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Article / Book Information

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Title(English)	Metabolic engineering of Ralstonia eutropha for production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) from biomass
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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

専攻： Department of	生物プロセス　専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　工学　）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Ralstonia eutropha has been well known as an efficient producer of polyhydroxyalkanoates (PHAs), which have been attracted as biodegradable thermoplastics. However, this bacterium can produce hard and brittle poly(3-hydroxybutyrate) [P(3HB)] from natural biomass. To improve polymer properties, our research group has constructed several recombinant strains for poly((*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)] synthesized through β-oxidation from soybean oil. Nevertheless, there has been only little information regarding β-oxidation pathway in *R. eutropha*. In this study, effects of disruption of bifunctional (*S*)-specific 2-enoyl-CoA hydratase/(*S*)-3-hydroxyacyl-CoA dehydrogenase encoded by *fadB*s on P(3HB-*co*-3HHx) biosynthesis through β-oxidation in *R. eutropha* were investigated.

Gene disruption analyses for three *fadB* homologs (*fadB1*, *fadB2*, and *fadB'*) in *R. eutropha* revealed that at least one of *fadB1* and *fadB'* was essential for growth and determination of PHA biosynthesis properties on soybean oil, while *fadB2* was presumed to be silent. *fadB1* was the better target gene than *fadB'* for modification of β-oxidation pathway, as the *fadB1* disruption led to slight increase of 3HHx composition of the PHA copolymer produced from soybean oil without significant impairment of PHA productivity. Thus, the *fadB1* disruption was further introduced into the previously engineered *R. eutropha* strains harboring *phaJ* encoding (*R*)-specific enoyl-CoA hydratase. The resulting strains showed 6~21% increase of the net amount of 3HHx unit in P(3HB-*co*-3HHx) synthesized from soybean oil. The engineered β-oxidation was expected to be useful in combination with other metabolic modifications for biosynthesis of PHA copolyesters from vegetable oils.

Another metabolic engineering was conducted for P(3HB-*co*-3HHx) production from unrelated carbon source, fructose. In this study, two kinds of *ccr* from *Streptomyces cinnamonensis* (ccr_{Sc}) and *Methylobacterium extroquens* (ccr_{Me}) were expressed in *R. eutropha*. It was clarified that overexpression of *ccr* and disruption of *phaB1* encoding a major NADPH-acetoacetyl-CoA reductase were important factors for biosynthesis of P(3HB-*co*-3HHx) from fructose. This biosynthesis might be enable by increase of flux from acetoacetyl-CoA to butyryl-CoA driven by Ccr due to the weakened consumption of acetoacetyl-CoA by the *phaB1* deletion. The Δ*phaB1* strain MF01ΔphaB1 expressing *ccr* produced P(3HB-*co*-2.4~6.7 mol% 3HHx) with 34~22 wt% PHA content from fructose, and the additional disruption of *phaB3* increased the 3HHx composition up to 17.8~19.2 mol% although the PHA content was decreased to ~7 wt%.

(*R*)-enoyl-CoA hydratase encoded by $phaJ4a_{Re}$ shows higher activity to 2-hexenoyl-CoA than to crotonyl-CoA. The co-overexpression of $phaJ4a_{Re}$ in the *phaB1*-deleted strains resulted in significant

increase of both 3HB and 3HHx units in the PHA fraction; production of 31 wt% P(3HB-*co*-5.3 mol% 3HHx) and 24 wt% P(3HB-*co*-7.2 mol% 3HHx) were achieved by MF01ΔphaB1 and MF01ΔΔphaB1B3 harboring the co-expression vector pBBRPP-ccr_{Me}J4a, respectively.

It can be supposed that formation of ethylmalonyl-CoA from butyryl-CoA by the carboxylase activity of Ccr competed with 3HHx-CoA formation from crotonyl-CoA. Therefore, site-directed mutagenesis at the predicted CO_2-binding site, Asn77 and/or Glu157 to Ala, were conducted to suppress the undesired carboxylase activity. Although the E157A mutant showed lower carboxylase activity with slightly higher reductase activity compared to the wild type enzyme, the function of the E157A mutant seemed to be weaker in *R. eutropha* than the intact Ccr_{Me}. The author further attempted to apply putative *trans*-2-enoyl-CoA reductase genes from *Methylobacillus flagellates* (ter_{Mf}) and *Pseudomonas putida* (ter_{Pp}), because Ter was reported to show NADH-dependent reductase activity to crotonyl-CoA without carboxylase and reverse oxidizing activities. However, despite the actual crotonyl-CoA reductase activity was detected in recombinant forms of Ter_{Mf}, the copolyester biosynthesis properties of *R. eutropha* MF01ΔphaB1 was not improved by introduction of the *ter* genes. This was assumed to be due to inefficient functions of the heterologous enzymes in *R. eutropha*.