Fatty-Acid Production in Yeast Through Reversal of the β-Oxidation Cycle

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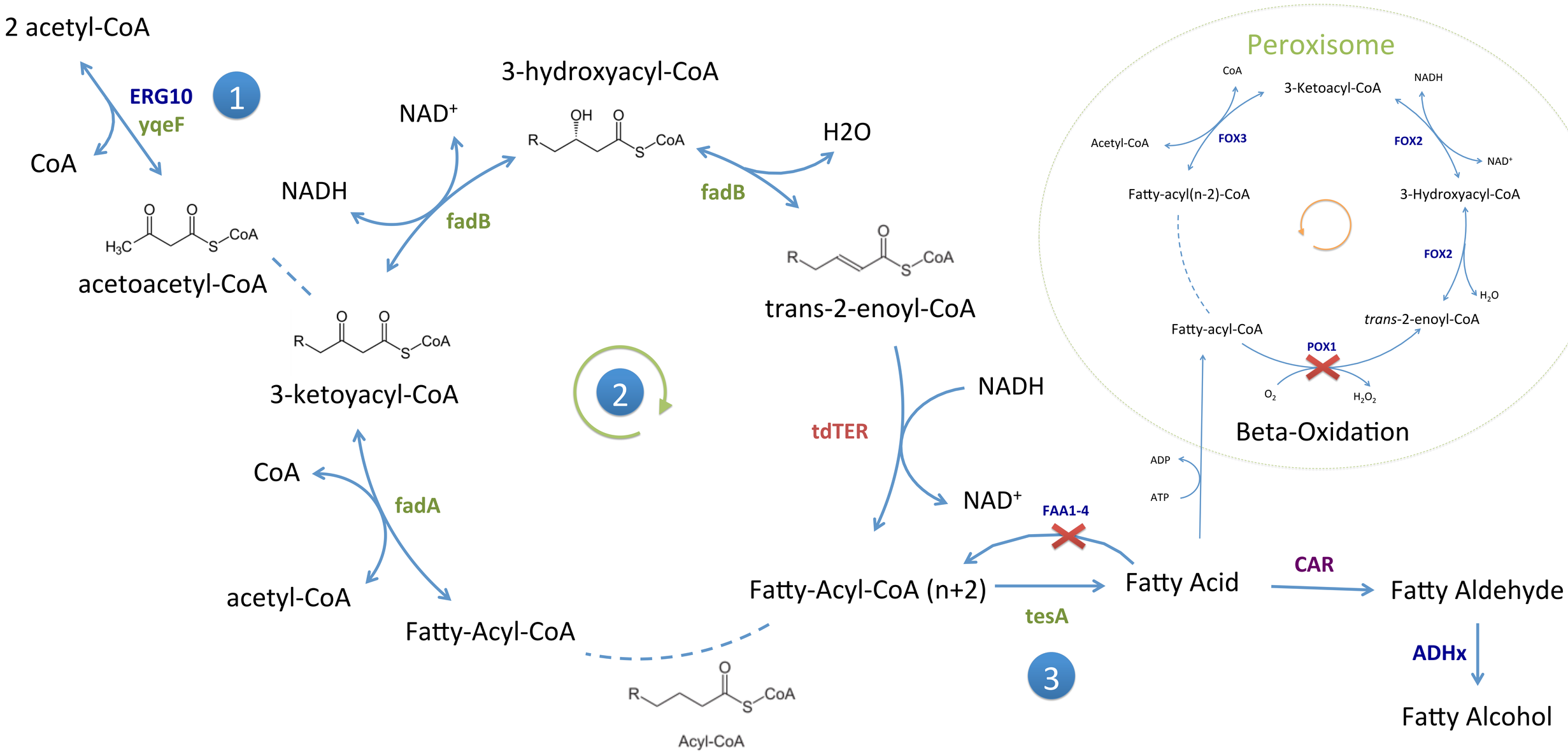
Introduction

Production of advanced renewable biofuels is regarded as a hotspot for industrially relevant applications for metabolic engineering of microbial cell factories. However, most of these fuels are derived from fatty-acids, and usually the kind of fatty acid produced is as important as its production titer. This way, we find extremely important the implementation of new fatty acid production routes in yeast. In this work, we:

- Built a new *S. cerevisiae* platform for the production of medium-chain fatty acids based on a reversal of the fatty-acid beta-oxidation cycle;
- Coupled this strategy with a pathway to produce fatty alcohols from free fatty acids.

The Reversed Beta-Oxidation pathway has been successfully demonstrated in *E. coli*[1,2] but never in yeast. The pathway is conceptually divided in 3 sections: The Initiation step (1) where 2 acetyl-CoA molecules are condensed into acetoacetyl-CoA; the Elongation step (2) where for each cycle a fatty-acyl-CoA molecule is elongated by 2 carbons at the cost of 1 acetyl-CoA and 2 NADH; and the Termination step (3), where the acyl-CoA molecule is converted into the fatty acid and subsequently to the desired final product.

As a proof of concept of the potential of this pathway for the production of fuels and chemicals, we plugged to this platform a pathway to convert free fatty acids into fatty alcohols.



Pathway Design

In order for the pathway to work, a background strain in which the peroxisomal beta-oxidation is inactive is needed. For that, we deleted POX1, the first gene of the beta-oxidation pathway. We also deleted FAA1 and FAA4 in order to prevent re-conversion of the produced fatty acids to acyl-CoA. We then overexpressed the pathway in the cytosol using plasmid expression. We expressed either ERG10 (*S. cerevisiae*) or yqeF (*E. coli*) to initiate the cycle by converting 2 acetyl-CoA to acetoacetyl-CoA, since fadA only works with longer acyl-CoAs. For the elongation step, the E. coli complex fadA + fadB was used together with the trans-Enoyl Reductase from *T. denticula.* To terminate the elongation, we overexpressed the thioesterase tesA which converts most fatty-acyl-CoAs into fatty acids. To provide a sink to the produced fatty acids, a Carboxylic Acid Reductase from *M. marinum* was expressed on a separate plasmid to enable the conversion of fatty acids into fatty aldehyde, which are then converted to fatty alcohols by the action of endongenous alcohol dehydrogenases.

Experimental

To overexpress the pathway, plasmids pRBye (P_{TPI}-ERG10 P_{TEF1}-fadA P_{PGK1}-fadB P_{TH3}-tdTER P_{HXT7}-tesA) and pRBee (P_{TPI}-yqeF P_{TEF1}-fadA P_{PGK1}-fadB P_{TDH3}-tdTER P_{HXT7}-tesA) were transformed together with a pCAR plasmid (P_{TDH3}-CAR) on a CEN.PK113-11C *ΔFAA1 ΔFAA4 ΔPOX1* strain. Strains were cultured in shake flasks for 72h, cells were harvested and the intracellular accumulated fatty alcohols were extracted[3] and analyzed by GC-MS.

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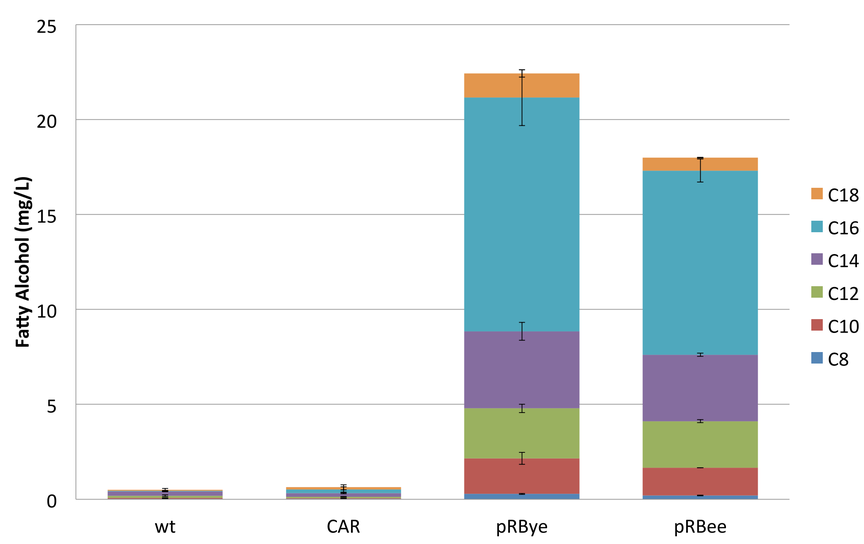
Results and Conclusions

Preliminary results show that while expressing the Carboxylic Acid Reductase gene alone does not seem to increase fatty alcohol production, expressing a Reversed Beta Oxidation pathway together with this fatty alcohol conversion route enables the production of fatty alcohols of different chain lengths. More surprisingly, around 40% of the produced fatty alcohols are shorter than C16, which is remarkable considering that S. cerevisiae produces mainly fatty acids of chain length around 16 and 18 carbons.



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References:
1) Dellomonaco, C, et al. *Nature* 2011, 476, 355–359
2) Clomburg, JM, et al. *ACS Synth. Biol*. 2012, 1, 541–554
3) Khoomrung, S., et al. *Appl Microbiol Biotechnol* 2012, 94(6), 1637-46

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