Profi ling in fluences of gene overexpression on heterologous resveratrol production in Saccharomyces cerevisiae

Duo Liu1,2*, Bingzhi Li1,2*, Hong Liu1,2, Xuejiao Guo1,2, Yingjin Yuan (✉)1,2

1 Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
2 SynBio Research Platform, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin University, Tianjin 300072, China

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2017

Abstract Metabolic engineering of heterologous resveratrol production in Saccharomyces cerevisiae faces challenges as the precursor L-tyrosine is stringently regulated by a complex biosynthetic system. We overexpressed the main gene targets in the upstream pathways to investigate their influences on the downstream resveratrol production. Single-gene overexpression and DNA assembly-directed multigene overexpression affect the production of resveratrol as well as its precursor p-coumaric acid. Finally, the collaboration of selected gene targets leads to an optimal resveratrol production of 66.14/C6 3.74 mg L−1, 2.27 times higher than the initial production in YPD medium (4% glucose). The newly discovered gene targets TRP1 expressing phosphoribosylanthranilate isomerase, ARO3 expressing 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, and 4CL expressing 4-coumaryl-CoA ligase show notable positive impacts on resveratrol production in S. cerevisiae.

Keywords resveratrol, aromatic amino acid, DNA assembly, metabolic engineering, gene overexpression

1 Introduction

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) has shown a range of potential benefits such as antitumor, anti-inflammatory and antiaging functions [1,2]. The predominant commercial resveratrol is extracted from Polygonum cuspidatum, always leading to variable compositions and purities [2]. The demand for lower cost and high purity resveratrol promotes its heterologous biosynthesis in engineered microbes [3].

In a first study, resveratrol was produced in Saccharomyces cerevisiae based on feeding p-coumaric acid and the expression of two heterologous enzymes, 4-coumaryl-CoA ligase (4CL) and resveratrol synthase (VST1, or named as stilbene synthase, STS) [4]. The 4CL from Populus trichocarpa, Nicotiana tabacum, Arabidopsis thaliana and the STS from Vitis vinifera, Arachis hypogaea were further tested separately [4–7]. The precursor p-coumaric acid could be synthesized from two sources, L-phenylalanine and L-tyrosine, in microbes. The pathway from L-phenylalanine requires three enzymes, phenylalanine ammonia lyase (PAL), cinnamic acid hydroxylase (C4H) and cytochrome P450 reductase (CPR) [8–10]. In contrast, the synthesis from L-tyrosine is governed by only one enzyme, tyrosine ammonia lyase (TAL) [7,11]. These primary pathways in laboratory strains of S. cerevisiae generate relatively low production of resveratrol from 0.29 mg·L−1 to 5.25 mg·L−1. The utilization of synthetic protein scaffold improved the production of resveratrol to 14.4 mg·L−1 [12].

Both the precursors, L-phenylalanine and L-tyrosine, belong to aromatic amino acids and share many common synthesizing steps in yeast. Some previous works have explored some key points in the complex regulation of these biosynthetic pathways. The mutants ARO4K229L and ARO7G141S were expressed to deregulate the two steps in the whole pathways [13,14]. The genes PDC5 and ARO10 were knocked out to cut off other branch products [14,15]. To synthesize necessary precursor, malonyl-CoA was also upregulated [7,14]. The heterologous enzyme such as E. coli shikimate kinase II (aroL) was also used to improve the amino acid production [14]. The highest production of resveratrol was 531.41 mg·L−1 in a fed-batch fermentation by adding these combinatorial genetic constructions.
These published works mostly focused on rational engineering of the relative pathways. However, such efforts proved prone to introducing new metabolic bottlenecks in the pathway of L-tyrosine synthesis in *E. coli* [16]. In this work, we describe an approach combining single-gene overexpression and DNA assembly-directed multigene overexpression to screen the direct upstream metabolic reactions (Fig. 1). The *de novo* synthesis of resveratrol in yeast is controlled by many reaction steps including branch pathways. The synthesis of each amino acid is stringently regulated at transcriptional and post-translational levels. The effects of the upstream genes on the synthesis of end products are uncertain. We design and construct the pathway containing new enzyme combinations for primary synthesis of resveratrol, and screen the upstream enzyme targets affecting production. The effects of the selected genes on resveratrol production are analyzed.

![Fig. 1 Schematic illustration of gene overexpression for improving resveratrol production in *S. cerevisiae*.](image_url)

(a) The upstream biosynthetic pathways of L-PHE, L-TYR, and L-TRP are chosen to screen the gene targets that are beneficial to resveratrol production in yeast; (b) metabolic engineering faces the difficulty when multiple highly biosynthetic regulated reactions are operated; (c) each single gene in charge of the related reactions in the upstream pathways is overexpressed; (d) the yeast *in vivo* DNA assembly process is utilized to combine multigene overexpression. Abbreviations: E4P, erythrose 4-phosphosphate; PEP, phosphoenol pyruvate; DAHP, 3-deoxy-D-arabino-heptulosononic acid 7-phosphate; DHQ, 3-dehydroquininate; DHS, 3-dehydro-shikimate; SHIK, shikimate; SHP, shikimate-3-phosphate; EP3P, 5-enolpyruvyl shikimate-3-phosphate; CHA, chorismate; PPA, prephenate; PYY, phenylpyruvate; HPP, para-hydroxy-phenyl pyruvate; l-PHE, L-phenylalanine; l-TYR, L-tyrosine; ANA, anthranilate; PRA, N-(5-phospho-D-ribosyl) anthranilate; CDRP, 1-(2-carboxyphenylamino)-1-deoxy-D-ribulose-5-phosphate; IGP, indoleglycerol phosphate; UMP, uridine monophosphate; GSH, glutathione; GTP, guanosine triphosphate; GDP, guanosine diphosphate; PPY, prephenate; PPA, prephenate; PYY, phenylpyruvate; TAL, tyrosine ammonia lyase; 4CL, 4-coumaryl-CoA ligase; STS, stilbene synthase.