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Review

Engineered *Escherichia coli* as new source of flavonoids and terpenoids

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ABSTRACT

During the last two decades, the engineered biosynthesis of several natural products by microbial sources has made enormous progresses. Noteworthy, *Escherichia coli* has been used as microbial power plant for the artificial biosynthesis of different types of molecules for both biological and clinical applications. Among natural products, especially flavonoids and terpenoids are particularly attractive because of the high variety of their biochemical activities including anti-oxidant, anti-inflammatory, anti-viral, anti-bacterial, anti-obesity and anti-cancer properties.

Here we are reviewing the characteristics of *E. coli* engineering and its "cell factory" properties to synthesize natural products. This review focuses on the exploitation of *E. coli* biochemical networking for the specific synthesis of flavonoids and terpenoids.

Contents

Abbreviations: GI, gastrointestinal; Top1, eukaryotic topoisomerase; AMPK, AMP-activated protein kinase; NF, nuclear factor; LDL, low-density-lipoprotein; IL, interleukin; PPAR, peroxisome proliferator-activated receptor; IPP, isopentenyl diphosphate; DMAPP, isomer dimethylallyl diphosphate; EIEC, enteroinvasive *E. coli*; PAIs, pathogenicity islands; IBD, inflammatory bowel disease; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EHEC, enterohemorrhagic *E. coli*; EAEC, enteroaggregative *E. coli*; UPEC, uropathogenic *E. coli*; PAL, phenylalanine ammonia lyase; 4CL, coumarate:coenzyme A ligase; CHS, chalcone synthase; RBS, ribosome-binding sequence; CHI, chalcone isomerase; ACC, acetyl-CoA carboxylase (ACC); ACS, acetyl-CoA synthase; IFS, isoflavonone synthase; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; BCM(D)O, β-carotene mono(di)oxygenase.

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1. Introduction

Higher plants display several secondary metabolites including alkaloids, isoprenoids (terpenoids) and phenolic compounds (phenylpropanoids and flavonoids). For several decades, numerous plant-derived metabolites, such as flavonoids and terpenoids, have been widely used in human nutrition and have been discussed as potential molecules for prevention and therapy of a variety of multifactorial human diseases, such as obesity-related conditions and cancer (Balsano & Alisi, 2009; Corcoran, McKay, & Blumberg, 2012; Xia & Weng, 2010).

Flavonoids are phenolic compounds, chemically characterized by two benzene rings joined by a linear carbon chain, which are copious in food and nutraceuticals (Tsao, 2010). Several experimental evidences indicate that regular consumption of foods containing flavonoids may exert a potent antioxidant and antiinflammatory activity reducing the risk of several chronic conditions including certain forms of cancer (Russo, Del Bufalo, & Cesario, 2012; Surh, 2003) and obesity-related diseases such as metabolic syndrome, diabetes and cardiovascular disease (Panchal, Poudyal, & Brown, 2012; Pfeuffer et al., 2011; Qi et al., 2010).

Terpenoids are the most diverse class of natural compounds consisting of more than 40,000 structurally different molecules, which have been isolated from animal and microbial species as well as from a wide variety of plants (Sacchettini & Poulter, 1997). Therefore, terpenoids are a large group of phytochemicals, used by Chinese and Indian traditional medicine, and are currently being explored as anticancer agents in experimental models and phase I/II clinical trials (Liby, Yore, & Sporn, 2007; Tanaka, Shnimizu, & Moriwaki, 2012).

These therapeutic properties and the consequent pharmaceutical value of many flavonoids and terpenoids have led to intense efforts in last ten-fifteen years toward understanding and engineering the corresponding biosynthetic pathways able to by-pass the natural supply. In fact, at present these products are mainly obtained by extraction from plants that accumulate them at low levels deserving several problems including high costs of extractive methods and a low yields. Many attempts for using plant metabolic engineering to increase the amounts of secondary metabolites have not gave big results because of the complex and strict biosynthesis regulation of the plant-derived metabolites. Furthermore, the complexity and chirality of most of these metabolites have limited the development of cost-effective chemical synthesis methods. Therefore, recently concerted efforts from several disciplines, such as biochemistry, structural biology, genetics, and metabolic engineering, have discovered alternative and promising approaches for economical biosynthesis of "unnatural" flavonoids and terpenoids. Metabolic engineering of biosynthetic pathways for these compounds in genetically modified heterologous hosts, such as microorganisms and higher plants, is an emerging field. An efficient biosynthesis of flavonoids and terpenoids has been performed using recombinant bacteria such as *Escherichia coli* (Ajikumar et al., 2008; Du, Shao, & Zhao, 2011; Misawa, 2011). *E. coli* is, among bacteria, the microorganism of choice for many biotechnological applications because not only it displays a high tolerance to solvents but it is also the most frequently manipulated by genetic engineering for several purposes.

This review describes novel or recent approaches and achievements in metabolic engineering of *E. coli* and its "cell factory" pathway, toward efficient biosynthesis of functional flavonoids and terpenoids.

2. Flavonoids

2.1. Chemical characteristics and properties

Flavonoids are a large group of natural compounds that belong to the polyphenols family. They have the C6–C3–C6 general structural backbone, in which the two C6 units (Ring A and Ring B) are of phenolic nature (Fig. 1). Due to rearrangement, methylation, methoxylation, alkylation, oxidation, *C*- and *O*-glycosylation, and hydroxylation in the Ring C flavonoids can form over 9000 members exhibiting antioxidant, antibacterial, antiviral, and anti-cancer activities (Leonard et al., 2008). Total of flavonoids can be divided into different subgroups, such as:

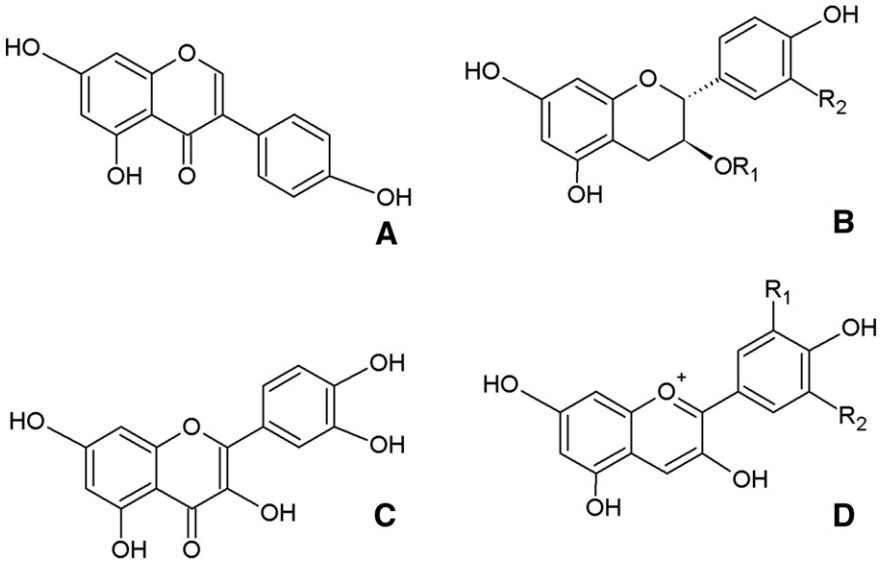

Fig. 1. Molecular structure of some representative flavonoids: (A) geninstein, (B) catechins, (C) quercetin and (D) anthocyanins.