論文 / 著書情報
Article / Book Information

題目(和文)	アミノ酸由来側鎖分岐型ユニットを含むポリヒドロキシアルカン酸の生合成
Title(English)	Biosynthesis of Polyhydroxyalkanoate Containing Branched Side-Chain Unit Derived from Amino Acid
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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	物質科学創造　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 ）

Polyhydroxyalkanoate (PHA) is a kind of polyester synthesized by various microorganisms from renewable carbon source. Poly(3-hydroxybutyrate) [P(3HB)], the most commonly seen PHA, is able to be synthesized by various bacteria, but it shows brittle and rigid properties. For commercial use of PHA, improving these unsuitable properties is strongly required. Incorporating second monomer unit such as 3-hydroxy-4-methylvalerate (3H4MV) is effective method to improve the property of P(3HB); however, it is difficult to increase 3H4MV fraction without using costly and toxic fatty acid precursors to date. The aim of this study is development of novel methods to biosynthesize PHA copolymers containing high 3H4MV fraction from sugars or sugar-derived precursors based on two approaches as described below.

In Chapter 1, general introductions about PHA, P(3HB-*co*-3H4MV) biosynthesis and amino acid metabolism were described.

In Chapter 2, it was demonstrated that 3H4MV fraction in PHA copolymer can be increased by feeding amino acid in culture medium. Among *Ralstonia eutropha* PHB^{-}4 recombinants cultured with branched amino acids, the increase in 3H4MV fraction was observed only when 10 g/L leucine was supplied. In addition, leucine analog resistant strains, especially strain 1F2, which was generated by chemical random mutagenesis, showed slightly higher 3H4MV fraction than the parent strain. Moreover, by combining strain 1F2 and 10 g/L leucine supplementation, 3H4MV fraction was increased up to 3.1 mol%, while the parent strain showed 0.9 mol% 3H4MV under the same cultivation condition. However, this difference could not be explained only by the regulation of leucine feedback system. It was suggested that they might have other mutation points specifically in the 3H4MV biosynthesis-related genes.

In Chapter 3, further investigation was performed to characterize *R. eutropha* 1F2 strain (leucine analog resistant strain) and the author attempted to elucidate 3H4MV biosynthesis pathway from sugars. To determine the mutation points in strain 1F2, genomic sequencing was performed and the sequences of *R. eutropha* PHB^{-}4 and strain 1F2 were compared. From genome analysis, it was found that A36T mutation had introduced in acetohydroxy acid synthase regulator subunit (IlvH). The IlvH is well known to regulate via feedback inhibition with end-product valine, thus strain 1F2 is not only leucine analog resistant strain but also valine over-producing strain. Also, enhanced valine production of strain 1F2 was confirmed. Based on these observations, the author proposed a 3H4MV biosynthesis pathway, where 3H4MV is synthesized by condensation of isobutyryl-CoA, one of the intermediates of valine degradation pathway, and acetyl-CoA by the action of beta-ketothiolase (BktB). In fact, over-expression of BktB in strain 1F2 led to the increase in 3H4MV fraction even from sugars as a sole carbon source. Furthermore, over 1 g/L of valine was detected in culture supernatant of strain 1F2 which cultured with fructose and 10 g/L leucine supplementation. This result suggested that the addition of leucine increased the fluxes toward valine and 3H4MV biosynthesis by reducing flux toward leucine biosynthesis.

In Chapter 4, the other approach was performed to increase 3H4MV fraction. The author attempted to construct an artificial 3H4MV biosynthesis pathway from leucine using *Clostridium difficile*'s leucine metabolism-related enzymes (LdhA and HadAIBC). An obligate anaerobic bacterium *C. difficile* has a unique metabolic pathway to convert leucine to 4M2PE-CoA which can be converted to 3H4MV by PhaJ. To synthesize 3H4MV copolymer, the LdhA and HadAIBC and PHA biosynthesis-related enzymes were co-expressed in the codon usage-improved *Escherichia coli* BL21 codonplus (DE3)-RIL. Under microaerobic culture conditions, this recombinant *E. coli* was able to synthesize P(3HB-*co*-12.2 mol% 3H4MV) from glucose with the supplementation of 1 g/L leucine. Furthermore, the author tested the feasibility of the 3H4MV copolymer synthesis from glucose. Recombinant *E. coli* BL21 codonplus (DE3)-RIL also produced P(3HB-*co*-12.6 mol% 3H4MV) by using the culture supernatant of leucine overproducer *E. coli* strain NS1391 as the culture medium. Furthermore, the recombinant *E. coli* NS1391 was able to synthesize P(3HB-*co*-3.0 mol% 3H4MV) from glucose without any leucine supplementation.

In conclusion, the author developed novel methods of biosynthesis high 3H4MV-fraction copolymer from sugars or sugar-derived precursors in this study. In *R. eutropha*, 3H4MV biosynthesis pathway was elucidated though the characterization of strain 1F2 and achieved to enhance 3H4MV fraction from sugars as a sole carbon source. In *E. coli*, this is the first report to biosynthesize 3H4MV copolymer using *E. coli* as a host strain and achieve its 3H4MV fraction over 10 mol% only from sugars. Based on these results, it may possible to further increase in 3H4MV fraction and also regulate the composition of 3H4MV copolymer.

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。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).