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Biocatalytic production of (2*S*,3*S*)-2,3-butanediol from diacetyl using whole cells of engineered *Escherichia coli*

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ABSTRACT

(2*S*,3*S*)-2,3-Butanediol ((2*S*,3*S*)-2,3-BD) is a crucial chiral compound that acts as an excellent building block in asymmetric synthesis of highly valuable chiral compounds. However, the low concentration and optical purity of (2*S*,3*S*)-2,3-BD produced in previous studies limited its applications. In the present work, the gene encoding 2,3-butanediol dehydrogenase from an *Enterobacter cloacae* ssp. *dissolvens* strain SDM was cloned and expressed in *Escherichia coli*. Whole cells of the recombinant *E. coli* was used to produce (2*S*,3*S*)-2,3-BD from diacetyl. Under optimal conditions, high-optical-purity (2*S*,3*S*)-2,3-BD (purity >99%) was obtained with concentrations of 16.1 g l^{-1} and 26.8 g l^{-1} in batch and fed-batch conversions, respectively. Thus, the process might be a promising alternative for the production of (2*S*,3*S*)-2,3-BD.

1. Introduction

2,3-Butanediol (2,3-BD) is a crucial vicinal diol with 3 stereoisomers: *meso*-2,3-BD, (2*R*,3*R*)-2,3-BD, and (2*S*,3*S*)-2,3-BD. As a vital platform chemical, 2,3-BD can be used to produce valuable derivatives, such as methyl ethyl ketone and 1,3-butadiene (Garg and Jain, 1995; Syu, 2001; Ji et al., 2011). Optically active isomers can act as antifreeze agents (Yan et al., 2009). Optically pure 2,3-BD can also be used as an excellent building block in asymmetric synthesis of chiral compounds that contain 2 vicinal stereogenic centers (Liu and Högberg, 2001). Therefore, it is desirable to develop a practical technique for the production of optically pure 2,3-BD (Zeng and Sabra, 2011).

Several microorganisms, such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Serratia marcescens* and *Paenibacillus polymyxa* are known to mainly produce *meso*-2,3-BD or (2*R*,3*R*)-2,3-BD in glucose fermentation (Qin et al., 2006; Ji et al., 2008, 2009; Celińska and Grajek, 2009; Gao et al., 2010; Zhang et al., 2010). Until now, the native microorganism with the ability to produce optically pure (2*S*,3*S*)-2,3-BD from glucose has not been found. Researchers previously reported that (2*S*,3*S*)-2,3-BD could be produced by the engineered *Escherichia coli* strains (Ui et al., 2001, 2004; Xiao et al., 2010), but the concentration and optical purity of (2*S*,3*S*)-2,3-BD were not high enough in the studies for economical industrial production.

The major 2,3-BD biosynthesis pathway in microorganisms is shown in Fig. 1. 2,3-Butanediol dehydrogenase (2,3-BDH) (also called acetoin reductase or diacetyl reductase) can catalyze stereoselective reaction between acetoin (AC) and 2,3-BD (Ji et al., 2011; Xiao and Xu, 2007). Especially, the reported (2*S*,3*S*)-2,3-BDHs can stereoselectively catalyze diacetyl (DA) to (3*S*)-AC and then to (2*S*,3*S*)-2,3-BD (Carballo et al., 1991; Giovannini et al., 1996; Rattray et al., 2000; Takusagawa et al., 2001; Ji et al., 2011). DA can be produced by certain microorganisms (Hugenholtz et al., 2000; Zhao et al., 2009) or by chemical synthesis or catalysis (Chen et al., 2004; Stecher et al., 1968), and could then be catalyzed to optically pure (2*S*,3*S*)-2,3-BD by (2*S*,3*S*)-2,3-BDH.

In this paper, a *bdh* gene encoding 2,3-BDH from *Enterobacter cloacae* ssp. *dissolvens* SDM was cloned and expressed in *E. coli*, and stereospecificity of the enzyme was confirmed. The engineered *E. coli* cells were then used to produce (2*S*,3*S*)-2,3-BD from DA as shown in the dashed box of Fig. 1.

2. Methods

2.1. Enzymes and chemicals

Restriction enzymes were purchased from TaKaRa Bio Inc. (China). FastPfu DNA polymerase and T_4 DNA ligase were purchased from Transgen Biotech (China) and MBI (USA), respectively. (2*R*,3*R*)-2,3-BD (98.0%), (2*S*,3*S*)-2,3-BD (99.0%), and *meso*-2,3-BD (98.0%) were purchased from ACROS (The Kingdom of Belgium). Isopropyl-β-D-1-thiogalactopyranoside (IPTG), dithiothreitol (DTT), and phenylmethanesulfonyl fluoride (PMSF) were obtained from

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