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Short Communication

A novel strategy for succinate and polyhydroxybutyrate co-production in *Escherichia coli*

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ABSTRACT

Based on the metabolic analysis, a succinate and polyhydroxybutyrate (PHB) co-production pathway was designed and engineered in *Escherichia coli* MG1655. Batch cultivation of the engineered *E. coli* revealed that it was able to accumulate both extracellular succinate and intracellular PHB simultaneously. PHB accumulation not only improved succinate production, but also reduced pyruvate and acetate secretion. With a consumption of 45 g l^{-1} glucose, *E. coli* QZ1112 was shown to accumulate 24.6 g l^{-1} succinate and 4.95 g l^{-1} PHB in batch fermentation. The PHB content reached 41.3 wt.% of its cell dry weight, which suggested that the cell debris can be used as value added by-product.

1. Introduction

Many chemicals and materials, including two important products with application potential, succinic acid and polyhydroxyalkanoates (PHAs), can be generated biologically from renewable substrates (Ragauskas et al., 2006; Sauer et al., 2008). PHAs are normally accumulated intracellularly by various bacteria and archaea under stressed conditions as carbon and energy storage materials (Anderson and Dawes, 1990; Lee, 1996). Because of their biodegradable and biocompatible properties, PHAs have been extensively studied as potential "green" substitutes for petroleum derived polymers in drug delivery, agriculture, fibers industry and consumer products (Aldor and Keasling, 2003; Steinbüchel and Füchtenbusch, 1998). Polyhydroxybutyrate (PHB), the best known PHA, has been intensively studied as a model product in the development of biotechnological strategies (Suriyamongkol et al., 2007). Succinate, which is a four-carbon dicarboxylic acid, is secreted extracellularly by many anaerobic bacteria, including *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens* and *Fibrobacter succinogenes* (Gokarn et al., 1997; Lee et al., 2002; Van der Werf et al., 1997). Succinate has also attracted much attention due to its value as a precursor to various commodity chemicals used in industries like food, pharmaceutical, detergent and polymer (Sauer et al., 2008).

Escherichia coli, a model micro-organism, had been successfully engineered for production of a wide variety of recombinant proteins and many important materials over the past decades. Although natural *E. coli* accumulates little succinate (under anaerobic conditions) and no PHA, it was considered as one of the best candidates for succinate and PHA production (Li et al., 2007b; Sanchez et al., 2005). The production efficiency of succinate (Isar et al., 2006; Lin et al., 2005) and PHB (Li et al., 2007b) in *E. coli* had been improved greatly by employing various metabolic engineering strategies. Many engineered *E. coli* strains with high production titer were obtained through these strategies (Jantama et al., 2008; Zhang et al., 2009).

In the present study, we designed a novel strategy for co-production of succinate (extracellular) and polyhydroxybutyrate (intracellular) in *E. coli*. Compared with single product process, this co-production strategy can utilize the substrate more effectively, accumulate fewer by-products and make full use of cell debris.

2. Methods

2.1. Strains and plasmids

E. coli MG1655 (F^- λ^-) served as parent strain for the construction of *E. coli* QZ1111 with five mutations (Δ*sdhA*, Δ*ptsG*, Δ*poxB*, Δ*pta* and Δ*iclR*). Stress-induced plasmid pQKZ103 (Kang et al., 2008) was used to express *phbCAB* genes from *Ralstonia eutropha* in *E. coli* QZ1111.

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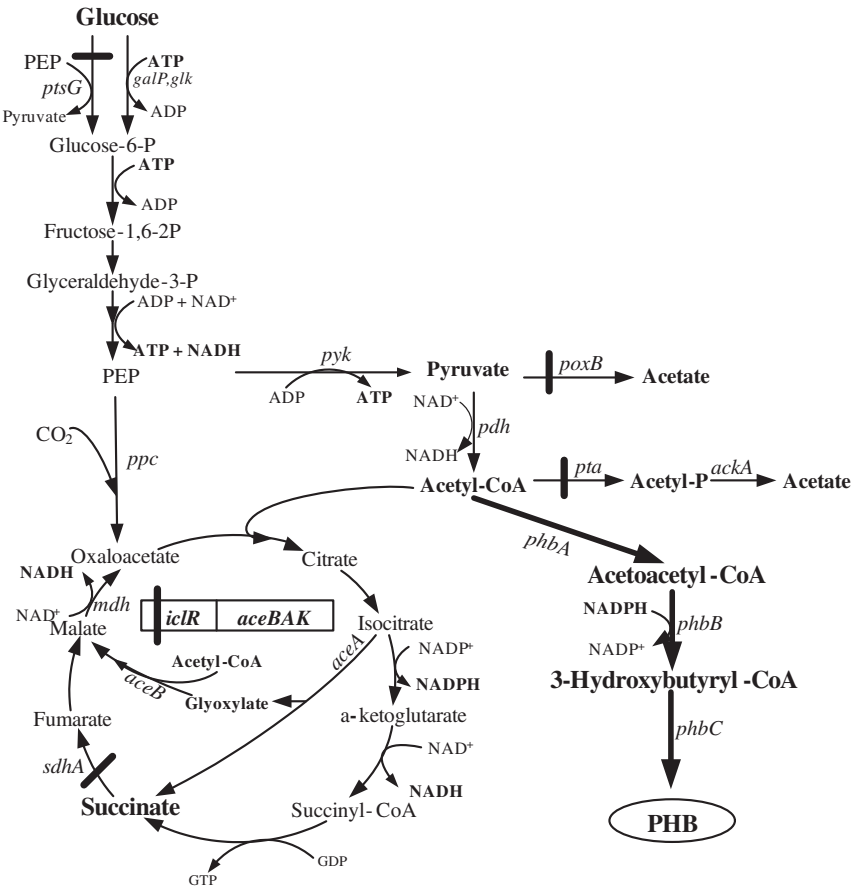

Fig. 1. The designed metabolic pathways for the co-production of succinate and PHB in *E. coli* QZ1112. The bars indicated the knockout site. PHB synthesis pathway was marked with bold black lines. PEP: phosphoenolpyruvate.

2.2. Cultivation medium and cultivation conditions

LB medium (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, and 10 g l^{-1} NaCl) with 10 g l^{-1} glucose and 2 g l^{-1} $NaHCO_3$ was used as cultivation medium. Ampicillin and/or kanamycin were added at a concentration of 100 μg/ml and 50 μg/ml when necessary. Aerobic batch fermentation was performed in 5 l bioreactor (Zhenjiang China). A 5% (v/v) inoculum was used. The pH was measured and controlled at 7.0 using a glass electrode with 2 M NaOH. During the fermentation, the dissolved oxygen was monitored using a polarographic oxygen electrode and was maintained above 80% saturation.

2.3. Analytical methods

Optical density (OD) was measured at 600 nm with a spectrophotometer; the culture was diluted to the linear range with 0.15 M NaCl. For analyzing the extracellular metabolites, 1 ml of culture was centrifuged (12,000*g* for 2 min at 4 °C) and the supernatant was then filtered through a 0.22 μm syringe filter for HPLC analysis. The HPLC system was equipped with a cation exchange column (HPX-87H, BioRad Labs), a UV detector (Shimadzu SPD-10A) and a differential refractive index (RI) detector (Shimadzu RID-10A). A 0.5 ml/min mobile phase using 5 mM H_2SO_4 solution was applied to the column. Standards were prepared for glucose, succinate, acetate, and pyruvate for both the RI detector and UV detector, and calibration curves were created. The column was operated at 65 °C. Succinate, glucose, and acetate were measured by the differential refractive index (RI) detector, while pyruvate was measured by the UV detector at 210 nm (Lin et al., 2005).

Cell concentration was defined as the amount (dry weight) of cells per liter of culture broth. PHB was quantitatively analyzed by gas chromatography (GC) (Li et al., 2007a). The PHB content was defined as the percent ratio of PHB concentration to cell concentration. The concentration of NADP(H), NAD(H) and the ratio of NADPH/$NADP^+$ was measured by $NADP^+$/NADPH Quantification Kit (BioVision). Specifically, the flask cultures at 12 h were harvested and measured.

3. Results and discussion

3.1. Designing and construction of a succinate and PHB production pathway

To achieve this strategy, we performed the following genetic modification of the host. First, we developed in *E. coli* the succinate production pathway accompanied with the aim for acetyl-CoA accumulation. In *E. coli*, acetyl-CoA generated from pyruvate mainly enters the TCA for energy and cell intermediates production (Vadali et al., 2004). Succinate, as an intermediate of the TCA cycle, is formed under aerobic conditions by succinyl-CoA synthetase and subsequently converted to fumarate by succinate dehydrogenase (SdhABCD). As a result, succinate does not accumulate in wild-type *E. coli* culture under aerobic condition.

To realize succinate accumulation, inactivation of *sdhA* gene to block the conversion of succinate to fumarate in TCA cycle is necessary. Meanwhile, polyhydroxybutyrate (PHB) accumulation in