropha*
 - 1.2.1 CO₂ fixation pathway in *R. eutropha*
 - 1.2.2 PHA synthesis pathway in *R. eutropha*
- 1.3 Isopropanol
 - 1.3.1 The uses and applications of isopropanol
- 1.4 Microbial production of isopropanol
 - 1.4.1 Isopropanol production by wild clostridial species
 - 1.4.2 Isopropanol production by genetically engineered clostridial strains
 - 1.4.3 Isopropanol production in genetically engineered non-clostridial hosts
- 1.5 Objectives of this study

Chapter 2 Construction of isopropanol-producing strains of *Ralstonia eutropha* and optimization of cultivation conditions

- 2.1 Introduction
- 2.2 Materials and Methods
 - 2.2.1 Bacterial strains and cultivation conditions
 - 2.2.2 General genetic engineering
 - 2.2.3 Plasmids and strains construction
 - 2.2.4 Isopropanol production
 - 2.2.5 Analytical procedures
 - 2.2.5.1 Determination of solvent concentrations
 - 2.2.5.2 Determination of glucose concentration
 - 2.2.6 Enzyme assay
- 2.3 Results
 - 2.3.1 Introduction of clostridial isopropanol biosynthesis genes into *R. eutropha* H16GΔC
 - 2.3.2 Effects of inactivation of NADPH-dependent acetoacetyl-CoA reductases on isopropanol production
 - 2.3.3 Effects of inactivation of NAD⁺-(S)-3-hydroxyacyl-CoA dehydrogenases on isopropanol production
 - 2.3.4 Effects of aeration condition on isopropanol production

- 2.3.5 Optimization of medium composition for isopropanol production
- 2.3.6 Enzyme assay
- 2.4 Discussion

Chapter 3 Isopropanol production by further engineering of *Ralstonia eutropha* in single- and two-stage cultivations

- 3.1 Introduction
- 3.2 Materials and Methods
 - 3.2.1 Bacterial strains and cultivation conditions
 - 3.2.2 General genetic engineering
 - 3.2.3 Plasmid construction
 - 3.2.4 Isopropanol production
 - 3.2.4.1 Single-stage cultivation
 - 3.2.4.2 Two-stage cultivation
 - 3.2.5 Analytical procedures
 - 3.2.5.1 Determination of solvents and glucose
 - 3.2.6 Enzyme assay
- 3.3 Results
 - 3.3.1 Replacement of promoter for expression of *adh_{Cb}-adc_{Ca}* in the plasmid-based strain
 - 3.3.2 Insertion of *adh_{Cb}-adc_{Ca}* at *phaP1* locus on the chromosome
 - 3.3.3 Isopropanol production by two-stage cultivation
 - 3.3.4 Effect of further metabolic engineering of the strain IP-015
- 3.4 Discussion

Chapter 4 Reutilization of CO₂ emitted through glucose degradation into isopropanol by engineered *R. eutropha*

- 4.1 Introduction
- 4.2 Materials and Methods
 - 4.2.1 Bacterial strains and cultivation conditions
 - 4.2.2 General genetic engineering
 - 4.2.3 Strain construction
 - 4.2.3.1 Construction of $\Delta\Delta cbbLS$ strains
 - 4.2.4 ¹³C-labelling cultivation
 - 4.2.5 Analytical procedures
 - 4.2.5.1 Determination of solvent concentrations and glucose
 - 4.2.5.2 Determination of ¹³C abundance
- 4.3 Results
 - 4.3.1 ¹³C abundance in solvents produced by engineered *R. eutropha* grown on [1-¹³C₁]-glucose
 - 4.3.2 Solvent production by $\Delta\Delta cbbLS$ strains
- 4.4 Discussion

Chapter 5 General Conclusions