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Biosynthesis of medium-chain-length poly(3-hydroxyalkanoates) by metabolically engineered *Escherichia coli* strains

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Abstract

Metabolically engineered *Escherichia coli* strains harboring a plasmid containing a novel artificial polyhydroxyalkanoate (PHA) operon consisting of the *Aeromonas* PHA biosynthesis related genes and *Ralstonia eutropha* reductase gene were developed for the production of poly(3-hydroxybutyrate-co-hydroxyhexanoate) [P(3HB-co-3HHx)] from dodecanoic acid. By applying stepwise reduction of dissolved oxygen concentration (DOC) during the fermentation, the final dry cell weight, PHA concentration, and PHA content of 79 g/L, 21.5 g/L, and 27.2 wt%, respectively, were obtained in 40.8 h, which resulted in the PHA productivity of 0.53 g/L/h. The 3HHx fraction slowly increased during the fed-batch culture to reach a final value of 10.8 mol%. The 3HHx fraction in the copolymer could be increased by three fold when the *Aeromonas hydrophila orf1* gene was co-expressed with the PHA biosynthesis genes.

Introduction

Polyhydroxyalkanoates (PHAs) have been considered to be good biodegradable substitutes for petroleum-derived synthetic plastics because of their similar material properties to synthetic polymers and complete biodegradability after disposal[1]. Short-chain-length PHA (SCL-PHAs) are partially crystalline thermoplastic materials, while medium-chain-length PHA (MCL-PHAs) are sticky, elastic and amorphous materials. therefore, SCL-MCL copolymer will possess mechanical properties desired for commercial purpose. Recently, several bacteria producing PHAs containing both SCL- and MCL- monomer units were isolated[2,3]. P(3HB-co-3HHx) copolymer could be produced in PHA-negative bacterial strains by transferring the PHA biosynthesis genes of *A. caviae*[4,5]. The strategies for the production of SCL-MCL copolymers in large quantities for commercial applications have not been developed yet. Recombinant *E. coli* equipped with the PHA biosynthetic machinery has several advantages over wild type PHA producing bacteria, which include fast growth, ease of purification of PHAs and the lack of intracellular depolymerases degrading PHAs[6].

In this study, we developed several metabolically engineered *E. coli* strains harboring different plasmids containing novel artificial PHA operons consisting of the *Aeromonas* PHA biosynthesis related genes and *Ralstonia eutropha* reductase gene. We also developed a strategy for the high cell density fed-batch cultivation in order to achieve high level production of P(3HB-co-3HHx) from dodecanoic acid. Finally, the effect of co-expressing the *Aeromonas orf1* gene on the copolymer production was examined.

Materials and methods

Bacterial strains and plasmids. All strains and plasmids used in this study are listed in Table 1. *E. coli* XL1-Blue was used as a host strain for general cloning purposes (Table 1) and *E. coli* LS5218 for the production of PHA.

Culture conditions. Two wild type *Aeromonas* strains were cultured in Luria-Bertani (LB) medium at 30℃. *E. coli* XL1-Blue was grown at 37℃ in LB medium. Recombinant *E. coli* LS5218 strains were cultivated in chemically defined MR medium[2,3] supplemented with dodecanoic acid (Junsei Co., Tokyo, Japan) as a carbon source at 37℃ for the production of PHA. For the cultivation of recombinant *E. coli* strains, ampicillin (Ap, 50 mg/L) was added. Fermentations were carried out in a 2.5 L jar fermentor (Korea Fermentor Company, Incheon, Korea) containing 0.9 L of MR medium containing 5 g/L of dodecanoic acid or in a 6.6 L jar fermentor (Bioflo 3000, New Brunswick Scientific Co., Edison, NJ) containing 1.6 L of MR medium containing 5 g/L of dodecanoic acid.

Results and discussion

Recombinant *E. coli* LS5218 strains harboring various plasmids were cultivated in flasks (Table 2). When the recombinant *E. coli* LS5218 harboring prTrp3A-CnJB was cultured in a MR medium containing 5 g/L dodecanoic acid, the final P(3HB-co-3HHx) content and 3HHx fraction of 7 wt% and 5.9 mol%, respectively, were obtained in 96 h (Table 2). The recombinant *E. coli* LS5218 harboring prTrp3A-CnJBOF1 co-expressing phasin produced P(3HB-co-3HHx) up to 10 wt% of DCW with the 3HHx fraction of 18.9 mol% (Table 2), which was 3-times higher than that obtained without expression of phasin.
A new strategy was applied for the fed-batch culture of the recombinant *E. coli* LS5218 (prTrp3A-CnJBOF1) to achieve higher cell density and higher PHA content, and to enhance PHA productivity. The DOC was maintained at 40% during the lag and initial growth phase, and then reduced to 10% during the active and late growth phase (ca. DCW of 40 g/L). During the stationary phase, the DOC was further reduced to 5%. When the DOC level was decreased to 5 %, cell growth was severely inhibited, and the

PHA content and 3HHx fraction were increased. The final DCW, PHA concentration, PHA content and 3HHx fraction obtained in 40.8 h were 79 g/L, 21.5 g/L, 27.2 wt%, and 10.8 mol%, respectively, resulting in the PHA productivity of 0.53 g of PHA/L/h (Fig. 1).

In this study, we developed metabolically engineered *E. coli* strains which can produce P(3HB-co-3HHx) from fatty acid, and also developed a fermentation strategy for the high level production of P(3HB-co-3HHx) from dodecanoic acid. By applying appropriate DOC limitation, a high level of P(3HB-co-3HHx) could be produced with a relatively high productivity.

Table 1 Bacterial strains and plasmids used in this study

strains and plasmids	relevant characteristics	source or references
A. salmonicida achromogenes	wild type	this study
A. hydrophila	wild type	this study
E.coli XL1-blue	*recA1, endA1, gyrA96, thi, hsdR17, suppE44, relA1,* l^-, *lac*$^-$, *F'[proAB lacIq lacZrM15, Tn10 (tet)*^{r}*]*	Stratagene[a]
E.coli LS5218	*fadR601 atoC2 (Con)*	CGSC[b]
prTrp3A-CnJB	pBluescript SK(-) derivative. Ap^r; *rtrp* promoter; $phaC_{Asa}$, $phaJ_{Ah}$, $phaB_{Re}$	this study
prTrp3A-CnJBOF1	pBluescript SK(-) derivative; Ap^r; *rtrp* promoter; *orf1*, $phaC_{Asa}$, $phaJ_{Ah}$, $phaB_{Re}$	this study

[a]Strategene Cloning Systems, La Jolla, CA.

[b]*E. coli* genetic stock center, Yale University, New Haven, Conn.

Table 2 Flask culture of recombinant *E. coli* LS5218 strains[a]

Plasmid	DCW (g/L)	PHA conc. (g/L)	PHA content (g/L)	Composition (mol%)	
				3HB	3HHx
PrTrp3A-CnJB	2.5	0.2	7.0	94.1	5.9
prTrp3A-CnJBOF1	2.9	0.3	10.0	81.1	18.9

[a]Cells were cultured for 96 h in MR medium containing 5 g/L dodecanoic acid as a carbon source at 37℃.

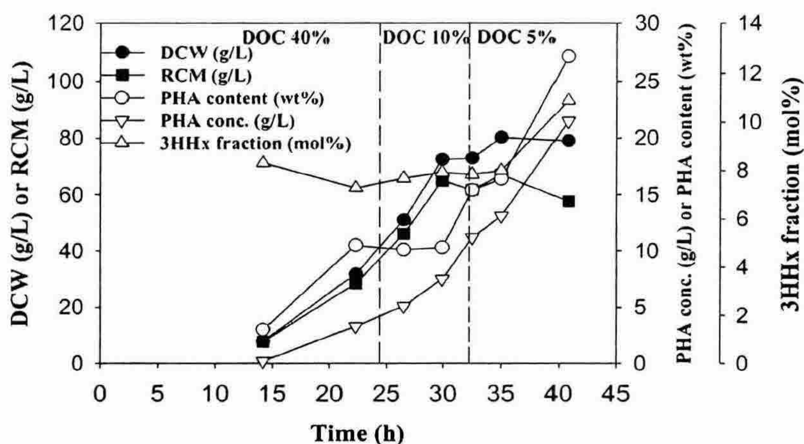

Fig. 1. Fed-batch culture of metabolically engineered *E. coli.*

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