FEMS Yeast Research, 15, 2015, 1–11

doi: 10.1111/1567-1364.12187
Minireview

MINIREVIEW

Yeast synthetic biology for high-value metabolites

Zhubo Dai[1], Yi Liu[1], Juan Guo[2], Luqi Huang[2,*] and Xueli Zhang[1,*]

[1]Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China and [2]National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

*Corresponding author. Luqi Huang, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China. Tel.: +8610 64014411; Fax: +8610 64013996; E-mail: huangluqi@263.net
Xueli Zhang, Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 West 7th Ave, Tianjin Airport Economic Park, Tianjin 300308, China. Tel.: +8622 84861983; Fax: +8622 84861983; E-mail: zhang_xl@tib.cas.cn
Editor. Hyun Ah Kang

ABSTRACT

Traditionally, high-value metabolites have been produced through direct extraction from natural biological sources which are inefficient, given the low abundance of these compounds. On the other hand, these high-value metabolites are usually difficult to be synthesized chemically, due to their complex structures. In the last few years, the discovery of genes involved in the synthetic pathways of these metabolites, combined with advances in synthetic biology tools, has allowed the construction of increasing numbers of yeast cell factories for production of these metabolites from renewable biomass. This review summarizes recent advances in synthetic biology in terms of the use of yeasts as microbial hosts for the identification of the pathways involved in the synthesis, as well as for the production of high-value metabolites.

Key words: high-value metabolites; synthetic biology; *Saccharomyces cerevisiae*

INTRODUCTION

Certain secondary metabolites, such as morphine, taxol, artemisinin, coenzyme Q_{10}, docosahexaenoic acid (DHA), and carotenoids, which exist in plants, mammals, microalgae, and microorganisms (Facchini *et al.*, 2012; Siddiqui *et al.*, 2012), are regarded as high-value metabolites (Chang and Keasling, 2006; Siddiqui *et al.*, 2012; Ye and Bhatia, 2012; Zhou *et al.*, 2014). In nature, these metabolites play important roles in an organism's function, including providing protection against biotic and abiotic stresses, radiation, and acting as regulatory molecules (Marienhagen and Bott, 2013). Many of these metabolites are known to play a key role in human health and the treatment of diseases, such as pain control (morphine; Chappell, 2008), treatment of cancer (taxol and vincristine; Chappell, 2008; Ye and Bhatia, 2012), eradication of parasites (artemisinin; Ye and Bhatia, 2012), and treatment of heart diseases (carotenoids and DHA; Adarme-Vega *et al.*, 2013; Mata-Gomez *et al.*, 2014). During the last 30 years, up to 50% of approved drugs have been derived either directly or indirectly from natural metabolites, and in the area of cancer alone, about 48.6% of anticancer drugs are either natural metabolites *per se* or are derivatives of natural metabolites (Li and Vederas, 2009; Newman and Cragg, 2012).

However, many of these metabolites are present in low quantities in their natural sources. For instance, the natural concentration of taxol is only about 0.02% of the dry weight yield from pacific yew trees (Ye and Bhatia, 2012), and the vincristine content in *Catharanthus roseus* is only 0.0003% of the dry weight yield (Kuboyama *et al.*, 2004). These low yields and lengthy production time have hindered their widespread industrial utilization (Chang and Keasling, 2006). The chemical synthesis approach for the production of these metabolites is also hampered by diminished yield, due to the multiple transformation steps required to synthesize these structurally complex molecules (Nicolaou *et al.*, 1994; Wuts, 1998; Kuboyama *et al.*, 2004).

Yeast is extensively used in food and beverage production and is generally recognized as safe (GRAS). *Saccharomyces cerevisiae* is also an important eukaryotic model microorganism for fundamental molecular biology research, and its genome has been sequenced completely (Goffeau, 2000). Recently, lots

Received: 30 April 2014; **Accepted:** 15 July 2014

of synthetic biology tools have been developed for engineering this organism as cell factories for production of high-value metabolites, including the rapid assembly of biosynthetic pathways (Shao and Zhao, 2009), modulation of expression of heterologous genes (Dai *et al.*, 2012; Zhou *et al.*, 2012), and localization of enzymes to a special subcellular region or scaffold (Farhi *et al.*, 2011; Avalos *et al.*, 2013). Moreover, *S. cerevisiae* can provide a similar physical and physiological environment for the functional expression of diverse heterologous enzymes [e.g. cytochrome P450s and uridine diphosphate glycosyltransferases (UGTs)] from plants and mammals, as they allow endomembrane localization and post-translational modifications, such as protein glycosylation (Eckart and Bussineau, 1996; Pompon *et al.*, 1996).

In the last few years, the discovery of genes involved in high-value metabolites pathways combined with advances in synthetic biology has allowed successful construction of increasing number of yeast cell factories for production of high-value metabolites (Table 1). This review summarizes recent advances in synthetic biology in terms of the use of yeasts as microbial hosts for the identification of the pathways involved in the synthesis, as well as for the production of high-value metabolites.

PATHWAY IDENTIFICATION

Identification of biosynthetic pathways of high-value metabolites is crucial for construction of yeast cell factories to produce these compounds. Several strategies have been developed for pathway identification, resulting in elucidation of key genes of multiple high-value metabolites biosynthetic pathways (Table 2).

In the early stage, to identify the synthetic pathway for production of the target metabolite, proteins for catalyzing the target biochemical reactions needed to be identified. Production of target metabolite was firstly confirmed by *in vitro* enzymatic assay using the crude enzyme extract of the natural organism. Specific proteins for catalyzing the target biochemical reactions were then purified and used for characterization. This strategy has been used in the identification of the amorphadiene synthase (ADS) gene in the artemisinin biosynthetic pathway from *Artemisia annua* (Bouwmeester *et al.*, 1999), as well as the taxadiene synthase (TDS) gene in the taxol biosynthetic pathway from *Taxus brevifolia* (Hezari *et al.*, 1995).

With the development of sequencing technology, gene-mining approach has been commonly used for pathway identification. Expressed sequence tag (EST) databases from cDNA libraries of an organism of interest were obtained, and candidate

Table 1. Yeast cell factories for high-value compounds.

Compound family	Metabolites	Host strains	Fed substrate	Titer or yield
Terpenoids				
Artemisinin	Amorphadiene	*S. cerevisiae*	Ethanol	40 g L^{-1*} (Westfall *et al.*, 2012)
	Artemisinic acid	*S. cerevisiae*	Ethanol	25 g L^{-1*} (Paddon *et al.*, 2013)
Taxol	Taxadiene	*S. cerevisiae*	Glucose	8.7 mg $L^{-1\dagger}$ (Engels *et al.*, 2008)
Tanshinone	Miltiradiene	*S. cerevisiae*	Glucose	488 mg L^{-1*} (Dai *et al.*, 2012)
	Ferruginol	*S. cerevisiae*	Glucose	10.5 mg $L^{-1\dagger}$ (Guo *et al.*, 2013)
Oleanane-type	β-amyrin	*S. cerevisiae*	Glucose	107 mg $L^{-1\dagger}$ (Dai *et al.*, 2014)
	Oleanolic acid	*S. cerevisiae*	Glucose	71 mg $L^{-1\dagger}$ (Dai *et al.*, 2014)
	3-O-Glc-echinocystic acid	*S. cerevisiae*	Galactose	Detected† (Moses *et al.*, 2014)
Dammarane-type	Dammarenediol-II	*S. cerevisiae*	Glucose	1548 mg L^{-1*} (Dai *et al.*, 2013)
	Protopanaxadiol	*S. cerevisiae*	Glucose	1189 mg L^{-1*} (Dai *et al.*, 2013)
	Protopanaxatriol	*S. cerevisiae*	Glucose	15.9 mg $L^{-1\dagger}$ (Dai *et al.*, 2014)
	Ginsenoside CK	*S. cerevisiae*	Galactose	1.4 mg $L^{-1\dagger}$ (Yan *et al.*, 2014)
Carotenoids	β-carotene	*S. cerevisiae*	Glucose	18 mg g^{-1}, DCW† (Reyes *et al.*, 2014)
	Astaxanthin	*X. dendrorhous*	Glucose	9 mg g^{-1}, DCW† (Gassel *et al.*, 2014)
Steroids	Hydrocortisone	*S. cerevisiae*	Glucose/Ethanol	11.5 mg $L^{-1\dagger}$ (Szczebara *et al.*, 2003)
	Cholesterol	*S. cerevisiae*	Glucose	1 mg $g^{-1\ddagger}$, WCW (Souza *et al.*, 2011)
Flavonoids	Naringenin	*S. cerevisiae*	*p*-coumaric acid	28.3 mg $L^{-1\dagger}$ (Yan *et al.*, 2005)
	Genistein	*S. cerevisiae*	Naringenin	7.7 mg $L^{-1\dagger}$ (Trantas *et al.*, 2009)
	Kaempferol	*S. cerevisiae*	Naringenin	4.6 mg $L^{-1\dagger}$ (Trantas *et al.*, 2009)
	Quercetin	*S. cerevisiae*	Naringenin	0.38 mg $L^{-1\dagger}$ (Trantas *et al.*, 2009)
Stilbenes	Resveratrol	*S. cerevisiae*	*p*-coumaric acid	391 mg $L^{-1\dagger}$ (Sydor *et al.*, 2010)
Alkaloids	Reticuline	*S. cerevisiae*	Norlaudanosoline	164.5 mg $L^{-1\dagger}$ (Hawkins and Smolke, 2008)
	Sanguinarine	*S. cerevisiae*	Norlaudanosoline	Detected‡ (Fossati *et al.*, 2014)
	Strictosidine	*S. cerevisiae*	Secologanin and tryptamine	2 g $L^{-1\dagger}$ (Geerlings *et al.*, 2001)
	Glucosinolates	*S. cerevisiae*	Galactose	1.07 mg $L^{-1\dagger}$ (Mikkelsen *et al.*, 2012)
Others	Eicosapentaenoic acid	*Y. lipolytica*	Glucose	15%, DCW† (Xue *et al.*, 2013)
	Penicillins	*H. polymorpha*	α-aminoadipic acid/ phenylacetic acid	Detected‡ (Gidijala *et al.*, 2009)
	6-MSA	*S. cerevisiae*	Glucose	554 mg $L^{-1\S}$ (Wattanachaisaereekul *et al.*, 2008)

*Fed-batch bioreactors, †Flasks or tubes, §Batch bioreactors,‡Culture conditions were not mentioned.

Table 2. Identification of high-value metabolite biosynthetic pathway genes by various approaches.

Compound	Metabolite	Enzymes	Approaches used	References
Artemisinin	Amorphadiene	ADS	Enzyme extract of the plant (*Artemisia annua*), *in vitro*	Bouwmeester *et al.* (1999)
	Artemisinic acid	CYP71AV1/ ADH1/ALDH1	cDNA library sequencing data, *in vivo* (yeast)	Ro *et al.* (2006), Teoh *et al.* (2009), Paddon *et al.* (2013)
Tanshinone	Miltiradiene	CPS/KSL	cDNA library sequencing data, *in vivo* (*E. coli*)	Gao *et al.* (2009)
	Ferruginol	CYP76AH1	Next-generation sequencing data, *in vivo* (yeast)	Guo *et al.* (2013)
Glycyrrhizin	β-amyrin	bAS	cDNA library (hybridized), *in vivo* (yeast)	Hayashi *et al.* (2001)
	11-oxo-β-amyrin	CYP88D6	cDNA library sequencing data, *in vivo* (yeast)	Seki *et al.* (2008)
	Glycyrrhetinic acid	CYP72A154	cDNA library sequencing data, *in vivo* (yeast)	Seki *et al.* (2011)
Ginsenoside CK	Dammarenediol-II	DDS	cDNA (homology-based PCR), *in vivo* (yeast)	Tansakul *et al.* (2006)
	Protopanaxadiol	CYP716A47	cDNA library sequencing data, *in vivo* (yeast)	Han *et al.* (2011)
	Protopanaxatriol	CYP716A53v2	cDNA library sequencing data, *in vivo* (yeast)	Han *et al.* (2012)
	Ginsenoside CK	UGTPg1	cDNA library sequencing data, *in vivo* (yeast)	Yan *et al.* (2014)

genes can be identified through sequence alignment analysis with the aid of bioinformatics tools. These candidate genes were then overexpressed, and the related proteins were assayed for functional characterization (Seki *et al.*, 2008; Gao *et al.*, 2009; Guo *et al.*, 2013). This strategy has been used for identification of two cytochrome P450 monooxygenase genes, CYP88D6 and CYP72A154, of the glycyrrhizin biosynthetic pathway. These two enzymes were identified by transcript profiling-based selection from a collection of licorice ESTs, followed by *in vitro* enzymatic activity assays. CYP88D6 was shown to catalyze the oxidation of β-amyrin at C-11 to 11-oxo-β-amyrin, while CYP72A154 catalyzed the oxidation of 11-oxo-β-amyrin at C-30 to glycyrrhetinic acid (Seki *et al.*, 2008, 2011). Through introducing β-amyrin synthase, CYP88D6, and CYP72A154 into *S. cerevisiae*, the engineered yeast could also catalyze the oxidation of β-amyrin to glycyrrhetinic acid *in vivo*, further demonstrating the functions of these two enzymes (Seki *et al.*, 2011).

In vitro functional characterization of candidate enzymes usually needs the feeding of appropriate substrates. However, most of these substrates are either very expensive or not commercially available (Facchini *et al.*, 2012), which limits characterization of the target enzymes. To solve this problem, yeast can be used as host organisms for *in vivo* characterization of candidate enzymes. The candidate genes were imported into yeast hosts, which have been engineered to produce the related substrates. Candidate enzymes can be demonstrated to be responsible for catalyzing the reaction if designed product was produced in the engineered yeast.

Recently, a combination of next-generation sequencing, computational algorithms, and synthetic biology was used to discover biosynthetic genes involved in high-value metabolites synthesis, such as in the PhytoMetaSyn Project (based in Canada; Facchini *et al.*, 2012; Xiao *et al.*, 2013) and the Medicinal Plants Genomic Resource (MPGR) project (based in the USA; Gongora-Castillo *et al.*, 2012a,b; Yeo *et al.*, 2013). These projects aimed to use a combination of a genomics pipeline that integrates massively parallel DNA sequencing, targeted metabolomics, advanced bioinformatics, and 'plug-and-play' functional genomics in yeast, to identify the corresponding pathway-related genes of an organism efficiently (Facchini *et al.*, 2012). This strategy was used by Guo *et al.* to identify six candidate CYP genes that were coregulated with diterpene synthase gene in the tanshinones biosynthetic pathway in both the rhizome and danshen hairy roots. Furthermore, using *S. cerevisiae* as a host organism, one of the encoded proteins, CYP76AH1, was demonstrated to be able to catalyze a unique four-electron oxidation cascade on miltiradiene to produce ferruginol (Guo *et al.*, 2013).

CONSTRUCTION OF YEAST CELL FACTORIES

Based on pathway identification and synthetic biology tools, lots of yeast cell factories have been constructed in recent years for production of varied high-value metabolites. Below, we focus on describing the yeast cell factories for production of terpenoids derived from the mevalonic acid (MVA)/2-C-methyl-D-erythritol-4-phosphate (MEP) pathways, flavonoids, stilbenes, alkaloids, and other compounds such as eicosapentaenoic acid (EPA) and 6-methylsalicylic acid (6-MSA; Table 1, Figs. 1 and 2; Keasling, 2008, 2012; Carothers *et al.*, 2009; Nielsen and Keasling, 2011; Lienert *et al.*, 2014; Singh, 2014).

TERPENOIDS

Terpenoids are the most diverse class of natural products and consist of more than 50 000 structurally diverse compounds, which are derived from two common building blocks, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP; Chang and Keasling, 2006). Terpenoids are synthesized through either the MVA pathway in all eukaryotic cells and in the cytoplasm and mitochondria of plants, or through the MEP

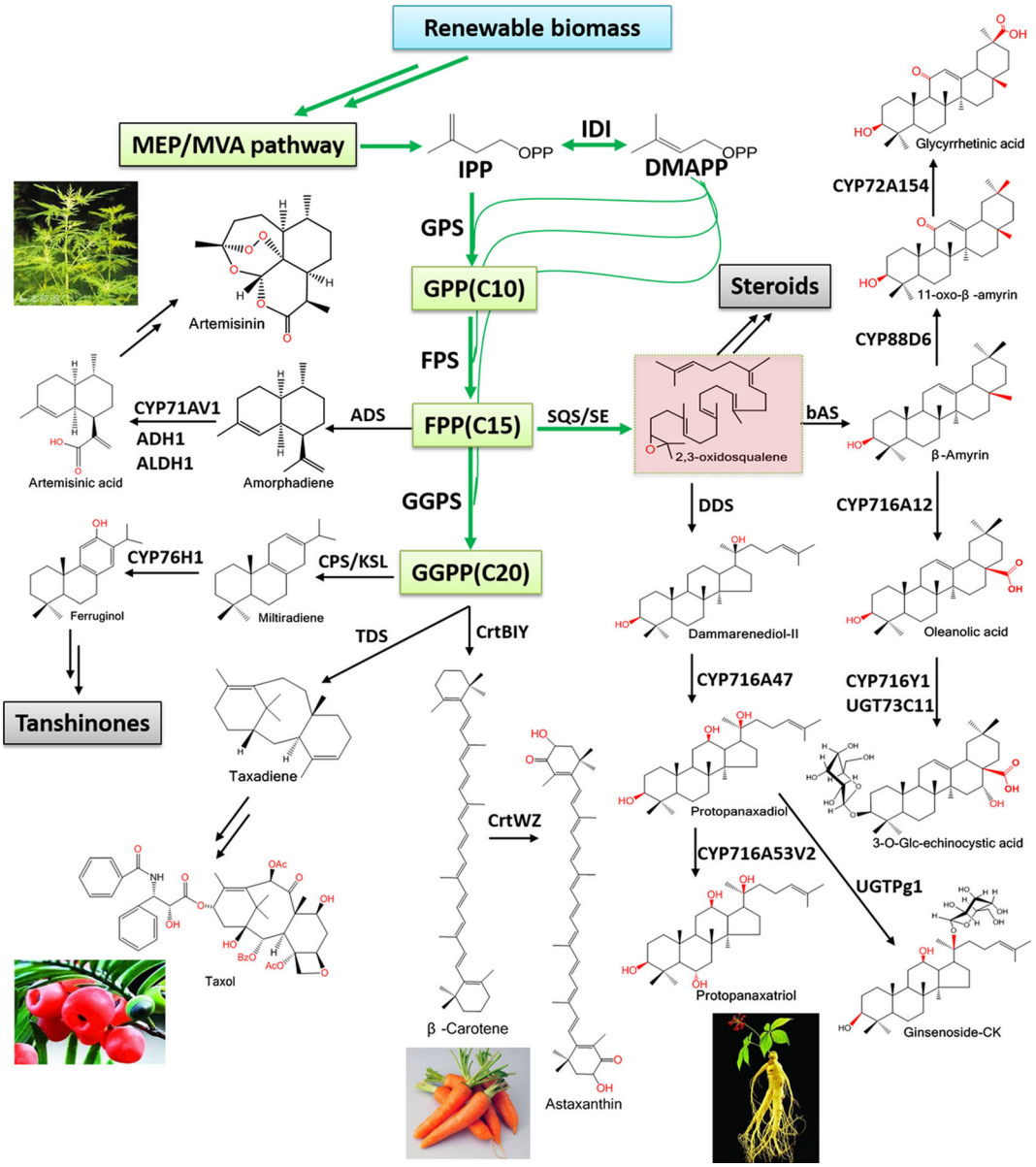

Figure 1. Biosynthesis of isoprenoids from MVA/MEP pathway. Single arrows represent the one-step conversions, while double arrows represent multiple steps. The cytochrome P450 reductase (CPR) was also expressed for functional expression of cytochromes P450s. GPP(C10), geranyl diphosphate; FPP(C15), farnesyl diphosphate; GGPP(C20), geranylgeranyl diphosphate; GPS, geranyl diphosphate synthase; FPS, farnesyl diphosphate synthase; GGPS, geranylgeranyl diphosphate synthase; CYP71AV1, artemisinic acid synthase; ALDH1, aldehyde dehydrogenase; ADH1, alcohol dehydrogenase; CPS, copalyl diphosphate synthase; KSL, copalyl diphosphate kaurene synthase-like; CYP76AH1, ferruginol synthase; CrtBIY, bifunctional phytoene synthase/lycopene cyclase(crtYB) and phytoene desaturase (crtI); CrtWZ, β-carotene ketolase (CrtW) and hydroxylase(CrtZ); bAS, β-amyrin synthase; CYP88D6, 11-oxo-β-amyrin synthase; CYP72A154, glycyrrhetinic acid synthase; CYP716A12, oleanolic acid synthase; CYP716Y1, C-16α hydroxylase(catalyzes the C-16α hydroxylation of oleanane- and ursane-type triterpenes); UGT73C11, 3-O-glucosyltransferase (3-O-Glc-echinocystic acid synthase); DDS, dammarenediol-II synthase; CYP716A47, protopanaxadiol synthase; CYP716A53V2, protopanaxatriol synthase; UGTPg1, ginsenoside compound K synthase.

pathway in bacteria, other prokaryotes, and plastids of plants (Ro *et al.*, 2006; Ajikumar *et al.*, 2010; Dai *et al.*, 2011; Li and Pfeifer, 2014). Terpenoids include monoterpenes, sesquiterpenes, diterpenes, triterpenes, and carotenoids (tetraterpenes), which exert a wide range of functions in human health, and have been applied extensively in the production of pharmaceuticals, nutraceuticals, cosmetics, and biomaterials (rubber; Courtney and Gilchrist, 1980; Chang and Keasling, 2006; Misawa, 2011; Li and Pfeifer, 2014). In yeast, these compounds are synthesized through the MVA pathway, and most of the IPP and DMAPP precursors enter the ergosterol synthetic pathway (Dai *et al.*, 2013). *S. cerevisiae* has been considered as an attractive host for terpenoids production because it harbors sufficient pools of precursors, and it has been chosen as the microbial host for the production of artemisinin, tanshinone, ginsenosides, and carotenoids.

Artemisinin

Artemisinin, a sesquiterpene lactone with an endoperoxide group, is used as an antimalarial drug and has been extracted from *A. annua*. The farnesyl diphosphate (FPP)

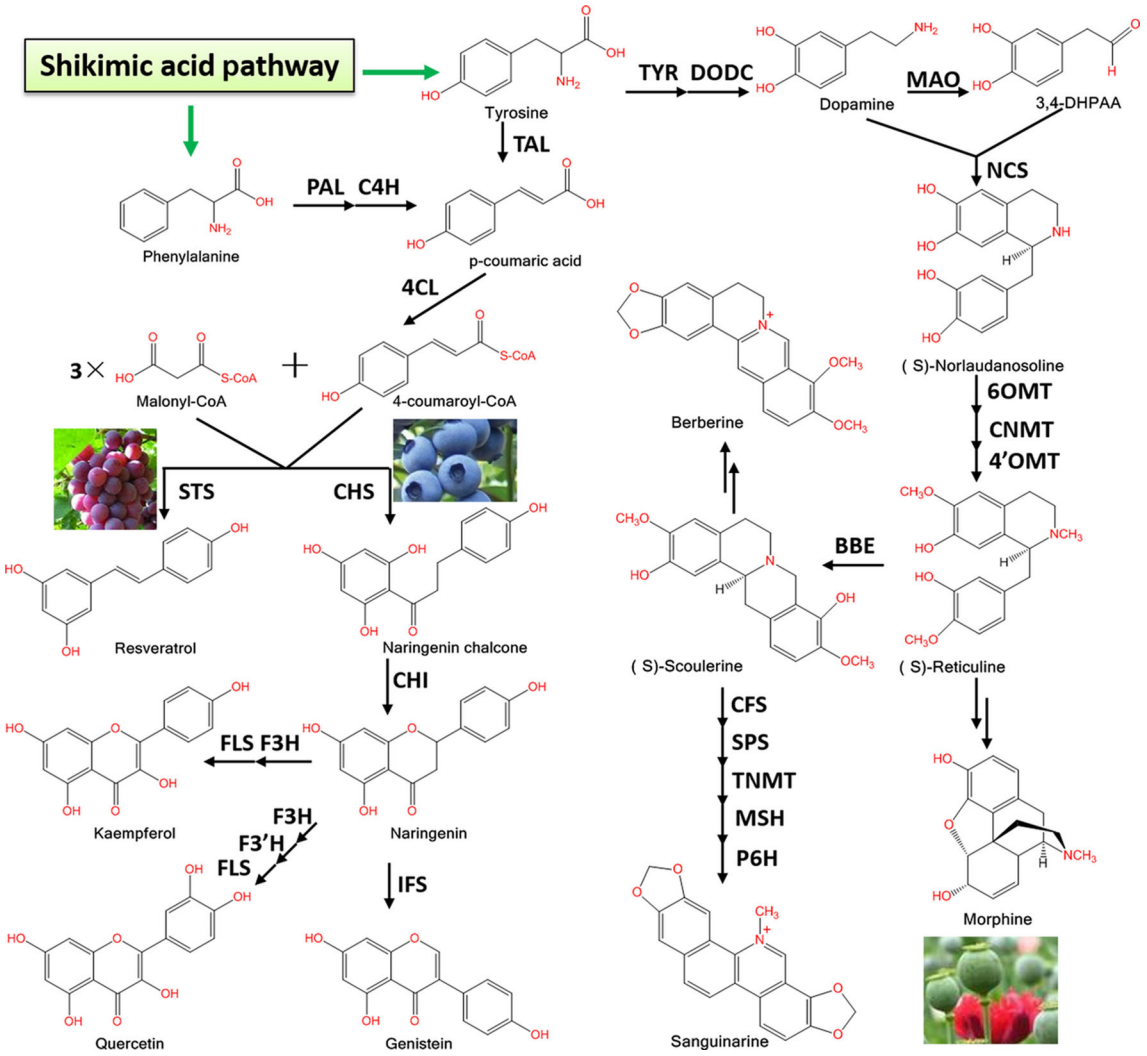

Figure 2. Biosynthesis of flavonoids, stilbenes, and benzylisoquinoline alkaloids (BIAs) from shikimic acid pathway. Single arrows represent the one-step conversions, while double arrows represent multiple steps. The cytochrome P450 reductase (CPR) was also expressed for functional expression of cytochromes P450s. C4H, cinnamate-4-hydroxylase; TAL, tyrosine ammonia lyase; 4CL, 4-coumarate-CoA-ligase; STS, stilbene synthase; F3H, flavanone 3b-hydroxylase; F3′H, flavonoid 3-hydroxylase; FLS, flavonol synthase; TYR, tyrosinase; DODC, DOPA decarboxylase; MAO, monoamine oxidase; NCS, norcoclaurine synthase; 3,4-DHPAA, 3,4-dihydroxyphenylacetaldehyde; BBE, berberine bridge enzyme which catalyze three methylation reactions to convert (R,S)-norlaudanosoline to (R,S)-reticuline; CFS, cheilanthifoline synthase; SPS, stylopine synthase; MSH, (S)-cis-*N*-methylstylopine 14-hydroxylase; P6H, protopine 6-hydroxylase; DBOX, dihydrobenzophenanthridine oxidase.

precursor can be catalyzed to amorphadiene by the ADS enzyme in *A. annua*, and cytochrome P450 CYP71AV1 is responsible for the three subsequent oxidation steps required to convert amorphadiene to artemisinic acid (Ro *et al.*, 2006; Lenihan *et al.*, 2008). Within this synthetic pathway, alcohol dehydrogenase (ADH1) can participate in the oxidation of artemisinic alcohol to artemisinic aldehyde, while artemisinic aldehyde dehydrogenase (ALDH1) oxidizes artemisinic aldehyde to artemisinic acid (Fig. 1; Teoh *et al.*, 2009; Paddon *et al.*, 2013). Recently, the complete artemisinic acid biosynthetic pathway, including AaCPR1 and cytochrome AaCYB5, was assembled in engineered *S. cerevisiae*, in which the mevalonate pathway was upregulated and the competing pathway, which converts FPP to ergosterol, was limited by a push-and-pull strategy (Paddon *et al.*, 2013). In the final engineered strain, a fermentation titer of 25 g L^{-1} of artemisinic acid was achieved (Paddon *et al.*, 2013).

Tanshinones

Diterpenoids are derived via the cyclization and further modification of geranylgeranyl diphosphate (GGPP), which include valuable metabolites taxol (Ajikumar *et al.*, 2010), ginkgolides (Leonard *et al.*, 2010) and tanshinones (Dai *et al.*, 2012; Fig. 1). Although researchers have obtained high titers of taxadiene, which is the precursor of taxol, by engineering, the heterologous pathway in *Escherichia coli* (Ajikumar *et al.*, 2010), using yeast as the host, generates a lower yield of only 8.7 mg L^{-1} (Engels *et al.*, 2008).

Miltiradiene is the precursor of tanshinones, which are a group of active diterpenoids found in the medicinal herb *Salvia miltiorrhiza* Bunge (Gao *et al.*, 2014), and exhibits Diverse pharmacological activities, including antibacterial, antioxidant, neuroprotective, cardioprotective, and antitumor effects (Zhou *et al.*, 2005). Modular pathway engineering strategy was applied for constructing *S. cerevisiae* cell factories to produce

miltiradiene. Through overexpression of the truncated hydroxyl-3-methylglutaryl coenzyme A synthase (tHMGR), the fusion of *S. miltiorrhiza* copalyl diphosphate (CPP) synthase (SmCPS) and *S. miltiorrhiza* CPP kaurene synthase-like (SmKSL), and the fusion of GGPP synthase (BTS1) and farnesyl diphosphate synthase (ERG20), a *S. cerevisiae* cell factory was obtained which produced 365 mg L^{-1} miltiradiene in a 15-L bioreactor (Zhou *et al.*, 2012). After functional characterization of CYP76AH1, this P450 module together with the SmCPR1 (NADPH-cytochrome P450 reductase 1 from *S. miltiorrhiza*) module were further introduced to the yeast host which can produce miltiradiene. The engineered strain produced ferruginol at a yield of 10.5 mg L^{-1} after 48 h of shake-flask fermentation (Guo *et al.*, 2013). In addition, through integrating *SmCPS* and *SmKSL* genes into the yeast chromosome and providing sufficient supplies of FPP and GGPP precursors by combinatorial overexpression of *tHMGR, upc2.1* (a semi-dominant mutant allele that enhances the activity of sterol uptake control protein 2, UPC2), *EGR20, BTS1*, and *Sulfolobus acidocaldarius* GGPP synthase (SaGGPPS) genes, another *S. cerevisiae* cell factory was obtained which produced 488 mg L^{-1} miltiradiene in fed-batch fermentation (Dai *et al.*, 2012).

Ginsenosides

Triterpenoids are a diverse group of metabolites, which mainly include dammarane-, oleanane-, ursane-, and lupane-type triterpenes that are associated with a variety of pharmacological activities (Augustin *et al.*, 2011; Fukushima *et al.*, 2011; Geisler *et al.*, 2013; Fig. 1). Ginsenosides are a group of active triterpenoids mainly found in *Panax ginseng* C.A. Meer and *Panax quinquefolius* L (Leung and Wong, 2010). According to their chemical structure, these compounds are divided into dammarane-type tetracyclic and oleanane-type pentacyclic ginsenosides (Han *et al.*, 2011). Protopanaxadiol (PPD) is the aglycon of several dammarane-type ginsenosides and also has anticancer activity (Musende *et al.*, 2012). For microbial production of PPD, dammarenediol-II synthase and PPD synthase genes of *P. ginseng*, together with a NADPH-cytochrome P450 reductase gene of *Arabidopsis thaliana*, were introduced into *S. cerevisiae*. Precursor supplies were increased by overexpressing the genes in the upstream pathway for squalene and 2,3-oxidosqualene synthesis. In addition, PPD synthase activity was increased by codon optimization. These modifications led to a 262-fold increase in PPD production. Using two-phase extractive fermentation, the engineered yeast cell factory produced 1189 mg L^{-1} PPD, together with 1548 mg dammarenediol-II (Dai *et al.*, 2013). Recently, the UGT-encoding gene (*UGTPg1*) from *P. ginseng* for converting PPD to ginsenoside compound K (CK), which is the C-20 position glycosylation product of PPD, was identified. After introducing *UGTPg1* gene together with the PPD-biosynthetic genes into *S. cerevisiae*, the resulting yeast cell factory could produce 1.4 mg L^{-1} ginsenoside CK (Yan *et al.*, 2014).

Oleanane-type triterpenoids are pharmacologically important chemicals with a variety of biological activities (Yendo *et al.*, 2010; Pollier and Goossens, 2012). All oleanane-type triterpenoids are derived from β-amyrin, and oleanolic acid is a representative compound of this family, which exhibits hepatoprotective effects, as well as antioxidant and anticancer activities (Pollier and Goossens, 2012). For microbial production of β-amyrin, β-amyrin synthase from *Glycyrrhiza glabra* was introduced into *S. cerevisiae* followed by overexpression of tHMGR, squalene synthase, and squalene epoxidase genes to increase precursor supply. The resulting strain BY-βA-G produced 107 mg L^{-1} β-amyrin (Dai *et al.*, 2014). The oleanolic acid synthase gene, together with the *A. thaliana* gene encoding NADPH-cytochrome P450 reductase (AtCPR), were further introduced into this β-amyrin-producing strain, resulting in strain BY-OA that produced 71 mg L^{-1} oleanolic acid (Dai *et al.*, 2014).

Recently, CYP716Y1 which encodes a cytochrome P450 monooxygenase was identified from *Bupleurum falcatum*. This enzyme can catalyze the C-16α hydroxylation of oleanane- and ursane-type triterpenes. After introducing this enzyme together with oxidosqualene cyclase, a P450 enzyme that catalyzes a multistep oxidation at C-28, and glycosyltransferase from other plant species into *S. cerevisiae*, a yeast cell factory for production of oleanane-type triterpene saponins of 3-O-Glc-echinocystic acid was obtained (Moses *et al.*, 2014).

Carotenoids

Carotenoids are tetraterpenoid pigments derived from two units of geranylgeranyl diphosphate and are produced by diverse organisms, including plants and numerous fungi and bacteria (Mata-Gomez *et al.*, 2014; Fig. 1). There are over 600 known carotenoids, such as lycopene, β-carotene, and astaxanthin (Mata-Gomez *et al.*, 2014). Most carotenoids exhibit significant antioxidant activities and are used in food and feed additives, cosmetics, and pharmaceuticals (Vachali *et al.*, 2012).

Several research groups have been constructing yeast cell factories for β-carotene production. The β-carotene biosynthetic pathway genes from *Erwinia uredovora* were introduced into *S. cerevisiae*, resulting in an engineered strain producing 0.1 mg g^{-1} DCW of β-carotene (Yamano *et al.*, 1994). Verwaal *et al.* (2007) succeeded in constructing an engineered *S. cerevisiae* strain capable of producing high levels of β-carotene, up to 5.9 mg g^{-1} DCW, by integrating the phytoene synthase/lycopene cyclase (crtYB) and phytoene desaturase (crtI) gene from *Xanthophyllomyces dendrorhous* into *S. cerevisiae*, followed by additional overexpression of *tHMG1* and *BTS1* genes from *S. cerevisiae* and *crtE*, *crtYB*, and *crtI* genes from *X. dendrorhous*. Li *et al.* (2013) improved β-carotene production in *S. cerevisiae* by 200% through codon optimization of *crtI* and *crtYB* genes from *X. dendrorhous* together with utilization of HMGR from *Staphylococcus aureus*. Reyes *et al.* (2014) successfully improved carotenoid production from 6 to 18 mg g^{-1} DCW in an engineered β-carotene-producing yeast by applying adaptive evolution method. The molecular mechanisms for increased carotenoids production were further characterized by comparative transcriptome analysis. It was found that upregulation of genes related with lipid biosynthesis and MVA pathways was responsible for increased carotenoids production (Reyes *et al.*, 2014).

Astaxanthin is a commercially important feed ingredient in salmon and trout farming (Markou and Nerantzis, 2013). Its health-promoting activities in humans are due to its high antioxidative potential (Wu *et al.*, 2014). The alga *Haematococcus pluvialis* naturally contains astaxanthin at about 1.5–3% DCW (Johnson and Schroeder, 1996; Gassel *et al.*, 2014), and the yeast *X. dendrorhous* is one of the rare microbial organisms that can synthesize astaxanthin. Wild-type strains of *X. dendrorhous* yields *c.* 200-400 μg g^{-1} DCW of astaxanthin (Johnson and Schroeder, 1995; Visser *et al.*, 2003). Using classical mutagenesis and genetic pathway engineering of *X. dendrorhous*, an efficient cell factory was obtained which produced 9 mg g^{-1} DCW astaxanthin (Gassel *et al.*, 2014). In addition, *S. cerevisiae* cell factories for astaxanthin production was constructed by introducing astaxanthin synthetic genes from *X. dendrorhous* into *S. cerevisiae*, leading to a yield of 29 μg g^{-1} DCW (Ukibe *et al.*, 2009).

Steroids

Steroids are molecules with four joined carbon rings and are synthesized in plants, animals, and fungi (Souza *et al.*, 2011; Siddiqui *et al.*, 2012). Steroids include the dietary lipid cholesterol, the sex hormones estradiol and testosterone, and the drug hydrocortisone (Heftmann, 1974). An erg6-mutant yeast strain, which produced ergosterol as the major sterol, has been reported to synthesize trace amounts of cholesterol (Xu and Nes, 1988). Recently, researchers have constructed yeast cell factories for production of different steroids.

For production of cholesterol, two genes, *ERG5* and *ERG6*, which are involved in the ergosterol pathway, were disrupted. By overexpression of the enzymes dehydrocholesterol 7-reductase (DHCR7) and dehydrocholesterol 24-reductase (DHCR24) from *Danio rerio*, the cholesterol pathway was constructed in engineered yeast. Similar DHCR7 and DHCR24 enzymes from *Xenopus laevis*, humans, and fish were compared, and the fish enzymes provided the best results. Engineered yeast strain RH6829 was obtained, which produced *c.* 1 mg g^{-1} wet weight of cholesterol (Souza *et al.*, 2011).

Hydrocortisone is an anti-inflammatory drug used widely in formulations for topical ointments and is also administered orally or intravenously as a prescription drug (Menkin, 1954). A yeast cell factory for total biosynthesis of hydrocortisone was realized by introducing 13 genes from plant and mammalian into *S. cerevisiae*. Hydrocortisone was produced as the major steroid of this engineered yeast strain, with a yield of 11.5 mg L^{-1} (Szczebara *et al.*, 2003).

FLAVONOIDS AND STILBENOIDS

Flavonoids and stilbenes are two classes of high-value phenylpropanoid metabolites that are considered as nutritional compounds (Siddiqui *et al.*, 2012; Zhou *et al.*, 2014). Although yeasts do not naturally synthesize flavonoids and stilbenes, they produce the necessary precursors (tyrosine and phenylalanine) for flavonoid and stilbene biosynthesis (Fig. 2). This makes the yeast a suitable host cell for the production of flavonoids and stilbenes through the heterologous expression of genes from plant and fungi.

Naringenin

Flavanones (e.g. naringenin) are precursors for synthesis of isoflavones (e.g. genistein), flavones (e.g. apigenin), and flavonols (e.g. kaempferol, quercetin; Siddiqui *et al.*, 2012; Zhou *et al.*, 2014). For microbial production of naringenin, four plant-derived enzymes which can convert *p*-coumaric acid to naringenin, including cinnamate-4-hydroxylase (C4H) from *A. thaliana*, 4-coumarate-CoA-ligase (4CL) from *Petroselinum crispum*, and chalcone isomerase (CHI) and chalcone synthase (CHS) from *Petunia hybrid* were introduced into *S. cerevisiae*. The resulting strain produced 28.3 mg L^{-1} naringenin when fed with *p*-coumaric acid (Yan *et al.*, 2005).

Three other different flavonoids (genistein, kaempferol, and quercetin) were also synthesized in yeast through pathway engineering approaches. By overexpression of isoflavone synthase (IFS), *C4H, 4CL, CHS,* and *CHI* gene from *Glycine max*, NADPH-cytochrome P450 reductase and phenylalanine-ammonia lyase (PAL) from *Populus trichocarpa* and *Populus deltoides*, the engineered *S. cerevisiae* strain could yield *c.* 7.7 mg L^{-1} genistein from naringenin (Trantas *et al.*, 2009). By overexpression of eight plant genes that are required for the biosynthesis of flavonols (kaempferol and quercetin), the engineered *S. cerevisiae* strains could produce 4.6 mg L^{-1} kaempferol and 0.38 mg L^{-1} quercetin, respectively, when 0.5 mM naringenin was added (Trantas *et al.*, 2009).

Resveratrol

Resveratrol is a well-known stibenoid with various health benefits. Several research groups have been constructing yeast cell factories for resveratrol production (Becker *et al.*, 2003; Beekwilder *et al.*, 2006; Zhang *et al.*, 2006; Sydor *et al.*, 2010). Initially, resveratrol was produced in yeast by co-expression of 4CL and stilbene synthase (STS), with feeding of *p*-coumaric acid as the substrate (Becker *et al.*, 2003; Beekwilder *et al.*, 2006). Subsequent research found that resveratrol production increased when a 4CL::STS fusion protein was used, but the titer of resveratrol was only 5.25 mg L^{-1} (Zhang *et al.*, 2006). The pathway for synthesizing resveratrol from *p*-coumaric acid was constructed in different *S. cerevisiae* host strains by overexpressing the *4CL* gene from *A. thaliana* and the *STS* gene from *Vitis vinifera*. The highest resveratrol yield, 391 mg L^{-1}, was obtained using rich medium with a Brazilian wild-type *S. cerevisiae* strain (Sydor *et al.*, 2010).

ALKALOIDS

Alkaloids have a rich structural diversity (12 000 alkaloids are known) and have been exploited for medicinal purposes for thousands of years (Facchini *et al.*, 2012; Glenn *et al.*, 2013). Recently, there has been marked progress in the heterologous biosynthesis of alkaloids in yeast, specifically in the production of the benzylisoquinoline alkaloids (BIAs) (S)-reticuline (Fig. 2), which is the precursor of sanguinarine, morphine, and berberine (Hawkins and Smolke, 2008; Fossati *et al.*, 2014), and the production of the monoterpene indole alkaloids strictosidine, which is the precursor of vinblastine and vincristine, two potent and widely prescribed anticancer agents that are currently produced solely through harvest from the leaves of *C. roseus* (Murata *et al.*, 2008; Leonard *et al.*, 2009; Glenn *et al.*, 2013). *S. cerevisiae* cell factories for production of (S)-reticuline from (R,S)-norlaudanosoline were obtained by overexpression of norcoclaurine 6-O-methyltransferase (6-OMT), coclaurine *N*-methyltransferase (CNMT), and 3′-hydroxy-*N*-methylcoclaurine 4′-O-methyltransferase (4′-OMT) genes from a mixture of plant and human sources. The optimized cell factories yielded (S)-reticuline levels of 164.5 mg L^{-1}, with 5 mM norlaudanosoline as fed substrate (Hawkins and Smolke, 2008). Fossati *et al.* (2014) have also reconstituted a 10-gene plant pathway in *S. cerevisiae* that allows for the production of dihydrosanguinarine and its oxidized derivative, sanguinarine, from the commercial precursor norlaudanosoline. Geerlings *et al.* have constructed a *S. cerevisiae* cell factory for production of strictosidine by introducing the strictosidine synthase (STR) and strictosidine β-glucosidase (SGD) enzymes from the medicinal plant *C. roseus*. Upon feeding of tryptamine and secologanin, this *S. cerevisiae* cell factory produced 2 g L^{-1} strictosidine in the medium (Geerlings *et al.*, 2001). Additionally, glucosinolates, which are not classically considered as alkaloids, and which are sulfur-containing and nitrogen-containing compounds that are derived from glucose and various amino acids, have been produced in yeast. Mikkelsen *et al.* (2012) introduced a seven-step pathway for indolyl glucosinolate production from *A. thaliana* into yeast, resulting in the first successful production of glucosinolates in a yeast host.

OTHERS

Besides the compounds mentioned above, there are many other high-value metabolites that were produced by yeast cell factories, including cyclooligomer depsipeptide (Yu *et al.*, 2013), vitamin C (Branduardi *et al.*, 2007), penicillin (Gidijala *et al.*, 2009), 6-MSA (Wattanachaisaereekul *et al.*, 2008), and EPA (Xue *et al.*, 2013).

6-MSAs are polyketides, which are produced through the successive condensation of small carboxylic acids. These compounds represent a large group of secondary metabolites with a broad range of structures and biological activities, such as antibiotic effects. Through overexpressing the 6-methylsalicylic acid synthase gene (*6-MSAS*) from *Penicillium patulum* and phosphopantetheinyl transferase gene (*PPTase*) from *Aspergillus nidulans*, as well as replacing the native promoter of the acetyl-CoA carboxylase gene (*ACC1*) with a strong and constitutive promoter to increase malonyl-CoA supply, a *S. cerevisiae* cell factory was obtained which produced 554 mg L^{-1} 6-MSA (Wattanachaisaereekul *et al.*, 2008).

Omega-3 long-chain polyunsaturated fatty acids, including α-linolenic acid (ALA, C18:3, ω-3), EPA (C20:5, ω-3), and DHA (C22:6, ω-3), have been recognized as being beneficial to human health and have been widely used in pharmaceuticals, nutraceuticals, animal feed, and cosmetics (Ye and Bhatia, 2012; Adarme-Vega *et al.*, 2013). EPA and DHA are mainly extracted from marine fish, requiring expensive separation and enrichment process. These two compounds have also been produced from marine microalgae, krill, and bioengineered plants, by utilizing algal, bacterial, and yeast genes involved in the PUFA biosynthetic pathway (Adarme-Vega *et al.*, 2013). Although DHA has been commercially produced from microalgae, such as *Crypthecodinium cohnii* and *Schizochytrium* sp. (Barclay *et al.*, 1994; Wynn, 2013), commercial microbial production of EPA was not realized that can replace fish-based oil. Genetic engineering of *S. cerevisiae* for EPA production has resulted in yields of $< 1\%$ of the total fatty acids (TFA) as EPA (Tavares *et al.*, 2011). On the other hand, the oleaginous yeast *Yarrowia lipolytica*, which naturally can produce and store 40% of its dry cell weight as fatty acids (Papanikolaou and Aggelis, 2002), was also engineered for EPA production. Through introducing 21 heterologous genes encoding five different activities, a *Y. lipolytica* cell factory was obtained which produced EPA at 15% of the dry cell weight. The engineered yeast strain produces various lipids, with EPA constituting 56.6% of the TFA (Xue *et al.*, 2013).

PERSPECTIVES

The use of yeasts as biosynthetic host strains for the production of high-value metabolites from renewable biomass has created an alternative way for production of these compounds in place of extraction from natural sources. Although exciting developments has been generated in recent years, there are several challenges that must be overcome:

(1) Identification of novel genes, particularly those in the downstream regions of the synthetic pathways, remains a challenge. Most high-value metabolite precursors are modified by cytochrome P450s and glycosyltransferases. However, there are lots of similar enzymes in the natural organism, making the identification process difficult.
(2) Expression of a long pathway limits the production efficiency. Statistically, the high-value metabolites from central metabolic precursors decreased exponentially with increasing number of enzymatic steps for biosynthesis (Varman *et al.*, 2011).
(3) Expensive intermediate metabolites need to be supplemented for production of chemicals with complex structures, such as alkaloids (Leonard *et al.*, 2009). Synthetic pathways for production of these expensive intermediates from central metabolites need to be introduced and optimized so that the target high-value metabolites can be produced directly from cheap carbon sources.
(4) Heterologous pathways introduced to host cells may lead to the accumulation of intermediates that are toxic to the host cells. Fine-tuning the expression level of each gene within the synthetic pathway needs to be performed to avoid the accumulation of these toxic intermediates.
(5) Many steps in alkaloid biosynthesis require methylation. To reach high-level production of alkaloids in yeast, intracellular availability of S-adenosyl-L-methionine needs to be increased (Leonard *et al.*, 2009).
(6) Many high-value metabolites are toxic to the yeast host strains. Therefore, it is necessary to mitigate toxicity for overproduction of these products through global perturbation strategies (Leonard *et al.*, 2009)
(7) Host strain selection is a crucial factor. The host strain should be suitable for production of the target metabolite (e.g. *Y. lipolytica* is well suited to fatty acid production and accumulation). In addition, the host strain should also be GRAS, especially when it will be engineered to produce pharmaceutical compounds and food additives.
(8) New synthetic biology methods that can perform quick assembly of numerous genes and simultaneous modulation of multiple gene expressions need to be developed to facilitate construction and optimization of yeast cell factories.
(9) Finally, many high-value metabolites have complex structures, and it is very difficult to identify their biosynthetic pathways in a short time. Yeast cell factories for production of intermediates can be combined with chemical semi-synthetic strategies to achieve commercial production of these complex high-value metabolites.

ACKNOWLEDGEMENTS

This research was supported by grants from the Tianjin Key Technology R&D Program of Tianjin Municipal Science and Technology Commission (12ZCZDSY14700), the National High Technology Research and Development Program (2012AA02A704), the National Basic Research Program of China (2011CBA00800), and the National Natural Science Foundation of China (81202864). Xueli Zhang was supported by the Hundred Talent Program of the Chinese Academy of Sciences.

Conflict of interest statement. None declared.

REFERENCES

Adarme-Vega TC, Thomas-Hall SR, Schenk PM Towards sustainable sources for omega-3 fatty acids production. *Curr Opin Biotechnol* 2013;**26C**:14–8.

Ajikumar PK, Xiao WH, Tyo KE, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli*. *Science* 2010;**330**:70–4.

Augustin JM, Kuzina V, Andersen SB, Bak S Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry* 2011;**72**:435–57.

Avalos JL, Fink GR, Stephanopoulos G Compartmentalization of metabolic pathways in yeast mitochondria improves the production of branched-chain alcohols. *Nat Biotechnol* 2013;**31**:335–41.

Barclay WR, Meager KM, Abril JR Heterotrophic production of long-chain omega-3-fatty-acids utilizing algae and algae-like microorganisms. *J Appl Phycol* 1994;**6**:123–9.

Becker JV, Armstrong GO, van der Merwe MJ, Lambrechts MG, Vivier MA, Pretorius IS Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine-related antioxidant resveratrol. *FEMS Yeast Res* 2003;**4**:79–85.

Beekwilder J, Wolswinkel R, Jonker H, Hall R, de Vos CH, Bovy A Production of resveratrol in recombinant microorganisms. *Appl Environ Microbiol* 2006;**72**:5670–2.

Bouwmeester HJ, Wallaart TE, Janssen MH, van Loo B, Jansen BJ, Posthumus MA, Schmidt CO, De Kraker JW, Konig WA, Franssen MC Amorpha-4,11-diene synthase catalyses the first probable step in artemisinin biosynthesis. *Phytochemistry* 1999;**52**:843–54.

Branduardi P, Fossati T, Sauer M, Pagani R, Mattanovich D, Porro D Biosynthesis of vitamin C by yeast leads to increased stress resistance. *PLoS ONE* 2007;**2**:e1092.

Carothers JM, Goler JA, Keasling JD Chemical synthesis using synthetic biology. *Curr Opin Biotechnol* 2009;**20**:498–503.

Chang MC, Keasling JD Production of isoprenoid pharmaceuticals by engineered microbes. *Nat Chem Biol* 2006;**2**:674–81.

Chappell J Production platforms for the molecular pharming of alkaloid diversity. *P Natl Acad Sci USA* 2008;**105**:7897–8.

Courtney JM, Gilchrist T Silicone rubber and natural rubber as biomaterials. *Med Biol Eng Comput* 1980;**18**:538–40.

Dai Z, Cui G, Zhou SF, Zhang X, Huang L Cloning and characterization of a novel 3-hydroxy-3-methylglutaryl coenzyme A reductase gene from *Salvia miltiorrhiza* involved in diterpenoid tanshinone accumulation. *J Plant Physiol* 2011;**168**:148–57.

Dai Z, Liu Y, Huang L, Zhang X Production of miltiradiene by metabolically engineered *Saccharomyces cerevisiae*. *Biotechnol Bioeng* 2012;**109**:2845–53.

Dai Z, Liu Y, Zhang X, Shi M, Wang B, Wang D, Huang L Metabolic engineering of *Saccharomyces cerevisiae* for production of ginsenosides. *Metab Eng* 2013;**20**:146–56.

Dai Z, Wang B, Liu Y, Shi M, Wang D, Zhang X, Liu T, Huang L Producing aglycons of ginsenosides in bakers'yeast. *Sci Rep* 2014;**4**:3698.

Eckart MR, Bussineau CM Quality and authenticity of heterologous proteins synthesized in yeast. *Curr Opin Biotechnol* 1996;**7**:525–30.

Engels B, Dahm P, Jennewein S Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards taxol (paclitaxel) production. *Metab Eng* 2008;**10**:201–6.

Facchini PJ, Bohlmann J, Covello PS, De Luca V, Mahadevan R, Page JE, Ro DK, Sensen CW, Storms R, Martin VJJ Synthetic biosystems for the production of high-value plant metabolites. *Trends Biotechnol* 2012;**30**:127–31.

Farhi M, Marhevka E, Masci T, Marcos E, Eyal Y, Ovadis M, Abeliovich H, Vainstein A Harnessing yeast subcellular compartments for the production of plant terpenoids. *Metab Eng* 2011;**13**:474–81.

Fossati E, Ekins A, Narcross L, Zhu Y, Falgueyret JP, Beaudoin GA, Facchini PJ, Martin VJ Reconstitution of a 10-gene pathway for synthesis of the plant alkaloid dihydrosanguinarine in *Saccharomyces cerevisiae*. *Nat Commun* 2014;**5**:3283.

Fukushima EO, Seki H, Ohyama K, Ono E, Umemoto N, Mizutani M, Saito K, Muranaka T CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis. *Plant Cell Physiol* 2011;**52**:2050–61.

Gao W, Hillwig ML, Huang L, Cui G, Wang X, Kong J, Yang B, Peters RJ A functional genomics approach to tanshinone biosynthesis provides stereochemical insights. *Org Lett* 2009;**11**:5170–3.

Gao W, Sun HX, Xiao H, *et al.* Combining metabolomics and transcriptomics to characterize tanshinone biosynthesis in *Salvia miltiorrhiza*. *BMC Genomics* 2014;**15**:73.

Gassel S, Breitenbach J, Sandmann G Genetic engineering of the complete carotenoid pathway towards enhanced astaxanthin formation in *Xanthophyllomyces dendrorhous* starting from a high-yield mutant. *Appl Microbiol Biotechnol* 2014;**98**:345–50.

Geerlings A, Redondo FJ, Contin A, Memelink J, van der Heijden R, Verpoorte R Biotransformation of tryptamine and secologanin into plant terpenoid indole alkaloids by transgenic yeast. *Appl Microbiol Biotechnol* 2001;**56**:420–4.

Geisler K, Hughes RK, Sainsbury F, *et al.* Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants. *P Natl Acad Sci USA* 2013;**110**:E3360–7.

Gidijala L, Kiel JA, Douma RD, Seifar RM, van Gulik WM, Bovenberg RA, Veenhuis M, van der Klei IJ An engineered yeast efficiently secreting penicillin. *PLoS ONE* 2009;**4**:e8317.

Glenn WS, Runguphan W, O'Connor SE Recent progress in the metabolic engineering of alkaloids in plant systems. *Curr Opin Biotechnol* 2013;**24**:354–65.

Goffeau A Four years of post-genomic life with 6,000 yeast genes. *FEBS Lett* 2000;**480**:37–41.

Gongora-Castillo E, Childs KL, Fedewa G, *et al.* Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species. *PLoS ONE* 2012a;**7**:e52506.

Gongora-Castillo E, Fedewa G, Yeo Y, Chappell J, DellaPenna D, Buell CR Genomic approaches for interrogating the biochemistry of medicinal plant species. *Methods Enzymol* 2012b;**517**:139–59.

Guo J, Zhou YJ, Hillwig ML, *et al.* CYP76AH1 catalyzes turnover of miltiradiene in tanshinones biosynthesis and enables heterologous production of ferruginol in yeasts. *P Natl Acad Sci USA* 2013;**110**:12108–13.

Han JY, Kim HJ, Kwon YS, Choi YE The Cyt P450 enzyme CYP716A47 catalyzes the formation of protopanaxadiol from dammarenediol-II during ginsenoside biosynthesis in *Panax ginseng*. *Plant Cell Physiol* 2011;**52**:2062–73.

Han JY, Hwang HS, Choi SW, Kim HJ, Choi YE Cytochrome P450 CYP716A53v2 catalyzes the formation of protopanaxatriol from protopanaxadiol during ginsenoside biosynthesis in *Panax ginseng*. *Plant Cell Physiol* 2012;**53**:1535–45.

Hawkins KM, Smolke CD Production of benzylisoquinoline alkaloids in *Saccharomyces cerevisiae*. *Nat Chem Biol* 2008;**4**:564–73.

Hayashi H, Huang P, Kirakosyan A, Inoue K, Hiraoka N, Ikeshiro Y, Kushiro T, Shibuya M, Ebizuka Y Cloning and characterization of a cDNA encoding beta-amyrin synthase involved in glycyrrhizin and soyasaponin biosyntheses in licorice. *Biol Pharm Bull* 2001;**24**:912–6.

Heftmann E Recent progress in the biochemistry of plant steroids other than sterols (saponins, glycoalkaloids, pregnane derivatives, cardiac glycosides, and sex hormones). *Lipids* 1974;**9**:626–39.

Hezari M, Lewis NG, Croteau R Purification and characterization of taxa-4(5),11(12)-diene synthase from Pacific yew (*Taxus brevifolia*) that catalyzes the first committed step of taxol biosynthesis. *Arch Biochem Biophys* 1995;**322**:437–44.

Johnson EA, Schroeder W Astaxanthin from the yeast *Phaffia rhodozyma*. *Stud Mycol* 1995;**38**:81–90.

Johnson EA, Schroeder WA Microbial carotenoids. *Adv Biochem Eng Biotechnol* 1996;**53**:119–78.

Keasling JD Synthetic biology for synthetic chemistry. *ACS Chem Biol* 2008;**3**:64–76.

Keasling JD Synthetic biology and the development of tools for metabolic engineering. *Metab Eng* 2012;**14**:189–95.

Kuboyama T, Yokoshima S, Tokuyama H, Fukuyama T Stereocontrolled total synthesis of (+)-vincristine. *P Natl Acad Sci USA* 2004;**101**:11966–70.

Lenihan JR, Tsuruta H, Diola D, Renninger NS, Regentin R Developing an industrial artemisinic acid fermentation process to support the cost-effective production of antimalarial artemisinin-based combination therapies. *Biotechnol Prog* 2008;**24**:1026–32.

Leonard E, Runguphan W, O'Connor S, Prather KJ Opportunities in metabolic engineering to facilitate scalable alkaloid production. *Nat Chem Biol* 2009;**5**:292–300.

Leonard E, Ajikumar PK, Thayer K, Xiao WH, Mo JD, Tidor B, Stephanopoulos G, Prather KL Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. *P Natl Acad Sci USA* 2010;**107**:13654–9.

Leung KW, Wong AS Pharmacology of ginsenosides: a literature review. *Chin Med* 2010;**5**:20.

Li Y, Pfeifer BA Heterologous production of plant-derived isoprenoid products in microbes and the application of metabolic engineering and synthetic biology. *Curr Opin Plant Biol* 2014;**19C**:8–13.

Li JWH, Vederas JC Drug discovery and natural products: end of an era or an endless frontier?. *Science* 2009;**325**:161–5.

Li Q, Sun Z, Li J, Zhang Y Enhancing beta-carotene production in *Saccharomyces cerevisiae* by metabolic engineering. *FEMS Microbiol Lett* 2013;**345**:94–101.

Lienert F, Lohmueller JJ, Garg A, Silver PA Synthetic biology in mammalian cells: next generation research tools and therapeutics. *Nat Rev Mol Cell Biol* 2014;**15**:95–107.

Marienhagen J, Bott M Metabolic engineering of microorganisms for the synthesis of plant natural products. *J Biotechnol* 2013;**163**:166–78.

Markou G, Nerantzis E Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnol Adv* 2013;**31**:1532–42.

Mata-Gomez LC, Montanez JC, Mendez-Zavala A, Aguilar CN Biotechnological production of carotenoids by yeasts: an overview. *Microb Cell Fact* 2014;**13**:12.

Menkin V On the anti-inflammatory mechanism of hydrocortisone (compound-F). *Science* 1954;**120**:1026–8.

Mikkelsen MD, Buron LD, Salomonsen B, Olsen CE, Hansen BG, Mortensen UH, Halkier BA Microbial production of indolylglucosinolate through engineering of a multi-gene pathway in a versatile yeast expression platform. *Metab Eng* 2012;**14**:104–11.

Misawa N Pathway engineering for functional isoprenoids. *Curr Opin Biotechnol* 2011;**22**:627–33.

Moses T, Pollier J, Almagro L, Buyst D, Van Montagu M, Pedreno MA, Martins JC, Thevelein JM, Goossens A Combinatorial biosynthesis of sapogenins and saponins in *Saccharomyces cerevisiae* using a C-16alpha hydroxylase from *Bupleurum falcatum*. *P Natl Acad Sci USA* 2014;**111**:1634–9.

Murata J, Roepke J, Gordon H, De Luca V The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization. *Plant Cell* 2008;**20**:524–42.

Musende AG, Eberding A, Wood CA, Adomat H, Fazli L, Hurtado-Coll A, Jia W, Bally MB, Guns EST A novel oral dosage formulation of the ginsenoside aglycone protopanaxadiol exhibits therapeutic activity against a hormone-insensitive model of prostate cancer. *Anticancer Drugs* 2012;**23**:543–52.

Newman DJ, Cragg GM Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012;**75**:311–35.

Nicolaou KC, Yang Z, Liu JJ, *et al*. Total synthesis of taxol. *Nature* 1994;**367**:630–4.

Nielsen J, Keasling JD Synergies between synthetic biology and metabolic engineering. *Nat Biotechnol* 2011;**29**:693–5.

Paddon CJ, Westfall PJ, Pitera DJ, *et al*. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* 2013;**496**:528–32.

Papanikolaou S, Aggelis G Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresour Technol* 2002;**82**:43–9.

Pollier J, Goossens A Oleanolic acid. *Phytochemistry* 2012;**77**:10–5.

Pompon D, Louerat B, Bronine A, Urban P Yeast expression of animal and plant P450s in optimized redox environments. *Methods Enzymol* 1996;**272**:51–64.

Reyes LH, Gomez JM, Kao KC Improving carotenoids production in yeast via adaptive laboratory evolution. *Metab Eng* 2014;**21**:26–33.

Ro DK, Paradise EM, Ouellet M, *et al*. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 2006;**440**:940–3.

Seki H, Ohyama K, Sawai S, Mizutani M, Ohnishi T, Sudo H, Akashi T, Aoki T, Saito K, Muranaka T Licorice beta-amyrin 11-oxidase, a cytochrome P450 with a key role in the biosynthesis of the triterpene sweetener glycyrrhizin. *P Natl Acad Sci USA* 2008;**105**:14204–9.

Seki H, Sawai S, Ohyama K, *et al*. Triterpene functional genomics in licorice for identification of CYP72A154 involved in the biosynthesis of glycyrrhizin. *Plant Cell* 2011;**23**:4112–23.

Shao Z, Zhao H DNA assembler, an *in vivo* genetic method for rapid construction of biochemical pathways. *Nucleic Acids Res* 2009;**37**:e16.

Siddiqui MS, Thodey K, Trenchard I, Smolke CD Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. *FEMS Yeast Res* 2012;**12**:144–70.

Singh V Recent advancements in synthetic biology: current status and challenges. *Gene* 2014;**535**:1–11.

Souza CM, Schwabe TM, Pichler H, Ploier B, Leitner E, Guan XL, Wenk MR, Riezman I, Riezman H A stable yeast strain efficiently producing cholesterol instead of ergosterol is functional for tryptophan uptake, but not weak organic acid resistance. *Metab Eng* 2011;**13**:555–69.

Sydor T, Schaffer S, Boles E Considerable increase in resveratrol production by recombinant industrial yeast strains with use of rich medium. *Appl Environ Microbiol* 2010;**76**:3361–3.

Szczebara FM, Chandelier C, Villeret C, *et al*. Total biosynthesis of hydrocortisone from a simple carbon source in yeast. *Nat Biotechnol* 2003;**21**:143–9.

Tansakul P, Shibuya M, Kushiro T, Ebizuka Y Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. *FEBS Lett* 2006;**580**:5143–9.

Tavares S, Grotkjaer T, Obsen T, Haslam RP, Napier JA, Gunnarsson N Metabolic engineering of *Saccharomyces cerevisiae* for

production of eicosapentaenoic acid, using a novel delta 5-desaturase from *Paramecium tetraurelia*. *Appl Environ Microbiol* 2011;**77**:1854–61.

Teoh KH, Polichuk DR, Reed DW, Covello PS Molecular cloning of an aldehyde dehydrogenase implicated in artemisinin biosynthesis in *Artemisia annua*. *Botany* 2009;**87**:635–42.

Trantas E, Panopoulos N, Ververidis F Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. *Metab Eng* 2009;**11**:355–66.

Ukibe K, Hashida K, Yoshida N, Takagi H Metabolic engineering of *Saccharomyces cerevisiae* for astaxanthin production and oxidative stress tolerance. *Appl Environ Microbiol* 2009;**75**:7205–11.

Vachali P, Bhosale P, Bernstein PS Microbial carotenoids. *Methods Mol Biol* 2012;**898**:41–59.

Varman AM, Xiao Y, Leonard E, Tang YJ Statistics-based model for prediction of chemical biosynthesis yield from *Saccharomyces cerevisiae*. *Microb Cell Fact* 2011;**10**:45.

Verwaal R, Wang J, Meijnen JP, Visser H, Sandmann G, van den Berg JA, van Ooyen AJ High-level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous*. *Appl Environ Microbiol* 2007;**73**:4342–50.

Visser H, van Ooyen AJJ, Verdoes JC Metabolic engineering of the astaxanthin-biosynthetic pathway of *Xanthophyllomyces dendrorhous*. *FEMS Yeast Res* 2003;**4**:221–31.

Wattanachaisaereekul S, Lantz AE, Nielsen ML, Nielsen J Production of the polyketide 6-MSA in yeast engineered for increased malonyl-CoA supply. *Metab Eng* 2008;**10**:246–54.

Westfall PJ, Pitera DJ, Lenihan JR, *et al.* Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. *P Natl Acad Sci USA* 2012;**109**:E111–8.

Wu WQ, Wang X, Xiang QS, Meng X, Peng Y, Du N, Liu ZG, Sun QC, Wang C, Liu XB Astaxanthin alleviates brain aging in rats by attenuating oxidative stress and increasing BDNF levels. *Food Funct* 2014;**5**:158–66.

Wuts PG Semisynthesis of taxol. *Curr Opin Drug Discov Devel* 1998;**1**:329–37.

Wynn JP Taking the fish out of fish oil. *Nat Biotechnol* 2013;**31**:716–7.

Xiao M, Zhang Y, Chen X, *et al.* Transcriptome analysis based on next-generation sequencing of non-model plants producing specialized metabolites of biotechnological interest. *J Biotechnol* 2013;**166**:122–34.

Xu SH, Nes WD Biosynthesis of cholesterol in the yeast mutant Erg6. *Biochem Biophys Res Commun* 1988;**155**:509–17.

Xue Z, Sharpe PL, Hong SP, *et al.* Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nat Biotechnol* 2013;**31**:734–40.

Yamano S, Ishii T, Nakagawa M, Ikenaga H, Misawa N Metabolic engineering for production of beta-carotene and lycopene in *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 1994;**58**:1112–4.

Yan Y, Kohli A, Koffas MA Biosynthesis of natural flavanones in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 2005;**71**:5610–3.

Yan X, Fan Y, Wei W, Wang P, Liu Q, Wei Y, Zhang L, Zhao G, Yue J, Zhou Z Production of bioactive ginsenoside compound K in metabolically engineered yeast. *Cell Res* 2014;**24**:770–3.

Ye VM, Bhatia SK Metabolic engineering for the production of clinically important molecules: omega-3 fatty acids, artemisinin, and taxol. *Biotechnol J* 2012;**7**:20–33.

Yendo AC, de Costa F, Gosmann G, Fett-Neto AG Production of plant bioactive triterpenoid saponins: elicitation strategies and target genes to improve yields. *Mol Biotechnol* 2010;**46**:94–104.

Yeo YS, Nybo SE, Chittiboyina AG, *et al.* Functional identification of valerena-1,10-diene synthase, a terpene synthase catalyzing a unique chemical cascade in the biosynthesis of biologically active sesquiterpenes in *Valeriana officinalis*. *J Biol Chem* 2013;**288**:3163–73.

Yu D, Xu F, Zi J, Wang S, Gage D, Zeng J, Zhan J Engineered production of fungal anticancer cyclooligomer depsipeptides in *Saccharomyces cerevisiae*. *Metab Eng* 2013;**18**:60–8.

Zhang YS, Li SZ, Li J, Pan XQ, Cahoon RE, Jaworski JG, Wang XM, Jez JM, Chen F, Yu O Using unnatural protein fusions to engineer resveratrol biosynthesis in yeast and mammalian cells. *J Am Chem Soc* 2006;**128**:13030–1.

Zhou L, Zuo Z, Chow MS Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. *J Clin Pharmacol* 2005;**45**:1345–59.

Zhou YJ, Gao W, Rong Q, *et al.* Modular pathway engineering of diterpenoid synthases and the mevalonic acid pathway for miltiradiene production. *J Am Chem Soc* 2012;**134**:3234–41.

Zhou JW, Du GC, Chen J Novel fermentation processes for manufacturing plant natural products. *Curr Opin Biotechnol* 2014;**25**:17–23.