Efficient (3R)-Acetoin Production from meso-2,3-Butanediol Using a New Whole-Cell Biocatalyst with Co-Expression of meso-2,3-Butanediol Dehydrogenase, NADH Oxidase, and Vitreoscilla Hemoglobin

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Introduction

Acetoin (AC) is a volatile platform compound with various potential industrial applications. AC contains two stereoisomeric forms: (3S)-AC and (3R)-AC. Optically pure AC is an important potential intermediate and widely used as a precursor to synthesize novel optically active materials. In this study, chiral (3R)-AC production from meso-2,3-butanediol (meso-2,3-BD) was obtained using recombinant Escherichia coli cells co-expressing meso-2,3-butanediol dehydrogenase (meso-2,3-BDH), NADH oxidase (NOX), and hemoglobin protein (VHB) from Serratia sp. T241, Lactobacillus brevis, and Vitreoscilla, respectively. The new biocatalyst of E. coli/pET-mbdh-nox-vgb was developed and the bioconversion conditions were optimized. Under the optimal conditions, 86.74 g/l of (3R)-AC with the productivity of 3.61 g/l/h and the stereoisomeric purity of 97.89% was achieved from 93.73 g/l meso-2,3-BD using the whole-cell biocatalyst. The yield and productivity were new records for (3R)-AC production. The results exhibit the industrial potential for (3R)-AC production via whole-cell biocatalysis.

Keywords: meso-2,3-butanediol dehydrogenase, meso-2,3-butanediol, (3R)-acetoin, NAD⁺ regeneration, vitreoscilla hemoglobin, whole-cell biocatalysis
catalytic efficiency and stereoselectivity [12–16].

(2R,3R)-2,3-BD, meso-2,3-BD, and diacetyl (DA) are three potential substrates for (3R)-AC production by enzyme catalysis. Two (2R,3R)-2,3-butanediol dehydrogenases ((2R,3R)-2,3-BDHs) showed the abilities of catalyzing the reduction of DA into (3R)-AC in the presence of NADH [17, 18]. However, (3R)-AC as an intermediate product was readily reduced into (2R,3R)-2,3-BD by the same enzyme, which led to less accumulation of (3R)-AC. Since (2R,3R)-2,3-BDH is a reversible enzyme, it can also use (2R,3R)-2,3-BD as a substrate to produce (3R)-AC in the presence of NAD⁺. Xiao et al. [5] developed a whole-cell biocatalytic method using two enzymes ((2R,3R)-2,3-BDH and NADH oxidase (NOX)) for the biocconversion of (2R,3R)-2,3-BD into (3R)-AC. Under the optimal conditions, (3R)-AC with a maximum yield of 41.8 g/l was achieved from (2R,3R)-2,3-BD in the whole-cell biocatalytic reaction [5]. Moreover, several glycerol dehydrogenases (GDHs) also catalyzed the oxidation of (2R,3R)-2,3-BD into (3R)-AC when NAD⁺ was used as a cofactor [19–22]. Yamada-Onodera et al. [23] developed a recombinant E. coli expressing the GDH enzyme of Hansenula polymorpha DI-1 for the biocconversion of (2R,3R)-2,3-BD into (3R)-AC. However, (3R)-AC with a low yield of 9.68 g/l was produced by the recombinant strain without other additives to regenerate NAD⁺ from NADH [23].

Previous study indicated that meso-2,3-BD as substrate was also converted into (3R)-AC by (S)-selective alcohol dehydrogenase. However, only 48 mM (3R)-AC was obtained in the reaction system owing to low catalytic efficiency [10]. Recent reports showed that three meso-2,3-BDHs from Klebsiella pneumoniae XJ-Li, Serratia marcescens H30, and Serratia sp. T241 could efficiently catalyze the oxidation of meso-2,3-BD into (3R)-AC [24, 25]. Compared with reported (2R,3R)-2,3-BDHs, these reported meso-2,3-BDHs possessed lower $K_m$ and higher $k_{cat}$, which showed the potential for biocatalysis. Among them, meso-2,3-BDH from Serratia sp. T241 exhibited higher catalytic efficiency compared with the other two meso-2,3-BDHs. Considering that a large amount of meso-2,3-BD could be obtained by S. marcescens H30 and K. pneumoniae SDM [26, 27], it is feasible that meso-2,3-BD is used for the production of (3R)-AC. In this study, a new whole-cell system was developed for efficient production of (3R)-AC from meso-2,3-BD via co-expression of meso-2,3-BDH, NOX, and Vitreoscilla hemoglobin (VHB) in E. coli cells (Fig. 1). A high concentration of (3R)-AC was obtained by the whole-cell biocatalyst.

**Materials and Methods**

**Enzymes and Biocatalysts**

Pfu DNA polymerase and the ligation kit were purchased from TaKaRa Biotech (China). Restriction endonucleases were from NEB (China). Protein and DNA markers, and competent cells were obtained from Tiangen Biotech (China). PCR primers were synthesized in Sangon Biotech (China). The standards, including (2S,3S)-2,3-BD (97.0%), (2R,3R)-2,3-BD (97.0%), meso-2,3-BD (99.0%), and (3S/3R)-AC (47.3%/52.7%), were obtained from Sigma-Aldrich (China). The substrate of 2,3-BD (93.73% meso-2,3-BD, 2.23% (2R,3R)-2,3-BD, and 4.04% (2R,3R)-2,3-BD) was purchased from Sinopharm (China).

**Construction of the Recombinant Strains**

The resources used in this study, including strains, plasmids, and primers, are shown in Table 1. *Serratia* sp. T241 and *Lactobacillus brevis* were cultured in LB medium at 30°C, and the genomic DNA was obtained using the OMEGA Bacterial Genomic DNA Kit (China). The VHB gene (vgb) source was from the pBR322-vgb plasmid constructed previously in our laboratory. The mbdh gene (GenBank Accession No. AEF50077) encoding meso-2,3-BDH from *Serratia* sp. T241 was PCR-amplified using primers (P1/P2) with BamHI and HindIII sites. The purified PCR product was double-digested using the restriction enzymes BamHI and HindIII and inserted into expression plasmid pET28a, generating the recombinant plasmid pET-mbdh. To construct the co-expressing plasmids of pET-mbdh-nox and pET-mbdh-nox-vgb, a series of primers (P3–P12) were designed as shown in Table 1. The mbdh and nox (GenBank Accession number AAN04047) gene fragments, obtained by PCR amplification from *Serratia* sp. T241 and *L. brevis* genomic DNA using the primers P3/P4 and P5/P6, were spliced by overlap extension PCR, generating the full-length product mbdh-nox. The spliced fragment was digested with restriction enzymes BamHI and HindIII.