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Strategies of Isoprenoids Production in Engineered Bacteria

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Summary

Isoprenoids are the largest family of natural products, over 40,000 compounds have been described, which have been widely used in various fields. Currently, the isoprenoids products are mainly produced by natural extraction or chemical synthesis, however limited yield and high cost is far behind the increasing need. Most bacteria synthesize the precursors of isoprenoids through the methylerythritol 4-phosphate (MEP) pathway, microbial synthesis of isoprenoids by fermentation becomes more attractive mainly in term of environmental concern and renewable resources. In this review, the strategies of isoprenoids production in bacteria by synthetic biology are discussed. Introducing foreign genes associated with desired products made it possible to produce isoprenoids in bacteria. Furthermore, the yield of isoprenoids is increased by the strategies of over-expression of native or foreign genes, introducing heterologous mevalonate (MVA) pathway, balancing of the precursors and inactivating the competing pathway, these methods were used separately or simultaneously.

Key words: isoprenoids, methylerythritol 4-phosphate (MEP) pathway, mevalonate (MVA) pathway, microbial synthesis, biosynthesis

Introduction

Isoprenoids, also called terpenoids, are one of the largest groups of natural compounds, which are essential in all living organisms. Isoprenoids are involved in a wide variety of vital biological functions, including electron transportation, cell respiration, photosynthesis, membrane biosynthesis, signaling and growth regulation (Bouvier *et al*. 2005; Heuston *et al*.

2012). In addition, more and more isoprenoids have been utilized for pharmaceuticals, nutraceuticals, biofuels, flavors, cosmetics, and food additives (Ro *et al*. 2006; Ajikumar *et al*. 2010; Danner *et al*. 2011; Westfall *et al*. 2012).

All isoprenoids are biosynthesized from a basic five-carbon precursor unit, isopentenyl diphosphate (IPP), and its isomer dimethylallyl diphosphate (DMAPP). The IPP and DMAPP can be synthesized by two different pathways: the mevalonate (MVA) pathway and the methylerythritol 4-phosphate (MEP) pathway (Figure 1, Table 1). The MVA pathway is present in most eukaryotes, archaea, a few bacteria, cytosol and mitochondria of plants, whereas the MEP pathway is discovered in most bacteria, chloroplasts of plant, algae and apicomplexan parasites (Rohmer 1999). The two pathways are quite different in term of the precursors, reaction steps, energy consumption, key enzymes, etc (Table 2) (Goldstein and Brown 1990; Lichtenthaler 2000).

The MVA biosynthetic pathway starts with two consecutive condensation reactions of three molecules of acetyl-CoA to form the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) (Figure 1). Then HMG-CoA is reduced to mevalonate (MVA). Following two consecutive phosphorylation steps, MVA is converted to mevalonate-5-diphosphate, which is then catalyzed to yield IPP. The isopentenyl diphosphate isomerase (IDI) is responsible for the inter-conversion of IPP and DMAPP (Goldstein and Brown 1990).

The MEP pathway relies on seven enzymes to produce IPP and DMAPP (Figure 1). The reaction begins with the condensation of pyruvate and glyceraldehydes-3-phosphate (G3P) to produce 1-deoxy-D-xylulose-5-phosphate (DXP). Followed by a reduction and isomerization, 2C-methyl-D-erythritol-4-phosphate (MEP) is formed from the DXP, which is catalyzed by

1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR/IspC). Subsequently, the MEP serves as a substrate for 2C-methyl-D-erythritol-4-phosphate cytidylyltransferase (IspD) and is converted into 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME), and then the CDP-ME is phosphorylated to 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate (CDP-MEP) by 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (IspE). The cyclization of CDP-MEP to 2C-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP) is catalyzed by 2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (IspF). In the last two steps of the MEP pathway, the MEcPP is converted into 4-hydroxy-3-mehtyl-butenyl 1-diphosphate (HMBPP) by 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (IspG), finally, the HMBPP is catalyzed by 4-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate reductase (IspH) to yield IPP and DMAPP (Lichtenthaler 2000; Rohmer 2008).

Since many isoprenoids are involved in important physiological process, which could be applied into biomedical and commercial fields. Currently, the production of isoprenoids by natural extraction and chemical synthesis is behind the needs owning to the limited quantity, low efficiency and environmental pollution (Clardy and Walsh 2004; Ajikumar *et al*. 2008). Therefore, many researchers explored innovative approaches to produce and increase the yield of the desired molecule in the microbial system. Microbial system has several advantages for isoprenoids production: *i*) low cost; *ii*) easily genetic manipulation; *iii*) large-scale production; *iv*) no pollution. In recent years, a few isoprenoids products were overproduced in engineered microbes, including isoprene, lycopene, artemisinic acid, taxadiene, carotenoid, etc (Table 3).

In this review, we summarize the strategies for isoprenoids production in engineered bacteria, including over-expression of genes in the MEP pathway, introducing foreign genes for desired products, introducing heterologous MVA pathway, balancing of the precursors, inhibiting the competing pathway and the other optimization methods.

Extending the MEP pathway for desired products

Most bacteria synthesize IPP and DMAPP by MEP pathway. IPP and DAMPP are precursors for the synthesis of isoprenoids. Introducing foreign genes associated with isoprenoids synthesis made it possible to generate desired products in bacteria. Many researchers explored synthetic biology methods of isoprenoids production by introducing heterologous isoprenoids synthase into bacteria (Zhao *et al.* 2011; Yang *et al*. 2012; Liu *et al*. 2013; Bentley *et al.* 2014). The isoprene synthase (*ispS*) gene catalyzes the conversion of DMAPP to the isoprene (Silver and Fall 1991), so the *ispS* gene was introduced into *E. coli* and a synthetic pathway of isoprene was constructed (Bentley *et al*. 2014). Zhao, *et al*. introduced *ispS* gene from *Populus nigra* (*pAispS*) into *E. coli* BL21 (DE3), the engineered bacteria could produce 94 mg l^{-1} isoprene comparing to 9 mg l^{-1} isoprene produced by the parent strain (Zhao *et al*. 2011; Yang *et al*. 2012)**.** The *ispA* gene encodes farnesyl diphosphate synthase, which could enhance the metabolic flux into geranyl pyrophosphate (GPP) by catalyzing the conversion of DMAPP/IPP. Zhang, *et al*. engineered the *E. coli* by harboring *IspA/SabS1* or *GPPS2/SabS1*, the recombinant bacteria could produced the sabinene at the concentration of 0.96 mg l^{-1} and 2.07 mg l^{-1}, respectively (Zhang *et al*. 2014). In the same way, carotenoid, lycopene and the other isoprenoids could be produced in the *E. coli* (Yuan *et al*. 2006; Ajikumar *et al*. 2010; Leonard *et al*. 2010; Wang *et al*. 2010; Albermann 2011).

Over-expression of key enzymes in the MEP pathway

As the IPP and DMAPP are the precursors for the isoprenoids synthesis, increasing the supply of IPP and DMAPP could improve isoprenoids accumulation. A number of works focused on the over-expression of key enzymes in the MEP pathway to increase isoprenoids production (Matthew and Wurtzel 2000; Kim and Keasling 2001; Rodriguez-Villalon *et al*. 2008; Lv *et al*. 2013). Several studies showed that over-expression of DXS, the enzyme catalyzing the first step in the MEP pathway, improved lycopene production up to 8-fold higher than the

control (Rodriguez-Villalon *et al*. 2008). Over-expression of DXS and DXR, the enzyme next to DXS in the pathway, further increased lycopene yields by 1.4–2 fold (Kim and keasling 2000). The amplification and over-expression of *idi*, *dxs*, *ispD* and *ispF* genes improved lycopene synthesis from 0.89 to 5.39 mg g-1 DCW in *E. coli* (Zhou *et al*. 2013). An even larger effect on the carotenoid production was observed when the *idi* gene was over-expressed (Yoon *et al*. 2007a).

Besides the over-expression of native genes, sometimes over-expression of heterologous MEP genes is also helpful for isoprenoids production. Zhao, *et al*. found that over-expression of heterologous DXS/DXR from *B. subtilis* in *E. coli* resulted in the enhancement of isoprene production (from 94 to 314 mg l^{-1}), which is about two times higher than that by over-expressing the native DXS/DXR in *E. coli* (Zhao *et al*. 2011)**.**

In the construct for genes expression, the gene order needs to be considered. According to the biosynthetic pathway, a sequential expression pattern could be optimized for efficient synthesis of the products in the metabolic pathway (Lv *et al*. 2013).

Introducing MVA pathway into bacteria

Most bacteria do not possess the MVA pathway, however, recent studies indicated that the heterologous MVA pathway could be constructed in bacteria. The heterologous MVA pathway can function in parallel with the MEP pathway, providing another large cellular metabolite pool (acetyl-CoA), this will increase the supply of the intermediate metabolites, IPP and DMAPP (Yoon *et al*. 2009; Morrone *et al*. 2010; Zurbriggen *et al*. 2012; Bentley *et al*. 2014; Zhang *et al*. 2014). Two strategies have been used to improve the efficiency of the MVA pathway on isoprenoids production in bacteria: *i)* Introducing the whole heterologous

MVA pathway into the bacteria; *ii*) Dividing the MVA pathway into two parts: the "upper pathway" and the "lower pathway" (Figure 1). These two parts could be introduced simultaneously or separately, moreover, the "upper pathway" and "lower pathway" could be derived from different microorganisms.

Martin *et al*. were the first to express the MVA pathway from *Saccharomyces cerevisiae* in *E. coli* for the production of amorphadiene, the sesquiterpene olefin precursor to artemisinin. Peak amorphadiene production from the complete mevalonate pathway reached ~6.5 μg caryophyllene equivalent/ml/OD_{600}, This represents 76- and 21-fold improvements over that for the native strains and the strain with engineered DXP pathway (0.086 and 0.313 μg caryophyllene equivalents/ml/OD_{600}, respectively) (Martin *et al*. 2003). The prokaryotic MVA pathway of *Streptomyces* sp. CL190 was introduced into *E. coli* for the production of lycopene, the yield was increased by over 2-fold compared to the *ackA-pta*, *nuo* mutant strain. (Vadali *et al*. 2005). Yoon *et al*. combined both the upper and lower portions of the MVA pathway from different microorganisms. The recombinant *E.coli* harboring the top portions of MVA pathway from *E. faecalis*, the bottom MVA pathway from *S. pneumonia* and β-carotene synthesis genes produced high amount of β-carotene (Yoon *et al*. 2009). Yang *et al*. replaced the upper pathway of MVA from *S. cerevisiae* by that of *Enterococcus faecalis*, and mutated an alanine 110 of the MvaS to glycine. The final genetic strain YJM25 contained the optimized MVA pathway and the isoprene synthase from *Populus alba*, which could accumulate the isoprene up to 6.3 g l^{-1} after 40 h fermentation (Yang *et al*. 2012). Otherwise, this strategy was also successfully applied into the production of Coenzyme Q10 (Zahiri *et al*. 2006).

Balancing of the precursors and blocking of the competing pathway

Studies indicated that the precursor balancing is important for the MEP pathway in the production of metabolites at high-level, which plays a major role at the alteration of metabolic fluxes in the central pathway (Farmer and Liao 2001). It is known that the synthesis of IPP/DMAPP in the MEP pathway requires pyruvate and G3P in equal amounts. However, in *E. coli*, the glucose is mainly metabolized by the Embden-Meyerhof pathway (EMP), which leads to an imbalanced distribution of the pyruvate and G3P. Liu, *et al*. revealed that this problem could be resolved by enhancing the Entner-Doudoroff Pathway (EDP), which generates the same moles of pyruvate and G3P, the isoprene titer and yield were more than three and six times higher than those yielded with the EMP module, respectively (Liu *et al*. 2013).

Inactivating of various competing pathway is another strategy for increasing the desired product. Model-based systematic method has been used to identify gene knock-out targets of central metabolic pathways to increase the precursors or cofactors supply of the MEP pathway (Alper *et al*. 2005b; Choi *et al*. 2010). By stoichiometric models, the gene deletion of *aceE* (Pyruvate dydrogenase), *talB* (transaldolase B) and *gpmB* (phosphogly-cerate mutase B), as well as gene amplification of *fbaA* (fructose bispho-sphatealdolase) and *tpi* (triose phosphate isomerase) had been identified, the inactivation and amplification of these genes will lead to more supply of pyruvate and G3P for synthesis of IPP/DMAPP, which is experimentally validated for improving lycopene production (Alper *et al*. 2005b). Zhou, *et al*. knocked out the central carbon metabolic gene *zwf* (glucose-6-phosphate dehydrogenase) and resulted in the enhancement of lycopene production above 130 % relative to control (Zhou *et al*. 2013). Inactivating various competing pathways at the nodes of pyruvate and acetyl-CoA synthesis also increased lycopene production in *E. coli* (Vadali *et al*. 2005).

Other optimization methods

The strategies for engineering the bacteria are described above, there are some other aspects need to be considered, such as the energy/cofactor supply, the culture conditions, the host selection, and so on.

ATP and NADPH are two important cofactors for the production of isoprenoids compounds, increasing the supplies of ATP and NADPH is helpful for improving β-carotene production (Zhao *et al.* 2013). In addition, ribosome-binding sites (RBS), promoters also need to be considered in the construct for isoprenoids synthesis (Kim and Keasing 2000; Alper *et al*. 2005a; Meynial-Salles *et al*. 2005; Zurbriggen *et al*. 2012).

In the fermentation, culture conditions could affect the growth and metabolism of engineered bacteria, such as carbon source, organic nitrogen source, induction temperature, inducer concentration, etc. These are important factors and have been optimized for increasing the yield of the sabinene (Zhang *et al*. 2014).

Improvements for Industrial Application

In the past decades, researchers have devoted considerable attention to produce isoprenoids and increase the yield in engineered microbes (Leonard *et al*. 2010; Zurbriggen *et al*. 2012).Various practices have confirmed this is a promising approach to produce isoprenoids, further optimization is still under way for industrial application.

One concern is on the cell growth of the engineered bacteria. Gene knock-out or gene amplification usually is applied to inactivate the competing pathway or enhance metabolic flux to the desired pathway. But those methods could lead to cell death or the retardation of

cell growth. For example, the knockout of *gdhA*, *aceE* and *fdhF* gene is helpful to increase lycopene yield by 37%, but the rate of cell growth decreased by 43%, finally, the titer of lycopene decreased (Alper *et al.* 2005c). On the other hand, incorporating multi genes into bacteria will cause genetic burden and affect the cell growth. Gene expression controlled by different regulatory parts with varied strength may provide solutions to these problems (Alper *et al*. 2005a; Meynial-Salles *et al*. 2005; Lu *et al*. 2012). Moreover, bioinformatics analysis and functional genomics could be used to predict or identify genes regulating the isoprenoids synthesis (Udwary *et al*. 2007). Meanwhile, the toxicity of the product to the host is another reason for the low cell mass. This issue could be resolved by optimizing the fermentation process, such as increasing cell density to elevate the yield of products, removing isoprenoids products during the production (Chen *et al*. 2009). Otherwise, the expression of efflux pumps, heat shock proteins, membrane modifying proteins and activating of general stress response genes may be applied to improve the tolerance of the host (Piper 1995; Jiang *et al*. 2013).

The other concern is on the instability of the recombinant plasmid. In spite of the great progression of isoprenoids production made in engineered bacteria, these results were achieved in lab scale by introducing recombinant plasmids into the bacteria, usually a selectable marker is required to retain the presence of the plasmid during the cell growth. However, the poor genetic stability of the plasmid and the need of the selective pressure hinder the transformation from lab scale to commercial production. In contrast, gene integration offers a stable manipulation, the transgenes could be integrated into the genome, these cells could be used for industrial production without requiring the presence of any selectable markers (Kuhlman and Cox 2010).

Conclusion

Isoprenoids are the valuable chemicals and could be applied into various industrial fields. Production of isoprenoids in engineered bacteria is a promising approach to meet the increasing needs, it is of incomparable advantages mentioned above. In the past decade, dozens of isoprenoids are produced in engineered bacteria at exciting yield, it is reasonable to expect successful manufacture in the factory. As the development of the microbial biotechnology, in the future, more and more isoprenoids products will be available at low cost.

Authors' contributions

Y. L and G. W contributed to literature search, analysis, and manuscript preparation. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Table 1 Abbreviation in Figure 1

HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
MVA	Mevalonate
MVA-5-P	Mevalonate-5-phosphate
MVA-5-PP	Mevalonate diphosphate
AtoB	Acetoacetyl-CoA synthase
HmgS	Hydroxymethylglutaryl-CoA synthase
HmgR	Hydroxymethylglutaryl-CoA reductase
MVK	Mevalonate kinase
PMK	Phosphomevalonate kinase
PMD	Mevalonate diphosphate decarboxylase
G3P	glyceraldehyde-3-phosphate
DXP	1-deoxy-D-xylulose-5-phosphate
MEP	2C-methyl-D-erythritol-4-phosphate, methylerythritol 4-phosphate
CDP-ME	4-diphosphocytidyl-2C-methyl-D-erythritol
CDP-MEP	4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate
MEcPP	2C-methyl-D-erythritol-2,4-cyclodiphosphate
HMBPP	4-hydroxy-3-mehtyl-butenyl 1-diphosphate
IPP	Isopentenyl diphosphate
DMAPP	Dimethylallyl diphosphate
DXS	1-deoxy-D-xylulose-5-phosphate synthase
DXR	1-D-deoxy-D-xylulose 5-phosphate reductoisomerase
IspD	2C-methyl-D-erythritol-4-phosphate cytidylyltransferase

IspE	4-diphosphocytidyl-2C-methyl-D-erythritol kinase
IspF	2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase
IspG	1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase
IspH	4-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate reductase
IDI	Isopentenyl diphosphate isomerase
GPP	Geranyl diphosphate
FPP	Farnesyl diphosphate
GGPP	Geranylgeranyl diphosphate

Table 2 Comparison of MEP pathway and MVA pathway

	MEP pathway	**MVA pathway**
Precursors	Pyruvate/G3P	Acetyl-CoA
Reaction steps	7 steps	6 steps
Key enzymes	Dxs, Dxr, IspD, IspE, IspF, IspG, IspH	AtoB, HumgS, HumgR, MVK, PMK, PMD
Energy consumption	1.255 mol of glucose/1 mol of IPP 2.151 mol of glycerol/1 mol of IPP	1.5 mol of glucose/1 mol of IPP 3 mol of glycerol/1 mol of IPP
Distribution	Most bacteria, cyanobacteria, plant chloroplasts, algae, apicomplexan parasites	Most eukaryotes, archaea, a few bacteria, cytosol and mitochondria of plants
Inhibitor	Fosmidomycin	Mevinolin

Table 3 Summary of Isoprenoids Produced in Bacteria

Isoprenoids produced	Host	Approach	Yield	References
Isoprene	*E .coli*	Heterologous expression of *ispS*; Over-expression of native *dxs/dxr* or *B. subtilis dxr/dxr*.	314mg l^{-1}	(Zhao *et al*. 2011)
Isoprene	*E .coli*	5 Feeding modules	220mg l^{-1}	(Liu *et al*. 2013)
isoprene	*E .coli*	Over-expression of native MEP genes; Introducing MVA pathway; Heterologous expression of *ispS*; Other optimization methods (superoperon, specific RBS, nucleotide spacers).	320mg l^{-1}	(Zurbriggen *et al*. 2012)
Isoprene	*E .coli*	Introducing MVA upper pathway from *Enterococcus faecalis* (*mvaS*A110G); Introducing MVA lower pathway from *Saccharomyces cerevisiae*; Heterologous expression of *ispS*.	6.3 g l^{-1}	(Yang *et al*. 2012)
Isoprene	*C. synechocystis*	Introducing MVA pathway; Heterologous expression of *ispS*; chromosomal integration.	2.5-fold	(Bentley *et al*. 2014)
Isoprene	*E .coli*	Heterologous expression of *ispS*; Over-expression of native *dxs/dxr/idi* in various orders.	2.727 mg g^{-1}	(Lv *et al*. 2013)
lycopene	*E .coli*	Balancing of the precursors	25mg g^{-1} DCW	(Farmer and Liao 2001)
lycopene	*E .coli*	Over-expression of native *dxs*; Other optimization methods (promoters, vectors, strains).	16.8mg l^{-1}	(Kim and Keasing 2000)
lycopene	*E. coli*	Stoichiometric calculations; Single, double and triple gene knockouts to increase precursor availability.	6.6mg g^{-1} DCW	(Alper *et al*. 2005b)
lycopene	*E. coli*	Central metabolic genes knockout; Amplification of MEP pathway genes.	7.55 mg g^{-1} DCW	(Zhou *et al*. 2013)
lycopene	*E. coli*	Introduction of the lycopene synthesis genes; Introducing MVA lower pathway and exogenous mevalonate supplementation; Genes introduced from different strains	59mg l^{-1}	(Yoon *et al*. 2007b)
lycopene	*E. coli*	Introduction of the lycopene synthesis genes; Introducing MVA pathway; Inactivating of the competing pathway.	4.28mg l^{-1}	(Vadali *et al*. 2005)
lycopene	*E. coli*	Introduction of the lycopene synthesis genes; Introducing MVA lower pathway and Mevalonate as a substrate; Other optimization methods (culture conditions).	102mg l^{-1} (22mg g^{-1} DCW)	(Yoon *et al*. 2006)
lycopene	*E. coli*	Systematic (model-based) methods; Combinatorial (transposition-based) methods; Gene knockout.	18mg g^{-1}DCW	(Alper *et al*. 2005c)
lycopene	*E. coli*	Systematic and combinatorial approaches; Knockout and over-expression gene targets.	16 mg g^{-1} DCW	(Jin and Stephanopoulos 2007)
β-cartoene	*E. coli*	Introduction of the b-carotene biosynthesis genes; Over-expression of native MEP genes; Chromosomal Promoter engineering.	6mg g^{-1}DCW	(Yuan *et al*. 2006)
β-carotene	*E. coli*	Introduction of the b-carotene biosynthesis genes; Introducing MVA upper pathway and lower pathway	465mg l^{-1}	(Yoon *et al*. 2009)

		from different host; Other optimization methods (carbon source, strains).		
β-carotene	*E. coli*	Introduction of the b-carotene biosynthesis genes; Over-expression of *dxs/idi* genes; Introducing MVA lower pathway; Other optimization methods (carbon source, mevalonate supplementation).	503mg l^{-1} , 49.3mg g^{-1} DCW	(Yoon *et al*. 2007a)
β-carotene	*E. coli*	Increase ATP and NADPH; Engineering b-carotene synthesis module; Engineering MEP module; Engineering TCA and PPP modules; Other optimization methods (strain, culture condition).	2.1g l^{-1} (60mg g^{-1} DCW)	(Zhao *et al*. 2013)
carotenoid	*E. coli*	Over-expression of *dxs/dxr/idi*.	1.6mg g^{-1} DCW	(Albrecht *et al*. 1999)
abietadiene	*E. coli*	Over-expression of native MEP genes; Introducing MVA pathway.	>100ml l^{-1}	(Morrone *et al*. 2010)
sabinene	*E. coli*	Introducing of the GPP and sabinene synthase genes; Engineering MEP and MVA pathway; Other optimization methods (culture medium, process conditions).	2.65g l^{-1}	(Zhang *et al*. 2014)
taxadiene	*E. coli*	Engineering MEP pathway; Introducing heterologous downstream terpenoid-forming pathway.	1g l^{-1}	(Ajikumar *et al*. 2010)
Farnesol(FOH)	*E. coli*	Introduction of the biosynthesis genes; Introducing MVA pathway.	135.5mg l^{-1}	(Wang *et al*. 2010)
levopimaradiene	*E. coli*	Over-expression of MEP genes; Introducing and engineering heterologous downstream terpenoid-forming pathway.	700mg l^{-1}	(Leonard *et al*. 2010)
astaxanthin	*E. coli*	Introduction of the biosynthesis genes; Over-expression of *idi*.	1.25mg g^{-1} DCW	(Wang *et al*. 1998)
amorphadiene	*E. coli*	Introduction of the biosynthesis genes; Introducing MVA pathway.	24ug ml^{-1}	(Martin *et al*. 2003)
zeaxanthin	*E. coli*	Introduction of the biosynthesis genes; Other optimization methods (gene order, promoter).	820mg g^{-1} DCW	(Nishizaki *et al*. 2007)
abietadiene	*E. coli*	Over-expression of native MEP genes; Introducing MVA pathway.	>100ml l^{-1}	(Morrone *et al*. 2010)