論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Bioconversion of Agarose in Red Seaweed by Engineered Microbial Cells for the Production of Bioethanol
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出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第10251号, 授与年月日:2016年3月26日, 学位の種別:課程博士, 審査員:中崎 清彦,日野出 洋文,丹治 保典,江頭 竜一,吉村 千洋
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第10251号, Conferred date:2016/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨
THESIS SUMMARY

専攻： Department of	International Development Engineering 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （Philosophy）
学生氏名： Student's Name	Syazni Zainul Kamal	指導教員（主）： Academic Advisor(main)	Prof. Kiyohiko Nakasaki
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Red seaweeds have emerged to be promising biomass for the production of bioethanol, because carbohydrate that form the major component of red seaweeds, glucan and galactan, can be hydrolyze into fermentable sugars. However, because red seaweeds contain low content of glucan, the conversion of only the glucan from seaweeds is not sufficient to produce high concentration of bioethanol. Therefore, it is also necessary to produce bioethanol from galactan component of red seaweeds. In spite of many researches on saccharification and fermentation (SSF) of glucan, there are no report for agarose (galactan) saccharification and fermentation by engineered microbial cells for the production of bioethanol yet.

An α-agarase gene, *agaNash* gene, was identified from the genome of *Cellvibrio* sp. OA-2007. Firstly, an α-neoagarooligosaccharide hydrolase, AgaNash, was purified from strain OA-2007. The N-terminal amino acid sequence of AgaNash was determined. The *agaNash* gene, which encodes AgaNash, was obtained by comparing the N-terminal amino acid sequence of AgaNash with that deduced from the nucleotide sequence of the full-length OA-2007 genome. The *agaNash* gene combined with the *Saccharomyces cerevisiae* signal peptide α-mating factor was transformed into the YPH499 strain of *S. cerevisiae* to generate YPH499/pTEF-MF-agaNash, and the recombinant yeast was confirmed to produce AgaNash, though it was mainly retained within the recombinant cell. To enhance AgaNash secretion from the cell, the signal peptide was replaced with a combination of the signal peptide and a threonine- and serine-rich tract (T-S region) of the *S. diastaticus STA1* gene. The new recombinant yeast, YPH499/pTEF-STA1SP-agaNash, successfully secrete AgaNash and hydrolyze neoagarobiose thereby producing galactose, which is a fermentable sugar for the yeast, and ethanol, directly from neoagarobiose.

Then, an expression system for endo-type β-agarase was constructed for its extracellular secretion in *Brevibacillus*. *agaA* gene, which encodes agarase AgaA, derived from strain OA-2007 was cloned in pNY326 vector with secretion signal peptide upstream of the gene to construct pNY326-agaA. The resultant plasmid was transformed into *Brevibacillus choshinensis* SP3 to generate *B. choshinensis* SP3/pNY326-agaA. The recombinant *B. choshinensis* SP3/pNY326-agaA was confirmed to secrete agarase AgaA extracellularly and hydrolyzed agarose to produce neoagarooligosaccharides. Simultaneous saccharification and fermentation (SSF) of agarose was carried out by co-culture of *B. choshinensis* SP3/pNY326-agaA and YPH499/pTEF-STA1-agaNash. An Efficient production of bioethanol was observed by conducting SSF.

Lastly, a novel β-agarase gene, *agaMY* gene, with exo-acting hydrolysis mode was identified from strain OA-2007. The *agaMY* gene consisted 2364 bp nucleotide sequence, was cloned in pUC19 vector. The recombinant plasmid, pUC19-agaMY was transformed into *Escherichia coli* DHα, to generate *E. coli* DHα/pUC19-agaMY. The recombinant agarase AgaMY was confirmed to have the ability to hydrolyze neoagarotetraose and neoagarohexaose to release neoagarobiose. The complete bioconversion of agarose to produce bioethanol was conducted by employing the engineered microbial cells that was constructed in this study. Hydrolysis of agarose by *B. choshinensis* SP3/pNY326-agaA resulted to the production of neoagarooligosaccharides. By addition of crude enzyme of recombinant AgaMY into neoagarooligosaccharides solution, the concentration of neoagarobiose doubled, resulted from neoagarohexaose and neoagarotetraose decomposed to neoagarobiose. By introducing YPH499/pTEF-STA1-agaNash, bioethanol was produced from neoagarobiose with higher concentration.