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Short communication

Production of ω-hydroxy octanoic acid with *Escherichia coli*

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ABSTRACT

The present proof-of-concept study reports the construction of a whole-cell biocatalyst for the *de novo* production of ω-hydroxy octanoic acid. This was achieved by hijacking the natural fatty acid cycle and subsequent hydroxylation using a specific monooxygenase without the need for the additional feed of alkene-like precursors. For this, we used the model organism *Escherichia coli* and increased primarily the release of the octanoic acid precursors by overexpressing the plant thioesterase FatB2 from *Cuphea hookeriana* in a β-oxidation deficient strain, which lead to the production of 2.32 mM (8.38 mg g_{cww}^{-1}) octanoic acid in 24 h. In order to produce the corresponding ω-hydroxy derivative, we additionally expressed the engineered self-sufficient monooxygenase fusion protein $CYP153A_{Maq}$(G307A)-CPR_{BM3} within the octanoic acid producing strain. With this, we finally produced 234 μM (0.95 mg g_{cww}^{-1}) ω-hydroxy octanoic acid in a 20 h fed-batch set-up.

1. Introduction

The current access to medium-chain fatty acids (C_6–C_{12}), which are building blocks for the production of emulsifiers, adhesives, or coatings and in the preparation of cosmetics or medicines, is mostly restricted to the Shell Higher Olefine Process (SHOP) (Cornils and Herrmann, 2006; Keim, 2013; Weissermel and Arpe, 1997a). Major drawbacks of this process are that it relies on fossil oil usage, is carried out at elevated temperatures and pressures, and requires the use of toxic and dangerous catalysts (Weissermel and Arpe, 1997b). Biocatalysts can overcome some of these hurdles due to the selectivity of enzymes, the possibility to use renewable feed stocks, and mild reaction conditions (Schrewe et al., 2013). Common approaches consist of either the genetic engineering of microorganisms to convert sustainable unrelated carbon sources (*de novo* biosynthesis) (Bokinsky et al., 2011; Dellomonaco et al., 2011; Steen et al., 2010; Sung et al., 2015) or the conversion of fatty acid precursors to hydroxylated derivatives using oxygenases (Bae et al., 2014; Gatter et al., 2014; Otte et al., 2014; Scheps et al., 2013). Notably, short and mid chain ω-hydroxy fatty acids and their corresponding dicarboxylic acid derivatives have interesting pharmaceutical activities (Sieber and Hegel, 2014) and represent precursor for new copolymers (Chung et al., 2015; Ceccorulli and Scandola, 2005; Rai et al., 2012). However, to our knowledge the *de novo* production of ω-hydroxy fatty acids with chain lengths shorter than C_{12} has not been reported so far.

2. Materials and methods

2.1. Strains, culture conditions, and plasmid construction

Strains, culture condition, oligonucleotide sequences, and a detailed description for plasmid constructions used in the study are given in the Supplementary material.

2.1.1. Production of octanoic acid

For experiments in 250 mL Erlenmeyer batch cultures, 50 mL TB medium (Sambrook and Russell, 2001) were inoculated with Δ*fadD* harboring pJEM[*trx*-FatB2] to an OD_{600} of 0.05 and grown at 37 °C with shaking at 180 rpm (HT Multitron, Infors AG, Switzerland). When an OD_{600} of 10 was reached, protein production was induced with 500 μL sterile rhamnose solution (final concentration of 0.2%). After incubation at 30 °C for 24 h, fatty acids were extracted and analyzed (Supplementary material).

For fed-batch experiments with 1 L operating volume (Multifors 2, Infors AG, Switzerland), 900 mL TB medium were inoculated to an OD_{600} of 0.05–0.1 with Δ*fadD* harboring pJEM[*trx*-FatB2]. The cells were grown at 37 °C with 600 rpm stirring and an airflow of 2 vvm to an OD_{600} of 10. Subsequently, cells were induced for *trx*-FatB2 production with addition of rhamnose and grown for 24 h at 30 °C with a constant feed of glycerol (4 g h^{-1}). Dissolved oxygen and pH

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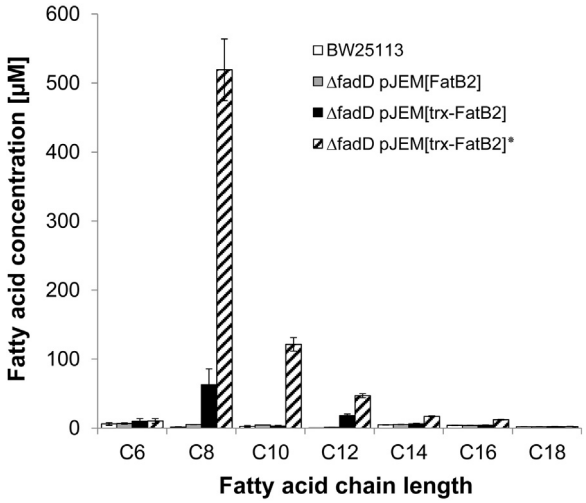

Fig. 1. Fatty acid profiles of strains BW25113, Δ*fadD*[FatB2], Δ*fadD*[trx-FatB2] under standard conditions, and the Δ*fadD*[*trx*-FatB2] strain under optimised conditions (marked with*). Data represent the mean value of biological triplicates. Error bars represent the corresponding standard deviation.

was monitored throughout the process and pH was maintained at 7.4 using NH_4OH (25%) and H_3PO_4 (10%).

2.1.2. Production of ω-hydroxy octanoic acid

Strain Δ*fadD* harboring pJEM[*trx*-FatB2] and pBAD18-CYP (encoding CYP153A$_{Maq}$(G307A)-CPR$_{BM3}$) were grown at 37 °C with shaking at 180 rpm. Proteins were induced simultaneously with sterile rhamnose and arabinose (final concentrations of 0.2%) at an OD_{600} of 1. After incubation for 24 h at 30 °C, fatty acids were extracted and analyzed (Supplementary material). For fed-batch experiments, the parameters described above were used; with the difference that arabinose was additionally added to the cultures.

3. Results and discussion

For ω-hydroxy octanoic acid production, a strain deleted in the acyl-CoA synthetase (Δ*fadD*), the first enzyme within the β-oxidation pathway (Pech-Canul et al., 2011), was used for construction (Baba et al., 2006). To shift the natural fatty acid spectrum towards octanoic acid and to increase the overall content of free fatty acids, we expressed a codon optimized and N-terminal *trx*-fusion protein of the plant thioesterase FatB2 (pJEM[*trx*-FatB2]) from *Cuphea hookeriana* (Dehesh et al., 1996). This enzyme has been previously shown to exhibit a high preference towards C_8 and C_{10} fatty acid-ACPs as a substrate for its hydrolysing activity (Dehesh et al., 1996). When we compared different strains in batch cultures, we found free octanoic acid concentrations of 1.4 µM (0.03 mg g_{cww}^{-1}, BW25113), 5.1 µM (0.12 mg g_{cww}^{-1}, Δ*fadD*), and 63 µM (1.52 mg g_{cww}^{-1}, Δ*fadD* pJEM[FatB2]) (Fig. 1). By use of the *trx*-construct and optimization of inducer concentrations, temperature, induction time point, and medium, the production of octanoic- and decanoic acid could be further increased to 518 µM (6.23 mg g_{cww}^{-1}) and 119 µM (1.71 mg g_{cww}^{-1}), respectively. We then adapted our approach to 1 L bioreactors in a fed-batch mode of operation (Fig. 2a). With this, we finally achieved the production of 2.32 mM (8.38 mg g_{cww}^{-1}) and 327 µM (1.4 mg g_{cww}^{-1}) of octanoic and decanoic acid, respectively. In our opinion, the observed increase in the specific production rate of octanoic acid in fed-batch cultures can mainly be explained by (i) the control of pH during growth, (ii) the continuous feed of glycerol, and thus (iii) the increased optical density during production.

Notably, other studies have reported total fatty acid concentrations up to 4.8 g L^{-1} in batch cultures. However, most of these reports are only of limited use for comparison, as they

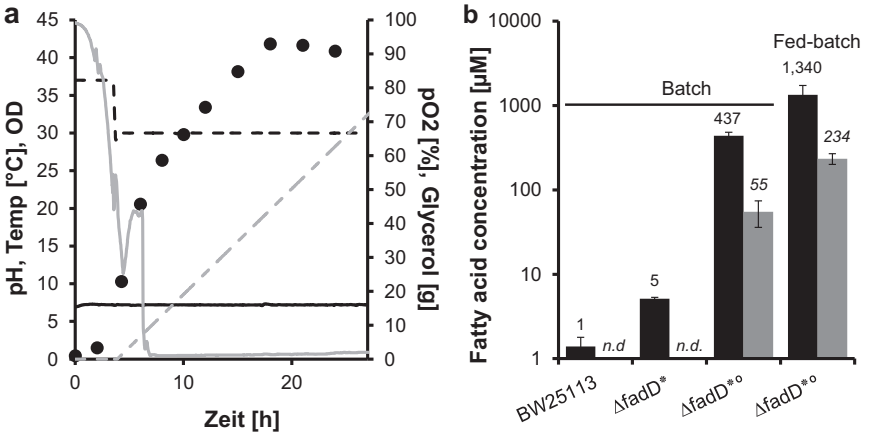

Fig. 2. (a) Various process parameters during the fed-batch mode for octanoic acid production. The recorded values are: pH (black solid line, primary axis), temperature (black dotted line, primary axis, in °C), OD_{600} (black dots, primary axis), pO_2 (gray solid line, secondary axis, in%), total amount of glycerol (gray dotted line, secondary axis, in g). (b) Comparison of octanoic acid (black bars) and ω-hydroxy octanoic acid (grey bars) produced by strains BW25113, Δ*fadD* harbouring pJEM[FatB2] (marked with*), and Δ*fadD* harbouring pJEM[*trx*-FatB2] and pBAD18-CYP (marked with*) in batch and fed-batch cultures under optimal conditions. Bars represent the mean of three independent experiments. The error bars the corresponding standard deviation.