Genome-wide screening for improved fatty acid production in yeast

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1. Introduction

Project Goal: identify genetic mutations for improving fatty acid production in yeast

- **Microbial cell factory** is proposed as a sustainable and renewable alternative to the petrochemical industry (**Fig. 1.1**)
- ***Saccharomyces cerevisiae*,** the baker's yeast**,** is a widely-used microbial host for producing fuel ethanol
- It is highly desirable to expand the scope of fermentation products by yeast, such as **fatty acid-derived** value-added chemicals (**Fig. 1.2**)

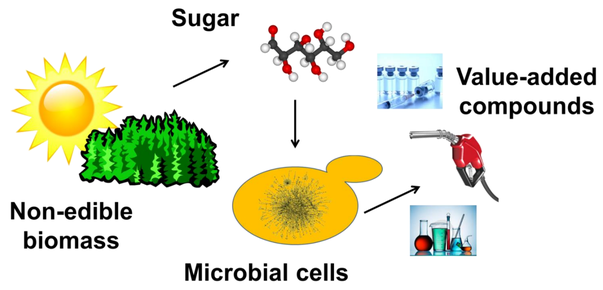

Fig. 1.1 The promise of microbial cell factory [1]

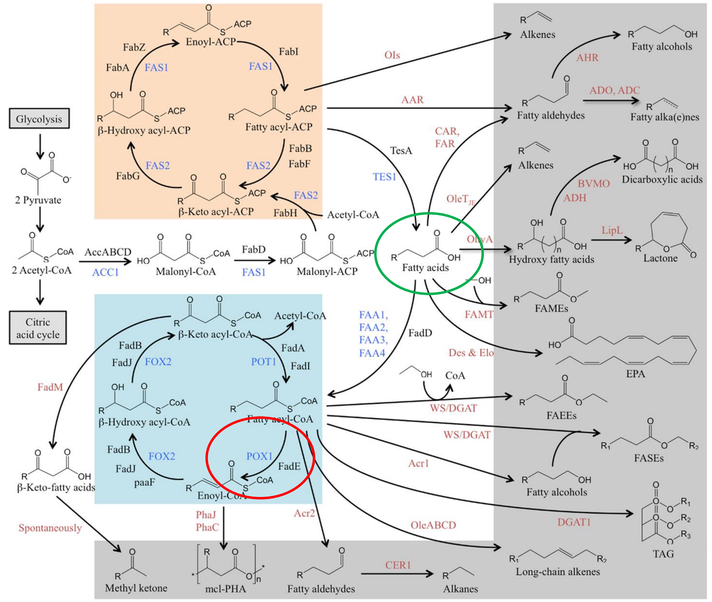

Fig. 1.2 Metabolic pathways of fatty acids and derivatives (orange: synthesis; blue: catabolism; gray: derived chemicals) [2]

Directed evolution

- With limited knowledge of complex biological systems, directed evolution can help to understand and improve a target phenotype using iterative mutagenesis and high-throughput screening

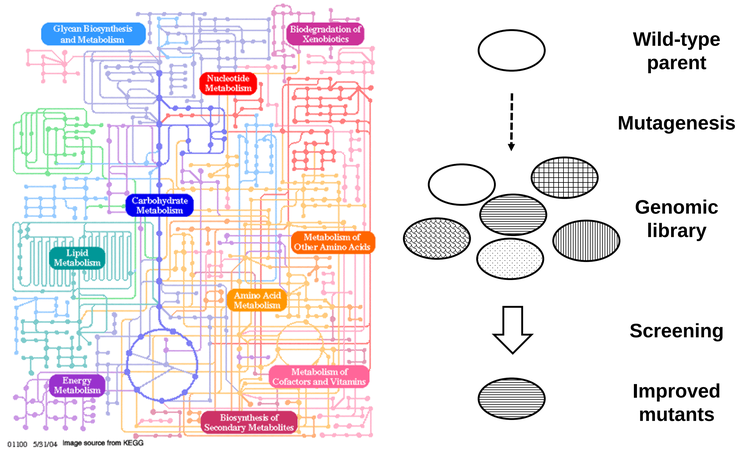

Fig. 1.3 Scheme of metabolic networks [3] and genome-wide screening [4]

2. Designs and results

Creation of a fatty-acid responsive biosensor

- Acyl-CoA oxidase (Pox1p) is the first enzyme for beta-oxidation of fatty acids, and its expression is activated with high levels of fatty acids
- To monitor cellular fatty acid concentrations, a biosensor was constructed by fusing the gene encoding the green fluorescence protein (GFP) to the promoter of the Pox1p protein (**Fig. 2.1**)

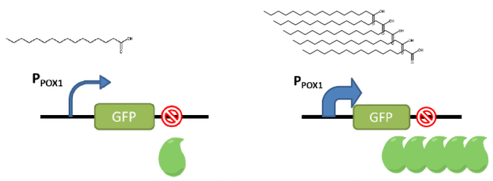

Fig. 2.1 Scheme of the Pox1-GFP biosensor.

- To validate this biosensor, the cells were incubated with exogenously added oleic acid and GFP fluorescence was monitored (**Fig. 2.2**)

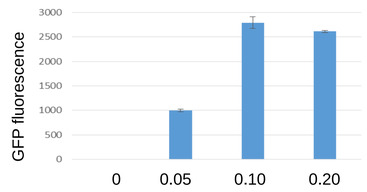

Fig. 2.2 GFP signals with exogenously added C18 fatty acid (g/L)

Fluorescence-activated cell sorting (FACS)

- A genome-wide mutant library was created in a strain with the Pox1 biosensor
- FACS was used to screen desirable mutants (**Fig. 2.3**)

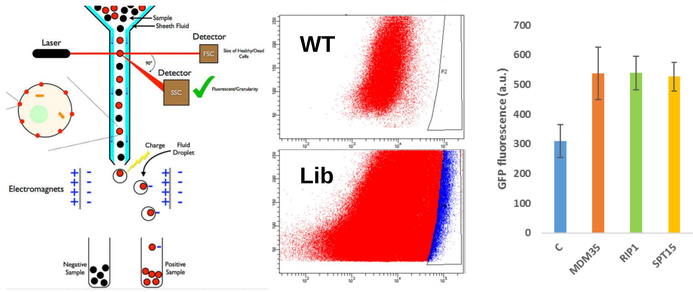

Fig. 2.3 Principles of FACS [5]; cell sorting for the brightest mutants; selected mutant strains with enhanced GFP fluorescence

- Total fatty acids were isolated and quantified using GC-MS (**Fig. 2.4**)

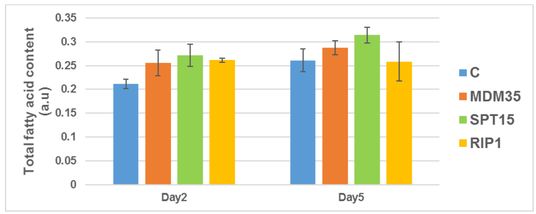

Fig. 2.4 Improved total fatty acid levels in identified mutant strains

Summary

- A fatty acid-responsive biosensor was successfully constructed
- A genome-wide mutant library was created and screened using FACS with the help of the biosensor
- Three knockdown mutations were identified to improve fatty acid content
- The production of fatty acid derivatives (e.g. hexadecanol) in the isolated mutants is being tested

Reference

[1] T Si, PhD thesis, University of Illinois at Urbana-Champaign; [2] *Front Bioeng Biotechnol*, 2014, 2:78; [3] http://www.genome.jp/KEGG/pathway.html; [4] T Si, H Xiao, H Zhao, *Biotechnol Adv*, 2015, 33 (7):1420-1432; [5] S. Sabban, PhD thesis, the University of Sheffield

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