재조합 대장균에서의 Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate)의 합성

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Production of Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) in non-mutant *Escherichia coli*

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INTRODUCTION

Polyhydroxyalkanoic acid (PHA) is an intracellular energy reserve material accumulated by a variety of bacteria under certain unbalanced growth conditions[1]. Among these polyesters, the copolymer of 3-hydroxybutyric and 3-hydroxyvaleric acids, P(3HB-co-3HV), has been of particular interest because it is more flexible than the P(3HB) homopolymer with better material properties[2].

Use of recombinant *E. coli* for the production of PHA has several advantages such as fast growth, a large amount of polymer accumulation, an ability to use various carbon sources, well-established high cell density culture techniques, and the lack of depolymerases[3].

Recently, Slater et al.[4] reported synthesis of P(3HB-co-3HV) using a mutant *E. coli* LS5218 (*fadR atoC*). They suggested that *E. coli fadR atoC* mutant, which can constitutively express the enzymes involved in short-chain fatty acid utilization, is adequate for the synthesis of P(3HB-co-3HV) copolymer. because of an inefficient system for the uptake and degradation of propionate in wild type *E. coli*. However, the uptake pathway of propionate is different from that of short-chain fatty acids such as valerate.

In this paper we studied that the difference of degradative metabolism of propionate and valerate, and the accumulation of P(3HB-co-3HV) by induction process in flask cultures in non-mutant *E. coli*. This work indicates that the restriction of various host choice caused by using a mutant strain can be removed by appropriate induction method and contributes to develop optimal P(3HB-co-3HV) production system in recombinant *E. coli*.

MATERIALS AND METHODS

Bacterial strains and plasmid DNA: Four *E. coli* strains (XL1-Blue, JM109, HB101, DH5α) are used in this study. A stable high copy number plasmid pSYL105 containing the *A. eutrophus* PHA biosynthesis genes has been previously described.[5,6]

Culture condition: A defined medium used was R medium[7] at pH 6.8 with glucose and propionate or valerate as carbon sources. This medium was supplemented with 2 g/L tryptone and ampicillin was added at a concentration of 100 μg/ml. Experiments were conducted in a 250 ml flask containing 100 ml medium at 37°C and 250 rpm.

Both the glucose and odd number fatty acids used as carbon sources had inhibitory effects on cell growth and P(3HB-co-3HV) formation. Therefore, glucose and odd number fatty acids were added at the optical density at 600nm of 0.8.

Analytical procedures: Cell growth was monitored by measuring the OD_{600} with a spectrophotometer (Beckman DU-65, USA). To measure cell mass (concentration), a 5-10 ml of culture broth was centrifuged and dried to a constant weight at 80 °C.

PHA concentration was determined by gas chromatography (Varian 3300, USA) with n-butyrate as an internal standard.

RESULTS

Growth Characteristics of *E. coli* Grown on Propionate and Valerate

The growth behavior of the *E. coli* XL1-Blue and JM109 in the defined medium containing propionate and valerate, respectively, as the sole carbon source is illustrated in Figure 1. Tryptone of 2 g/L was added to reduce lag period in the defined medium. Both XL1-Blue and JM109 grows with a relatively short lag when cultured on propionate, whereas exhibits a long lag time of 20-30 hours on valerate. 20 hours. These results indicate that the production of P(3HB-co-3HV) in recombinant *E. coli* can be likelihood through adaptation process without the use of mutant strain, and propionate is more suitable carbon source as a inducer for 3HV incorporation into polymer.

Effect of Propionate Concentration on P(3HB-co-3HV) Synthesis

The effect of propionate concentration on the 3HV fraction of the copolymer in *E. coli* XL1-Blue was investigated (Figure 2). The 3HV fraction increased from 4.3% at 10mM propionate to 9.4% at 40mM propionate with an increase in propionate concentration. Total PHA concentration and PHA content reached a maximum of 1.56 g/L and 57.8% at 20mM propionate, and decreased with further increase in propionate concentration. Higher levels of propionate were toxic to the growth of *E. coli* and PHA production.

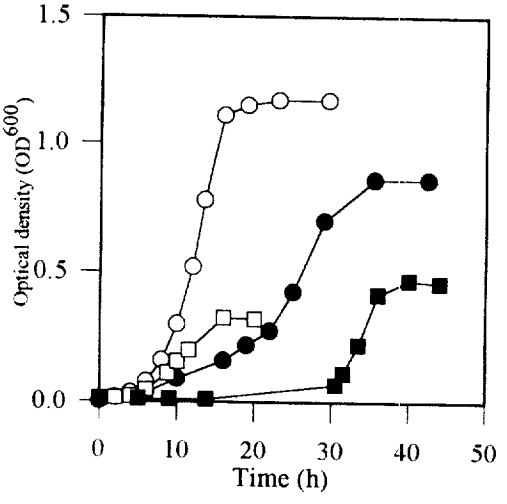

Figure 1. Growth curve of the *E. coli* XL1-Blue and JM109
○: XL1-Blue on propionate, ●: XL1-Blue on valerate,
□: JM109 on propionate, ■: JM109 on valerate

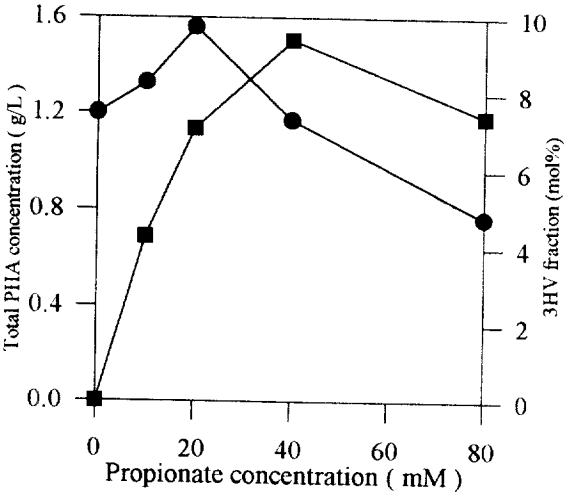

Figure 2. Effect of propionate concentration on P(3HB-co-3HV) synthesis.

Synthesis of P(3HB-co-3HV) in various *E. coli* strains

Initial test of P(3HB-co-3HV) synthesis by four *E. coli* strains was carried out in the LB and the defined medium containing 2 g/L tryptone. The cell mass, PHA concentration, PHA content, and 3HV fraction are compared in Table 1. More PHA concentration and PHA content were obtained in a defined medium than in LB medium for all strains. XL1-Blue and JM109 accumulated P(3HB-co-3HV) copolymer at a higher rate than HB101 and DH5α. The highest cell mass, PHA concentration, and PHA content were obtained with XL1-Blue; with R medium plus 2 g/L tryptone these values were 2.55 g/L, 1.39 g/L, and 54.5 %, respectively. Relatively low cell mass and PHA content seems to be due to the inhibition effect of glucose and propionate, which could be relieved by delaying the addition time of both carbon sources. When cultured on valerate, the 3HV incorporation was not induced and cell mass was reduced for all strains.

Decrease of Growth Temperature of 32 °C

We have previously shown[8] that high P(3HB-co-3HV) copolymer concentration can be obtained by decreasing the growth temperature, which resulted in less accumulation of acetate. Figure 3 shows the PHA concentration as a function of acetate induction concentration at 32 °C. When cultured on propionate, both 3HB and 3HV concentration increased significantly when induced with 10 mM acetate than without induction. The highest PHA concentration, PHA content, and 3HV fraction when induced with acetate of 10 mM were 5.86 g/L, 73.0 %, and 10.1 %, respectively. The results of oleate supplementation in the medium on the copolymer production was compared with those obtained without oleate addition (Figure 3). The highest PHA concentration and PHA content when supplemented with oleate were 5.32 g/L and 72.8 % at 10 mM acetate induction. The 3HV fraction increased fourfold by adding 1 g/L oleate without acetate induction. We also examined the effect of propionate induction, instead of acetate, on P(3HB-co-3HV) synthesis. In general, cell mass and PHA concentration reduced obviously when induced on propionate compared with on acetate. The 3HV fraction was as high as 33.0 % at propionate of 30 mM induction.

Table 1. Formation of P(3HB-co-3HV) in recombinant *E. coli* strains in LB and defined medium containing propionate.

Strain	Medium	Dry Cell Weight (g/L)	PHA concentration (g/L)	PHA content (wt%)	3HV fraction (mol%)
XL1-Blue	LB	1.85	0.60	32.4	1.6
	R + tryptone	2.55	1.39	54.5	6.5
JM109	LB	0.85	0.17	20.0	-[a]
	R + tryptone	1.68	0.83	49.4	7.2
HB101	LB	1.02	0.12	11.8	-
	R + tryptone	1.21	0.29	25.6	6.5
DH5α	LB	0.95	0.03	3.2	-
	R + tryptone	1.10	0.04	3.6	-

[a]detected as much as 3HV concentration of < 0.01 g/L

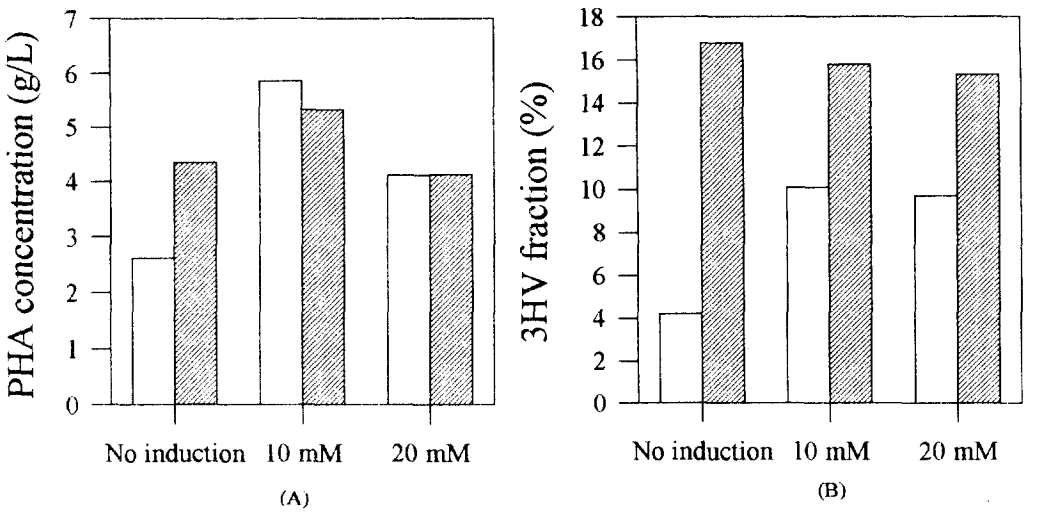

Figure 3. Effect of oleate supplementation on (A) PHA concentration, (B) 3HV fraction. X-axis represents acetate induction concentration.; □: without oleate supplementation, ▨: with oleate supplementation.

DISCUSSION

We have studied on P(3HB-co-3HV) copolymer production through induction process in recombinant *E. coli*. The main purpose of this study is to compare the uptake and degradation pathways of propionate and valerate, which have been known to induce 3HV incorporation into polymer, and to examine the factors to affect P(3HB-co-3HV) synthesis through induction process but not mutant selection in *E. coli*.

Based on our results, the most plausible pathway for the transport and degradation of propionate was described. We proposed that the transport and activation system of propionate may be similar to that of acetate. In this system, propionate is activated to propionyl-CoA by the *ack*, *pta* gene product, and the transport of propionate may be facilitated by the glyoxylate shunt enzymes. By the study on propionate utilization system, we have successfully performed the P(3HB-co-3HV) synthesis in recombinant *E. coli* without mutant selection. And it makes possible more effective P(3HB-co-3HV) production in various *E. coli* strains. Based on these results, we are currently developing an available fermentation strategy to produce a high concentration of P(3HB-co-3HV) copolymer with high productivity by recombinant *E. coli*.

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