Cofactor self-sufficient whole-cell biocatalysts for the production of 2-phenylethanol

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1. Introduction

2-Phenylethanol (2-PE), an aromatic alcohol with a pleasant rose-like fragrance, is widely used in the cosmetic and food industries. 2-PE is traditionally extracted from rose, the yield is typically low (Hazelwood et al., 2008). There are three reactions involved in this synthetic pathway (Fig. 1a). First, aromatic transaminase converts l-Phe to phenylpyruvate (PPA) using 2-oxoglutarate (2-OG) as an amine receptor. Second, PPA is converted to phenylacetaldehyde (PAAL) by phenylpyruvate decarboxylase (PDC). Finally, aldehyde reductase or alcohol dehydrogenase (ADH) converts PAAL to 2-PE, at the expense of the cell’s reducing power. Great efforts have been made on yeast-based production of 2-PE, encompassing metabolic engineering, fermentation and process engineering, leading to the production of about 6 g/L 2-PE by yeast fermentation (Eshkol et al., 2009; Etschmann and Schrader, 2006; Kim et al., 2014; Morrissey et al., 2015; Stark et al., 2002). Furthermore, since cell growth is inhibited by the accumulated 2-PE (Etschmann and Schrader, 2006), in situ product removal (ISPR) has been used to extract 2-PE in the aqueous phase to circumvent the problem of product inhibition, which is proven to be a useful approach. Extractants such as polypropylene glycol 1200 and Hytrel® 8206 were used to remove 2-PE from the fermentation broth, and 10.2–20.4 g/L 2-PE was achieved in 30–36 h (Etschmann and Schrader, 2006; Gao and Daugulis, 2009). However the yeast fermentation process usually takes 30–50 h or even a few nights.
duced from 100 mM Phe (Hwang et al., 2009). However, the yield of bioconversion of phenylalanine and 38 mM (4.7 g/L) 2-PE was produced to optimize the 2-PE biosynthetic pathway in cetaldehyde synthase and phenylacetaldehyde reductase from plants (Koma et al., 2012). To further improve the efficiency of the pathway, well-characterized genes encoding phenylacetaldehyde synthase and phenylacetaldehyde reductase from plants were introduced to optimize the 2-PE biosynthetic pathway in E. coli (Achmon et al., 2014; Sakai et al., 2007). The shikimic acid pathway was redesigned to increase the supply of the intermediate (PPA) and 2-PE production from glucose was improved to 2.33 mM (0.285 g/L) (Achmon et al., 2014; Madje et al., 2012). For 2-PE biosynthesis, the efficiency (Hummel and Groger, 2014). The efficient strategy has the potential to enhance the production of a number of desirable chemicals with respect to cofactor regeneraiton and balance.

2. Materials and methods

2.1. Chemicals and reagents

L-Phe, 2-OG, L-Glu and zeolite were purchased from Shanghai ShengGong Bio-chemical Co. Ltd. (Shanghai, China). For high-performance liquid chromatography (HPLC) analysis, standards for L-Phe, PPA, PAAL, 2-PE and D-PLA were purchased from Sigma-Aldrich (Steinheim, Germany), Hytrel® 8206 was purchased from DuPont (Delaware, USA). Restriction endonucleases, DNA polymerases, T4 DNA ligase, and Gibson kits were purchased from New England Biolabs (USA).

2.2. Bacterial strains and culture conditions

All strains and plasmids used in this study are listed in Supplementary Table S1. P1 transduction based on Keio collection strains (Baba et al., 2006) enabled gene deletion. The mutant strains were verified by PCR and further confirmed by sequencing (Shenggong, China). E. coli strains were grown at 37 °C on a shaker at 220 rpm in LB (Luria–Bertani) medium with streptomycin (50 μg/mL) added as required. For protein expression, overnight cultures were inoculated into an auto-induction ZYM medium (Studier, 2005) with 1% inoculum and incubated under constant shaking at 20 °C (for 2-PE) or 37 °C (for D-PLA) for 16 h.

2.3. Cloning and expression

The genes encoding AbPDC, ZmPDC, PAR, PeGHD and hGHD were codon-optimized based on the E. coli codon preference; the other genes for 2-PE and D-PLA production were amplified from corresponding genomic DNA. The primers are listed in Supplementary Table S2. The...