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Production of 1,3-propanediol using a novel 1,3-propanediol dehydrogenase from isolated *Clostridium butyricum* and co-biotransformation of whole cells

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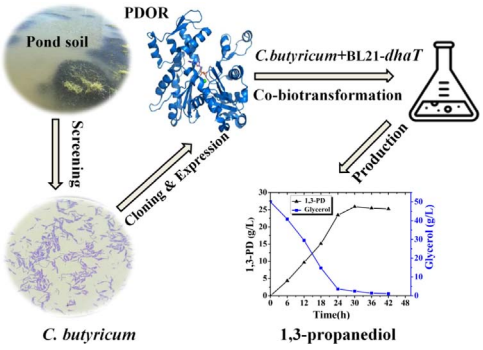
GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a newly strain named *Clostridium butyricum* YJH-09 were isolated from the sample of pond soil and identified through physiological, biochemical and 16S rDNA analysis. Then, the *dhaT* gene encoding a novel 1,3-propanediol dehydrogenase (PDOR) was cloned from this strain and expressed in *Escherichia coli* BL21(DE3). Subsequently, the recombinant PDOR was purified and the optimal pH and temperature, specific activities and kinetic parameter were investigated. Furthermore, the whole cells of *Clostridium butyricum* YJH-09 mixed with BL21-*dhaT* were used to produce 1,3-PD through co-biotransformation. As results, 25.88 g/L of 1,3-PD was generated with 0.54 g/g yield from 50 g/L glycerol in 30 h, and the 1,3-PD production was increased more than 2-fold compared with wild type strain alone. This research would offer useful information for further development of the biosynthesis of 1,3-PD.

1. Introduction

Glycerol is a valuable platform chemical. About 10% (w/w) of glycerol is generated as a byproduct in the producing of biodiesel which is usually produced from animal fats and oils (Johnson and Rehmann, 2016). Today, 75% glycerol supply is produced by renewable resources biodiesel in the world (Chatzifragkou et al., 2014; Saxena et al., 2009). One of the most economical applications of glycerol is converted to high value intermediate such as 1,3-propanediol (1,3-PD). 1,3-PD is a promising versatile chemical compound which has a wide range of applications in cosmetic, food, solvent, pharmaceutical industries. Moreover, 1,3-PD has been used as a monomer for the synthesis of

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various polyesters and feedstock for immence importance compounds (Silva et al., 2009). With the increasing development of important applications of 1,3-PD, the demand for its production has been burgeoning around the world. So far, 1,3-PD can be produced via chemical methods or bio-methods. However, high pressure, high temperature, expensive catalysts are required in the chemical methods, and also toxic intermediates will be produced by this method (Chatzifragkou et al., 2011b). In the bio-methods, 1,3-PD can be biosynthesized under mild conditions utilizing renewable resource, which would offers environmental benefits and cost-competitive product. Therefore, bio-methods have gained much research interests (Lee et al., 2015).

Many microorganisms such as *Klebsiella pneumoniae* (Zhao et al., 2009b), *Citrobacter freundii* (Celinska et al., 2015), *Lactobacillus reuteri* (Baeza-Jiménez et al., 2011) can utilize glycerol as substrate to produce 1,3-PD. Glycerol can be metabolized by those bacteria through a dismutation process coupled oxidation and reduction branch (Chatzifragkou et al., 2011a). In the reductive branch, glycerol is dehydrated to 3-HPA, a reaction catalyzed by coenzyme B_{12}-dependent glycerol dehydratase (GDHt), which is then reduced to 1,3-PD by NADH-dependent 1,3-PD dehydrogenase (PDOR). Till now, the bacteria mentioned above showed a good ability to produce 1,3-PD by fermentation of bio-methods (Guo et al., 2017; Mu et al., 2006; Vivek et al., 2016). However, there are some limitations in the production of 1,3-PD through fermentation. Besides the growth inhibition due to high concentration of substrate, the accumulation of the toxic intermediate 3-HPA and other undesirable by-products, the coenzyme B_{12}-dependent GDHt is another vital factor which will increase the cost of 1,3-PD production.

The previous study showed that a novel glycerol dehydratase from *Clostridium butyricum* is a coenzyme B_{12}-independent enzyme (Raynaud et al., 2003). In addition, *C. butyricum* has been regarded as non-pathogenic bacteria compared with other pathogenic strain such as *K. pneumoniae*, *C. freundii* and *Clostridium perfrigens*. Therefore, *C. butyricum* is a potential candidate for producing 1,3-PD by bio-methods. It was reported that PDOR is a key enzyme that plays crucial roles in the biosynthesis pathway of 1,3-PD from glycerol (Jiang et al., 2016; Zheng et al., 2006). However, the activity of PDOR is not enough for glycerol conversion to 1,3-PD (Hao et al., 2008). As a result, the activity of PDOR will be inhibited in metabolic pathway due to the accumulation of 3-HPA, which then results in the decreasing of 1,3-PD production (Barbirato et al., 1997). Thus, it's necessary to improve the activity of PDOR through the genetic engineering technology (Hao et al., 2008). On the other hand, PDOR is a kind of NADH-dependent enzyme, that means besides improving PDOR activity, increasing the $NADH/NAD^+$ ratio in metabolic pathway may be helpful for the production of 1,3-PD (Zhao et al., 2009a). In addition, biotransformation using whole cell is one of optional strategies of bio-methods, which can accelerate the conversion of glycerol to 1,3-PD.

Therefore, a recombinant strain with a novel PDOR from isolated *C. butyricum* was used for co-biotransformation of glycerol to 1,3-PD coupled with *C. butyricum* by whole cell in this study. In addition, the effect of exogenous NADH on the production of 1,3-PD was also investigated. This study is helpful for the production of 1,3-PD high-efficiently in future.

2. Materials and methods

2.1. Strain, plasmid, culture medium and conditions

E. coli BL21 (DE3) (Stratagene, USA) was used as the host strain for heterologous expression. pET-30a(+) (Invitrogen, USA) was used as expression plasmid. pMD18-T (TakaRa) was used as a cloning vector. Reinforced Clostridium Medium (RCM, g/L), containing Yeast extract powder 3.0, Beef extract 10.0, Tryptone 10.0, Glucose 10.0, Starch 1.0, NaCl 5.0, Sodium acetate 3.0, Cysteine hydrochloride 0.15, Ager 15.0, was used for bacterial growth and enrichment. TSN medium (g/L) for screening was composed of as the following: Tryptone 10.0, Yeast extract 3.0, Sodium sulfite 10.0, Iron(III) citrate tribasic hydrate 0.5, Novobiocin sodium salt 0.02, Polymyxin B sulfate 0.05. *C. butyricum* was initially grown in RCM at 37 °C for 48 h, and then was grown in TSN at 37 °C for 48 h. Thereafter, *C. butyricum* was cultured at RCM agar plates at 37 °C for 48 h. LB medium supplemented with 50 μg/mL of Kanamycin (LB-Kan) additionally was used to select recombinant strain.

2.2. Screening and isolation

Samples of soil for screening *C. butyricum* strains were collected from of the soil (10 g) of a pond in Zhenjiang, China. A heat-shock was conducted in order to stimulate germination of spores in a water bath at 80 °C for 10 min. The soil samples were added into 250 mL RCM medium and incubated for 48 h, and then transferred to TSN medium under anaerobic condition for another 48 h. The sample was serially diluted and transferred to the agar plates of RCM for preliminary screening (Dąbrowski et al., 2012).

2.3. Identification of strains

The preliminary identification was conducted by observing morphology colonies, and then isolation was carried out through the physiological, and biochemical assay. The strains, which had been identified as *Clostridia,* were chosen for further analysis via the 16S rDNA sequence (Guo et al., 2017). The sense primer is 5′-AGAGTTTGATCCTGGCTCAG-3′, and the anti-sense primer is 5′-TACGGCTACCTTGTTACGACTT-3′. These universal primers of bacteria were used in colony PCR for 16S rDNA amplification with the following program: 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 60 s, and the final extension was conducted at 72 °C for 10 min. The amplified DNA fragment was checked by electrophoresis in 0.8% agarose gel. The 16S rDNA sequence analysis was performed with BLAST program of National Center for Biotechnology Information (NCBI).

2.4. Cloning of dhaT gene

According to the 1,3-PD operon region of *C. butyricum* 2CR371.5 from NCBI and the characteristics of restriction sites of expression vector pET30a(+), the primer *dhaT*-F: 5′-GTACCGACGACGACGAC AAGATGAGAATGTATGATTATTT-3 and *dhaT*-R: 5′-CAGTGGTGGT GGTGGTGGTGTTAATAAGCAGCCTTAAAAA-3′ were designed. Above primers were used to clone the *dhaT* gene of *C. butyricum* YJH-09. PCR procedure was performed with denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 2 min, 55 °C for 30 s, and extension at 72 °C for 90 s, with final extension for 10 min at 72 °C. Subsequently, the PCR fragment was digested with *Nco*I and *Xho*I and linked to linearized pET30a(+). The recombinant plasmid named as pET-*dhaT* was confirmed by PCR test and sequenced by Genscript Biotech (Nanjing, China).

2.5. Expression of dhaT gene

The plasmid pET-*dhaT* was transferred into competent cells of *E. coli* BL21(DE3) for expression, and the recombinant strain was named as BL21-*dhaT*. Then, BL21-*dhaT* was inoculated and cultivated with LB-Kan medium at 37 °C. When the cultivation optical density (A600) was about 0.4–0.6, isopropyl-β-D-thiogalactopyranoside (IPTG) was supplemented into the medium with the final concentration of 0.1 mM. Then cultivation was continued under low temperature (25 °C) over night. Subsequently, the cells were centrifuged for 15 min at 8000 rpm, and washed twice with potassium phosphate buffer (100 mM potassium phosphate, 300 mM potassium chloride, pH 7.4). Thereafter, the resuspended cells were disrupted by ultrasonication and then were centrifuged to remove the cell debris.