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種別(和文)	要約
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Metabolic engineering of *Ralstonia eutropha* for production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from biomass

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Introduction

A bacterial copolyesters poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)] has been attracted as a flexible bioplastic, whereas efficient microbial production and compositional regulation is required to be developed for the practical applications. This study focused on engineering of *R. eutropha* for efficient production of P(3HB-*co*-HHx) from renewable carbon sources without additional supplement of precursor compounds.

Our research group has constructed several recombinant strains of *R. eutropha* H16 for P(3HB-*co*-3HHx) biosynthesis through β-oxidation of soybean oil (Fig. 1). 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* was investigated. In addition, P(3HB-*co*-3HHx) production from an unrelated carbon source, fructose was conducted.

Results and discussion

1) Modification of β-oxidation pathway in *R. eutropha* for P(3HB-*co*-3HHx) production from soybean oil

Three *fadB* homologs (*fadB1*, *fadB2* and *fadB'*) were disrupted in *R. eutropha* strain NSDG. Gene disruption of these *fadB* genes revealed that at least one of *fadB1* and *fadB'* was essential for growth of *R. eutropha* on soybean oil, while, *fadB2* was presumed to be silent from growth and PHA production. Based on the results of single gene disruption, *fadB1* was the better target gene than *fadB'* for modification of β-oxidation pathway, as *fadB1* did not give significant reduction of PHA productivity with increase of 3HHx composition of PHA copolymer produced by *R. eutropha* strain NSDG from soybean oil. Thus, the *fadB1* disruption was further introduced into previously engineered *R. eutropha* strains harboring *phaJ* encoding (*R*)-specific enoyl-CoA hydratase. The resulting strains could enhance 3HHx composition about 1-1.5 mol% in P(3HB-*co*-3HHx) from soybean oil. The engineered β-oxidation was expected to be useful in combination with other metabolic modifications for biosynthesis of PHA copolyesters from biomass resources.

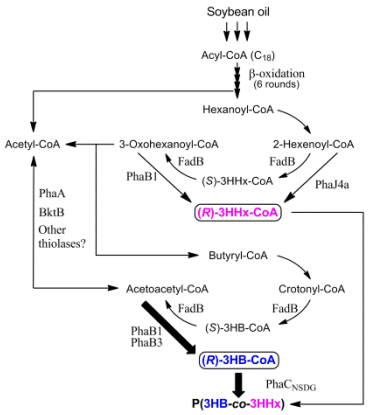

Fig. 1 Proposed P(3HB-*co*-3HHx) biosynthesis pathway from soybean oil in *R. eutropha* strain NSDG

2) P(3HB-*co*-3HHx) production by recombinant *R. eutropha* from fructose

Previous report has demonstrated that introduction of crotonyl-CoA reductase (*ccr*) gene from *Streptomycis cinnamonenis*, PHA synthase and (*R*)-specific enoyl-CoA hydratase genes from *Aeromonas caviae phaC-phaJ*$_{Ac}$) in *R. eutropha* PHB^{-}4 could promote P(3HB-*co*-3HHx) biosynthesis from fructose. However, the 3HHx fraction in the resulting PHA was less than 2mol%, so further metabolic modification has been required for production of flexible biopolymers. In this study, two kinds of *ccr* were examined in *R. eutropha* strain MF01 previously engineered by replacing the original *phaC1-phaA* within *pha* operon on chromosome 1 by *phaC*$_{NSDG}$-*bktB*$_{Re}$

encoding PHA synthase and β-ketothiolase with broad specificities. Moreover, several modifications were introduced into the metabolic pathway for provision of 3HHx-CoA from acetyl-CoA. Finally, the engineered strains were demonstrated to synthesize P(3HB-*co*-3HHx) with 7~11 mol% 3HHx fraction from fructose. This study was expected to contribute establishment of microbial production of useful bioplastics from inexpensive sugars.

Publication

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