Bioresource Technology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Production of 1,3-diols in *Escherichia coli*

Naoya Kataoka [a,b,*], Alisa S. Vangnai [c,d], Thunyarat Pongtharangkul [e], Toshiharu Yakushi [a,b], Kazunobu Matsushita [a,b]

[a] Division of Agricultural Sciences, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 753-8515, Japan
[b] Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi 753-8515, Japan
[c] Biocatalyst and Environmental Biotechnology Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[d] Center of Excellence in Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok 10330, Thailand
[e] Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

HIGHLIGHTS

- A platform pathway for 1,3-diol production was constructed in *Escherichia coli*.
- The engineered *E. coli* produced 4.43 mM 1,3-pentanediol.
- The engineered *E. coli* produced 702 μM 4-methyl-1,3-pentanediol.
- The engineered *E. coli* produced 36.5 μM 1,2,4-butanetriol.

ARTICLE INFO

Article history:
Received 1 March 2017
Received in revised form 13 May 2017
Accepted 15 May 2017
Available online xxxx

Keywords:
1,3-pentanediol
4-methyl-1,3-pentanediol
1,2,4-butanetriol
Escherichia coli
Synthetic pathway

ABSTRACT

To expand the diversity of chemical compounds produced through microbial conversion, a platform pathway for the production of widely used industrial chemicals, 1,3-diols, was engineered in *Escherichia coli*. The pathway was designed by modifying the previously reported (*R*)-1,3-butanediol synthetic pathway to consist of *pct* (propionate CoA-transferase) from *Megasphaera elsdenii*, *bktB* (thiolase), *phaB* (NADPH-dependent acetoacetyl-CoA reductase) from *Ralstonia eutropha*, *bld* (butyraldehyde dehydrogenase) from *Clostridium saccharoperbutylacetonicum*, and the endogenous alcohol dehydrogenase(s) of *E. coli*. The recombinant *E. coli* strains produced 1,3-pentanediol, 4-methyl-1,3-pentanediol, and 1,2,4-butanetriol, together with 1,3-butanediol, from mixtures of glucose and propionate, isobutyrate, and glycolate, respectively, in shake flask cultures. To the best of our knowledge, this is the first report of microbial production of 1,3-pentanediol and 4-methyl-1,3-pentanediol.

1. Introduction

Biotechnological production of chemical compounds has been applied successfully with natural products, typified by primary and secondary metabolites such as alcohols, organic and amino acids, alkaloids, and terpenes (Chang and Keasling, 2006; Jang et al., 2012; Lee et al., 2011). On the other hand, the biological synthesis of non-natural compounds has had limited success because their production strictly relies on the establishment of synthetic pathways (Atsumi et al., 2008; Cheong et al., 2016; Choi et al., 2016; Choi and Lee, 2013; Martin et al., 2013; Sheppard et al., 2014; Yim et al., 2011). 1,3-Diols, except for 1,3-propanediol, are non-natural compounds, some of which are great interest for industrial applications. For instance, 1,3-pentanediol, 4-methyl-1,3-pentanediol, and 1,2,4-butanetriol are industrial chemicals that are widely used as a humectant, a key intermediate for the synthesis of cytotoxic drugs, and a precursor of a novel energetic plasticizer in propellant, respectively (Frankenfeld et al., 1970; Hanessian et al., 2004; Niu et al., 2003). In the current study, we aimed to assemble a synthetic pathway for these compounds and explore the possibility of their fermentative production.

Our previous study described the engineering of a synthetic pathway for the production of (*R*)-1,3-butanediol in *Escherichia coli* (Kataoka et al., 2013). The engineered pathway begins with the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, which is catalyzed by the thiolase PhaA from *Ralstonia eutropha*. This is followed by stereospecific reduction by PhaB from *R. eutropha* to generate (*R*)-3-hydroxybutyryl-CoA. The CoA moiety

* Corresponding author at: Division of Agricultural Sciences, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 753-8515, Japan.
E-mail address: nkataoka@yamaguchi-u.ac.jp (N. Kataoka).

http://dx.doi.org/10.1016/j.biortech.2017.05.082
0960-8524/

is then detached by butyraldehyde dehydrogenase (Bld) from *Clostridium saccharoperbutylacetonicum*, and the aldehyde generated in this step is finally reduced to the target primary alcohol by the endogenous alcohol dehydrogenase(s) of *E. coli*. Here, we describe the construction of a novel synthetic pathway based on the (*R*)-1,3-butanediol synthetic pathway to produce 1,3-diols, and show its general versatility by the production of 1,3-pentanediol, 4-methyl-1,3-pentanediol, and 1,2,4-butanetriol.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were commercial analytical grade. 1,3-Pentanediol and 4-methyl-1,3-pentanediol were synthesized by Tokyo Chemical Industry Co. (Tokyo, Japan). All restriction enzymes and the ligase (Ligation high Ver. 2) were kindly donated by Toyobo Co. (Osaka, Japan). Herculase II Fusion DNA Polymerase and In-Fusion HD Cloning Kits were purchased from Agilent Technologies (Santa Clara, CA, USA) and Takara Bio (Shiga, Japan), respectively. Oligonucleotides were synthesized by FASMAC Co. (Kanagawa, Japan).

2.2. Bacterial strains

E. coli BW25113 F', constructed in our previous study (Kataoka et al., 2014), was used as the host strain. *E. coli* NEB 5-alpha F'I^q (New England Biolabs, Ipswich, MA, USA) was used to propagate all plasmids.

2.3. Plasmid construction

Primers used in this study are shown in Table S1. pNK6 ($P_{A1lacO-1}$::*bktB bld phaB*) was designed to contain three consecutive structural genes, using the restriction sites shown in parenthesis: *bktB* (*Eco*RI-*Bgl*II) and *bld-phaB* (*Bgl*II-*Sal*I). *bktB* was amplified from the genomic DNA of *R. eutropha* ATCC 17697 using primers BKTBf and BKTBr. *bld* and *phaB* were amplified simultaneously from pNK3 ($P_{A1lacO-1}$::*phaA bld phaB*) using primers BLDf and PHABr. The *bktB* and *bld-phaB* amplicons were digested with *Eco*RI-*Bgl*II and *Bgl*II-*Sal*I, respectively, and the digested fragments were cloned into *Eco*RI-*Sal*I-digested pNK (Kataoka et al., 2013), generating pNK6. To construct pNK8 ($P_{A1lacO-1}$::*bktB bld phaB pct*), *pct* was amplified from the genomic DNA of *Megasphaera elsdenii* DSM 20460 using primers PCTf and PCTr. The *pct* amplicon was then directionally cloned into *Hin*dIII-digested pNK6 using an In-Fusion HD Cloning Kit to generate pNK8.

2.4. Fermentation media and culture conditions

Fermentations were performed in modified M9 medium (6.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 2.0 g NH_4Cl, 1.0 g $(NH_4)_2SO_4$, 2 mM $MgSO_4$, and 0.1 mM $CaCl_2$ per liter of water) supplemented with 10 g/L yeast extract, 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-KOH (pH 7.2), and the desired concentration of glucose (111 mM for the production of 1,3-pentanediol and 4-methyl-1,3-pentanediol, and 55.5 mM for the production 1,2,4-butanetriol). Ampicillin (100 μg/mL) was included when appropriate.

For the production experiments, cells were inoculated into a 300-mL flask with baffles containing 20 mL of Luria-Bertani medium supplemented with 11.1 mM glucose, and then pre-cultured at 30 °C on a rotary shaker at 150 rpm until early stationary phase (~5 h post-inoculation). Aliquots of the culture (2%, v/v) were transferred into a 300-mL flask with baffles containing 40 mL of the production medium. Cultures were carried out at 30 °C on a rotary shaker at 150 rpm. When cells reached an optical density at 600 nm (OD_{600}) of 0.3–0.6, isopropyl-β-D-thiogalactopyranoside (IPTG) and 20 mM sodium propionate, 20 mM potassium isobutyrate, or 40 mM sodium glycolate were added to induce production of 1,3-pentanediol, 4-methyl-1,3-pentanediol, and 1,2,4-butanetriol, respectively. IPTG at a concentration of 1 μM was used for production of the first two 1,3-diols, while 10 μM IPTG was used to induce production of 1,2,4-butanetriol. The induction time represented the beginning of production. Flasks were then incubated for a further 48 h.

2.5. Analytical procedure

Growth, residual glucose, and levels of extracellular metabolites (α-ketoglutarate, acetate, and 1,3-butanediol) were measured as described elsewhere (Kataoka et al., 2013; Kataoka et al., 2015; Kataoka et al., 2017). Extracellular metabolites were analyzed using a high-performance liquid chromatography (HPLC) system equipped with a refraction index detector, a diode array detector at 210 nm, and an ion exclusion column (RSpak KC-811, 8.0 mm internal diameter × 300 mm length; Shodex, Showa denko KK, Kawasaki, Japan). Analyses were conducted at 60 °C, with 0.1% (w/v) H_3PO_4 as the mobile phase at a flow rate of 0.7 mL/min.

3. Results and discussion

3.1. Design of a platform pathway for the production of 1,3-diols

In our previous report (Kataoka et al., 2013), the synthetic pathway for the production of (*R*)-1,3-butanediol, a 1,3-diol, was successfully constructed in *E. coli*. Based on this pathway, a platform pathway for the production of other 1,3-diols was redesigned in the current study (Fig. S1). To expand the product spectrum, the original synthetic pathway was modified by replacing *phaA* with *bktB*, because BktB has specificity for long-chain CoA molecules (Slater et al., 1998), and *pct* was added to convert exogenously supplied organic acids to their CoA-derivatives. Although propionate is a natural substrate for Pct, Pct is active toward several short-chain organic acids such as isobutyrate, glycolate, and lactate (Choi et al., 2016; Martin et al., 2013; Taguchi et al., 2008). Using this design, the new synthetic pathway for 1,3-diol production was constructed, and consisted of *bktB*, *pct*, *phaB*, and *bld* under the control of the IPTG-inducible $P_{A1lacO-1}$ promoter, and expressed in *E. coli* BW25113 F'. In this system, endogenous alcohol dehydrogenase (s) was responsible for the final reaction step (Fig. S1).

3.2. Production of 1,3-pentanediol

We first attempted to produce 1,3-pentanediol because it can be synthesized from propionate, the natural substrate of Pct. To validate the functional expression of the enzymes and the production of 1,3-pentanediol, *E. coli* BW25113 F' harboring pNK8 was cultured in medium supplemented with glucose and propionate. Glucose and propionate were simultaneously consumed and exhausted after 48 h of fermentation, while 1,3-pentanediol was produced to a maximum concentration of 4.43 mM (Fig. 1A, Table 1). In addition to the target product, other end products, including 1,3-butanediol, 3-hydroxybutyrate, 1-propanol, acetate, and α-ketoglutarate were produced (Table 1). It was because BktB is highly active in the condensation of two acetyl-CoA molecules to synthesize acetoacetyl-CoA (Slater et al., 1998), which is the natural substrate of PhaB. As a consequence, 1,3-butanediol and 3-hydroxybutyrate were produced as the reduced and hydrolyzed forms of 3-hydroxybutyryl-CoA, respectively. This finding agrees with a report showing that an *E. coli* strain expressing *bktB*, *phaB*

Please cite this article in press as: Kataoka, N., et al. Production of 1,3-diols in *Escherichia coli*. Bioresour. Technol. (2017), http://dx.doi.org/10.1016/j.biortech.2017.05.082