Bioresource Technology 114 (2012) 561–566

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Biosynthesis of long chain hydroxyfatty acids from glucose by engineered *Escherichia coli*

Xiangwei Wang [a,b], Lingling Li [a,c], Yanning Zheng [a,b], Huibin Zou [a], Yujin Cao [a], Hui Liu [a], Wei Liu [a], Mo Xian [a,*]

[a] Chinese Academy of Sciences, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] College of Food Science, Sichuan Agricultural University, Yaan 625014, China

ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 23 February 2012
Accepted 24 February 2012
Available online 4 March 2012

Keywords:
Long chain hydroxyfatty acid
Escherichia coli
Thioesterase
Fatty acid hydroxylase
fadD knocking out

ABSTRACT

This study devised a pathway in *Escherichia coli* for direct production of long chain hydroxyfatty acids (HFAs) from glucose. This is first report on the biosynthesis of HFAs from renewable sugar, without the need of exogenous fatty acids. By employing thioesterases BTE and 'TesA to tailor the composition of free fatty acids (FFAs) and using fatty acid hydroxylase $P450_{BM3}$ to convert FFAs to HFAs, high-specificity production of C12 and C14 HFAs was achieved. By further knocking out the endogenous *fadD* gene of *E. coli*, an engineered strain capable of producing 117.0 mg/L HFAs was finally obtained, representing a high HFA production in shake flask. This study indicated an attractive metabolic strategy for the biosynthesis of HFAs directly from renewable carbohydrates resources.

1. Introduction

Long chain hydroxyfatty acids (HFAs) as the intermediates are widely used in the production of biodegradable plastics, cyclic lactones and pharmaceutical agents (Pohl et al., 2008; Rawlings, 2003; Vandamme and Soetaert, 2002). Given that HFAs have great commercial potentials, many endeavors have been made to produce HFAs chemically or biologically. Though HFAs can be prepared through a variety of organic synthesis, the expensive starting material as well as the complicated procedures precluded their industrial application in commodity plastics (Lie Ken Jie and Lam, 1977; Metzger, 2009; Metzger and Bornscheuer, 2006). Alternatively, HFAs can be produced through bio-routes with different biocatalysts like yeast (Lu et al., 2010), *Pseudomonas* (Wallen et al., 1962), *Bacillus* (Kuo et al., 2002; Lanser et al., 1992) and *Escherichia coli* (Boddupalli et al., 1992; Schneider et al., 1998a). However, all the studies towards bio-HFAs reported require exogenous addition of fatty acids as a substrate. The HFA biosynthesis will be more economic if the whole-cell biocatalyst can directly utilize renewable sugar as substrate. Therefore, it is of great importance to develop a whole-cell biocatalyst that can efficiently convert glucose to the desired HFA products.

E. coli is a candidate model for direct production of HFAs from sugars. First, it can utilize the inexpensive renewable feedstock and easily reach a high cell density. In addition, it has been previously genetically modified effortlessly to produce a variety of valuable platform chemicals and biofuels (Antoni et al., 2007; Hu et al., 2010; Kang et al., 2011). However, wild-type *E. coli* is incapable of accumulating fatty acids and further converting them to HFAs. To theoretically reach the goal of converting glucose to HFAs by metabolically engineered *E. coli* catalysts, it is needed to (1) overproduce free fatty acids (FFAs) by overexpressing a thioesterase, which can release feedback inhibition caused by fatty acyl-acyl carrier proteins (acyl-ACPs), (2) convert FFAs to HFAs with an exogenous fatty acid hydroxylase, (3) integrate the two pathways above mentioned into one metabolic route.

For the first step, in an effort to overproduce fatty acids in *E. coli*, it is essential to find a thioesterase capable of efficiently releasing FFAs from fatty acyl-ACPs. 'TesA, a leaderless version of the endogenous thioesterase TesA of *E. coli*, is capable of releasing feedback inhibition caused by long-chain fatty acyl-ACPs and giving significant levels of FFAs (Cho and Cronan, 1995; Jiang et al., 1994). BTE, a medium-chain acyl-ACP thioesterase of *Umbellularia californica*, is also used to accumulate of medium-chain fatty acids (Voelker and Davies, 1994). Furthermore, as it is well known, the released FFAs can be degraded via β-oxidation pathway (Overath et al., 1969; Perkins et al., 1963). In this pathway, the conversion of fatty acid to fatty acyl-CoA is the acknowledged rate-limiting step,

* Corresponding author. Address: No. 189 Songling Road, Laoshan District, Qingdao 266101, China. Tel./fax: 86 532 80662765.
E-mail address: xianmo@qibebt.ac.cn (M. Xian).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2012.02.119