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•Commentary•

De novo production of 24-epi-ergosterol in bioengineered yeast

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Plant natural products (PNPs), a rich source of structurally unique and functionally diverse bioactive compounds, comprise a large variety of natural product families including alkaloids, terpenoids, and phenylpropanoids [1]. PNPs possess a wide range of applications such as antibiotics, analgesics, and antiviral, antimalarial and antineoplastic agents [2, 3]. Classically, collection of PNPs relies on extraction and purification from natural producers. However, due to their low amounts in nature, the extraction process is often inefficient and costly. Chemical synthesis, on the other hand, is difficult due to the stereochemical complexity of PNPs. In recent years, advances in synthetic biology have accelerated the process of producing complex PNPs in yeast cell factories. *Saccharomyces cerevisiae* (yeast) is charaterized by a wealth of well-established tools available for genetic manipulation, a well-characterized core metabolic network, low cultivation cost, and compatibility with high cell density fermentation [4-7]. As a result, *S. cerevisiae* is increasingly used as a workhorse for enzyme characterization, biosynthetic pathway elucidation [8, 9], and *de novo* production for various high-value PNPs [10].

Recently, in an article entitled "Manipulation of sterol homeostasis for the production of 24-epi-ergosterol in industrial yeast" published in *Nature Communications* [11], JIANG and co-workers employed a synthetic biology toolkit to successfully construct a cell factory for a scalable production of 24-epi-ergosterol in *S. cerevisiae*, which serves as a synthetic precursor for semi-synthesis of plant hormone brassinolide (BL). BL, the most bioactive compound in natural brassinosteroids, is able to regulate many plant physiological activities but has an extremely low yield from the natural source [12]. Given that the biosynthesis of 24-epi-ergosterol has not been reported, the authors designed an artificial pathway for converting ergosta-5,7,22,24(28)-tetraene-3β-ol to 24-epi-ergosterol by introducing a $\Delta^{24(28)}$ sterol reductase (DWF1) from *Ajuga reptans* (ArDWF1). Although the yeast strain YQE224 bearing ArDWF1 produced larger amounts of 24-epi-ergosterol, the production level was still far from industrial applications. Thus, the authors sought to increase the production of 24-epi-ergosterol through protein and metabolic engineering. First, they performed directed evolution of DWF1 using a high-throughput screening method. As a result, the mutant strain produced 46.72 mg·L^{-1} of 24-epiergosterol, which was 3.46-fold higher than that of the control strain. Subsequently, higher-level production of 24-epi-ergosterol was achieved in another yeast strain YQE717 by engineering sterol homeostasis, with the titer and yield reaching 2.15 g·L^{-1} and 11.53 mg·gDCW^{-1}, respectively. Finally, the team used several promoters to enhance pathway gene expression that further increased the production of 24-epi-ergosterol with a titer of 2.76 g·L^{-1} and a yield of 19.27 mg·gDCW^{-1} through fed-batch fermentation.

In this work, JIANG and co-workers showcased an artificial pathway for the biosynthesis of 24-epi-ergosterol in *S. cerevisiae*. The large scalable production of 24-epi-ergosterol paves a practical pathway for semi-synthesis of plant - derived natural product BL. Currently, it remains difficult to introduce artificial pathways into microbial cell factories for a scalable production of those PNPs due to a lack of elucidation of biosynthetic pathways. Furthermore, an effective introduction of non-native substrates which are greatly different from native substrates is also challenging. Thus, the strategy launched here is particularly significant and will allow to synthesize un-natural sterols easier and faster. Notably, it is also becoming clear that a detailed understanding of sterol homeostasis between sterol acylation and sterol ester hydrolysis will ultimately facilitate rational pathway design.

Although protein and metabolic engineering have been

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greatly accelerated in recent years, engineering enzymes for altered small molecule production still heavily relies on screening, typically *via* LC or GC, resulting in a disproportionate amount of project time required for sample analysis. The successful application of the growth-associated high-throughput screening method in this work makes the protein engineering more efficient. As the field moves forward, future improvement to the bioengineered yeast platform and genetic manipulation systems will enhance the synthetic capability of cells to produce more valuable and complex PNPs.

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