High production of fatty alcohols in *Yarrowia lipolytica* by coordination with glycolysis

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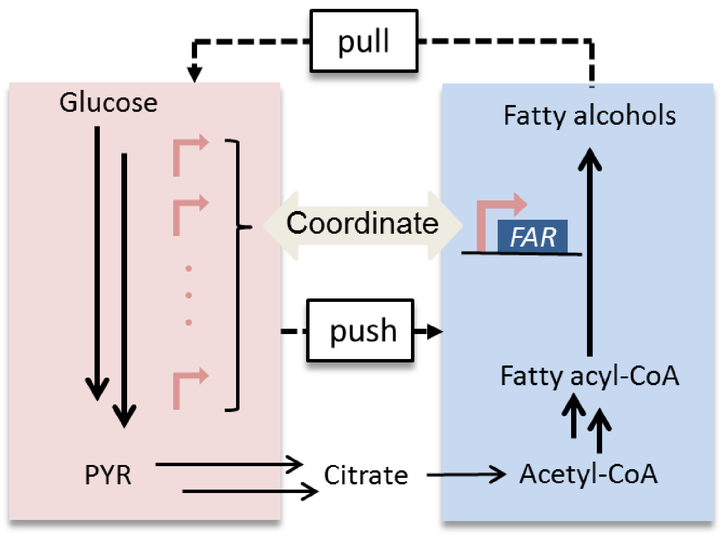
Highlights:

- Transcript profiling analysis identify genes in carbohydrate metabolism were up-regulated significantly in high fatty alcohol production strain.
- 11 glycolysis promoters were screened to correlate the fatty alcohols production with the up-regulated carbohydrate metabolism.
- The coordination system caused a ‘pull-and-push’ effect which dynamically enhanced the product synthesis flux.
- 5.75 g/L fatty alcohols production was achieved, which was the highest reported titer in shake flasks to data.

Design:



Here, we developed a novel and efficient strategy to coordinate fatty alcohol synthesis with glycolysis which achieved a ‘pull-and-push’ effect to improve fatty alcohol production. Transcript profiling indicated that genes in carbohydrate metabolism were up-regulated significantly in response to higher fatty alcohol production. Based on it, 11 glycolysis promoters were screened to express fatty acyl-CoA reductase (FAR) to corelate the fatty alcohol production with the up-regulated carbohydrate metabolism, and the fatty alcohol production reached to 557 mg/L when *FAR* was expressed by the promoter of P_{FBAin}. RNA-seq and qRT-PCR analysis demonstrated that a ‘pull-and-push’ effect caused by the coordination system dynamically enhanced the product synthesis flux from top to bottom, which was also testified and intensified by doubled glucose concentration. After manipulating structural and regulatory genes of lipid metabolism, the final strain achieved up to 5.75 g/L fatty alcohol production from modified YPD medium (containing 91 g/L glucose) in shake flasks.

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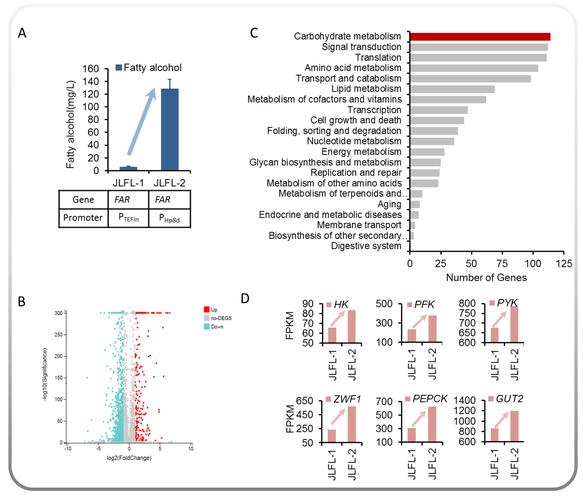
Results and Discussion:

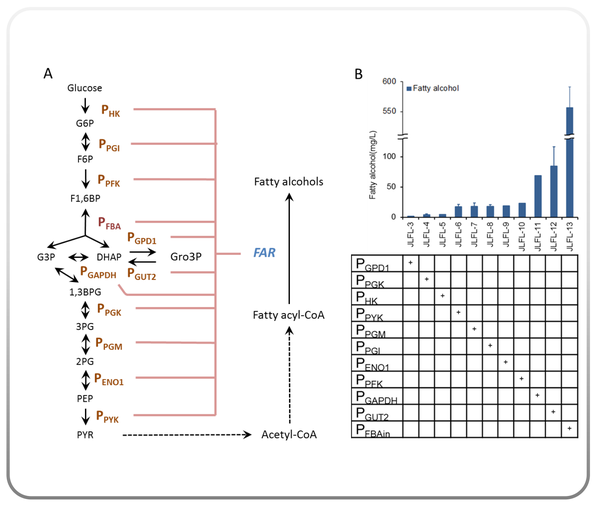
Glycolysis was pulled to up-regulated in high fatty alcohol production strain



Transcriptional profiles between different fatty alcohol-producing strains.

(A) Fatty alcohol production by JLFL-1 (P_{TEFin}-*FAR*) and JLFL-2 (P_{Hp8d}-*FAR*).
(B) Gene-set analysis.
(C) KEGG pathway enrichment analysis.
(D) Changes of FPKM of genes around glycolysis.

Coordination of fatty alcohol biosynthesis with glycolysis

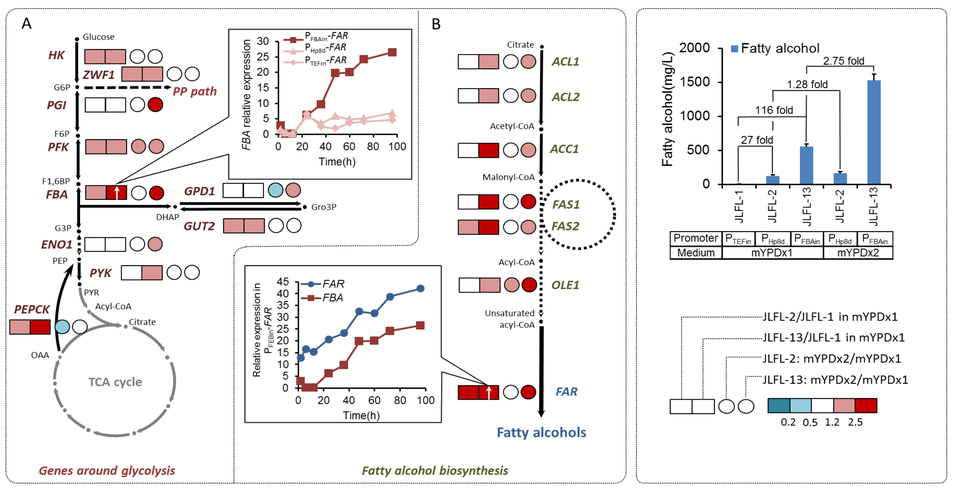


Effect of utilizing glycolysis promoters on fatty alcohol production.

(A) 11 glycolysis promoters were screened to express *FAR* to corelate the fatty alcohol production with glycolysis.

(B) Fatty alcohol production using different glycolysis promoters.

Coordination with glycolysis achieved a ‘pull-and-push’ effect for fatty alcohol biosynthesis



Transcriptional profiles of genes around glycolysis (A) and fatty alcohol biosynthesis (B) by strain JLFL-1 (P_{TEFin}-*FAR*), JLFL-2 (P_{Hp8d}-*FAR*) and JLFL-13(P_{FBAin}-*FAR*) cultured under two glucose concentrations. qRT-PCR was applied to investigate transcriptional changes of *FAR* and *FBA* during fatty alcohol accumulation (insert graph). The related titers were listed on the right. Up-regulated and down-regulated genes and genes without significant transcriptional difference were highlighted in red, blue, and white, respectively.

Acknowledgment:

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