



## Short Communication

# Biosynthesis of poly(3-hydroxypropionate) from glycerol by recombinant *Escherichia coli*



Qi Wang<sup>a,b</sup>, Peng Yang<sup>a,c</sup>, Changshui Liu<sup>a,b</sup>, Yongchang Xue<sup>c</sup>, Mo Xian<sup>a,\*</sup>, Guang Zhao<sup>a,\*</sup>

<sup>a</sup> Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

<sup>b</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>c</sup> School of Biological Engineering, Dalian Polytechnic University, Dalian 116034, China

## HIGHLIGHTS

- ▶ A P3HP biosynthetic pathway from glycerol was constructed in *Escherichia coli*.
- ▶ Utilization of glucose as cosubstrate improved P3HP production greatly.
- ▶ Fermentation process for P3HP production was simplified.
- ▶ The highest P3HP production from inexpensive carbon source was achieved.

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## ABSTRACT

Poly(3-hydroxypropionate) (P3HP) is a biodegradable and biocompatible thermoplastic. In this study, a P3HP biosynthetic pathway from glycerol was constructed in recombinant *Escherichia coli*. The genes for glycerol dehydratase and its reactivating factor (*dhaB123* and *gdrAB*, from *Klebsiella pneumoniae*), propionaldehyde dehydrogenase (*pduP*, from *Salmonella typhimurium*), and polyhydroxyalkanoate synthase (*phaC1*, from *Cupriavidus necator*) were cloned and expressed in *E. coli*. After culture condition optimization, the final engineered strain accumulated 10.1 g/L P3HP (46.4% of the cell dry weight) using glycerol and glucose as cosubstrates in an aerobic fed-batch fermentation. To date, this is the highest P3HP production without addition of any expensive precursor.

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## 1. Introduction

Poly(3-hydroxypropionate) (P3HP) is an interesting polymer exhibiting high rigidity, ductility, and exceptional tensile strength in drawn films, and was regarded as one of the alternatives to petrochemical-derived plastic (Andreeßen and Steinbüchel, 2010). Thus far, P3HP cannot be produced by any known organisms. The first attempt to produce PHA polymer containing 3-hydroxypropionate (3HP) arose in 1991 (Nakamura et al., 1991). Ever since then, a series of strains were reported to produce PHA copolymer containing 3HP dependently on structurally related precursors, such as 3HP and 1,3-propanediol (Ichikawa et al., 1996; Meng et al., 2012). However, the addition of these expensive precursors increased P3HP production cost.

To solve this problem, two artificial pathways were constructed recently for P3HP biosynthesis from inexpensive carbon sources,

glucose and glycerol, without addition of any precursor. Unfortunately, only 13 mg/L P3HP was produced by the recombinant *Escherichia coli* strain from glucose probably due to the low activity of enzymes in the P3HP pathway (Wang et al., 2012). In the case of glycerol conversion, a recombinant *E. coli* strain carrying glycerol dehydratase genes *dhaB12* of *Clostridium butyricum*, propionaldehyde dehydrogenase gene *pduP* of *Salmonella typhimurium*, and PHA synthase gene *phaC1* of *Cupriavidus necator* accumulated 1.42 g/L P3HP (Andreeßen et al., 2010). Due to this glycerol dehydratase is active only under strict anaerobic conditions, the fed-batch fermentation was carried out in an aerobic growth phase and an anaerobic P3HP production phase. Disodium fumarate and potassium sodium tartrate were used to maintain the anaerobic conditions. Compared with other PHA production, the P3HP yield is relatively low. Taken together with the complicated fermentation process and cost of disodium fumarate and potassium sodium tartrate, it is necessary to carry out further developments to achieve high P3HP yield from glycerol.

In this study, a new strategy converting glycerol to P3HP was developed (Fig. 1). We employed glycerol dehydratase of *Klebsiella*

\* Corresponding authors. Tel.: +86 532 80662767; fax: +86 532 80662765.

E-mail addresses: [xianmo@qibebt.ac.cn](mailto:xianmo@qibebt.ac.cn) (M. Xian), [zhaoguang@qibebt.ac.cn](mailto:zhaoguang@qibebt.ac.cn) (G. Zhao).

*pneumonia*, which is functional along with its reactivase GdrAB under both aerobic and anaerobic conditions and has been applied in production of 3HP and 1,3-propanediol (Huang et al., 2012; Rathnasingh et al., 2009). Under the optimized culture conditions, the recombinant cells produce 21.8 g/L cell dry weight containing 46.4% (wt/wt [cell dry weight]) P3HP in an aerobic fed-batch fermentation. To date, it is the highest P3HP production without addition of any expensive precursor.

## 2. Methods

### 2.1. Bacterial strains and plasmids construction

All bacterial strains, plasmids and primers used in this study were listed in Table S1. The plasmids pWQ02 and pWQ03 were constructed by inserting the *pduP* gene from *S. typhimurium* into pHP301 (Wang et al., 2012) and the *dhaB123* genes from *Klebsiella pneumoniae* into pACYCDuet-1, respectively. The *gdrAB* fragments from *K. pneumoniae* were cloned into pWQ03 and pACYCDuet-1 to generate pWQ04 and pWQ05, respectively.

### 2.2. Protein expression and enzymatic assays

The cell lysate of *E. coli* BL21(DE3) strains harboring empty vectors or expression plasmids was used for SDS-PAGE and enzyme assays. The activities of glycerol dehydratase and propionaldehyde dehydrogenase were determined *in vitro* as described by Toraya et al. (1977) and Walter et al. (1997), respectively.

### 2.3. Shake flask cultures

The strain was inoculated in minimal medium (MM), which contains 20 g glycerol, 1.5 g  $\text{KH}_2\text{PO}_4$ , 3 g  $(\text{NH}_4)_2\text{SO}_4$ , 1 g citric acid, 1 g citrate sodium, 1.9 g KCl, 3 g  $\text{MgSO}_4$ , 0.138 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 45 mg vitamin B<sub>1</sub>, and 1 ml of trace element solution per liter. The trace element solution contained (per liter): 0.37 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 2.47 g  $\text{H}_3\text{BO}_3$ , 1.58 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.29 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . 100 mg/L ampicillin and 34 mg/L chloramphenicol were supplemented when necessary. The cells were induced at  $\text{OD}_{600} \sim 0.6$  with 0.1 mM IPTG and further incubated at 30 °C. 5  $\mu\text{M}$  of vitamin B<sub>12</sub> (VB<sub>12</sub>) was added every 12 h during 48 h culture. The PHA extraction and analysis were performed as described previously (Wang et al., 2009; Brandl et al., 1988).

### 2.4. Fed-batch fermentation

Fed-batch cultures were carried out in a Biostat B plus MO5L fermentor (Sartorius Stedim Biotech GmbH, Germany) containing 3 L of MM as described above, except using 3 g/L glucose and 20 g/L glycerol as initial carbon sources. The feeding medium is 10 M glycerol. The pH was controlled at 7.0 and the dissolved oxygen (DO) concentration was maintained at 5% saturation. The cells were induced at  $\text{OD}_{600} \sim 0.6$  by adding 0.05 mM IPTG and 5  $\mu\text{M}$  VB<sub>12</sub>. IPTG and VB<sub>12</sub> were added every 12 h during 84 h fermentation.

## 3. Results and discussion

### 3.1. Gene cloning and enzyme activity assay

Seven genes including *dhaB123*, *gdrAB*, *pduP*, and *phaC1* were cloned into pACYCDuet-1 and pET-21a vectors under the T7 promoter, respectively (Fig. S1).

Glycerol dehydratase of *K. pneumoniae* (DhaB) catalyzes the conversion of glycerol to 3-hydroxypropionaldehyde. DhaB undergoes inactivation by glycerol and O<sub>2</sub>, and can be rescued by its reactivase GdrAB (Kajiura et al., 2001; Tobimatsu et al., 1999). Three plasmids, carrying *dhaB123*, *gdrAB*, *dhaB123* and *gdrAB*, respectively, along with empty vector, were tested in *E. coli* BL21(DE3) strain. DhaB1 and GdrA were observed as distinct bands with the expected molecular weights on SDS-PAGE (Fig. 2A). Crude extract containing both DhaB and GdrAB catalyzed the glycerol dehydration with an apparent activity of  $2.94 \pm 0.21$   $\mu\text{mol}/\text{min}/\text{mg}$  protein while other three did not show significant enzyme activity (Fig. 2B), indicating the essential role of GdrAB in glycerol dehydration.

Propionaldehyde dehydrogenase, PduP of *S. typhimurium* LT2 catalyzes the CoA-dependent propionaldehyde dehydrogenation (Walter et al., 1997). The crude cell extract containing PduP catalyzed the formation of 3HP-CoA with an average activity of  $1.22 \pm 0.12$   $\mu\text{mol}/\text{min}/\text{mg}$  protein.

### 3.2. P3HP accumulation and analysis

For P3HP production, control strain *E. coli* BL21(DE3)/pACYC-Duet-1/pET-21a and *E. coli* BL21(DE3)/pWQ02/pWQ04 were cultivated with glycerol as the sole carbon source. After 48 h cultivation, *E. coli* BL21(DE3)/pWQ02/pWQ04 cells produced 2.40 g/L cell dry weight (CDW) containing 6.5% (wt/wt CDW) P3HP while the control strain produced no P3HP. In addition, *E. coli* BL21(DE3)/pWQ02/pWQ03 strain did not synthesize P3HP, indicating the important role of glycerol dehydratase reactivating factor GdrAB in P3HP biosynthesis. NMR analysis clearly showed the polymer is P3HP (Fig. S2). The number-average molecular weight ( $M_n$ ) and weight-average molecular weight ( $M_w$ ) of the P3HP were  $1.12 \times 10^5$  Da and  $1.78 \times 10^5$  Da, respectively.

### 3.3. Comparison of P3HP production in different *E. coli* strains

Grown using glycerol as the sole carbon source, JM109(DE3) strain carrying pWQ02 and pWQ04 accumulated 0.38 g/L P3HP representing 33% of CDW, which is much higher than the P3HP yield in BL21(DE3) and BL21 star(DE3), although JM109(DE3) strain possessed the lowest CDW (Table 1). So, JM109(DE3) was selected as the host strain for P3HP production thereafter.

### 3.4. Optimization of culture conditions

Culture conditions play an important role in the microbial production of desired products, and optimization of culture conditions is a useful method to enhance the product quality and quantity. To improve P3HP yield, organic nitrogen source, carbon source and

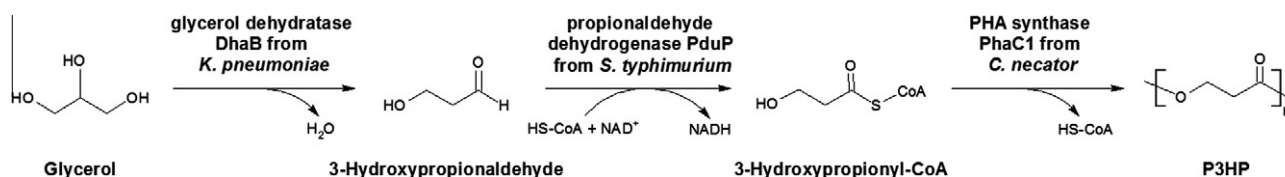
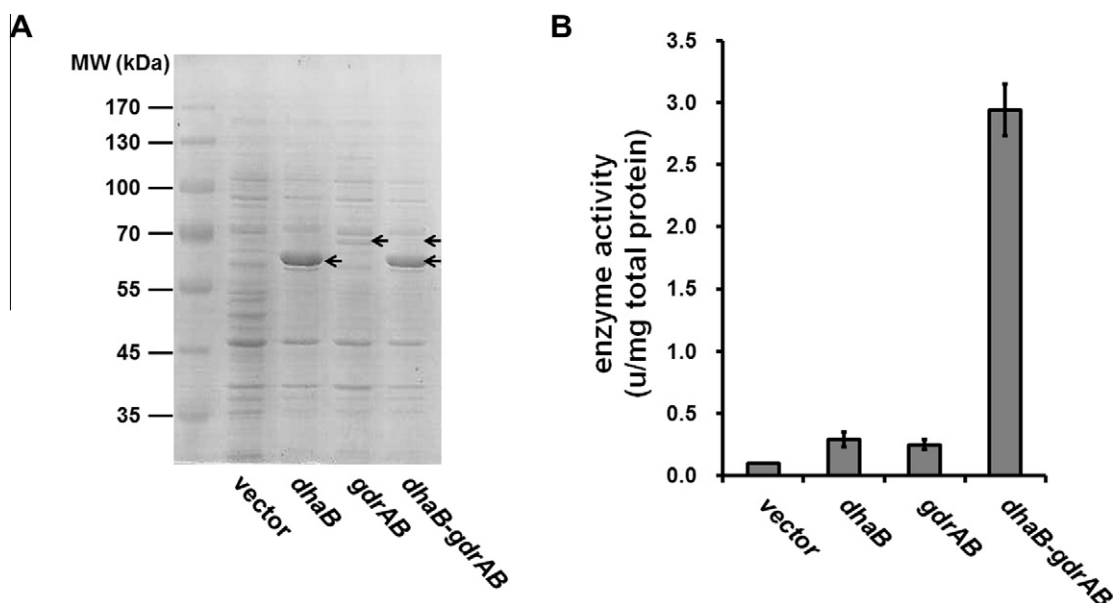


Fig. 1. Pathway for P3HP biosynthesis from glycerol in the engineered strain of *E. coli*.



**Fig. 2.** Expression (A) and enzyme activity (B) of glycerol dehydratase in *E. coli* cells. Crude cell extracts of *E. coli* BL21(DE3) strains carrying empty vector pACYCDuet-1, pWQ03 (*dhaB*), pWQ05 (*gdrAB*), and pWQ04 (*dhaB-gdrAB*) were used. The positions of overexpressed DhaB1 (60.7 kDa) and GdrA (63.6 kDa) are indicated by arrows.

**Table 1**  
P3HP production by different *E. coli* strains harboring pWQ02 and pWQ04 from glycerol.

Strain	CDW (g/L)	P3HP (g/L)	P3HP content (%)
<i>E. coli</i> BL21(DE3)	2.40 ± 0.13	0.16 ± 0.03	6.48
<i>E. coli</i> BL21 star(DE3)	2.17 ± 0.20	0.13 ± 0.02	5.80
<i>E. coli</i> JM109(DE3)	1.15 ± 0.08	0.38 ± 0.06	32.69

The experiment was performed under shake flask condition in triplicate.

IPTG concentration were optimized in this study using “one-factor-at-a-time” optimization strategy (Fig. S3).

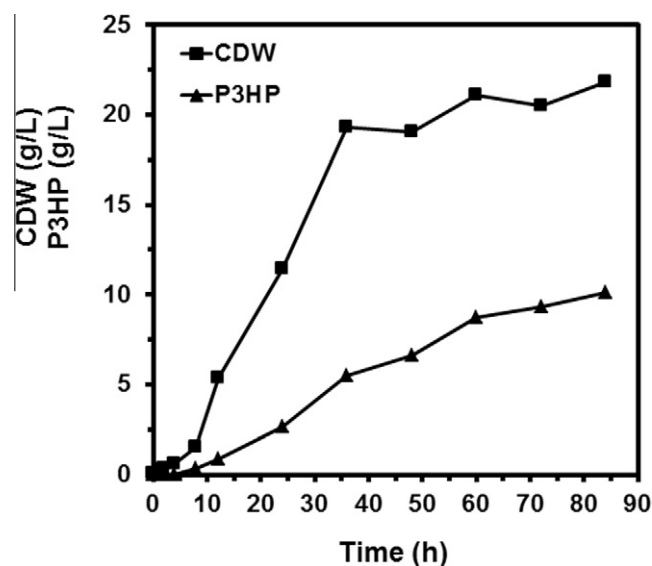
Finally, when *E. coli* JM109(DE3)/pWQ02/pWQ04 strain was cultivated in MM containing 20 g/L glycerol and 3 g/L glucose as cosubstrates and induced by 0.05 mM IPTG, the highest P3HP production of 1.54 g/L was achieved, which is 4-times higher than that before the culture conditions optimization. The P3HP content also increased up to 54% of CDW.

Interestingly, P3HP accumulation in recombinant *E. coli* strain is also in response to the nitrogen shortage as natural PHB accumulation bacteria, even though the expression of P3HP synthesis associated genes was controlled by IPTG induction. The possible explanation is that a larger proportion of metabolic flux was channeled into the P3HP biosynthesis pathway when cell growth was blocked by nitrogen limitation.

### 3.5. Fed-batch fermentation

We carried out the fed-batch fermentation based on results obtained with flask cultures. The fermentations were performed under aerobic condition using *E. coli* JM109(DE3)/pWQ02/pWQ04 and MM containing glucose and glycerol as above. Cell growth and P3HP accumulation were monitored over the course of fermentation (Fig. 3). After 84 h cultivation, 21.8 g/L CDW and 10.1 g/L P3HP (46.4% of CDW) were accumulated. To date, this is the highest P3HP production from glycerol.

Compared with the P3HP pathway constructed by Andreeßen et al. (2010), the glycerol dehydratase and its reactivatase of *K. pneumoniae*, were used in our engineered strain. So the fermentation process for P3HP production was simplified by throwing off



**Fig. 3.** Time profiles for CDW and P3HP production during an aerobic fed-batch fermentation of *E. coli* JM109(DE3)/pWQ02/pWQ04.

the anaerobic P3HP accumulation phase, and disodium fumarate and potassium sodium tartrate are not required anymore resulting in decreased cost. Furthermore, using glucose as a cosubstrate with glycerol has improved the P3HP production greatly. The reason could be attributed to the utilization of glucose catabolism for the generation of energy for biomass and glycerol was used for P3HP production resulting in the enhanced yield.

Despite the progress already obtained in microbial P3HP production, some problems still remained unsolved. For example, the addition of VB<sub>12</sub> resulted in a high cost. This problem could be solved by using strains producing VB<sub>12</sub> itself, like *K. pneumoniae*, as host strain for P3HP biosynthesis pathway. Another problem is the redox balance and disposal of reducing equivalents. A possible solution is to introduce the NADH-consuming PHB synthesis pathway. This could bring two advantages: (i) the surplus of NADH will

be oxidized to NAD<sup>+</sup>, and (ii) P(3HB-co-3HP) copolymer with some special characteristics will be produced from glycerol.

#### 4. Conclusion

In this study, we improved the microbial P3HP production from glycerol by using the glycerol dehydratase and its reactivating factor of *K. pneumoniae* and culture condition optimization. When *dhaB123*, *gdrAB*, *pduP*, and *phaC1* were coexpressed, the final engineered *E. coli* JM109(DE3) strain accumulated 10.1 g/L P3HP from glycerol in an aerobic fed-batch fermentation. It is the highest P3HP production from inexpensive carbon sources reported so far.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2013.01.096>.

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