Creating an oil yeast from brewing yeast

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Engineering microorganisms for sustainable production of fuels and chemicals are attractive because it is a renewable process and has potential to replace fossil based production. The cost-efficient production of fuels and chemicals needs to achieve high yields with low by-product production, which requires high metabolic flux toward the desired products. However, microorganisms have evolved robust natural habitat, and their tight regulation of metabolism make it challenging to rewire the flux [1]. For instance, as a potential cell factory, *Saccharomyces cerevisiae* has been engineered for the production of a variety of fuels, chemicals, and pharmaceuticals, whereas the inherent ethanol production hinders the overproduction of desired chemicals. *S. cerevisiae* is a Crabtree-positive organism, and ethanol production is predominate even under aerobic condition when glucose is in excess [2]. Completely blocking ethanol production leads to growth defects when using glucose as the sole carbon source, due to the lack of cytosolic acetyl-CoA for synthesis of cellular essential components such as lipids [2]. Therefore, it is very difficult to completely redirect the flux from ethanol accumulation toward the product of interest only through rational engineering. Recently, the group headed by Professor Jens Nielsen at Chalmers University of Technology, who has pioneered on yeast synthetic biology, solved this through combining metabolic engineering with adaptive evolution, which resulted a synthetic oil yeast with high fatty acid production and abolished ethanol accumulation [3]. This study represents an important milestone in engineering of yeast for production of fuels, chemicals, and pharmaceuticals and shows the great potential metabolic engineering in rewiring the natural metabolic networks.

This super yeast *S. cerevisiae* has been widely used for brewing and baking, and several group has tried to completely abolish the Crabtree effect for enhancing the production of chemicals other than ethanol. These previous studies tried to delete the pyruvate decarboxylase (PDC), the first step of ethanol biosynthetic pathway, to completely abolish the Crabtree effect [2,4,5]. And the PDC minus strains were then evolved to grow in excess glucose, but the growth rates were still low for industrial application [5]. Then Nielsen and coworkers constructed an alternative pyruvate dehydrogenase (PDH) bypass pathway in PDC minus strain to supply the cytosolic acetyl-CoA synthesis, and the evolved strain achieved better growth rate [6]. Though these work created Crabtree negative yeast which can grow in glucose without

ethanol production, the growth performance is still much lower than wild-type yeast and the redirection of the carbon flux to chemicals synthesis has not been demonstrated. In this recent report published in the Cell, Nielsen and coworkers completely reprogram yeast metabolism from ethanol fermentation to lipogenesis, which resulted the highest production of free fatty acids (FFAs) without ethanol accumulation [3].

In their work, an efficient FFA-producing pathway was established first. As pyruvate conversion to acetyl-CoA could be a limiting step for FFA, the carbon flux was then driven to improve the cytosolic acetyl-CoA supply. In addition to their previous work of citrate shuttle consisting of an ATP:citrate lyase the cleaves citrate to oxaloacetate and acetyl-CoA [7], the acetyl-CoA supply was further optimized through a series of approach including engineering the citrate synthesis and subcellular trafficking, as well as turning the tri-carboxylic acid cycle (Fig. 1). In addition, the NADPH supply was also enhanced by fine-tuning glycolysis and strengthening the PPP pathway with the aid of their previous established transhydrogenase cycle (Fig. 1) for converting excess NADH to NADPH [7]. These strategies helped to sufficient supply of NADPH and re-oxidation of excess cellular NADH and thus was beneficial for relieving NADH stress and Crabtree effect. Combining these approaches with growth restriction fermentation, the best strain can produce 33.4 g/L FFAs, which reached 0.1 g FFAs/g glucose corresponding to 30% of the theoretical yield.

Another innovative design of this work is the growth-coupled lipogenesis design, which might be helpful for blocking the ethanol biosynthetic pathway. As we mentioned that PDC deletion result in growth defect due to the lack of cytosolic acetyl-CoA for synthesis of cellular lipids and the block of NADH re-oxidation. The construction of the lipogenesis pathway supplied sufficient cytosolic acetyl-CoA and built the NADH re-oxidation cycle, which play important role for recovering and maintaining the cell growth. Although the growth deficiency was also observed after PDC deletion in the lipogenesis strain perhaps due to the metabolic imbalance, after the adaptive laboratory evolution (ALE), the growth on glucose was achieved.

The genome sequencing of the evolved strain identified the mutations in pyruvate kinase, which is considered as a key control point of glycolytic flux. It seems that the mutation of pyruvate kinase can downregulate glycolytic flux, and allow the cell to balance flux through
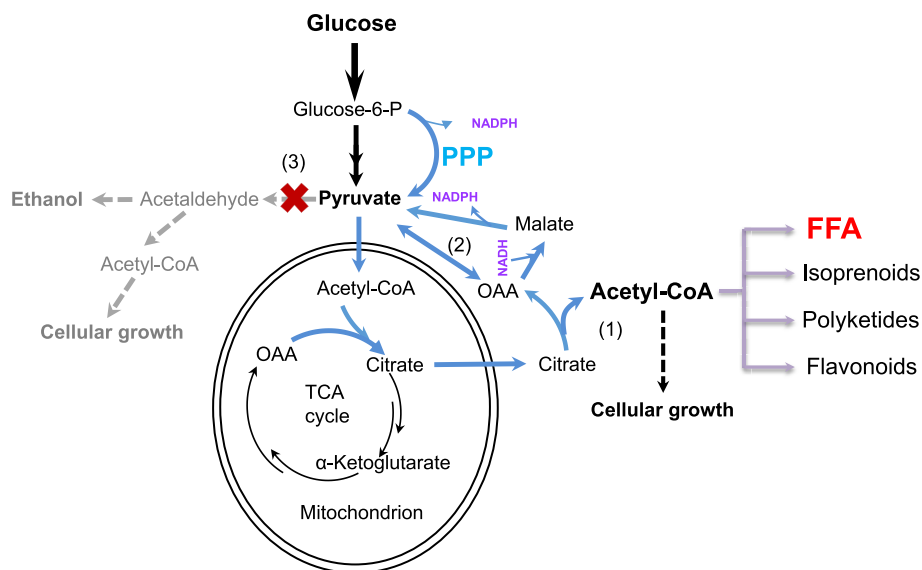
Fig. 1. Rewiring Yeast Metabolism from ethanol production to free fatty acids or other valuable chemicals production. The synthetic oil yeast was generated by driving the flux to acetyl-CoA (1), balancing the cofactor requirement (2), abolishing the ethanol production (3) and growth coupled adaptive evolution. The engineered strain could also be used for production of other valuable chemicals that are derived from cytosolic acetyl-CoA.

glycolysis and respiration, thereby relieve the Crabtree effect.

As discussed in the paper, from an evolutionary perspective, cellular metabolism has been evolved to establish tight regulation to convert carbon sources into biomass and metabolic products for optimizing their growth. This tight regulation makes it challenging to engineer metabolism for over-production of other metabolites. This work demonstrate combining metabolic engineering and adaptive evolution it is possible to complete replace the ethanol accumulation with FFAs production in *S. cerevisiae*.

The lack of information in regulation often results in flux imbalance when designing the cell factory through rational engineering approaches. If the metabolic design can be coupled with cellular fitness, evolution engineering provide us a feasible approach to overcome the regulatory constraints and reconstruct the metabolic balance [8]. This study redirected high carbon flux from ethanol to cytosolic acetyl-CoA and lay the basis for efficient production of other chemicals that are derived from acetyl-CoA as illustrated in Fig. 1. The work therefore represents an important landmark in yeast cell factory design and gives several important experience in engineering cellular metabolism.

Acknowledgments

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