Title

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

Running title

1,3-Propanediol production by engineered cyanobacteria

Authors

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

*Corresponding author

Affiliation and Address

Laboratory for Bioinformatics, Graduate School of Systems Biosciences, Kyushu University, 804

Westwing, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

TEL: +81-92-642-6751, FAX: +81-92-642-6744

E-mail: taizo@brs.kyushu-u.ac.jp

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 B₁₂-dependent manner worked in *S. elongatus* PCC 7942 without addition of vitamin B₁₂, suggesting that the intrinsic pseudovitamin B₁₂ served as a substitute of coenzyme B₁₂. 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.

Keywords: Cyanobacteria, Synthetic metabolic pathway, 1,3-propanediol production, Dihydroxyacetone phosphate, Pseudovitamin B₁₂

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 et al., 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 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 B₁₂-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 B₁₂ as a precursor of coenzyme B₁₂, indicating the potential of cyanobacteria to perform a coenzyme B₁₂-dependent enzymatic reaction without addition of vitamin B₁₂.

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 NaNO₃, 0.037 g/L CaCl₂·H₂O, 0.006 g/L ferric ammonium citrate, 0.001 g/L Na₂EDTA·2H₂O, 0.031 g/L K₂HPO₄, 0.074 g/L MgSO₄·7H₂O, 0.020 g/L Na₂CO₃, 0.004 g/L citric acid·H₂O, 2.5 mg/L H₃BO₄, 1.8 mg/L MnCl₂·4H₂O, 0.22 mg/L ZnSO₄·7H₂O, 0.39 mg/L NaMoO₄·2H₂O, 0.080 mg/L CuSO₄·5H₂O, 0.049 mg/L Co(NO₃)₂·6H₂O, 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 (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 (OD₇₃₀) 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 OD₇₃₀ of 1.0–2.0 were inoculated into 50 mL BG11 medium containing 1 mM IPTG to an initial OD₇₃₀ 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 OD₇₃₀ of 5.0. Cells were incubated

with rotary shaking at 150 rpm for 5 days. Vitamin B₁₂ (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 Table 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. The PCR product was digested by AvrII and BamHI and inserted into the AvrII-BamHI site of pTA371, generating pTA1004.

2.4.2. pTA1380 (*P_L*lacO1:: *gpd1-hor2*, NS II-targeting plasmid)

gpd1 and *hor2* were synthesized by DNA2.0 (Menlo Park, CA, USA) after codon optimization for *E. coli*. *gpd1* was amplified by PCR using primers T1331 and T1635. The PCR product was digested by Acc65I and BamHI. *hor2* was amplified by PCR using primers T1328 and T1329. The PCR product was digested by BamHI and MluI. The digested PCR products were inserted into the Acc65I-BamHI site of pZE12-MCS (Expressys), generating pTA956. The sequences of the synthesized genes (*gpd1* and *hor2*) were shown in supplemental data.

For constructing the NS II-targeting vector, pSA126 was digested by AatII and MluI and inserted into the AatII-MluI site of pZE22-MCS (Expressys; Ruelzheim, Germany), generating pTA424.

To construct a plasmid for homologous recombination into the NS II (Anderson et al., 2000) site of *S. elongatus* PCC 7942, the fragment of *P_L*lacO1:: *gpd1-hor2* was amplified from pTA956 by PCR using primers T2377 and T1329. The PCR product was digested by Acc65I and MluI and inserted into the Acc65I-MluI site of pTA424, generating pTA1380.

2.5. Construction of the 1,3-PDO-producing strain

The genes of the synthetic metabolic pathway for 1,3-PDO production were integrated into the *S. elongatus* PCC 7942 genome by homologous recombination using two plasmids (pTA1004 and pTA1380). *S. elongatus* PCC 7942 was used as a wild type strain and named TA1297. The genes for 1,3-PDO production from glycerol ($P_{\text{LlacO1}}::dhaB1-dhaB2-dhaB3-gdrA-gdrB-yqhD$) with spectinomycin resistance gene were integrated into the NS I site of TA1297, generating strain TA2211. For construction of the 1,3-PDO-producing strain TA2984, the genes for 1,3-PDO production from glycerol (see above) and glycerol production from DHAP ($P_{\text{LlacO1}}::gpd1-hor2$) with kanamycin resistance gene were simultaneously integrated into the NS I and NS II sites of TA1297, and the recombinant strain was selected for spectinomycin and kanamycin resistance. The chromosome segregation of each antibiotic-resistant strain was confirmed by PCR using primer sets T1198-1199 and T1052-1053, specific for NS I and NS II, respectively. All integrated genes were sequenced and confirmed.

2.6. Product analysis

Culture supernatants were centrifuged ($20,000 \times g$, 10 min) and filtered using Minisart RC4 (Sartorius; Goettingen, Germany). Concentrations of glycerol and 1,3-PDO in filtered samples were determined using a high-performance liquid chromatograph (LC-20AD, Shimadzu; Kyoto, Japan) equipped with an autosampler (AOC-20; Shimadzu), a SUGAR SP-G guard column (Shodex; Tokyo, Japan), a SUGAR SP0810 column (Shodex), and a refractive index detector (RID-10A; Shimadzu). Operating conditions were as follows: mobile phase, MilliQ water; flow rate, 1.0 mL/min; column temperature, 80°C.

3. Results and discussion

3.1. Design of a synthetic metabolic pathway for 1,3-PDO production

In our constructed synthetic metabolic pathway, 1,3-PDO is synthesized from cellular DHAP via a four-step reaction (Fig. 1). First, DHAP is converted into glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase (GPD1, encoded by *gpd1*) in an NADH-dependent manner. Next, the dephosphorylation catalyzed by glycerol-3-phosphatase (HOR2, encoded by *hor2*) produces glycerol from glycerol-3-phosphate. The genes encoding these enzymes were cloned from *Saccharomyces cerevisiae* and integrated into the NS II site of *S. elongatus* PCC 7942, using a kanamycin resistance gene-containing vector (Fig. 2). In the pathway, the intermediate between glycerol and 1,3-PDO is 3-hydroxypropionaldehyde (3-HPA) (Fig. 1). Therefore, glycerol dehydratase from *K. pneumoniae* (DhaB, encoded by *dhaB1*, *dhaB2*, and *dhaB3*) and aldehyde reductase from *E. coli* (YqhD, encoded by *yqhD*) were used to convert glycerol into 3-HPA and 3-HPA into 1,3-PDO, respectively. In a natural 1,3-PDO producer, for example, *K. pneumoniae*, the reduction of 3-HPA is catalyzed by NADH-dependent 1,3-propanediol oxidoreductase (DhaT, encoded by *dhaT*). Here, we chose YqhD for 3-HPA reduction in an NADPH-dependent manner for cofactor balance, as it was reported that NADPH is more abundant in cyanobacteria than NADH and the ratio of NADH to NADPH in *S. elongatus* PCC 7942 was estimated to be 0.15 (Vermaas, 2001; Tamoi et al., 2005; Takahashi et al., 2008). Therefore, it was assumed that NADPH dependence would be preferable for production. In addition to the enzyme-encoding genes, the glycerol dehydratase reactivating factor-encoding genes *gdrA* and *gdrB* (Tobimatsu et al., 1999) were also introduced. All genes involved in 1,3-PDO production from glycerol, linked to a spectinomycin resistance gene, were integrated into the NS I site of *S. elongatus* PCC 7942 (Fig. 2).

3.2. Toxic effects of glycerol and 1,3-PDO on cell growth

Toxic effects of intermediate and end-product, glycerol and 1,3-PDO, on cell growth were assessed (Fig. 3). Cell growth was slightly inhibited by 10 g/L 1,3-PDO (Fig. 3A), while there was no inhibition of growth by glycerol of any concentration up to 10 g/L (Fig. 3B). No chemicals produced by engineered cyanobacteria have ever reached a titer of more than 10 g/L (Savakis and Hellingwerf, 2015). Thus, it has been assumed that the toxic effects of either compound are negligible for *S. elongatus* PCC 7942.

3.3. 1,3-PDO production from glycerol

Prior to 1,3-PDO production directly from carbon dioxide, we characterized the last part of the pathway from glycerol to 1,3-PDO, using strain TA2211, into which the enzymes DhaB and YqhD had been introduced (Fig. 4).

The addition of glycerol enabled TA2211 to produce 1,3-PDO, corresponding to glycerol consumption (Fig. 4A, B). During 5 days of production with 10 nM of vitamin B₁₂, 2.92 ± 0.35 mM of 1,3-PDO was produced from 3.20 ± 0.29 mM of glycerol. The molar conversion ratio was higher than 90%, indicating that the consumed glycerol was catalyzed through the introduced pathway and that 3-HPA (Barbirato et al., 1996) was not accumulated. The growth profile of TA2211 with added glycerol was not different from that without glycerol, indicating that the consumed glycerol was mainly converted into 1,3-PDO. No 1,3-PDO production was detected during incubation of TA2211 without glycerol. On the other hand, a wild type strain TA1297, lacking the synthetic metabolic pathway, did not consume any glycerol and did not produce any 1,3-PDO even with addition of glycerol (Fig. 5A, B). These results indicate that *S. elongatus* PCC 7942 alone was unable to metabolize glycerol and that the introduced synthetic metabolic pathway worked successfully.

Previous reports indicated that glycerol dehydratase catalyzes the conversion of glycerol to 3-HPA in a coenzyme B₁₂-dependent manner (Daniel et al., 1999) and that the production of high-titered 1,3-PDO by engineered *E. coli* required successive addition of vitamin B₁₂ as a precursor of coenzyme B₁₂ (Emptage et al., 2003). As the same glycerol dehydratase was used for 1,3-PDO production in TA2211, 10 nM of vitamin B₁₂ was supplemented to the medium. However, it was found that the 1,3-PDO production by this strain was not dependent on vitamin B₁₂ supply, because the levels of 1,3-PDO production, glycerol consumption, and growth were not different when no vitamin B₁₂ was added (Fig. 4). According to previous reports, some cyanobacteria produce a compound structurally similar to vitamin B₁₂, known as pseudovitamin B₁₂ (Watanabe et al., 2001; Taga and Walker, 2008). The difference between these compounds is that 5,6-dimethylbenzimidazole in vitamin B₁₂ is replaced with adenine in pseudovitamin B₁₂. Although the existence of pseudovitamin B₁₂ in *S. elongatus* PCC 7942 has not been confirmed, the genes involved in pseudovitamin B₁₂ synthesis (*cobA*, *cobD*, *cobH*, *cobM*, *cobN*, *cobO*, *cobS*, *cobU*) were annotated in the genome sequence (Cyanobase; <http://genome.microbedb.jp/cyanobase/SYNPCC7942>). Production of 1,3-PDO by TA2211 without added vitamin B₁₂ suggests that the intrinsic pseudovitamin B₁₂ of *S. elongatus* PCC 7942 served as a substitute for coenzyme B₁₂.

3.4. 1,3-PDO production directly from carbon dioxide

After confirming the functions of the pathway from glycerol to 1,3-PDO (Fig. 4), 1,3-PDO production directly from carbon dioxide was attempted, using strain TA2984 into which the complete synthetic metabolic pathway from DHAP to 1,3-PDO had been introduced (Fig. 6). According to the results shown in Fig. 4, we omitted the supplement of vitamin B₁₂ as a cofactor for glycerol dehydratase in this pathway.

During 14 days of production under aeration with ambient air, the concentration of 1,3-PDO linearly increased and reached 1.88 ± 0.29 mM (Fig. 6A). This is the time that 1,3-PDO production directly from carbon dioxide, using engineered cyanobacteria, was accomplished. Glycerol, the intermediate compound in this synthetic metabolic pathway, was also detected in the culture supernatants at increasing concentrations up to 2.50 ± 0.58 mM (Fig. 6B). The production rates of 1,3-PDO and glycerol correspond to 0.15 mM/day and 0.18 mM/day, respectively. Assuming equal molecular conversion ratios in this synthetic metabolic pathway, the production rate of glycerol from DHAP was estimated to be 0.33 mM/day. The gap between the production rates of glycerol from DHAP and of 1,3-PDO from glycerol indicates that the latter part of the pathway, converting glycerol into 1,3-PDO, is the bottleneck and needs to be enhanced for efficient production of 1,3-PDO from DHAP. For example, combinatorial optimization of transcription and translation in a synthetic metabolic pathway by changing the promoter and/or the ribosomal binding site (Oliver et al., 2014) are necessary in the future research. Here, TA2984 showed the same growth profile as TA1297 (Fig. 6C and Fig. S1-A), indicating that the 1,3-PDO-producing pathway did not inhibit cellular growth.

For improving 1,3-PDO productivity, concentrations of 1% and 3% carbon dioxide were used for aeration and compared to ambient air only. Although the aeration with 1% and 3% carbon dioxide improved growth of TA2984 (Fig. 6C), this improved growth was inferior to that of TA1297 under each condition (Fig. S1). As the concentrations of products such as glycerol and 1,3-PDO did not reached toxic levels (Fig. 3), the mechanism of growth inhibition remains unclear. In general, there is a competitive relationship between growth and production in heterotrophic hosts using sugar as a substrate, and excessive enhancement of production often results in growth inhibition. In our case, however, growth inhibition was caused by an increase in carbon dioxide as the substrate. Therefore, it will be necessary to analyze and compare the metabolic profiles under different conditions of aeration.

Accumulation rates of glycerol, 0.94 mM/day (1%) and 0.90 mM/day (3%), were improved with carbon dioxide, compared to ambient air (Fig. 6B). The improved rates increased glycerol concentrations up to 13.14 ± 1.05 mM (1%) and 12.62 ± 1.55 mM (3%), being about 5-fold higher than that under ambient air aeration (2.50 ± 0.58 mM). This result indicates that the pool of DHAP utilized by the synthetic metabolic pathway increased and that an appropriate increase of carbon dioxide led to more production. Under these conditions, the titers of 1,3-PDO after 14 days reached 3.25 ± 0.61 mM (1%) and 3.79 ± 0.23 mM (3%) (Fig. 6A). Although the aeration with higher concentration of carbon dioxide increased the productivities of 1,3-PDO and glycerol, the increased rates of 1,3-PDO were lower than those of glycerol. This result also indicated that the latter pathway from glycerol to 1,3-PDO is the bottleneck. The incubation under 3% carbon dioxide aeration indicating the highest titer of 1,3-PDO, showed the two different production rates of 1,3-PDO with boundary at 6 days. The production rate before 6 days (0.43 mM/day) was about 3-fold higher than that under ambient air aeration. However, a drastically decreased rate (0.16 mM/day) was obtained after 6 days. Under 3% carbon dioxide aeration, the growth profile resembled the curve of 1,3-PDO production (Fig. 6A, C). The similar decrease of production rate and similarity between growth profile and production curve were also obtained in the

incubation under 1% carbon dioxide aeration. Coenzyme B₁₂ for glycerol dehydratase (DhaB) and NADPH for aldehyde reductase (YqhD) are necessary for conversion of glycerol into 1,3-PDO. Under photoautotrophic conditions, it is expected that NADPH is continuously supplied by the photosystem. Therefore, the supply of coenzyme B₁₂ would be the limiting factor for 1,3-PDO production. Previous reports showed that one of the roles of pseudovitamin B₁₂ in cyanobacteria (*Synechocystis* sp. PCC 6803, *A. platensis*) was the activation of methionine synthase (MS) (Tanioka et al., 2009; 2010). In these reports, the content of pseudovitamin B₁₂ and MS activity increased during the growth phase and decreased during the stationary phase. Therefore, moderate growth under ambient air aeration yielded a constant 1,3-PDO production rate. In contrast to ambient air, the improved but early arrested growth under 1% and 3% carbon dioxide aeration caused a decrease in production rate. Vitamin B₁₂ with a final concentration of 10 nM was supplemented to the culture at 6 days (Fig. S2). The supplementation of vitamin B₁₂ increased the titer of 1,3-PDO by 1.34-fold, suggesting that the deficiency of pseudovitamin B₁₂ in the cell is one of the reasons to decrease 1,3-PDO titer. The metabolic pathway of pseudovitamin B₁₂ synthesis would be a good target of genetic engineering for improvement of 1,3-PDO productivity. The incubation under 3% carbon dioxide aeration achieved highest titer of 1,3-PDO (3.79 ± 0.23 mM) and total carbon flux to the synthetic metabolic pathway [1.26 mM/day = 0.97 (glycerol) + 0.29 (1,3-PDO)]. Compared with a similar production rate (about 0.2 mM/day) of 1,2-PDO originating from DHAP reported by Li and Liao (2013), a larger flux was achieved in this study. However, the expected flux from DHAP to the synthetic metabolic pathway, as calculated from data of glycogen accumulation (Aikawa et al., 2012; 2014), is larger than our results. More genetic modifications of the host strain are necessary for more productivity improvement.

4. Conclusion

In this study, the 1,3-PDO production directly from carbon dioxide and independent of vitamin B₁₂ supply was achieved by engineering cyanobacteria (*S. elongatus* PCC 7942) with a synthetic metabolic pathway. The highest titer 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) was reached after 14 days of culture under 3% carbon dioxide aeration. Aeration with increasing carbon dioxide concentrations caused enhancement of DHAP available for the synthetic metabolic pathway.

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Figure legends

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.

Fig. 2. Construction of the 1,3-PDO-producing strain by homologous recombination.

The genes involved in 1,3-PDO production from glycerol were integrated into NS I site with *lacI* and spectinomycin resistance gene (represented *Spec^r* in figure). The genes involved in glycerol production from DHAP were integrated into NS II site with kanamycin resistance gene (represented *Kan^r* in figure).

Fig. 3. Toxic effects of 1,3-PDO (A) and glycerol (B) on the growth of *S. elongatus* PCC 7942.

Circle, square, and triangle symbols represent concentrations of 0, 5 g/L, and 10 g/L, respectively. Data are represented as means $\pm$ S.D. of three individual experiments.

Fig. 4. 1,3-PDO production from glycerol by strain TA2211 introduced synthetic metabolic pathway from glycerol to 1,3-PDO.

(A) 1,3-PDO production, (B) glycerol consumption, (C) growth.

Square and circle symbols represent experiments with and without 10 nM of vitamin B₁₂, respectively.

Data are represented as means $\pm$ S.D. of three individual experiments.

Fig. 5. Glycerol assimilation by wild type strain TA1297 and strain TA2211 introduced synthetic metabolic pathway from glycerol to 1,3-PDO.

(A) 1,3-PDO production, (B) glycerol consumption, (C) growth.

Triangle and circle symbols represent experiments by TA1297 and TA2211, respectively. Data are represented as means $\pm$ S.D. of three individual experiments. Data for TA2211 are same as those in Fig.

4.

Fig. 6. 1,3-PDO production directly from carbon dioxide by strain TA2984 introduced synthetic metabolic pathway from DHAP to 1,3-PDO.

(A) 1,3-PDO production, (B) glycerol accumulation, (C) growth.

Circle, square, and triangle symbols represent conditions of aeration with ambient air, 1%, and 3% of carbon dioxide, respectively. Data are represented as means $\pm$ S.D. of three individual experiments.

Figure

Fig. 1.

Fig. 2.

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Fig. 5.

Fig. 6.