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# Production of 3-hydroxypropionate homopolymer and poly(3-hydroxypropionate-*co*-4-hydroxybutyrate) copolymer by recombinant *Escherichia coli*

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ABSTRACT

Conversion of 3-hydroxypropionate (3HP) from 1,3-propanediol (PDO) was improved by expressing dehydratase gene (*dhaT*) and aldehyde dehydrogenase gene (*aldD*) of *Pseudomonas putida* KT2442 under the promoter of *phaCAB* operon from *Ralstonia eutropha* H16. Expression of these genes in *Aeromonas hydrophila* 4AK4 produced up to 21 g/L 3HP in a fermentation process. To synthesize homopolymer poly(3-hydroxypropionate) (P3HP), and copolymer poly(3-hydroxypropionate-co-3-hydroxybutyrate) (P3HP4HB), *dhaT* and *aldD* were expressed in *E. coli* together with the *phaC1* gene encoding polyhydroxyalkanoate (PHA) synthase gene of *Ralstonia eutropha*, and *pcs*' gene encoding the ACS domain of the tri-functional propionyl-CoA ligase (PCS) of *Chloroflexus aurantiacus*. Up to 92 wt% P3HP and 42 wt% P3HP4HB were produced by the recombinant *Escherichia coli* grown on PDO and a mixture of PDO+1,4-butanediol (BD), respectively.



## 1. Introduction

Bacterial polyhydroxyalkanoates (PHA) are natural biodegradable thermoplastics produced by various microorganisms as intracellular energy and carbon storage compounds (Rehm, 2010; Steinbüchel and Fuchtenbusch, 1998; Steinbüchel and Hein, 2001; Verlinden et al., 2007). The potential application of PHA as alternatives to petroleum-based plastics elevated the importance of PHA in the field of bioengineering (Rincones et al., 2009; Keshavarz and Roy, 2010; Chen, 2009). PHA are biodegradable and biocompatible polyesters with piezoelectricity and flexible mechanical properties (Chen, 2009; Chen and Wu, 2005). They are used in packaging, medicine, pharmacy, agriculture, and food industry (Chen, 2009; Rehm, 2010; Cheng et al., 2006). Microbial enhanced production of various PHA have been realized by metabolic engineered recombinant microorganisms (Chen et al., 2004; Park and Lee, 2005; Park et al., 2005; Jung et al., 2010; Tyo et al., 2009a, b).

3-Hydroxypropionic acid (3HP) is considered as a very important bio-renewable platform chemical (Cho et al., 2010; Straathof et al., 2005; van Maris et al., 2004). For example, bulk chemical acrylic acid can be produced from 3HP by dehydration both chemically and biochemically (Straathof et al., 2005; Lee et al., 2009). Yamada et al. (2001) reported the microbial production of acrylic acid from 3HP. 3HP can be derived from agricultural products including sugar, starch, or even cellulose in an anaerobic stoichiometric way (van Maris et al., 2004). Over 10 synthetic pathways were designed for producing 3HP from bio-renewable sources such as glucose or glycerol (Cho et al., 2010; Jiang et al., 2009). Notably, 39 g/L 3HP was produced from glycerol by overexpressing aldehyde dehydrogenase (ALDH), α-ketoglutaric semialdehyde dehydrogenase in *Escherichia coli* (Rathnasingh et al., 2009).

Bacterial synthesis of homopolymer P3HP was first reported in 2010 (Andreessen et al., 2010). In that report, P3HP reached 12% of cell dry weight (CDW) when the *pduP* from *Salmonella enterica* serovar Typhimurium LT2 encoding propionaldehyde dehydrogenase was expressed to function as both aldehyde dehydrogenase and CoA ligase PHA copolymers containing 3HP monomer were reviewed by Andreessen and Steinbüchel (2010), including poly(3HB-3HP), poly(3HB-3HP-2HP), poly(3HB-3HP-4HB-2HP) and poly(3HP-3HB-3HH-3HO) (Fukui et al., 2009; Ichikawa et al., 1996; Nakamura et al., 1991; Shimamura et al., 1994; Valentin et al., 2000).

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[1] Both authors contributed equally to this paper. ZQ did most of the experiments and analyzed the data, and SZY designed the research.

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Two key enzymes, including malonyl-CoA reductase (MCR) and propionyl-CoA synthetase (PCS) from the 3-hydroxypropionate cycle of phototrophic green non-sulfur eubacterium *Chloroflexus aurantiacus*, were identified (Alber and Fuchs, 2002; Herter et al., 2002; Hugler et al., 2002). Since the over-expression of all the *accABCD* genes in *E. coli* can increase the intracellular malonyl-CoA level (Davis et al., 2000), pathways that can produce 3HP from sugar were proposed shortly after the discovery of the 3-hydroxypropanoate cycle genes. A simplified version of this idea was first demonstrated in 2009 where poly(3HB-3HP) were produced in *Ralstonia eutropha* (Fukui et al., 2009). However, there was only a small portion of P3HP in that study. Thus, the poor yield of all previous studies might imply that either the 3HP-CoA ligase or the PHA synthase might have low activity for 3HP or 3HP-CoA.

In order to find the reasons, 3HP-CoA ligase or PHA synthase have to be supplied with sufficient precursors. Therefore, an alternative pathway of producing 3HP and P3HP from PDO was proposed based on the fact that genes *dhaT* and *aldD* from *Pseudomonas putida* KT2442 are very likely to convert 1,3-propanediol to 3-hydroxypropionate because they can convert 1,4-butanediol to 4-hydroxybutyrate for P4HB synthesis (Zhang et al., 2009).

In this study, a P3HP synthesis pathway combining *dhaT*, *aldD*, *pcs*' and *phaC1* was constructed for the first time in recombinant *E. coli* (Fig. 1). We aimed to exploit possibilities of producing 3HP, P3HP and novel copolymers of 3HP and 4HB from 1,3-propanediol or/and 1,4-butanediol.

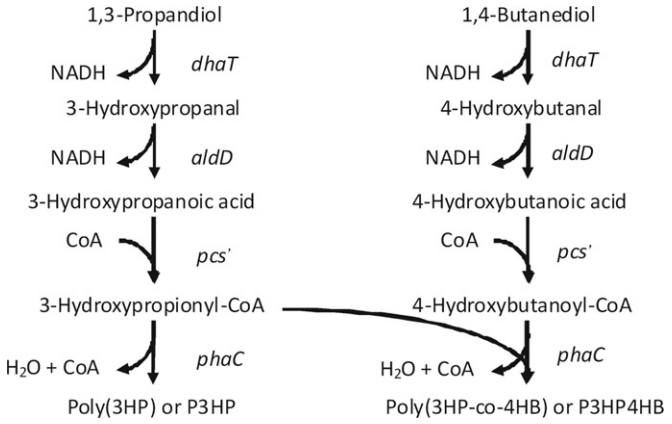


**Fig. 1.** Metabolically engineered pathways for production of P3HP and P3HP4HB in *E. coli*. Genes: *dhaT*, 1.3-propanediol dehydrogenase; *aldD*, aldehyde dehydrogenase; *pcs*', the gene encoding the ACS domain of propionyl-CoA synthase and functioning like 3-hydroxypropanoic acid CoA ligase; *phaC*, PHA synthase.

## 2. Materials and methods

### 2.1. Microorganisms, plasmids and genetic methods

For molecular cloning experiments, standard procedures were used. All cloning works were performed in *E. coli* JM109 or *E. coli* TOP10 from TaKaRa Bio Inc. (Shiga, Japan). Kits for plasmid isolation and DNA purification kits were from Qiagen (Shanghai, China), and enzymes for DNA restriction and modifying were from MBI Fermentas (Vilnius, Lithuania). Strains and plasmids used in this study are listed in Table 1, and primers for construction of new plasmids in this study in Table 2.

The expression vector pZQ01 was constructed by inserting the promoter of *phaCAB* operon from *Ralstonia eutropha* H16 ($P_{Re}$ promoter) into the plasmid pZL-dhaT-aldD, and the primers PREF and PRER were used to subclone the promoter (Fig. 2).

pZQ02 was constructed by subcloning the *dhaT* and *aldD* genes from pZL-dhaT-aldD into pET28-a which contains T7 promoter. Primers dhaTaldDF/dhaTaldDR were used for the PCR amplification (Fig. 2).

Primers *pcs'F* and *pcs'R* were used to clone the gene (named *pcs*' herein) encoding ACS domain from the *Chloroflexus aurantiacus* genome with a Shine-Dalgarno (SD) sequence added to its upstream and a TAG termination codon to its end. This PCR product was then digested by *Stu*I and *Eco*RI, and ligated with the gel electrophoresis-purified 5600 bp fragment, which was obtained by digesting pBHR68 with *Stu*I and *Eco*RI. The constructed plasmid was verified via DNA sequencing and named as pZQ03 (Fig. 2). It expresses the PHA synthase PhaC1, and the ACS domain.

**Table 2**
Oligonucleotides used in this study.

| Primers | Sequence |
|---|---|
| pcs'F | 5′-TATACAGGCCTAGGAGGATGGTCGATG |
| pcs'R | 5′-GCGTGAATTCCTATTCGATGATCTGCTGC (stuI/EcoRI) |
| dhaTaldDF | 5′-CGTTCGGGATCCCTAGAAGAAGC |
| dhaTaldDR | 5′-GTTGGCTAGCGCAGAGAGACAATC (NheI/BamHI) |
| PREF | 5′-ATAAGTCGACCTCCTATTTGATTGTCTCTCTGCCGTC |
| PRER | 5′-TTAAGGGCCCGATGCGAGCGCTGCATACCGTC (ApaI/SalI) |

All oligonucleotides were synthesized by AuGCT Biotech (Beijing, China). Restriction endonuclease digestion sites were underlined.

**Table 1**
Strains and plasmids used in this study.

| Strains/plasmids | Description | Reference/source |
|---|---|---|
| pBBR1MCS2 | Cloning vector, $Km^R$ | Kovach et al. (1995) |
| pET28-A | Expression vector, $Km^R$ | Novagen |
| pBHR68 | pkSE5.3 derived, containing *phaA phaB phaC* from *R. eutropha*, $Amp^R$ | Spiekermann et al. (1999) |
| pZL-dhaT | pBBR1MCS2 derived, *dhaT* under the control of *lac* promoter, $Km^R$ | Zhang et al. (2009) |
| pZL-aldD | pBBR1MCS2 derived, *aldD* under the control of *lac* promoter, $Km^R$ | Zhang et al. (2009) |
| pZL-dhaT-aldD | pBBR1MCS2 derived, *dhaT* and *aldD* under the control of *lac* promoter, $Km^R$ | Zhang et al. (2009) |
| pZQ01 | pBBR1MCS2 derived, *dhaT* and *aldD* under the control of $P_{Re}$ promoter, $Km^R$ | This study |
| pZQ02 | pET28A derived, *dhaT* and *aldD* under the control of T7 promoter, $Km^R$ | This study |
| pZQ03 | pBHR68 derived, *phaC* and *pcs*' under the control of *lac* promoter, $Amp^R$ | This study |
| *E.coli* S17-1 | *recA*, harbors the *tra* genes of plasmid RP4 in the chromosome; *proA*, *thi*-1 | Simon et al. (1983) |
| *E.coli* JM109 | Expression host | TransGen Biotech |
| *E.coli* Trans5α | Expression host | TransGen Biotech |
| *E.coli* Trans1-T1 | The fastest growing chemically competent strain currently available | TransGen Biotech |
| *E.coli* BL21 (DE3) | Expression host for pET vectors, containing IPTG-inducible T7 RNA polymerase gene | TransGen Biotech |
| *A. hydrophila* 4AK4 | Wild type. Host for producing 3-hydroxypropionic acid | Qiu et al. (2004) |