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Biosynthesis of odd-chain fatty alcohols in *Escherichia coli*

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ABSTRACT

Engineered microbes offer the opportunity to design and implement artificial molecular pathways for renewable production of tailored chemical commodities. Targeted biosynthesis of odd-chain fatty alcohols is very challenging in microbe, due to the specificity of fatty acids synthase for two-carbon unit elongation. Here, we developed a novel strategy to directly tailor carbon number in fatty aldehydes formation step by incorporating α-dioxygenase (αDOX) from *Oryza sativa* (rice) into *Escherichia coli* αDOX oxidizes C_n fatty acids (even-chain) to form C_{n-1} fatty aldehydes (odd-chain). Through combining αDOX with fatty acyl–acyl carrier protein (-ACP) thioesterase (TE) and aldehyde reductase (AHR), the medium odd-chain fatty alcohols profile (C_{11}, C_{13}, C_{15}) was firstly established in *E. coli*. Also, medium even-chain alkanes (C_{12}, C_{14}) were obtained by substitution of AHR to aldehyde decarbonylase (AD). The titer of odd-chain fatty alcohols was improved from 7.4 mg/L to 101.5 mg/L in tube cultivation by means of fine-tuning endogenous fatty acyl-ACP TE (TesA′), αDOX, AHRs and the genes involved in fatty acids metabolism pathway. Through high cell density fed-batch fermentation, a titer of 1.95 g/L odd-chain fatty alcohols was achieved, which was the highest reported titer in *E. coli*. Our system has greatly expanded the current microbial fatty alcohols profile that provides a new brand solution for producing complex and desired molecules in microbes.

1. Introduction

Microbial biosynthesis of fatty alcohols has attracted great attentions, since fatty alcohols are widely utilized in chemical industry and transportation sector but the synthesis process is unsustainable (Choi et al., 2014; Lennen and Pfleger, 2013; Thompson et al., 2014). The fatty alcohols profile that produced from fatty acids biosynthesis pathway is fixedly even-chain length (Fig. 1A), as native *Escherichia coli* fatty acids synthase system possesses strict specificity for two-carbon unit elongation (Janssen and Steinbuchel, 2014; Magnuson et al., 1993). However, the fatty alcohols, which used as detergents, plastics or cosmetics, are more diverse in structure (Choi et al., 2014; Nozzi et al., 2014). They compose of both odd and even chain and both linear and branched chain hydrocarbon compositions. Therefore, it is necessary to expand the recently developed fatty alcohols biosynthesis pathways in order to realize arbitrarily microbial production of desired and complex products.

A few works have expanded free fatty acid (FFA) or alkane profile through manipulation of *fabH* gene in upstream fatty acids biosynthesis pathway (Harger et al., 2013; Howard et al., 2013; Wu and San, 2014). Microbial alkanes are normally odd chain length as the last reaction is decarbonylation that takes off one carbon from the intermediate fatty aldehydes (Lee et al., 2015) (Fig. 1A). By incorporating FabH2 from *Bacillus subtilis* into *E. coli*, even chain alkanes were produced (Harger et al., 2013). However, the purity and titer of the expected even chain alkanes was rather disillusionary. For example, only 3.7 mg/L of C14 and 1.2 mg/L of C16 alkanes were detected, comprising 6% of total alkanes when no propionate was supplemented. Addition of 6.5 mM propionate only increased the proportion of even chain alkane from 6% to 27%, which indicated that introduction of FabH2 alone did not have the expected effect of significant changing the product profile. Although combination of heterogenous expression of propionyl-CoA synthase allowed high concentration (1205 mg/L) or high percentage (83.2%) of odd-chain fatty acids biosynthesis (Wu and San, 2014), extracellular propionate was still compulsory to be supplemented. These results suggest that fatty acids biosynthesis process is reluctant to be changed to directly produce unusual FFAs of odd or branched chain length. Besides, the metabolism distance from acetoacetyl-ACP catalyzed by FabH to the final products is too long that adds the uncertainty for targeted

Abbreviations: ACP, acyl carrier protein; TE, thioesterase; αDOX, α-dioxygenase; AHR, aldehyde reductase; AD, aldehyde decarbonylase; FFAs, free fatty acids

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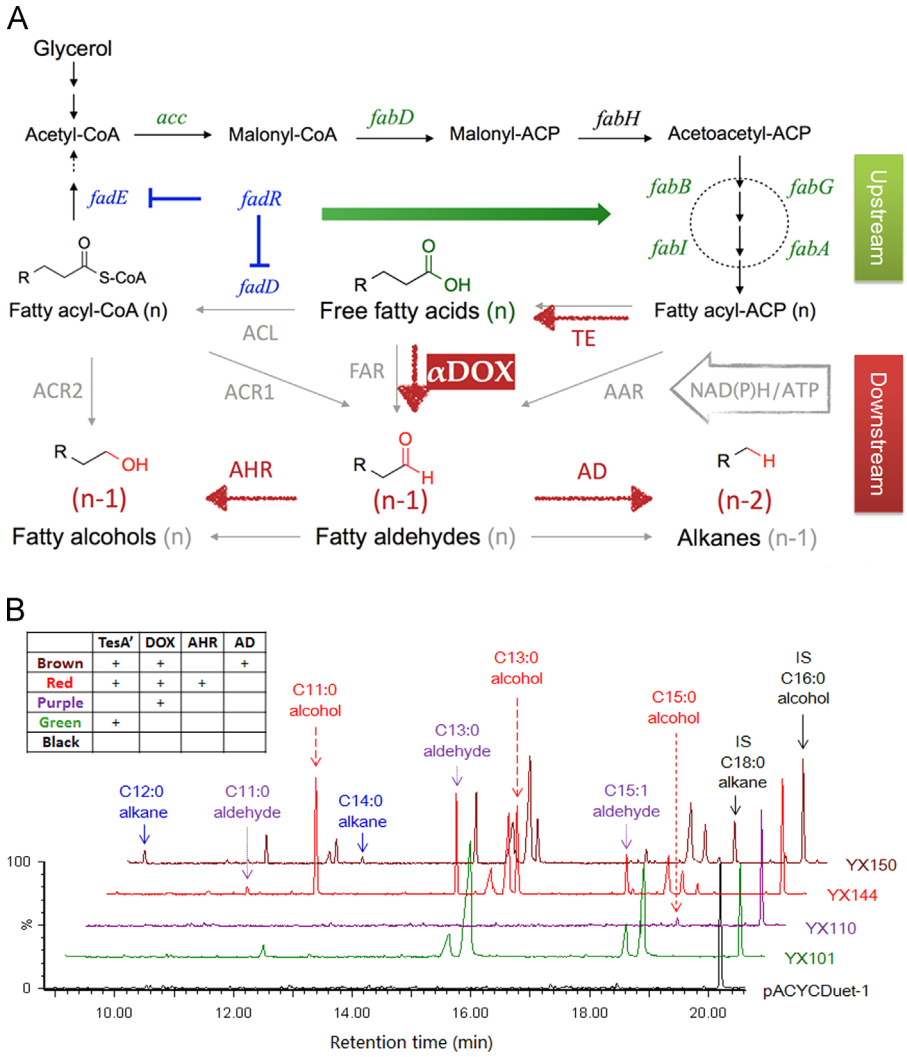

Fig. 1. Microbial production of odd-chain fatty alcohols and even-chain alkanes in *E. coli*. (A) Previous (gray) and our (red) fatty alcohols/alkanes biosynthesis pathways. The genes involved in fatty acids biosynthesis and degradation pathway are illustrated as green and blue color, respectively. The first steps of fatty alcohols production in previous pathways are reductive reactions, which need NAD(P)H/ATP as cofactors and cannot change carbon number during reactions. In our αDOX-dependent pathway, however, the fatty aldehydes formation step does not require energy and reducing power from host cells. And most importantly, the oxidized fatty aldehydes intermediates are one carbon shorter than the fatty acids precursors. This will lead to odd-chain fatty alcohols or even chain alkanes formation in a high purity and titer. Abbreviations: ACP, acyl carrier protein; TE, thioesterase; ACL, acyl-CoA ligase; AAR, acyl-ACP reductase; αDOX, α-dioxygenase; FAR, fatty acid reductase; ACR, fatty acyl-CoA reductase [ACR1 and ACR2]; AHR, aldehyde reductase; AD, aldehyde decarbonylase. (B) Gas chromatograms of odd-chain fatty alcohols and even-chain alkanes production in *E. coli* BL21(DE3) cells expressing TesA′, YX110, YX144 and YX150 plasmids, respectively. Simple expression of αDOX only resulted in the production of pentadecanol. Combined expression of αDOX, TesA′ and AHR (YX144), C_{11}, C_{13}, C_{15} aldehydes and alcohols were produced. Substitution of AHR to AD (YX150) resulted in C_{12} and C_{14} alkanes production. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

production. Thus, it is seemed more sensible to straight tailor downstream fatty aldehydes formation step for producing pure and high amount of unusual odd-chain fatty alcohols. It will be a brand new strategy to expand current microbial product profile in fatty alcohol or alkane biosynthesis area, since there is no such report yet either in bacteria (Yao et al., 2014) or in yeast (Runguphan and Keasling, 2014; Zhou et al., 2014).

Here, we explored a unique strategy to obtain odd-chain fatty alcohols or even-chain alkanes by incorporating α-dioxygenase (αDOX) from *O. sativa* (rice) into *E. coli*. α-Dioxygenases are the initial enzymes of α-oxidation in plants and oxidize long and medium-chain C_n fatty acids to 2-hydroperoxy fatty acids, which are then converted to C_{n-1} fatty aldehydes by spontaneous decarboxylation (Koeduka et al., 2000, 2002). Whereas most α-dioxygenases also retain peroxidase activity that directs 2-hydroperoxy fatty acids to form α-hydroxy fatty acids (Hamberg et al., 2005), αDOX from rice seems to lack that property (Koeduka et al., 2002). Besides, the *O. sativa* αDOX was able to convert C_{16}, C_{14}, C_{12} and C_{10} FFAs to the corresponding C_{n-1} fatty aldehydes in resting *E. coli* cells in the presence of exogenous supplemented FFAs (Kaehne et al., 2011). The substrate selectivity of αDOX meets fatty alcohols products demand quite well. These catalytic characteristics make DOX very promising for producing targeted fatty aldehydes without by-product that is applied for the next reduction or decarbonylation reaction. In addition, in the previous fatty alcohols or alkanes biosynthesis studies, fatty acyl-ACP/CoA or FFAs were catalyzed by reductases. These reductases used NAD(P)H as cofactor, and sometimes ATP was also required (Akhtar et al., 2013), which might lead to extra burden of reducing power or energy on host cells. On the contrary, αDOX does not need any such cofactor in its catalytic process due to the oxidase nature, and thus offers the opportunity to produce final product at maximum carbon and energy efficiency (Dellomonaco et al., 2010).

In the following work, we (i) developed an artificially αDOX based biosynthesis pathway with the aim of producing odd-chain fatty alcohols or even-chain alkanes in *E. coli*, (ii) demonstrated αDOX possessing broad compatibility with different TEs, (iii)