Production System for Biodegradable Polyester Polyhydroxybutyrate by *Corynebacterium glutamicum*

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Introduction

Poly-3-hydroxybutyrate [P(3HB)] is biologically produced polyester that has much attention as biodegradable polymer and can be produced from biorenewable resources. Despite the readily apparent benefits of using P(3HB) as replacement for petrochemical derived plastics, the use and distribution of P(3HB) have been limited by its cost of production. This problem is currently being addressed by the engineering of enzymes involved in the production of P(3HB) [1, 2]. Although several approaches by genetic engineering to improve P(3HB) productivity have been reported, they have been almost used in Gram-negative bacteria including *Escherichia coli, Ralstonia eutropha*, and *Pseudomonas* strains [3]. For medical or food contact applications of P(3HB), bacterial host selection for the production should be carefully considered, because Gram-negative bacteria have a poisonous substance, endotoxin. To avoid the harmful contaminants, the use of Gram-positive bacteria for P(3HB) production would be preferable. *Corynebacterium glutamicum* is an aerobic, Gram-positive, and nonsporulating bacterium that has been employed for the industrial production of several amino acids which have been used in food, feeds and pharmaceutical products for several decades [4,5].

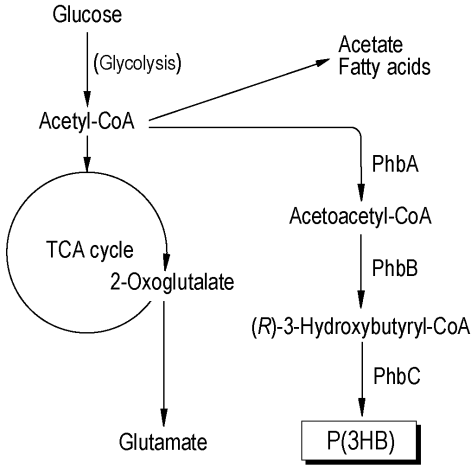

Figure 1. Metabolic linkage between glutamate synthetic pathway and P(3HB) synthetic pathway

An artificial P(3HB) synthetic pathway was newly introduced bty connecting with the glutamate synthetic pathway (See Fig. 1). Acetyl-CoA is a starting substance required for the P(3HB) biosynthetic pathways consisting of 3-hydroxybutyryl-CoA (3HB-CoA) synthesis [catalyzed by β-ketothiolase (PhbA) and NADPH-dependent acetoacetyl-CoA reductase (PhbB)] and polymerization of 3HB-CoA [catalyzed by P(3HB) synthase (PhbC)] to P(3HB).

Experimental

Bacterial strains and plasmids. *Escherichia coli* JM109 was used for genetic manipulation and *C. glutamicum* ATCC13869 was used as a host for the P(3HB) biosynthetic genes (*phbCAB*) expression. Two plasmids, pPGEM-*phbCAB* and pPS-*phbCAB*, were constructed to express P(3HB) biosynthetic gene operon under control of promoters from *R. eutropha* and *C. glutamicum*, respectively.

Results and discussion

Growth and PHB production in recombinant *C. glutamicum*. Maximum cell growth after 72 h cultivation was observed at 27°C and pH 7.5, while the highest P(3HB) production was obtained at 30°C and pH 7.5. Therefore, cultivation for the production of P(3HB) was perfomed at 30°C and pH 7.5. Time courses of cell growth and P(3HB) production were laid to overlap each other. Basically, synchronized pattern was obtained between cell growth and P(3HB) production during the cource of cultivation. The cell growth reached 12.5 mg/ml at 36 h, while P(3HB) content linearly increased up to 48 h and then reached the plateau level corresponding to about 22.5% (w/w) until 96 h cultivation.

Transmission electron micrograph (TEM). Intracellular P(3HB)s were directly observed as inclusion granules within the cell of *C. glutamicum* harboring pPS-*phbCAB* by TEM, as shown in Fig. 2. Shape and size of the fully grown cells which accumulate P(3HB) granules were hardly changed, on contrary to the filamentous morphologenesis often observed for recombinant *E. coli* producing P(3HB) [6].

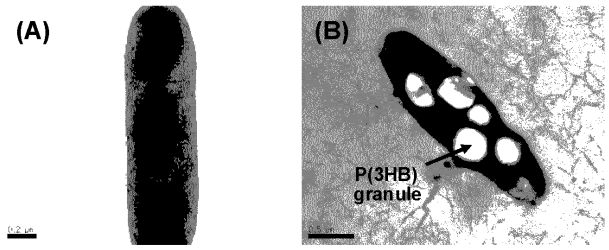

Figure 2. TEM images of recombinant *C. glutamicum* cells harboring pPGEM-*phbCAB* **(A)** and pPS-*phbCAB* **(B)**. P(3HB) accumulation was observed as granules in the cytoplasm of the cell **(B)**. Bars indicate 0.2 μm **(A)** and 0.5 μm **(B)**, respectively.

Gel permeation chromatography (GPC). GPC analysis of the synthesized P(3HB) prepared from 72 h cultivation cells revealed that the number-average molecular weight (Mn) and polydispersity were 2.1 x 10^5 and 1.63, respectively, which were different from those of P(3HB) (1.8 x 10^6 of Mn and 1.8 of polydispersity) synthesized in recombinant *E. coli* harboring *phbCAB* operon from *R. eutropha*.

Conclusions

This is the first report demonstrating the endotoxin-free production system of P(3HB) in recombinant *C. glutamicum* by constructing the artificial P(3HB) biosynthetic pathway channeling from acetyl-CoA. This beneficial system will facilitate the next generation researches such as biosynthesis of 3HB-based copolymers with desirable properties and double industrial production of biopolymers with amino acids from renewable carbon sources.

References

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