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REVIEW

Microbial production strategies and applications of lycopene and other terpenoids

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Abstract Terpenoids are a large class of compounds that have far-reaching applications and economic value, particularly those most commonly found in plants; however, the extraction and synthesis of these compounds is often expensive and technically challenging. Recent advances in microbial metabolic engineering comprise a breakthrough that may enable the efficient, cost-effective production of these limited natural resources. Via the engineering of safe, industrial microorganisms that encode product-specific enzymes, and even entire metabolic pathways of interest, microbial-derived semisynthetic terpenoids may soon replace plant-derived terpenoids as the primary source of these valuable compounds. Indeed, the recent metabolic engineering of an *Escherichia coli* strain that produces the precursor to lycopene, a commercially and medically important compound, with higher yields than those in tomato plants serves as a successful example. Here, we review the recent developments in the metabolic engineering of microbes for the production of certain terpenoid compounds, particularly lycopene, which has been increasingly used in pharmaceuticals, nutritional supplements, and cosmetics. Furthermore, we summarize the metabolic engineering strategies used to achieve successful microbial production of some similar compounds. Based on this overview, there is a reason to believe that metabolic engineering comprises an optimal approach for increasing the production of lycopene and other terpenoids.

Keywords Lycopene · Metabolic engineering · Biosynthesis · Synthetic biology · Terpenoid

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Introduction

Terpenoids, also referred to as isoprenoids, are a class of naturally produced chemicals comprised of thousands of compounds, including sterols, carotenoids, and quinines. Over the past several decades, many of these compounds, particularly those most commonly found in plants, have been utilized for a wide variety of pharmaceutical and industrial applications (Breitmaier 2006; Davies et al. 2015). While many terpenoids can generally be purified from plants, such extraction and/or purification methods are often technically challenging and/or expensive. In 2015 Nobel Prize was given to Youyou Tu, for her and her team to discover the artemisinin, a typical terpenoid which has good anti-malaria activity. Alternatively, terpenoids can be produced via chemical synthesis methods. Indeed, for some compounds, this approach is commonly used and cost-effective. For many other compounds, however, low levels of production make chemical synthesis too difficult or expensive, or simply impossible.

Microbial production is typically a more popular approach for terpenoid production owing to the relative ease and speed of microbial cultivation, the genetic tractability of these organisms, and the relatively straightforward bioprocess requirements for these methods. Many bioactive microbial compounds are secondary metabolites of their native

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producers, to some extent, they are produced at relatively low levels by these organisms (Story et al. 2010). The recent boom in metabolic engineering has been a major breakthrough in the production of these limited microbial products. Genetic manipulation in readily engineered, safe, industrial microorganismsis is becoming a classical route to maximally tap the potential of the microbes for certain compounds, and even for designing and creating novel genetic circuits (Quin and Schmidt-Dannert 2014). According to one market research report, the market value of biotechnologically produced chemicals will be approximately US$400 billion by the year 2030 (Lorenz and Zinke 2005).

Some readily engineered industrial microorganisms have been well characterized and are widely used for heterologous biosynthesis of secondary metabolites, especially *Escherichia coli* and some yeast species (Stephanopoulos et al. 2004). *E. coli* is an industrial workhorse and a model prokaryotic organism that plays an important role in modern biological engineering and industrial microbiology. Yeast as one of the most thoroughly researched eukaryotic microorganisms is widely used for making foods and valuable compounds. Given the prevalence of powerful genetic manipulation platforms, recent advances in genomic analyses, rapid development of synthetic biology, and the wide variety of tools and strategies currently available for metabolic engineering, the synthetic optimization of target biosynthetic pathways could facilitate the efficient, cost-effective production of high-value compounds.

Here, we review the current applications and recent advances in the metabolic engineering of microbes (especially *E. coli* and some yeast species) for the production of terpenoids, particularly lycopene, which has been increasingly used in pharmaceuticals, nutritional supplements, and cosmetics. We summarize the metabolic engineering strategies that were successfully used to achieve microbial production of some similar compounds and propose future strategies for enhancing biotechnological production of terpenoids in microbes.

The two major pathways for producing terpenoid precursors

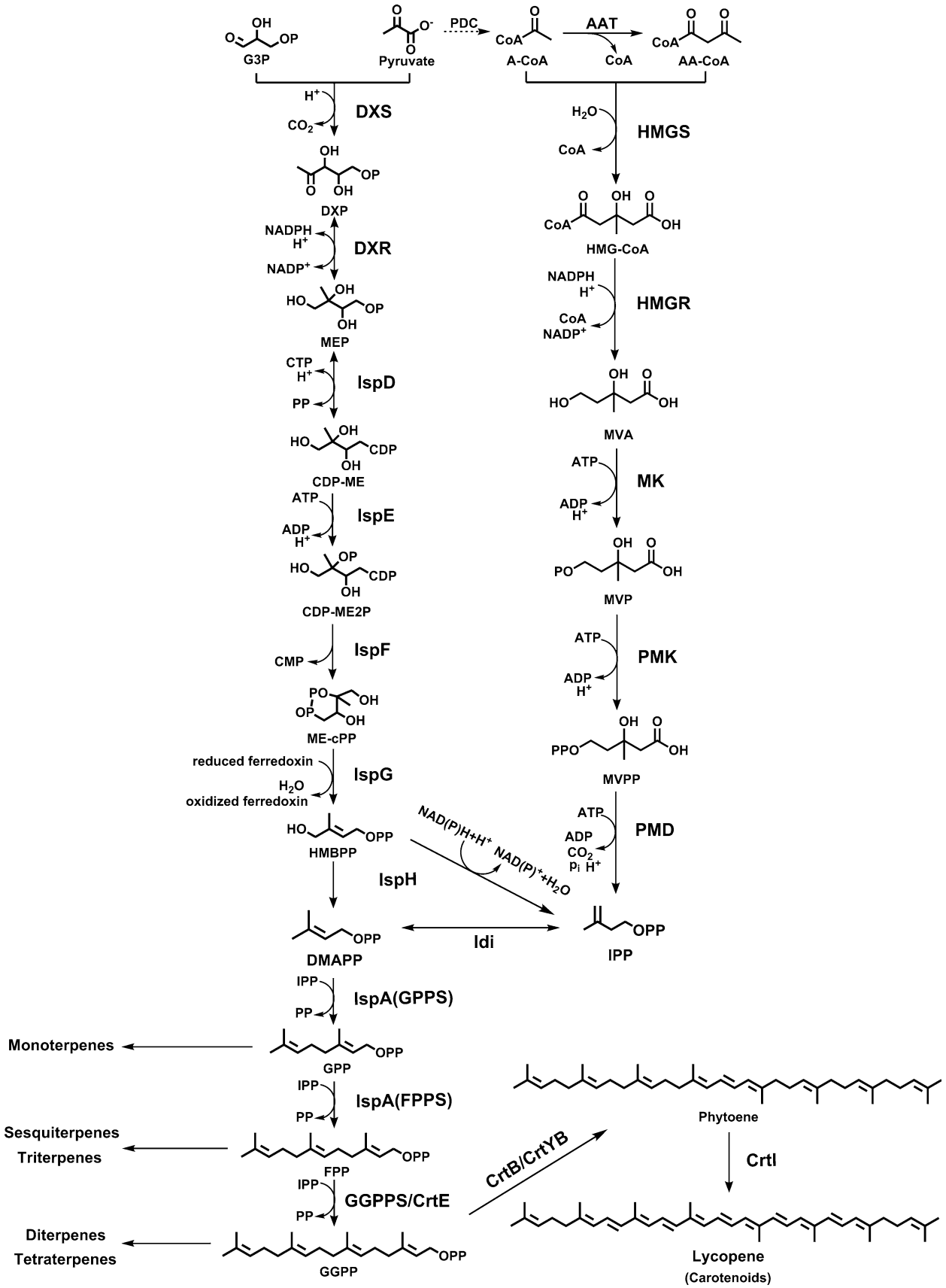
Terpenoids are comprised of a series of basic five-carbon (C5) building blocks, and they are typically classified into groups according to the number of carbons that they contain: hemiterpenoids (C5), monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), triterpenoids (C30), and the long-chained poly-terpenoids. In general, terpenoid biosynthesis pathways can be divided into two streams: the upstream pathway, which is responsible for the synthesis of the universal C5 building blocks is opentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), and the downstream pathway, which is responsible for the condensation and various modifications of the IPP and DMAPP precursors.

Fig. 1 Terpenoid biosynthetic pathways. *Metabolite abbreviations* G3P, D-glyceraldehyde-3-phosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; CDP-ME, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol; CDP-ME2P, 2-phospho-4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol; MEcPP, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; CoA, coenzyme A; A-CoA, acetyl-CoA; AA-CoA, acetoacetyl-CoA; HMG-CoA, (*S*)-3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonate; MVP, mevalonate-5-phosphate; MVPP, mevalonate diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; PP, diphosphate. *Enzyme abbreviations* DXS, 1-deoxyxylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; IspD, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase; IspE, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol kinase; IspF, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; IspG, (*E*)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase; IspH, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; PDC, pyruvate carboxylase; AAT, acetyl-CoA C-acetyltransferase; HMGS, 3-hydroxy-3-methylglutaryl CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; PMD, mevalonate diphosphate decarboxylase; Idi, isopentenyl diphosphate isomerase; GPPS, geranyl diphosphate synthase; IspA/FPPS, farnesyl diphosphate synthase; GGPPS/CrtE, geranylgeranyl diphosphate synthase; CrtB/CrtYB, phytoene synthase; CrtI, phytoene desaturase

Production of IPP and DMAPP can occur through either the mevalonate (MVA) pathway or the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway. The MVA pathway, which is found in most eukaryotes and archaea, as well as in certain bacterial species, utilizes acetyl-CoA to produce IPP, subsequently isomerized to generate DMAPP (Bloch et al. 1959). Meanwhile, the MEP pathway, which is found in plant chloroplasts, in most bacterial species, and in certain eukaryotic parasites (Boucher and Doolittle 2000), generates IPP and DMAPP via the condensation of pyruvate and glyceraldehyde-3-phosphate (Rohmer et al. 1993). As depicted in Fig. 1. *E. coli* utilizes the MEP pathway for native production of terpenoids; however, this pathway exhibits low metabolite flux (Ajikumar et al. 2008). In contrast, MVA is the primary metabolic pathway used for terpenoid production in yeast. Notably, in plants, these two pathways exist in parallel for primary and secondary metabolism, a system that could be advantageous for environmental adaptation and more efficient carbon utilization (Wagner and Krab 1995).

Metabolic engineering of the two pathways for terpenoid precursors overproduction

Engineering of the MVA and MEP pathways yielded increased production of the two terpenoid precursors. Specifically, the introduction of the MVA pathway from


G3P
Pyruvate
PDC
A-CoA
AAT
CoA
AA-CoA
DXS
DXP
DXR
MEP
IspD
CDP-ME
IspE
CDP-ME2P
IspF
ME-cPP
IspG
HMBPP
IspH
DMAPP
Idi
IPP
HMGS
HMG-CoA
HMGR
MVA
MK
MVP
PMK
MVPP
PMD
NAD(P)H+H+
NAD(P)++H2O
IspA(GPPS)
GPP
IspA(FPPS)
FPP
GGPPS/CrtE
GGPP
Monoterpenes
Sesquiterpenes
Triterpenes
Diterpenes
Tetraterpenes
CrtB/CrtYB
Phytoene
CrtI
Lycopene
(Carotenoids)

Saccharomyces cerevisiae into *E. coli* to bypass the MEP pathway resulted in a 36-fold increase in precursor production (Martin et al. 2003). Meanwhile, the expression of a complete MVA pathway (Martin et al. 2003), or even a partial MVA pathway coupled with mevalonate supplementation (Campos et al. 2001),was capable of providing a significantly higher flux route to the precursors than that achieved using the MEP pathway in *E. coli* (Martin et al. 2003). Conversely, heterologous expression of the MEP pathway to circumvent MVA pathway regulation in yeast has, to date, been unsuccessful (Vickers et al. 2014).

Metabolic engineering of the rate-limiting enzymes for terpenoid precursors overproduction

Among the genes in these two pathways, 1-deoxy-D-xylulose-5-phosphate synthase (encoded by the *dxs* gene) has the highest impact on precursor production, while IPP isomerase (encoded by *idi*) has been shown to play a significant role in the flux. Meanwhile, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (encoded by *dxr*) and 3-hydroxymethyl-3-glutaryl coenzyme A reductase (HMG-CoA reductase) are the rate-limiting enzymes of the MEP and MVA pathways, respectively. The *ispD* and *ispF* genes appear to form an operon and encode rate-limiting enzymes for the flux, whereas there is no rate limitation associated with the *ispE*, *ispG*, or *ispH* gene products (Verwaal et al. 2007; Lv et al. 2013). Indeed, overexpression of these rate-limiting enzymes resulted in increased flux and precursor production (Yuan et al. 2006), and further increases were achieved by enhancing the availability of the pathway precursor acetyl-CoA (Shiba et al. 2007).

IPP toxicity

IPP is the universal intermediate precursor of terpenoids, it is also the start point of downstream engineering, preventing IPP toxicity is an important issue to consider when engineering terpenoid biosynthesis. It was first proved to be toxic when a high-flux mevalonate pathway was assembled in *E. coli* (Martin et al. 2003). Some studies pointed that depleting the pool of IPP is harmful for the cell because IPP is needed for tRNA prenylation, and for quinone and cell wall biosynthesis (Caillet and Droogmans 1988; Maury et al. 2005), while cell growth and glucose consumption decreased when IPP concentrations were high (George et al. 2014). To achieve overproduction of target products, an abundance of precursors is necessary; however, downstream engineering is also critical to decrease the accumulation of IPP, and thereby reduce IPP-mediated toxicity, and to increase both the flux and target output.

Metabolic engineering in microorganisms for lycopene production

Lycopene is a carotenoid pigment that is naturally produced primarily by tomatoes, in which the lycopene content can be as high as 3–14 mg/100 g (Story et al. 2010). This highly polyunsaturated hydrocarbon contains 11 conjugated and 2 unconjugated double bonds, which are responsible for the sensitivity of the compound to temperature and light (Shi et al. 2008), as well as for its antioxidant effects and bioactivities, including its role in the prevention of cancer and tumor development (Giovannucci 1999), prevention of diabetes, cardiovascular protection (Rissanen et al. 2002), and alleviation of osteoporosis (Rao et al. 2007).

Biosynthesis of lycopene

Lycopene serves as a popular antioxidant, but perhaps more importantly, its pigment properties enable convenient colorimetric screening, which has facilitated the investigation and optimization of new tools for metabolic engineering. Lycopene is always synthesized from geranylgeranyl diphosphate (GGPP) or farnesyl diphosphate (FPP), which in turn are synthesized by GGPP synthase (GGPPS/CrtE) or FPP synthase (IspA), respectively. Notably, certain proteins perform the functions of both IspA and CrtE, such as the *gps* gene product of *Archaeoglobus fulgidus*. The condensation of two GGPP molecules by phytoene synthase (CrtB) leads to the formation of the colorless compound phytoene. Lycopene, the first colored compound produced during carotenoid biosynthesis, is then synthesized via the catalytic activity of phytoene desaturase (CrtI). In certain yeast and fungal species, such as *Xanthophyllomyces dendrorhous* and *Blakeslea trispora*, the phytoene synthase and phytoene desaturase activities are performed by a bifunctional enzyme.

Metabolic engineering of the rate-limiting enzymes for lycopene overproduction

A previous study indicated that GGPP synthesis is a rate-limiting step in the lycopene pathway (Kang et al. 2005), and the considerable sequence diversity among *crtE* genes(Sedkova et al. 2005; Nishizaki and Tsuge 2007) indicates that these enzymes exhibit distinct levels of activity. It is therefore essential to choose an efficient *crtE* gene to eliminate limitations in GGPP synthesis. Even so, various IspA proteins have been evaluated to achieve enhanced substrate availability (Ohnuma et al. 1996; Narita et al. 1999). Similarly, an *E. coli* genomic library was

screened via a shot-gun approach to isolate native genes that enhance lycopene accumulation when overexpressed, including *dxs*, *appY* (transcriptional activator of energy metabolism operons; relief from energy limitations), *crl* (transcriptional regulator associated with surface fiber formation; protection of the cell from the hydrophobicity of lycopene), and *rpoS* (Kang et al. 2005).

Removal of the competing pathways for lycopene overproduction

Notably, the deletion of genes involved in the lycopene pathway resulted in enhanced lycopene synthesis through increased precursor supply. Alper et al. (2005b) undertook a genome-wide stoichiometric flux balance analysis as an aid calculated metabolic fluxes and then scanned the genome for single and multiple gene knockouts that yield improved product yield. A strain encoding deletions in *gdhA*, *aceE*, and *fdhF* (*ΔgdhA*, *ΔaceE*, *ΔfdhF*) exhibited 6.6 mg/g cell dry weight lycopene production, an increase of approximately 40 % relative to the engineered parental strain(Alper et al. 2005b).

Promoters, copy number, gene order and genome integration for lycopene overproduction

In a previous study, promoters of varying strength were utilized for quantitative assessment of the effects of distinct gene expression levels on growth and product formation (Yuan et al. 2006).Likewise, Alper et al. (2005a) used a previously characterized library of promoters to evaluate the impact of different gene expression levels on growth and lycopene production (Alper et al. 2005a). With respect to the copy number of engineered plasmids, while most studies have found that higher copy numbers lead to higher production levels, a linear relationship between these factors has not been found (Tao et al. 2005). Moreover, because multi-copy vectors can impose a significant metabolic burden on replication rates, low-copy vectors should be considered for pathway optimization studies (Jones et al. 2000). As gene order can also affect lycopene production levels, evaluation of permutations in the order of genes within an operon provides an additional strategy for manipulating transcript levels (Nishizaki and Tsuge 2007). Lastly, in the scale up of fermentation, for decreasing the requirement for complex media or the cost of antibiotics to be used in bioprocesses, chromosomal integration of genes has been performed to improve stability, and integration of lycopene genetic genes into the *E. coli* chromosome, approximately 30 mg/g cdw of lycopene has been obtained (Chen et al. 2013), and varies of available integration tools makes engineering large DNA sequences possible (Sabri et al. 2013).

Pathway balancing and engineering for lycopene overproduction

In the above engineering strategies, over-expression of the *idi* gene relieves the production bottleneck in *E. coli* (Zhu et al. 2014); however, excess intracellular concentrations of IPP or DMAPP can lead to growth inhibition (Martin et al. 2003).Engineering biosynthetic pathways for the production of target compounds may always lead to imbalances in the pathways. For eliminating limitation of flux and inhibition of cell growth, some work for pathway balancing has been done. For example, in a recent study, we successfully redesigned the biosynthetic process for lycopene in *E. coli* by targeted engineering. In vitro reconstituted system guided us to rationally optimize each component by quantitatively overexpressing. With the whole pathway balancing, the strain exhibited 1.44 g/L lycopene production during fed-batch fermentation (Zhu et al. 2015).

Similarly, certain optimized mevalonate-producing strains were found to exhibit decreased transcript levels of both HMG-CoA synthase and HMG-CoA reductase, implying that gene deletion or overexpression is not always an ideal solution and that balanced expression should be considered when engineering metabolic networks (Lombard and Moreira 2011). Indeed, pathway balancing such as by enhancing mRNA stability or translation initiation to modulate flux has led directly to improvements in lycopene production levels (Smolke et al. 2001).

Another engineering strategy that is particularly worth mentioning is evolutionary pathway engineering. Umeno et al. described how pathway engineers have used laboratory evolution experiments to diversify biosynthetic pathways. This work has demonstrated the remarkable ability of biosynthetic enzymes to evolve and acquire new specificities, and has illuminated pathway features that facilitate the creation of new natural products via "unnatural" molecular scaffolds. Although evolutionary pathway engineering is in its infancy, it is capable of generating whole new families of natural product analogs. Some of these compounds may await discovery in nature, while others will probably never be produced by natural evolution (Umeno et al. 2005).

Cofactor engineering for lycopene overproduction

Efficient regeneration of NADPH and the supply of ATP are the limiting factors that constrain the productivity of biotransformation processes, optimization of the

expression levels of the cofactors NADPH and ATP comprises an effective approach for enhancing lycopene production. Modulating expression of genes encoding a-ketoglutarate dehydrogenase, succinate dehydrogenase and transaldolase B within central metabolic modules increased NADPH and ATP supplies, leading to a 76 % increase of lycopene yield, and ribosome binding site libraries were further used to modulate expression of genes. Indeed, this approach previously yielded 3.52 g lycopene per L (50.6 mg/g cdw) during fed-batch fermentation (Sun et al. 2014).

Host engineering for lycopene overproduction

The above engineering strategies were implemented in *E. coli*, while strategies for pathway engineering are transferrable to a variety of other microbes, including yeast species (Xue and Ahring 2011; Kiyota et al. 2014). Host selection depends on a number of different factors, including genetic tractability, native capacity for target compound production, potential toxicity of the end product, competition with inherent essential requirements, and potential/ability to carry out (desired or detrimental) biotransformations/modifications (Vickers et al. 2014). Furthermore, production capacities are highly variable between strains (Takahashi et al. 2007; Rodríguez-Villalón et al. 2008).Yeast metabolism is more complex than that of *E. coli*, which could potentially facilitate the production of more complicated target products. The first attempt at lycopene production in yeast was carried out by introducing the carotenogenic genes of *Pantoea ananatis* (formerly called *Erwinia uredovora*) into *S. cerevisiae* (Yamano et al. 1994).Meanwhile, in a subsequent study, genetic engineering of the edible yeast *Candida utilis* yielded 0.758 mg/g cdw lycopene, indicating that this organism comprises another promising source of this valuable compound (Miura et al. 1998).

Given that lycopene production is limited by enzymatic and regulatory factors, many of which might still be unknown, we could utilize different kinds of comprehensive combinations with respect to enzyme activity, gene expression, gene modification, and metabolite flux to improve the lycopene production. In recent years, analytical tools, including targeted engineering (Zhu et al. 2014), targeted proteomics (Redding-Johanson et al. 2011), multiplex automated genome engineering (MAGE) for large-scale programming (Wang et al. 2009), and metabolomics have been coupled with genetic standardization and modern cloning techniques to make this goal attainable (George et al. 2014).

Microbial production strategies and applications of other terpenoids

Compared with lycopene production, metabolic engineering can also be applied to meeting the increasing demand for other natural terpenoids, similarly, some pigmented compounds such as hydrocarbons carotenoids (β-carotene), or the oxygenated derivatives (astaxanthin). Indeed, these compounds have been successfully synthesized in non-carotenogenic microbes, such as *E. coli*, *S. cerevisiae*, *C. utilis*, and *Zymomonas mobilis*, by transforming these organisms with carotenoid genes from carotenogenic microbes (Misawa and Shimada 1998). Moreover, by combining genes from carotenoid biosynthetic branches of distinct microorganisms, novel carotenoids not found in any other pathway can also be synthesized (Sedkova et al. 2005).

Metabolic engineering of the endogenous MEP pathway or introduction of foreign MVA pathways could result in increased supplies of the IPP precursor for carotenoid production. Likewise, enhanced IPP production can be achieved by combining the MEP and MVA pathways (Yang and Guo 2014). To date, several genetic engineering approaches have been utilized to improve target production, including overexpression of the rate-limiting genes *crtW* and *crtZ* (Fray et al. 1995), integration of carotenogenic genes into the *E. coli* chromosome (Lemuth et al. 2011), cofactor tuning (Tao et al. 2007), and the use of synthetic regulatory circuits (Williams et al. 2013). In the long run of the metabolic engineering, a series of a high-flux terpenoid pathway was engineered and exuberantly in numerous strategy optimizations. And with the construction of cellular networks, metabolic engineering plays swinging effect on the production of interest product precisely and timely. The carotenoids produced via distinct metabolic engineering approaches, as well as their production levels, in *E. coli* and other microorganisms are listed in Table 1.

Conclusion and future perspectives

Traditional methods for terpenoid production typically rely on extraction of the compound from plants or chemical synthesis, both of which are associated with low yields, high cost and, in certain cases, the generation of environmental pollutants. In contrast, metabolic engineering of microorganisms comprises an environmental friendly, sustainable, and economical production approach that has become the method of choice for the production of various valuable compounds. Generally, these strategies involve

Table 1 Summary of the various metabolic engineering approaches and hosts used for the production of lycopene and other terpenoids

Major approaches		Host microorganism	Products	Content (mg/g cdw)	Culture conditions	References
Whole pathway engineering	MVA pathway engineering	*Escherichia coli*	Lycopene	22	NR	Yoon et al. (2006)
	Combined MEP and MVA	*E. coli*	Lycopene	32	Fed-batch fermentation	Kim et al. (2011)
	Whole pathway engineering in the chromosome	*E. coli*	Lycopene	33.4	Shake flask fermentation	Chen et al. (2013)
Removal of competing pathways	Knockout (*ΔgdhA*, *ΔaceE*, *ΔpyjiD*)	*E. coli*	Lycopene	6.6	Shake flask fermentation	Alper et al. (2005b)
Genome engineering	Genome engineering	*E. coli*	Lycopene	9	NR	Wang et al. (2009)
Pathway balancing	Targeted engineering	*E. coli*	Lycopene	43.7	Fed-batch fermentation	Zhu et al. (2015)
	Systematic and combinatorial methods	*E. coli*	Lycopene	18	Fed-batch fermentation	Alper et al. (2005a)
Cofactor engineering	NADPH, ATP engineering	*E. coli*	Lycopene	50.6	Fed-batch fermentation	Sun et al. (2014)
Overexpression of a rate-limiting enzyme	Whole pathway	*Saccharomyces cerevisiae*	Lycopene	5.9	Fed-batch fermentation	Xie et al. (2015)
	Whole pathway	*Pichia pastoris*	Lycopene	4.6	Fed-batch fermentation	Bhataya et al. (2009)
	Whole pathway	*Yarrowia lipolytica*	Lycopene	16	Fed-batch fermentation	Matthäus et al. (2014)
Promoters and RBSs engineering	Promoters and RBSs engineering	*Streptomyces avermitilis*	Lycopene	82	Shake flask fermentation	Bai et al. (2015)
Whole pathway engineering	MEP, ATP, PPP, TCA engineering	*E. coli*	β-Carotene	60	Fed-batch fermentation	Zhao et al. (2013)
	Whole pathway engineering with defined medium	*E. coli*	β-Carotene	72.6	Fed-batch fermentation	Nam et al. (2013)
	Whole pathway	*S. cerevisiae*	β-Carotene	7.4	Shake flask fermentation	Xie et al. (2014)
Whole pathway engineering	Whole pathway engineering in the chromosome	*E. coli*	Astaxanthin	1.4	Shake flask fermentation	Lemuth et al. (2011)
	Multi-dimensional search approach	*Xanthophyllomyces dendrorhous*	Astaxanthin	9.7	Fed-batch fermentation	Gassel et al. (2013)

NR Not reported

the introduction of heterologous pathways, the elimination of unnecessary but usable and practical native reactions, re-engineering of regulatory networks through rational or random approaches, cofactor tuning, balancing of metabolite flux, and regulation of the accumulation of toxic intermediates to increase strain stability and improve pathway efficiency.

Lycopene production in microbes provides proof-of-concept for metabolic engineering. With advances in genomics, computational biology, and synthetic biology (Stephanopoulos et al. 2004), synthetic microbes may soon replace plants as the primary source of various valuable terpenoids. Moreover, the generation of enhanced strains through metabolic engineering and the use of these organisms for large-scale production in fermentors is likely just the beginning of the applications for the industrial exploitation of recombinant microorganisms.

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Compliance with ethical standards

Conflicts of interest The authors declare no financial or commercial conflicts of interest.

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