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High-yield production of meso-2,3-butanediol from cellodextrin by engineered *E. coli* biocatalysts

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HIGHLIGHTS

- First *Escherichia coli* biocatalyst to convert cellodextrin to meso-2,3-butanediol (BDO).
- High yield of BDO from glucose and cellodextrin, 88% and 84%, respectively.
- Advantageous use of the engineered biocatalyst in production of BDO from cellulose.

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ABSTRACT

Escherichia coli has been engineered to produce a variety of biofuel and biorefinery products. However, it can only produce these products from simple sugars, requiring large amounts of enzymes to depolymerize cellulose into monomer sugars. Engineering *E. coli* to directly use cellodextrin, the partial hydrolysis product of cellulose, potentially could reduce the requirement of enzyme thereby the overall cost. Through a combination of gene deletion, introduction of a synthetic operon, and periplasmic expression of a *Saccharophagus* cellodextrinase, we engineered, for the first time, an *E. coli* biocatalyst capable of producing BDO from cellodextrin. The success of the engineering strategy is evidenced by the high BDO yield (>80%) from cellodextrin. We additionally demonstrate that the engineered biocatalyst can be advantageously used in a SSF process for BDO production from cellulose as the expression of cellodextrinase from a BDO producer augments the insufficient β-glucosidase activities in a commercial cellulase cocktail.

1. Introduction

2,3-Butanediol (BDO) is a commodity chemical that can be produced using microbial biocatalysts (Xiu and Zeng, 2008; Celinska and Grajek, 2009). BDO, along with its many derivatives, is remarkable in its wide range of applications. Not only it has an established market as anti-freezer, but it potentially could serve an emerging biofuel market of an enormous size, as a precursor to 2-butanol and to methyl ethyl ketone (MEK), a liquid fuel additive (Leroux and Lucas, (1951); Tran and Chambers, 1987; Muramatsu and Suzuki, 2011). Another notable application of BDO is in the synthetic rubber as it can be converted to 1,3-butadiene (Nielsen et al., 2010; Celinska and Grajek, 2009). Additionally, it has applications ranging from pharmaceutical to food additives and in the manufacturing of solvent and plastics (Grag and Jain, 1995; Syu, 2001; Liu et al., 2011).

Feasibility of its biological production was demonstrated back in the World War II era but was deemed to be uncompetitive to the petroleum route (Magee and Kosaric, 1987; Grag and Jain, 1995; Ji et al., 2011). BDO is a common microbial metabolite from *Enterobacteriaceae* family (Xu et al., 2012). In fact, *Klebsiella pneumonia* and *Klebsiella oxytoca* are considered among the best producers. The final product concentration exceeding 100 g/L and a yield close to 90% of theoretic value were reported for these strains (Zeng et al., 1991; Ma et al., 2009; Ji et al., 2010). However, these efficient microbial producers are classified as class 2 (or pathogenic), thus not suitable for large scale industrial applications (Celinska and Grajek, 2009). Constructing benign *Escherichia coli* catalysts for the production of BDO could avoid such concerns. Additionally, engineering *E. coli* biocatalyst offers unique opportunities to produce enantiomerically pure BDO for particular applications, whereas natural producers typically provide a mixture of BDO isomers.

Indeed, recent years witnessed significant progress in engineering *E. coli* for BDO production. In particular, *E. coli* biocatalyst was engineered to produce each of the three isomers of BDO (S,S-BDO, R,R-BDO, and meso-BDO). Co-expression of meso-BDH of

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