



Efficient fatty acid synthesis from methanol in methylotrophic yeast

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ARTICLE INFO

Keywords

Methanol
Free fatty acids
Methylotrophic yeast

ABSTRACT

Methanol is an attractive C1 feedstock with high abundance and low cost in bio-manufacturing. However, the metabolic construction of cell factories to utilize methanol for chemicals production remains a challenge due to the toxic intermediates and complicated metabolic pathways. The group of Zhou rescued methylotrophic yeast from cell death and achieved high-level production of free fatty acids from methanol through a combination of adaptive laboratory evolution, rational metabolic engineering and multi-omics analysis.

Methanol can be abundantly produced from CH₄ and CO₂ via electrocatalytic conversion or hydrogenation and has been considered a promising feedstock in bio-manufacturing owing to its low-price and renewability [1,2]. In addition, compared to starch-based materials such as glucose, methanol possesses higher reducing power, which can increase the titer and yield of reduced metabolites [3]. Accordingly, the metabolic engineering of industrial microorganisms to convert methanol into more valuable chemicals has become one of main research areas in the field of synthetic biology. Despite this promise of methanol-based bioprocesses, several challenges still remain. For instance, the methanol toxicity will damage the cell membrane integrity and lead to low product titer. Besides, the more toxic intermediate of formaldehyde inevitably generated during methanol metabolism may cause DNA-protein crosslinking problem, which inhibits cell activities and thus limits the efficiency of methanol bioconversion [4]. In addition, the regulation of methanol metabolism *in vivo* is strict and complex, presenting difficulties in the exploitation of methanol for biochemical production.

Recently, the group of Zhou successfully achieved the efficient synthesis of free fatty acids (FFA) from sole methanol by engineering the native methylotrophic yeast *Ogataea polymorpha* through a combination of adaptive laboratory evolution, rational metabolic engineering and multi-omics analysis (genomics, transcriptomics and lipidomics) (Fig. 1) [5]. In this work, the acyl-CoA synthase gene *faa1* was first disrupted by employing self-developed CRISPR-Cas9 tools [6] for the accumulation of FFA in *O. polymorpha*. However, the engineered strain HpFA01 (*Δfaa1*) lost the ability of utilizing methanol for growth. This phenomenon was

initially speculated to be caused by the competition of cofactor NADPH between FFA biosynthesis and cell growth, because the former will consume large amounts of NADPH. Thus, the pentose phosphate pathway (PPP) and gluconeogenesis were strengthened to increase the supply of NADPH and Xu5P (precursor for methanol assimilation). However, all the engineering strategies failed to restore cell growth in minimal methanol medium. The adaptive laboratory evolution (ALE) was supposed to be a powerful strategy, which allows the occurrence and selection of beneficial mutations [7,8]. Therefore, continuous passage cultivation of three independent colonies of strain HpFA01 was conducted by using minimal medium with gradually reduced ratios of glucose to methanol. After 35–60 days of ALE, the ability of using methanol as sole carbon source for cell growth and FFA production was all recovered in three groups of colonies.

The powerful ALE can not only enable the desired phenotype, but also provide an opportunity to identify the cryptic limiting factors. In this case, genome sequencing and lipidomics analysis of evolution mutants revealed that the recovery of cell growth on methanol was mainly attributed to the inactivation of *lp1* (encoding a putative lipase) and *izh3* (encoding a membrane protein related to zinc metabolism), which restored the homeostasis of phospholipids. It was found that the deletion of *faa1* remarkably decreased the levels of phospholipids, which in turn might damage cellular membrane integrity, especially peroxisome membranes. Subsequently, the Rim101 pathway which contains a sophisticated sensor complex sensed the alteration of phospholipids and mediated the necrosis of cells [11]. Fortunately, ALE-mediated inactivation of *lp1* and *izh3* could maintain phospholipid pools by blocking

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<https://doi.org/10.1016/j.synbio.2022.09.003>

Received 9 August 2022; Received in revised form 22 September 2022; Accepted 25 September 2022

Available online 30 September 2022

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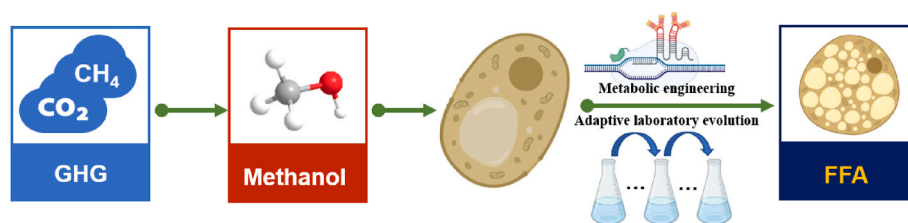


Fig. 1. Overproduction of FFA from sole methanol in methylotrophic yeast. Methanol can be abundantly produced from greenhouse gases (GHG) including methane and CO₂; however, the toxicity of methanol inhibits cell activity and thus limits the efficiency of methanol bioconversion. Gao et al. rescued methylotrophic yeast *O. polymorpha* from cell death by restoring phospholipid metabolism and achieved overproduction of free fatty acids from sole methanol.

the degradation of phospholipids and thus restored the cell growth on methanol. It was worth noting that the maximum OD₆₀₀ of the engineered strain (inactivation of *lpl1* and *izh3*) was only one third of the evolved strain, indicating that several hidden genetic modifications responsible for improved growth have not been excavated.

Finally, transcriptome analysis guided metabolic engineering was performed to enhance the supply of acetyl-CoA and NADPH in the best evolved mutant. The resulting strain HpFAM16u enabled a high level of FFA production (8.3 g/L), which was 2.8-fold higher than that of the control strain. Interestingly, cell growth and product synthesis were both improved after a series of genetic modifications. Further fed-batch cultivation of engineered strain achieved the highest FFA production of 15.9 g/L from sole methanol, which was comparable to results from sugar-based FFA production.

Constructing microbial cell factories capable of efficiently utilizing methanol as sole carbon source is urgently needed for the implementation of methanol-based bio-manufacturing. Rewiring the methanol assimilation metabolism to strengthen the capacity of methanol utilization and production synthesise in native methylotrophs has been considered as a promising direction [12]. However, genetic modifications of native methylotrophs were difficult and time-consuming due to the lack of efficient genetic tools [8]. Thus, massive efforts have been devoted to engineer synthetic methylotrophy in model organisms by heterologous expression of native or artificially designed methanol assimilation pathways [3,9]. Unfortunately, the efficiency of methanol bioconversion in synthetic methylotrophy was severely hampered by the complexities of natural methanol assimilation pathways and inefficiency of artificially designed methanol assimilation pathways. For instance, the methylotrophic *Escherichia coli*, which was constructed by metabolic engineering and ALE, was enabled to utilize methanol as sole carbon source for cell growth still suffer from slower growth rates and low methanol bioconversion efficiency [4,13]. In order to break the dilemma of methanol bioconversion, the group of Zhou developed a series of efficient and precise genome editing tools in native methylotrophs including *O. polymorpha* and *Pichia pastoris* [6,10]. Therefore, with the help of efficient genome editing tools and multi-omics analysis, they systematically revealed the mechanism of methanol assimilation stress in microbial cells and finally realized the efficient conversion of methanol to high value-added compounds. Looking forward, this study will provide a blueprint for the development of industrial methanol bioconversion.

Credit author statement

Shangjie Zhang wrote the manuscript. Wenming Zhang: reviewed

this paper and provided valuable suggestions. All authors read, edited, and approved the final manuscript. Min Jiang: reviewed this paper and provided valuable suggestions. All authors read, edited, and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by National Key R&D Program of China (2018YFA0901500), the National Natural Science Foundation of China (22078151, 21978130), Young Elite Scientist Sponsorship Program by CAST (YESS20200174) and Jiangsu Synergetic Innovation Center for Advanced Bio-Manufacture.

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