Synthetic and Systems Biotechnology xxx (2017) 1–8

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: http://www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology/

Production of anthocyanins in metabolically engineered microorganisms: Current status and perspectives

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ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form
24 September 2017
Accepted 26 October 2017

Keywords:
Anthocyanin
Enzyme engineering
Metabolic engineering
Microbial production

ABSTRACT

Microbial production of plant-derived natural products by engineered microorganisms has achieved great success thanks to large extend to metabolic engineering and synthetic biology. Anthocyanins, the water-soluble colored pigments found in terrestrial plants that are responsible for the red, blue and purple coloration of many flowers and fruits, are extensively used in food and cosmetics industry; however, their current supply heavily relies on complex extraction from plant-based materials. A promising alternative is their sustainable production in metabolically engineered microbes. Here, we review the recent progress on anthocyanin biosynthesis in engineered bacteria, with a special focus on the systematic engineering modifications such as selection and engineering of biosynthetic enzymes, engineering of transportation, regulation of UDP-glucose supply, as well as process optimization. These promising engineering strategies will facilitate successful microbial production of anthocyanins in industry in the near future.

Contents

1. Introduction . . . 00
2. Industrial applications of anthocyanins . . . 00
3. Biosynthesis of anthocyanins in plants . . . 00
4. Metabolic engineering of anthocyanin production in microorganisms . . . 00
   4.1. Enzyme screening and engineering for anthocyanin production . . . 00
   4.2. Regulation of cofactor/cosubstrate supply for anthocyanin overproduction . . . 00
   4.3. Engineering of specific transporters for anthocyanin production . . . 00
   4.4. Optimization of culture processes for anthocyanin production . . . 00
5. Future perspectives . . . 00
6. Conclusions . . . 00
Conflict of interest . . . 00
Acknowledgement . . . 00
References . . . 00

1. Introduction

As a member of the flavonoid group of polyphenols, anthocyanins are important chemicals in the plant kingdom as pigments, antioxidants, and antimicrobials (Fig. 1). With the rising interest in natural nutraceuticals, there is an increasing preference for natural food colorants such as anthocyanins, thus stimulating high demand

Abbreviations: ANS, anthocyanidin synthase; CHI, chalcone isomerase; CHS, chalcone synthase; 4CL, 4-coumaroyl-CoA ligase; DFR, dihydroflavonol 4-reductase; DSSC, dye-sensitized solar cell; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′, 5′-hydroxylase; FGT, flavonoid glucosyltransferase; F3GT, flavonoid 3-*O*-glucosyltransferase; UV, ultraviolet.

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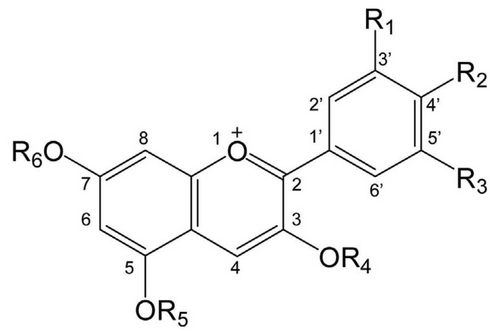
Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.synbio.2017.10.005



	Anthocyanins	R_1	R_2	R_3	R_4	R_5	R_6
1	Pelargonidin 3-*O*-glucoside	H	OH	H	Glc	H	H
2	Cyanidin 3-*O*-glucoside	OH	OH	H	Glc	H	H
3	Peonidin 3-*O*-glucoside	OMe	OH	H	Glc	H	H
4	Malvidin 3-*O*-glucosdie	OMe	OH	OMe	Glc	H	H
5	Cyanidin 3-*O*-galactoside	OH	OH	H	Gal	H	H
6	Malvidin-3-*O*-(6-*O*-acetyl-glucoside)	OMe	OH	OMe	A-Glc	H	H

Glc: glucosyl unit; Me: methyl; Gal: galactosyl unit; A-Glc: 6-*O*-acetyl-glucosyl unit.

Fig. 1. The structure of anthocyanins. Modifications (such as glycosylation, hydroxylation, methylation, and acylation) at C3' (R1), C4' (R2), C5' (R3), C3 (R4), C5 (R5) and C7 (R6) generate structural analogs. R1-R5 are functional groups derived from glycosyl, hydroxyl, methyl, and acyl units. Representative anthocyanins and their structures are listed.

for these compounds mainly as colorants and cosmetic additives [1–4].

The traditional means of producing anthocyanins is by extraction and purification from fruits, flowers, and other tissues of plants [5–7]. This approach has a couple of advantages such as diverse sources of readily available cheap feedstocks, sophisticated extraction and purification techniques, and decent market recognition (non-GMO products). And great success has been achieved in the development of novel extraction and purification procedures, understanding of anthocyanin biosynthesis in plants, and genetic engineering of plants for anthocyanin production [8–10]. However, anthocyanins isolated from plants exist as a heterogeneous mixture of multiple types of molecules with diverse chemical structures. In addition, anthocyanin production through plant extraction is neither stable nor sustainable, since plants produce anthocyanins with varied productivity, and the production relies on farmland and irrigation, and fluctuates depending on seasonal and environmental conditions [11–13]. An alternative is microbial production, which has already demonstrated great potential in the biosynthesis of plant-derived natural compounds. The intrinsic characteristics of microorganisms, such as fast growth and easy cultivation, and the sophisticated microbial manipulation techniques, including convenient genetic modifications and readily available bioinformatics tools, render microbial production of natural products facile, controllable, and cost-effective [14–16]. So far, many plant-derived compounds have been produced, including terpenoids, alkaloids, and flavonoids, in prokaryotic and eukaryotic microorganisms [15,17,18]. Microbial biosynthesis of natural flavonoids dates back to 2003 [19], and various flavonoid compounds, from flavanones to the more complicated anthocyanins, have been generated in engineered microorganisms [14]. Meanwhile, microbial production of non-natural flavonoids has also been made possible through the feeding of specific substrates [20,21]. In all these cases, the producing microbes are metabolically engineered for the optimal synthesis of the target products. Coupled with the advances in genome sequencing and DNA synthesis, structural biology and enzymology, and modeling of metabolic networks, metabolic engineering is playing a powerful and indispensable role in microbial synthesis of on-demand compounds with biochemical functions or industrial significance [22,23].

In this review, the recent progress on anthocyanin production in genetically modified microorganisms will be described, with a main focus on metabolic engineering strategies on improving *in vivo* production. Also, process optimization and *in vitro* storage of anthocyanins are overviewed, providing a comprehensive understanding of the engineering and bioproduction processes of these

Please cite this article in press as: Zha J, Koffas MAG, Production of anthocyanins in metabolically engineered microorganisms: Current status and perspectives, Synthetic and Systems Biotechnology (2017), https://doi.org/10.1016/j.synbio.2017.10.005