Production and characterization of ultra-high-molecular weight poly(3-hydroxybutyrate) by recombinant *Escherichia coli*

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Abstract

An efficient fermentation strategy for the high level production of ultra-high-molecular weight poly(3-hdyroxybutyrate) (PHB) was developed. Although the cell and PHA concentrations obtained by flask cultures at different initial pH (6.0 or 6.9) were almost same level, the molecular mass of PHB produced were quite different along with the initial pH. When a recombinant *Escherichia coli* XL1-Blue harboring pJC2 containing the *Alcaligenes latus* PHB biosynthesis genes was cultivated in flask culture (pH 6.0), the PHB having a very high molecular weight of 22 MDa could be produced while only below 1 MDa at initial pH 6.9. The ultra-high-molecular weight PHB could be synthesized to high concentration of 89.8 g/L resulting in the PHB productivity of 2.07 g/L-h by simple fed-batch culture. In this study, we report that PHB having various molecular mass can be produced by employing metabolically engineered *E. coli* strains harboring the plasmids of different copy numbers containing the *A. latus phbCAB* genes.

Introduction

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters, which are accumulated as carbon/energy or reducing power storage materials in various microorganisms usually under the limiting condition of nutritional elements such as N, P, S, O, or Mg in the presence of excess carbon source[1-3]. Poly[(R)-3-hydroxybutyrate] (PHB) is accumulated as distinct granules inside the cytoplasm. Some investigators reported that when the recombinant *E. coli* was cultured at pH 6.0, which is a suboptimal growth conditions, PHB having an extremely high molecular weight was obtained[4]. The mechanical properties of this ultra-high-molecular weight PHB were markedly improved compared with the PHB produced under normal condition[4]. The ultra-high-molecular weight PHB homopolymer has been suggested to be suitable for making a strong fiber with complete biodegradability[5]. However, the development of applications has been hampered due to the lack of a strategy for producing this kind of PHB in a large quantity.

Materials and methods

Bacterial strain and plasmids. *E. coli* XL1-Blue was used as host strain (*supE44 hsdR17 recA1 endA1 gyrA96 thi relA1 lacF'[proAB' lacIq lacZ△M15 Tn10(tet^r)]*). The plasmids pJC1, pJC2. pJC3, pJC4 and pJC5 containing the *A. latus* PHA biosynthesis genes were used in this study[3].

Culture conditions. Cells were routinely grown at 30℃ for 16 h in Luria-Bertani (LB) medium. Under these conditions, PHB was not accumulated in the cells. Flask cultures were carried out in a chemically defined MR medium[2]. Fed-batch cultures were carried out at 30℃ in a 6.6 L jar fermentor (Bioflo 3000, New Brunswick Scientific Co., Edison, NJ) initially containing 1.6 L of MR medium supplemented with 20 g/L of glucose and 10 mg/L of thiamine. Seed cultures were prepared by growing cells in a shaking incubator overnight at 30℃ and 250 rpm. Culture pH was controlled by addition of 28% (v/v) ammonia water. The dissolved oxygen concentration (DOC) was controlled as desired by the automatic change of agitation speed up to 1000 rpm and addition of pure oxygen. The feeding solution contains per liter: 700 g of glucose, 15 g of $MgSO_4 7H_2O$, 250 mg of thiamine. The pH-stat feeding strategy was employed for fed-batch cultures. When pH rose to higher than its setpoint (6.0 or 6.9) by 0.1, an appropriate volume of feeding solution was automatically added to increase the glucose concentration in the culture medium to 20 g/L.

Analytical methods. Cell growth was monitored by measuring the absorbance at 600_{nm} (OD_{600}) by spectrophotometer (DU Series 600, Beckman, CA). Cell concentration, defined as cell dry weight per liter of culture broth, was determined as described previously[3]. PHA concentration was determined by gas chromatography with n-benzoic acid as an internal standard[3]. The PHA content (wt%) was defined as the percentage of the ratio of PHA concentration to cell concentration. PHB was purified by chloroform extraction. Cells were collected by centrifugation and were washed with hot acetone. After drying, cells were mixed with 50 volumes of chloroform for 48 h at 30℃. Clear PHB solution was recovered by centrifugation, which was followed by polishing filtration. Finally, pure PHB was obtained by non-solvent precipitation and filtration. The number average molecular mass (M_n) of PHB sample was determined by gel permeation chromatography. Shodex SM-105 polystyrene and chloroform were used as a molecular weight standard and mobile phase, respectively.

Results and discussion

Five recombinant *E. coli* strains harboring different plasmids were cultivated in flasks at 30℃ and pH 6.0 or 6.9 in MR medium containing 20 g/L of glucose. As shown in Table 1, the highest cell and PHA concentrations of 6.71 g/L and 4.76 g/L, respectively, were obtained by employing *E. coli* XL1-Blue (pJC4). However, the molecular mass of PHB obtained with XL1-Blue (pJC2) was the highest among these recombinant *E. coli* strains. Also, the cell and PHA concentrations obtained at pH 6.0 were almost same as those obtained at pH 6.9, however, the molecular mass of PHB produced were much higher than that at pH 6.9. According to the previous reports[4,5], the major factors regulating the molecular mass of PHB have been suggested to be the activity of PHA synthase and a hypothesized chain transfer agent. In conclusion, the ultra-high-weight PHB could be synthesized to high concentration of 89.8 g/L resulting in the PHB productivity of 2.07 g/L by fed-batch fermentation at pH 6.0.

Table 1 Flask culture of recombinant *E. coli* strains at 30℃ and pH 6.9

	pJC1	pJC2	pJC3	pJC4	pJC5
Cell concentration (g/L)	4.69	5.21	6.45	6.71	6.05
PHB concentration (g/L)	1.83	2.76	4.64	4.76	4.54
PHB content (wt%)	39	53	72	71	75
Molecular mass (kDa)	870	970	890	910	900

Table 2 Flask culture of recombinant *E. coli* strains at 30℃ and pH 6.0

	pJC1	pJC2	pJC3	pJC4	pJC5
Cell concentration (g/L)	4.19	5.02	6.15	6.31	5.53
PHB concentration (g/L)	1.55	2.76	4.31	4.71	3.93
PHB content (wt%)	37	55	70	73	71
Molecular mass (MDa)	13	22	18	19	19

Acknowledgement

This work was supported by the Ministry of Science and Technology and by the LGCI, Ltd.

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