Bioresource Technology 118 (2012) 572–575

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Effect of ribose, xylose, aspartic acid, glutamine and nicotinic acid on ethyl (*S*)-4-chloro-3-hydroxybutanoate synthesis by recombinant *Escherichia coli*

Nan Li [a], Yueyuan Zhang [a], Qi Ye [a], Yuzhe Zhang [b], Yong Chen [a], Xiaochun Chen [a], Jinglan Wu [a], Jianxin Bai [a], Jingjing Xie [a,*], Hanjie Ying [a,*]

[a] *State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, PR China*
[b] *College of Materials Science and Engineering, Nanjing University of Technology, Nanjing 210009, PR China*

ARTICLE INFO

Article history:
Received 28 October 2011
Received in revised form 9 February 2012
Accepted 21 February 2012
Available online 1 March 2012

Keywords:
Ethyl (*S*)-4-chloro-3-hydroxybutanoate
Co-expression
Coenzyme regeneration
precursor

ABSTRACT

Most reductases which belong to the short chain dehydrogenase/reductase (SDR) superfamily require NAD (P) H for activity. Addition of this cofactor was still necessary for the production of ethyl (*S*)-4-chloro-3-hydroxybutanoate by *Escherichia coli* even when a cofactor regeneration system was constructed by co-expressing carbonyl reductase from *Pichia stipitis* (PsCRI) and glucose dehydrogenase from *Bacillus megaterium* (BmGDH). In an attempt to reduce dependence on the expensive cofactor, compounds directly or indirectly involved in NADP synthesis were added to the medium. Only glutamine and xylose enhanced the content of intracellular NADP (H) and the concentration of product. The concentration and yield of (*S*)-CHBE reached 730 mM and 48.7%, with 30 g/L of glutamine and 40 g/L of xylose, a 2.6-fold increase over the control without the addition of the two compounds.

1. Introduction

Ethyl (*S*)-4-chloro-3-hydroxybutanoate [(*S*)-CHBE] is a precursor for enantiopure intermediates for chiral drugs, including cholesterol-lowering hydroxymethylglutaryl-CoA reductase inhibitors like atorvastatin calcium (Asako et al., 2009). Compared to conventional chemical synthesis, the asymmetric reduction of ethyl 4-chloro-3-oxobutanoate (COBE) to (*S*)-CHBE by reductases has several advantages, including low cost, mild reaction conditions, high yield, and remarkable enantioselectivity (Nakamura et al., 2003; Matsuda et al., 2009). However, biocatalysis from COBE to (*S*)-CHBE by reductases can be quite challenging, since it often requires expensive cofactors such as NADH or NADPH. Because of the high cost of the pyridine cofactors, in situ cofactor regeneration is critical to the economic viability of industrial-scale biotransformations (van der Donk and Zhao, 2003; Liu and Wang, 2007). For example, NAD^+ can be recycled from NADH for production of (*S*)-CHBE using *Escherichia coli* co-expression of a carbonyl reductase and a formate dehydrogenase (Yamamoto et al., 2004). In the previous study, a cofactor regeneration system for enzymatic biosynthesis was constructed by co-expressing a carbonyl reductase from *Pichia stipitis* (PsCRI) and a glucose dehydrogenase from *Bacillus megaterium* (BmGDH) in *E. coli* Rosetta (DE3) plysS cells (Ye et al., 2010). (*S*)-CHBE was synthesized from COBE with the catalysis of PsCRI which required the NADPH for activity. The cofactor was consumed during the reaction, but by using the cofactor regeneration system, NADPH could be recycled from $NADP^+$ with BmGDH. However, a certain amount of NADP (H) was still needed for the start-up of the asymmetric reduction. In stereoselective reduction of COBE by *E. coli* transformant cells co-expressing the aldehyde reductase and glucose dehydrogenase genes, Shimizu et al. (1990) added 0.05 mM $NADP^+$ into a two-phase reaction system, but doing so on an industrial scale would be too expensive. Since increases in intracellular NAD (P) H concentrations have been observed upon provision of relatively inexpensive compounds directly or indirectly involved in the synthesis of this cofactor (Liu et al., 2006; Ma et al., 2007), the present study investigated if ribose, xylose, aspartic acid, glutamine and nicotinic acid improved (*S*)-CHBE biosynthesis by recombinant *E. coli* in a water/butyl acetate system.

2. Methods

2.1. E. coli strain and cultivation

Escherichia coli Rosetta (DE3) pLysS co-expressing PsCR from *P. stipitis* (CBS 6054) and BmGDH (DSM 2894, *E. coli* Rosetta pBP) (Ye et al., 2010) was maintained on Luria–Bertani (LB) agar slants and inoculated into 1-L flasks containing MC medium with (g/L):

* Corresponding authors. Present Address: College of Life Science and Pharmaceutical Engineering, Nanjing University of Technology, No. 5, Xin Mofan Road, Nanjing 210009, PR China. Tel.: +86 25 86990001; fax: +86 25 58133398.
E-mail addresses: xiej@njut.edu.cn (J. Xie), yinghanjie@njut.edu.cn, cxc_1981@sohu.com (H. Ying).

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doi:10.1016/j.biortech.2012.02.102