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Production of Poly(3-hydroxybutyrate) [P(3HB)] with High P(3HB) Content by Recombinant *Escherichia coli* Harboring the *Alcaligenes latus* P(3HB) Biosynthesis Genes and the *E. coli ftsZ* Gene

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Abstract Filamentation-suppressed recombinant *Escherichia coli* strain harboring the *Alcaligenes latus* polyhydroxyalkanoate (PHA) biosynthesis genes and the *E. coli ftsZ* gene was constructed and cultivated for the production of poly(3-hydroxybutyrate) [P(3HB)] with high concentration and high content. By the pH-stat fed-batch culture of this recombinant *E. coli* strain XL1-Blue(pJC5), the final cell concentration and P(3HB) concentration obtained in 44.25 h were 172.2 g cell dry weight/l and 141.9 g P(3HB)/l, respectively, resulting in productivity of 3.21 g P(3HB)/l-h. More importantly, the P(3HB) content obtained was 82.4 wt %, which was significantly higher than that obtained with the recombinant *E. coli* harboring only the PHA biosynthesis genes.

Key words: Poly(3-hydroxybutyrate), polyhydroxyalkanoate biosynthesis genes, filamentation-suppressed recombinant *Escherichia coli*

Poly(3-hydroxybutyrate) [P(3HB)], the widely studied and the best characterized member of polyhydroxyalkanoates (PHAs), is synthesized and intracellularly accumulated by numerous microorganisms [1, 8, 12, 20] for the energy and/or carbon storage material. Recently, problems concerning the global environment have created much interest in the development of biodegradable polymers. P(3HB) and other PHAs are good candidates for biodegradable plastics and elastomers since they possess material properties similar to those of synthetic polymers and are completely biodegradable when disposed [8, 9, 12, 18].

In order to understand the PHA biosynthetic pathways, PHA biosynthesis genes were cloned and closely analyzed at a molecular level in various bacteria. After the first attempt in cloning the PHA biosynthesis genes from *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*), more than 40 different types of PHA biosynthesis genes were cloned from different bacteria [21]. Cloning of various PHA biosynthesis genes has not only provided detailed information regarding the structure and organization of the PHA biosynthesis genes, but also created genetically engineered microorganisms for a more efficient production of this biodegradable polymer [4, 13, 15].

Alcaligenes latus has received much attention not only for being a candidate as a P(3HB) producer, but also for its ability to create P(3HB) with high yield [3, 15, 22]. Recently, the *A. latus* PHA biosynthesis genes were cloned in *Escherichia coli* strains [7]. By the pH-stat fed-batch culture of the recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes, the final cell concentration, P(3HB) concentration, and P(3HB) content obtained were 194.1 g/l, 141.6 g/l, and 73 wt %, respectively [7]. However, the P(3HB) content in recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes was lower than that of other P(3HB) producing recombinant *E. coli* strains harboring the *R. eutropha* PHA biosynthesis genes. From the economic viewpoint on the process for the production of P(3HB) by bacterial fermentation, it was shown that high P(3HB) content was the most important requirement for the economical production of P(3HB) [5]. It was reported that recombinant *E. coli* with amplified FtsZ activity could more efficiently synthesize P(3HB) in a chemically defined medium due to the suppression of filamentation [11]. Recombinant *E. coli* harboring the *R. eutropha* PHA biosynthesis genes and the *E. coli ftsZ* gene produced a larger amount of P(3HB) with higher P(3HB) content than recombinant *E. coli* strains harboring only the PHA biosynthesis genes [11]. In this paper, we describe the construction of filamentation-suppressed recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes and the *E. coli ftsZ* gene, and its use for the production of P(3HB) with high content.

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Culture Condition for P(3HB) Production

E. coli XL1-Blue (*supE44 hsdR17 recA1 endA1 gyrA96 thi⁻ relA1 lacF'[proAB⁺lacIqlacZΔM15Tn10(tet')]*) was used as a host strain in this study [7]. Plasmids pJC1, pJC2, pJC3, and pJC4 containing the *A. latus* PHA biosynthesis genes have been previously described [7]. For the flask culture of recombinant *E. coli*, chemically defined R medium was used [11]. Seed and fed-batch cultures of the recombinant *E. coli* were carried out in a chemically defined MR medium [23]. Separately sterilized glucose and thiamine were supplemented into the medium at final concentrations of 20 g/l and 10 mg/l, respectively. Fed-batch culture was carried out in a jar fermentor (6.6 l, Bioflo 3000, New Brunswick Scientific Co., Edison, NJ, U.S.A.) at 30°C consisting of 1.2 l of the initial MR medium. Detailed procedures were previously described [23].

Cell concentration was defined as cell dry weight per liter of the culture broth. P(3HB) concentration was determined by gas chromatography (HP5890, Hewlett-Packard, Wilmington, DE, U.S.A.) with benzoic acid as an internal standard [2]. Residual cell concentration was defined as cell concentration minus P(3HB) concentration. P(3HB) content was expressed as the percentage of the ratio of P(3HB) weight to the cell dry weight.

Construction of Plasmid Containing the *A. latus* PHA Biosynthesis Genes and the *E. coli ftsZ* Gene: Cell Growth and P(3HB) Synthesis in Recombinant *E. coli* Strain

Plasmid DNA isolation, agarose gel electrophoresis, and transformation of *E. coli* were carried out as described by

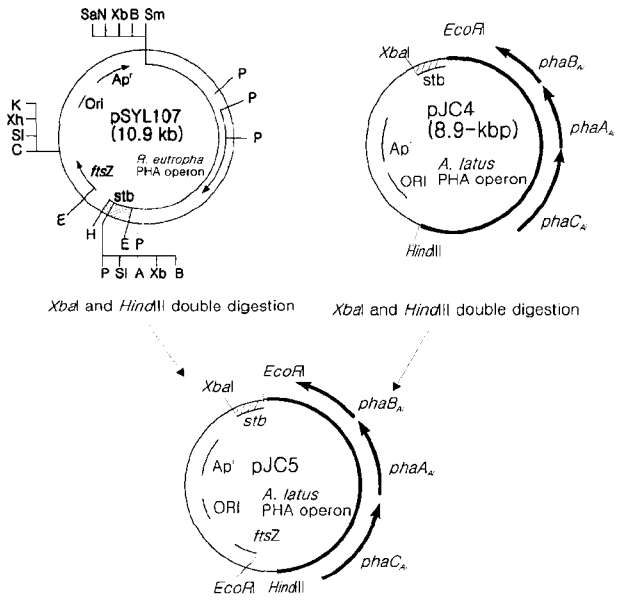

Fig. 1. Construction of plasmid pJC5.
The abbreviations are: Ap, ampicillin; r, resistance; ORI, origin of replication; stb, the *parB* locus of plasmid R1; *ftsZ*, *E. coli ftsZ* gene.

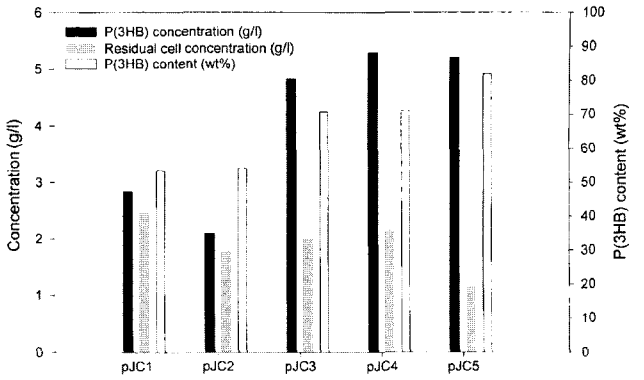

Fig. 2. Final P(3HB) concentration, residual cell concentration, and P(3HB) content obtained with five recombinant *E. coli* strains harboring the *A. latus* PHA biosynthesis genes after cultivation at 30°C for 66 h in a defined R medium containing 20 g/l of glucose.

Sambrook *et al.* [19]. A stable high copy number plasmid containing the *A. latus* PHA biosynthesis genes, pJC4, was digested with *Hin*dIII-*Xba*I, and ligated with the *Hin*dIII-*Xba*I fragment of pSYL107 [11] containing the *E. coli ftsZ* gene. The restriction map of the resulting plasmid, pJC5, is shown in Fig. 1.

Recombinant *E. coli* strains harboring 5 different plasmids (pJC1, pJC2, pJC3, pJC4, and pJC5) containing the *A. latus* PHA biosynthesis genes were cultivated for 66 h in a defined R medium supplemented with 20 g/l of glucose and 50 mg/l of ampicillin at 30°C (Fig. 2). XL1-Blue (pJC4) and XL1-Blue (pJC5) grew to higher cell concentrations and accumulated more P(3HB) than other recombinant strains. The final P(3HB) concentrations obtained with XL1-Blue (pJC4) and XL1-Blue (pJC5) were 5.30 and 5.21 g/l, respectively. However, the P(3HB) content obtained with XL1-Blue (pJC5) was much higher than that obtained with the recombinant *E. coli* XL1-Blue (pJC4). The P(3HB) contents obtained with XL1-Blue (pJC4) and XL1-Blue (pJC5) were 71.1 wt% and 81.9 wt%, respectively. By comparing the results obtained with pJC series of plasmids, it should be noted that a higher P(3HB) content could be achieved by cloning the *E. coli ftsZ* gene into the plasmid containing the *A. latus* PHA biosynthesis genes.

Production of P(3HB) by Fed-Batch Culture of Recombinant *E. coli* Harboring the *A. latus* PHA Biosynthesis Genes and the *E. coli ftsZ* Gene

To examine the production of P(3HB) by using the recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes along with the *E. coli ftsZ* gene, the fed-batch culture of recombinant *E. coli* XL1-Blue (pJC5) was carried out. Since it was previously shown that the pH in broth increased when glucose was consumed, the pH-stat feeding method was employed [23]. The time profiles of the concentrations of cell and P(3HB) and P(3HB) content

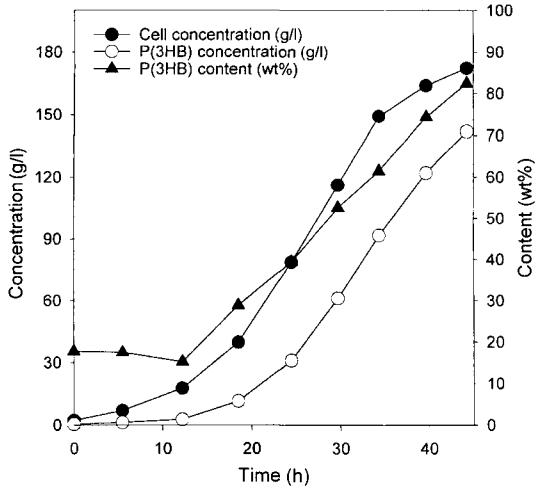

Fig. 3. Time profiles of cell concentration, P(3HB) concentration, and P(3HB) content during the pH-stat fed-batch culture of recombinant *E. coli* XL1-Blue (pJC5) in a chemically defined medium.

are shown in Fig. 3. The final cell concentration, P(3HB) concentration, and P(3HB) content were 172.2 g cell dry weight/l, 141.9 g P(3HB)/l, and 82.4 wt %, respectively, resulting in the productivity of 3.21 g P(3HB)/l-h.

Recently, it was reported that a P(3HB) concentration of 141.6 g/l with P(3HB) content of 73 wt % was obtained by the pH-stat fed-batch culture of recombinant *E. coli* XL1-Blue (pJC4) [7]. Although high concentration of P(3HB) was obtained with high P(3HB) productivity, the P(3HB) content was lower than that obtained with other recombinant *E. coli* strains harboring the *R. eutropha* PHA biosynthesis genes. Plasmid pJC4 contained the *A. latus* PHA biosynthesis genes but lacked the *E. coli ftsZ* gene. According to the previous study on the P(3HB) production by recombinant *E. coli* harboring the *R. eutropha* PHA biosynthesis genes, recombinant *E. coli* harboring the PHA biosynthesis genes and the *E. coli ftsZ* gene produced higher concentration of P(3HB) with higher P(3HB) content than that harboring only the PHA biosynthesis genes [11, 23]. Recombinant *E. coli* XL1-Blue (pJC5) harboring the *A. latus* PHA biosynthesis genes and the *E. coli ftsZ* gene produced higher concentration of P(3HB) with higher P(3HB) content compared with *E. coli* XL1-Blue (pJC4). This result suggests that co-expression of the *E. coli ftsZ* gene with the PHA biosynthesis genes enhances P(3HB) synthesis in recombinant *E. coli*. Also, the P(3HB) yield on glucose increased from 0.27 g P(3HB)/g glucose for XL1-Blue (pJC4) to 0.29 g P(3HB)/g glucose for XL1-Blue (pJC5). The high P(3HB) content and high P(3HB) yield on the carbon substrate significantly decrease the production cost of P(3HB) by bacterial fermentation [5]. Furthermore, P(3HB) granules were easily recovered from *E. coli* cells having high P(3HB) content by simple digestion [6].

There are more than 300 different microorganisms that are known to synthesize PHAs, but only a few bacteria such as *R. eutropha*, *A. latus*, and *Azotobacter vinelandii* have been employed for the production of P(3HB) [12, 15]. However, P(3HB) production by these native P(3HB) producers have a problem of high production cost for the commercialization of P(3HB) as the bulk plastic material [5]. In order to broaden the utilizable substrate range, to enhance PHA synthetic capacity, and to produce novel PHA, various recombinant bacteria have been developed [13]. *E. coli* has several advantages such as fast cell growth, established fermentation technology, and well-known metabolism, making it to become a suitable host strain for the production of P(3HB) [14, 16, 17]. Recombinant *E. coli* can also produce a high content of copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) to a high content [24, 25] and other PHAs [10]. In addition to these advantages, the results from this study suggest that more economical production of P(3HB) is possible because the newly developed recombinant *E. coli* strain allows accumulation of P(3HB) to a high content.

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