Metabolic Engineering ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Regular Article

Cyanobacterial production of 1,3-propanediol directly from carbon dioxide using a synthetic metabolic pathway

Yasutaka Hirokawa, Yuki Maki, Tsuneyuki Tatsuke, Taizo Hanai*

Laboratory for Bioinformatics, Graduate School of Systems Biosciences, Kyushu University, 804 Westwing, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form
25 December 2015
Accepted 26 December 2015

Keywords:
Cyanobacteria
Synthetic metabolic pathway
1,3-propanediol production
Dihydroxyacetone phosphate
Pseudovitamin B_{12}

ABSTRACT

Production of chemicals directly from carbon dioxide using light energy is an attractive option for a sustainable future. The 1,3-propanediol (1,3-PDO) production directly from carbon dioxide was achieved by engineered *Synechococcus elongatus* PCC 7942 with a synthetic metabolic pathway. Glycerol dehydratase catalyzing the conversion of glycerol to 3-hydroxypropionaldehyde in a coenzyme B_{12}-dependent manner worked in *S. elongatus* PCC 7942 without addition of vitamin B_{12}, suggesting that the intrinsic pseudovitamin B_{12} served as a substitute of coenzyme B_{12}. The highest titers of 1,3-PDO (3.79 ± 0.23 mM; 288 ± 17.7 mg/L) and glycerol (12.62 ± 1.55 mM; 1.16 ± 0.14 g/L), precursor of 1,3-PDO, were reached after 14 days of culture under optimized conditions in this study.

1. Introduction

The world's energy dependence on petroleum is unsustainable. Instead, the production of fuels and chemicals from renewable sources using microorganisms is a promising option for a sustainable future. Recently, various chemicals have been produced by microorganisms equipped with a synthetic metabolic pathway composed of multiple genes derived from other organisms (Zhang et al., 2011; McEwen and Atsumi, 2012). Such a synthetic metabolic pathway makes the host produce chemicals that it would not have produced naturally or would have produced at a lower titer. *Escherichia coli* and yeast have been widely used as hosts because of these species' ideal properties, including fast growth, the availability of well-established genetic tools, and well-characterized metabolic pathways. In contrast to these heterotrophic hosts, cyanobacteria possess the attractive property of photosynthesis, converting carbon dioxide into organic compounds using light energy. Cyanobacteria have been well studied in photosynthesis research, and are considered as ancestors of the chloroplast. Various techniques and tools for gene manipulation (Koksharova and Wolk, 2002; Clerico et al., 2007) and many sequenced genomes (Kotani et al., 1994; Holtman et al., 2005) have made cyanobacteria a useful host for bioengineering. Thus, the production of various chemicals directly from carbon dioxide is feasible in engineered cyanobacteria with synthetic metabolic pathways. Indeed, some valuable chemicals have been produced by engineered cyanobacteria, such as isobutyraldehyde, isobutanol (Atsumi et al., 2009), 1-butanol (Lan and Liao, 2011, 2012; Lan et al., 2013), 2-methyl-1-butanol (Shen and Liao, 2012), acetone (Zhou et al., 2012), ethylene (Takahama et al., 2003; Ungerer et al., 2012), ethanol (Gao et al., 2012), isoprene (Bentley and Melis, 2012), fatty acids (Liu et al., 2011), 3-hydroxybutyrate (Wang et al., 2013), 1,2-propanediol (Li and Liao, 2013), and 2,3-butanediol (Oliver et al., 2013; 2014). Previously, we achieved isopropanol production from carbon dioxide using *Synechococcus elongatus* PCC 7942 with a synthetic metabolic pathway (Kusakabe et al., 2013; Hirokawa et al., 2015).

Photosynthesis converts light energy into chemical energy (ATP, NADPH) in a photosystem and fixes carbon dioxide via the Calvin cycle, driven by ATP and NADPH. The fixed carbon is converted into cellular components and storage products through various metabolic pathways. It was reported that the cyanobacterial carbon flux in the Calvin cycle is larger than that in glycolysis and the tricarboxylic acid cycle under normal photoautotrophic conditions (Young et al, 2011; Alagesan et al., 2013; Knoop et al., 2013). Glycogen is synthesized from fructose-6-phosphate (F6P) in the Calvin cycle and is one of the major carbon storage products in cyanobacteria. Under optimized conditions, the accumulation rate of glycogen reached $0.29\ g\ L^{-1}\ day^{-1}$ in *Arthrospira platensis* (Aikawa et al., 2012) and $0.50\ g\ L^{-1}\ day^{-1}$ in *Synechococcus* sp. PCC 7002 (Aikawa et al., 2014). Compared with the production of chemicals previously achieved by engineered cyanobacteria (Savakis and

* Corresponding author. Fax: +81-92-642-6744.
E-mail address: taizo@brs.kyushu-u.ac.jp (T. Hanai).

http://dx.doi.org/10.1016/j.ymben.2015.12.008
1096-7176/

Hellingwerf, 2015), these rates are relatively high. It is expected that production originated from compounds in the Calvin cycle can achieve higher production rates and/or titers. Here, dihydroxyacetone phosphate (DHAP), one of the major compounds of the Calvin cycle and a precursor of fructose 6-phosphate (F6P), has been used as an initial substrate for 1,3-propanediol (PDO) production by engineered cyanobacteria.

1,3-PDO is one of the oldest fermentation products (Freund, 1881), and its usage as a monomer for polymer synthesis has drastically increased in industry. Polytrimethylene terephthalate (PTT), having fine elasticity and antifouling properties, is a polymer produced from 1,3-PDO with terephthalate and has been commercialized in the textile and carpet industries (Saxena et al., 2009; Liu et al., 2010). Difficulties and low productivity of chemical 1,3-PDO synthesis promote the development of 1,3-PDO bioproduction. Naturally, 1,3-PDO is produced by *Klebsiella pneumoniae* (Seo et al., 2009), *Citrobacter freundii* (Ainala et al., 2013), and *Clostridium acetobutylicum* (Gungormusler et al., 2010). The highest titer of 1,3-PDO production (129 g/L, 1.7 M) was achieved by engineered *E. coli* with an introduced synthetic metabolic pathway composed of genes from natural producers (Emptage et al., 2003). Algenol Biofuels Inc. has already published a patent application about cyanobacterial production of 1,3-PDO using engineered *Synechocystis* PCC 6803 and *Synechococcus* sp. PCC 7002 (Chin et al., 2014), but the productivity of 1,3-PDO and detailed information was not mentioned.

In this study, we constructed a synthetic metabolic pathway in *S. elongatus* PCC 7942 comparable to that constructed in *E. coli* (Fig. 1). In this pathway, cellular DHAP is first converted to glycerol via glycerol-3-phosphate, and the produced glycerol is subsequently converted to 1,3-PDO via 3-hydroxypropionaldehyde (3-HPA). This is the report of 1,3-PDO production directly from carbon dioxide using engineered cyanobacteria. Although the conversion of glycerol to 3-HPA is catalyzed by the coenzyme B_{12}-dependent glycerol dehydratase in this synthetic metabolic pathway, the engineered *S. elongatus* PCC 7942 was able to produce 1,3-PDO independently of extrinsic vitamin B_{12} as a precursor of coenzyme B_{12}, indicating the potential of cyanobacteria to perform a coenzyme B_{12}-dependent enzymatic reaction without addition of vitamin B_{12}.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise specified. Restriction enzymes, phosphatase (New England Biolabs; Ipswich, MA, USA), ligase (Rapid DNA Ligation Kit, Roche; Mannheim, Germany), and DNA polymerase (KOD Plus Neo DNA polymerase, TOYOBO Co., Ltd.; Osaka, Japan) were used for cloning. Oligonucleotides were synthesized by Life Technologies Japan, Ltd. (Tokyo, Japan).

2.2. Culture media

For cultivation of *S. elongatus* PCC 7942 (Life Technologies Corporation; Carlsbad, CA, USA), modified BG11 medium supplemented HEPES buffer for pH stabilization was used. Modified BG11 medium (hereafter referred to as BG11 medium) was composed of 1.5 g/L $NaNO_3$, 0.037 g/L $CaCl_2 \cdot H_2O$, 0.006 g/L ferric ammonium citrate, 0.001 g/L $Na_2EDTA \cdot 2H_2O$, 0.031 g/L K_2HPO_4, 0.074 g/L $MgSO_4 \cdot 7H_2O$, 0.020 g/L Na_2CO_3, 0.004 g/L citric acid $\cdot H_2O$, 2.5 mg/L H_3BO_4, 1.8 mg/L $MnCl_2 \cdot 4H_2O$, 0.22 mg/L $ZnSO_4 \cdot 7H_2O$, 0.39 mg/L $NaMoO_4 \cdot 2H_2O$, 0.080 mg/L $CuSO_4 \cdot 5H_2O$, 0.049 mg/L $Co(NO_3)_2 \cdot 6H_2O$, 20 mM HEPES-NaOH (pH 7.5). To prepare the plate medium, 1.5% (w/v) of Bacto Agar (Difco Laboratories; Franklin Lakes, NJ, USA) was added. Antibiotics

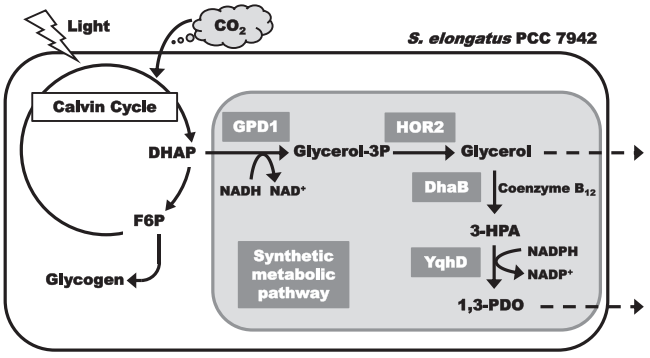

Fig. 1. The synthetic metabolic pathway for 1,3-PDO production in *S. elongatus* PCC 7942. The shadowed area represents the introduced pathway. Terms outlined in white on gray background represent the enzymes of the synthetic metabolic pathway. The abbreviations of F6P and 3-HPA indicate fructose-6-phosphate and 3-hydroxypropionaldehyde.

(10 μg/mL kanamycin and 20 μg/mL spectinomycin) were added to BG11 medium as appropriate.

2.3. Growth and production conditions

All cyanobacterial cultures were grown under fluorescent light (100 μmol photon m^{-2} s^{-1}) at 30 °C in a growth chamber (MLR-325H PJ; Panasonic; Osaka, Japan). Cell density (OD_{730}) was measured using Infinite 200 PRO (TECAN; Männedorf, Switzerland). For preculture, cells were inoculated into 20 mL BG11 medium in a 50-mL flask and incubated under fluorescent light with rotary shaking at 150 rpm (NR-30 shaker; TAITEC; Saitama, Japan). For 1,3-PDO production from carbon dioxide, cells of precultures at OD_{730} of 1.0–2.0 were inoculated into 50 mL BG11 medium containing 1 mM IPTG to an initial OD_{730} of 0.025. Cultures were grown under continuous aeration with ambient air at 1.0 vvm (volume per volume per minute) for two weeks. To optimize production conditions, carbon dioxide concentrations of 1% and 3% were tested. To prevent evaporation during aeration, ambient carbon dioxide-mixed air was passed through MilliQ water.

For 1,3-PDO production from glycerol, cells precultured in 50 mL BG11 medium under continuous aeration (1% carbon dioxide) were collected and resuspended into an appropriate volume of fresh BG11 medium containing 1 mM IPTG and 10 mM glycerol to an OD_{730} of 5.0. Cells were incubated with rotary shaking at 150 rpm for 5 days. Vitamin B_{12} (10 nM) was added as appropriate. A concentration factor of cultures caused by evaporation during production was calculated from the weight of each flask and compensated for in the data presented.

2.4. Plasmids constructed for 1,3-PDO production

Bacterial strains, plasmids, and primers used and constructed in this study are listed in Tables S1 and S2.

2.4.1. pTA1004 ($P_L$lacO1:: *dhaB1-dhaB2-dhaB3-gdrA-gdrB-yqhD*, PlacIq:: lacIq, NS I-targeting plasmid)

yqhD was amplified from *E. coli* BW25113 by PCR using primers T1633 and T1634. The PCR product was digested by BamHI and BglII and inserted into a BamHI site of pTA839 (Honjo et al., 2015), generating pTA945. The sequences of the synthesized genes (*dhaB1*, *dhaB2*, *dhaB3*, *gdrA*, *gdrB*) in pTA839 were shown in Supplemental data.

To construct a plasmid for homologous recombination into the NS I (Bustos and Golden, 1991) site of *S. elongatus* PCC 7942, the fragment of $P_L$lacO1:: *dhaB1-dhaB2-dhaB3-gdrA-gdrB-yqhD* was amplified from pTA945 by PCR using primers T1260 and T1634.

Please cite this article as: Hirokawa, Y., et al., Cyanobacterial production of 1,3-propanediol directly from carbon dioxide using a synthetic metabolic pathway. Metab. Eng. (2016), http://dx.doi.org/10.1016/j.ymben.2015.12.008