Bioresource Technology 104 (2012) 642–647

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Bio-isoprene production using exogenous MVA pathway and isoprene synthase in *Escherichia coli*

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ARTICLE INFO

Article history:
Received 13 July 2011
Received in revised form 10 October 2011
Accepted 12 October 2011
Available online 20 October 2011

Keywords:
Isoprene
MVA pathway
Isoprene synthase
Escherichia coli

ABSTRACT

In this paper, an original strategy is employed to biosynthesize the isoprene by heterologously co-expressing the *Saccharomyces cerevisiae* MVA pathway and isoprene synthase (IspS) from *Populus alba* in the *Escherichia coli* BL21 (DE3) strain, which was screened from three different IspS enzymes. The finally genetic strain YJM13 harboring the MVA pathway and $ispS_{Pa}$ gene could accumulate isoprene up to 2.48 mg/l and 532 mg/l under the flask and fed-batch fermentation conditions, respectively, which is about three times and five times to the control strain. The result proves to be higher than that in the report documents. In this way, a potential production system for isoprene from renewable sources via the MVA pathway in *E. coli* has been provided.

1. Introduction

Since the beginning of the 20th century, the demand for rubber has dramatically increased (Mooibroek and Cornish, 2000). Rubber was mainly derived from natural rubber (NR, produced by various tropical plants, especially the Brazilian rubber tree *Hevea brasiliensis*) or petroleum-based synthetic rubber (SR) (Tanaka and Sakdapipanich, 2001). In 2003, the global annual production of NR was approximately seven million tones, meeting only about 40% (wt/wt) of the total global rubber demand with the remaining 60% coming from various SRs produced from fossil-carbon sources-isoprene (Steinbuchel, 2003).

Isoprene is an important platform chemical, 95% of which was exploited for the production of synthetic rubber for tires and coating (Alianell et al., 2010). Furthermore, isoprene can also be wildly used in the fields of isoprenoid medicines, fragrances, and aviation fuel (Kesselmeier and Staudt, 1999; Lindberg et al., 2010; Sharkey, 2009). At present, industrial production of isoprene is derived entirely from petrochemical sources through chemical synthesis methods (Anhorn et al., 1961; De Malde, 1963; DiGiacomo et al., 1961; Reis, 1972; Ushio, 1972). As the petroleum reserve comes to exhaustion, the problem of material shortage is becoming a bottleneck for the production of industrial isoprene. Therefore, it becomes increasingly urgent to seek sustainable technologies for isoprene production without petroleum consumption.

There are two natural pathways for biosynthesis of dimethylallyl diphosphate (DMAPP) which is the precursor of isoprene: methylerythritol 4-phosphate (MEP) pathway and mevalonate (MVA) pathway (see Fig1, reviewed by Rodriguez-Concepcion and Boronat (2002)). MVA pathway mainly exists in eukaryotes, archaebacteria, and cytosols of higher plants, while the MEP pathway is used by many eubacteria, green algae, and chloroplasts of higher plants (Eroglu and Melis, 2010; Kuzuyama, 2002). Currently, with the rapid development of biocatalysis, isoprene production by means of biosynthetic method has become a hot spot. And some studies about genetic modification to enhance microbial production of isoprene or isoprenoid have been reported. Lindberg et al. has demonstrated that the cyanobacterium *Synechocystis* could produce 50 μg/g dry cell weight/day isoprene in the sealed *Synechocystis* culture headspace by introducing heterologous isoprene synthase gene (*ispS*). However, slow growth rate and low amount of biomass restricted the isoprene production by cyanobacterium *Synechocystis*. The genetic modification was made on *Bacillus subtilis*, which also employed the native MEP pathway to enhance isoprene production, leading to 40% increase of isoprene production compared with the wild-type strain (Xue and Ahring, 2011). However, the current productivity is still too low and economically infeasible in industrial application. To achieve high yield production of isoprene, Zhao et al. has constructed the engineered strain with over-expression of 1-deoxy-D-xylulose-5-phosphate (DXP) synthase gene (*dxs*) and DXP reductoisomerase gene (*dxr*) from *B. subtilis*, and nonnative isoprene synthase gene (*ispS*) from *Populus nigra*, which can cause an 3.3-fold increase on isoprene production (from 94 mg/l to 314 mg/l) (Zhao et al., 2011).

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doi:10.1016/j.biortech.2011.10.042

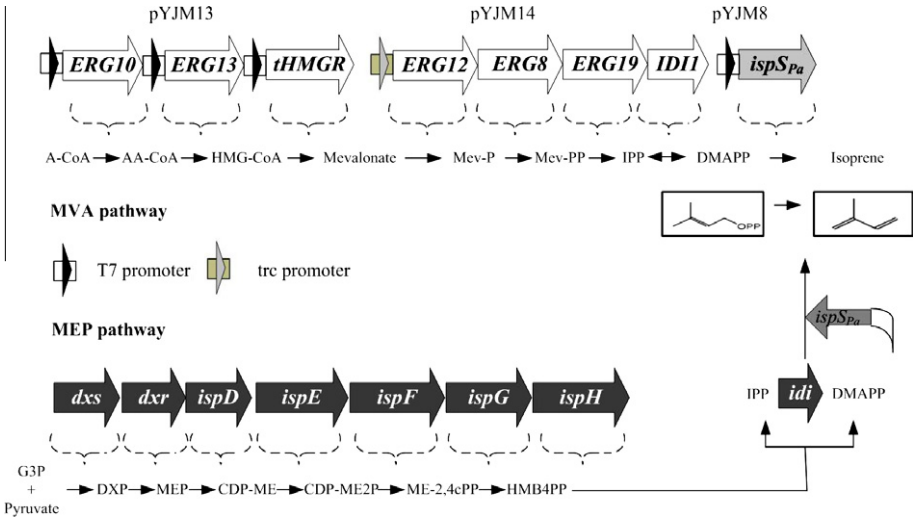

Fig. 1. Production of isoprene via the DXP or MVA pathways used in this study. Gene symbols and the enzymes they encode (all genes marked with white arrows were isolated from *S. cerevisiae*, the gene marked with light gray arrows derived from *P. alba* and all genes marked with gray arrows were from *E. coli*). MVA pathway: ERG10, acetoacetyl-CoA thiolase; ERG13, HMG-CoA synthase; tHMGR, truncated HMG-CoA reductase; ERG12, mevalonate kinase; ERG8, phosphomevalonate kinase; ERG19, mevalonate pyrophosphate decarboxylase; IDI1, IPP isomerase; ispSPa, *P. alba* isoprene synthase was optimized to the preferred codon usage of *E. coli*. MEP pathway: DXS, DXP synthase; DXR, DXP reductoisomerase; ispD, MEP cytidylyltransferase; ispE: CDP-ME kinase; ispF: ME-2,4cPP synthase; ispG: HMB4PP synthase; ispH: HMB4PP reductase; idi, IPP isomerase. Pathway intermediates. MVA pathway: A-CoA, acetyl-CoA; AA-CoA, acetoacetyl-CoA; HMG-CoA, hydroxymethylglutaryl-CoA; MeV-P, mevalonate 5-phosphate; MeV-PP, mevalonate pyrophosphate. IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; MEP pathway: G3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-ME2P, 4-diphosphocytidyl-2-C-methyl-D-erythritol -2-phosphate; ME-2,4cPP, 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate; HMB4PP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-pyrophosphate.

Despite of great efforts taken in isoprene production, this approach still remains ineffective due to regulation mechanisms present in the native host (Martin et al., 2003). To avoid this native control of MEP pathway, this paper gives an attempt to introduce the *Saccharomyces cerevisiae* MVA pathway in *E. coli* expressing the codon-optimized *ispS* gene from *Populus alba*. This study provided an alternative method for isoprene biosynthesis, which will play an important role in the industrial isoprene production in the near future.

2. Methods

2.1. Strains, medium and culture conditions

E. coli BL21(DE3) and *S. cerevisiae* were purchased from Invitrogen and ATCC, respectively. The LB medium was used for gene cloning and shake-flask fermentation. In fed-batch fermentation, each liter of medium contains glucose 20 g, K_2HPO_4 9.8 g, beef extract 5 g, ferric ammonium citrate 0.3 g, citric acid monohydrate 2.1 g, $MgSO_4$ 0.06 g, 1 ml trace element solution ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 0.37 g/l, $ZnSO_4\cdot 7H_2O$ 0.29 g/l, H_3BO_4 2.47 g/l, $CuSO_4\cdot 5H_2O$ 0.25 g/l, and $MnCl_2\cdot 4H_2O$·1.58 g/l) and 2% initial glucose was used for fed-batch culture. If necessary, appropriate antibiotics were added to the culture medium at the following concentration: ampicillin (100 μg/ml), kanamycin (50 μg/ml), and chloramphenicol (34 μg/ml).

2.2. Plasmid construction

Common procedures including genomic DNA preparation, restriction digestions, transformations, and other standard molecular biological techniques were carried out as previously described by Sambrook and Russell (2001). Polymerase chain reaction (PCR) was performed using *Pfu* DNA polymerase (TaKaRa, Dalian, China) based on the manufacturer's instruction.

2.2.1. Cloning of ispS genes from different organisms

The nucleotide sequences of *ispS* genes from *P. alba* ($ispS_{Pa}$, GenBank No. AB198180), *P. nigra* ($ispS_{Pn}$, GenBank No. AM410988), and *P. alba* × *Populus tremula* ($ispS_{PP}$, GenBank No. AJ294819) were analyzed (http://www.genscript.com/cgi-bin/tools/rare_codon_analysis) and optimized to the preferred codon of *E. coli* (http://www.jcat.de/) online. The codon-optimized *ispS* genes ($ispS_{Pa}$, $ispS_{Pn}$ and $ispS_{Pp}$) were synthesized by Genray Company with plasmid pGH as vector (named pGH/$ispS_{Pa}$, pGH/$ispS_{Pn}$, and pGH/$ispS_{Pp}$, respectively). The plasmid pYJM8 was constructed using PCR fragments containing the $ispS_{Pa}$ coding region generated with primers Pa-F (5′-CGCGGATCCAGATGTAGCGTGTCCACCGAA-3′) and Pa-R (5′-ACGCGTCGACTTAGCGTTCAAACGGCAGAATC-3′) and pGH/$ispS_{Pa}$ as template, which was digested with *Bam*HI and *Sal*I and then ligated between the *Bam*HI and *Sal*I sites of pACYCDuet-1. The plasmid pYJM9 was constructed using PCR fragments containing the $ispS_{Pp}$ coding region generated with primers Pp-F (5′-GGAAGATC TGAAGCCAGACGGTCTGCCA-3′) and Pp-R (5′-CCGCTCGAGTTATCTC TCAAAGGGTAGAATAG-3′) and pGH/$ispS_{Pp}$ as template, which was digested with *Bgl*II and *Xho*I and then ligated between the *Bgl*II and *Xho*I sites of pACYCDuet-1. The plasmid pYJM10 was constructed using PCR fragments containing the $ispS_{Pn}$ coding region generated with primers Pn-F (5′-GGAAGATCTGCGACCGAACTGC TGTGCCT-3′) and Pn-R (5′-CCGCTCGAGTTAACGTTCGAACGGCAGG ATC-3′) and pGH/$ispS_{Pn}$ as template, which was digested with *Bgl*II and *Xho*I and then ligated between the *Bgl*II and *Xho*I sites of pACYCDuet-1.

2.2.2. Construction of plasmid for upper pathway of MVA

The plasmid pYJM11 was constructed using PCR fragments containing the *ERG10* coding region generated with primers Erg10-F (5′-CGCGGATCCATGTCTCAGAACGTTTACATTGT-3′) and Erg10-R (5′-ATCGGAGCTCTCATATCTTTTCAATGACAATAG-3′) and *S. cerevisiae* chromosomal DNA as template, which was digested with *Bam*HI and *Sac*I and then ligated between the *Bam*HI and *Sac*I sites of pCOLADuet™-1. The plasmid pYJM12 was constructed using PCR

fragments containing the *ERG13* coding region generated with primers Erg13-F (5′-ACGCGTCGACATGAAACTCTCAACTAAACTTTG-3′) and Erg13-R (5′-CCCAAGCTTTTATTTTTTAACATCGTAAGATC-3′) and *S. cerevisiae* chromosomal DNA as template, which was digested with *Sal*I and *Hin*dIII and then ligated between the *Sal*I and *Hin*dIII sites of pYJM11. The PCR fragments containing the *tHMGR* coding region generated with primers GR-F (5′-CCGCGGCCGGCCATGGACCAATTGGTGAAAACTGA-3′) and GR-R (5′-CCGGACGTCTTAGGATTTAATGCAGGTGACGGA-3′) and *S. cerevisiae* chromosomal DNA as template, was digested with *Fse*I and *Aat*II and then ligated with pJYM12 digested with the same enzymes to create pYJM13.

2.2.3. Construction of plasmid for lower pathway of MVA

Plasmid pYJM14 was constructed on pTrcHis2B by introducing the *ERG8*, *ERG12*, *ERG19* and *IDI1* from *S. cerevisiae*. The four genes were ligated into the plasmid pTrcHis2B using the method established in the lab (manuscript in preparation)

2.3. Expression of $IspS_{Pa}$ in E. coli and assay of enzymatic activity

A single colony of *E. coli* BL21 (DE3) harboring pYJM8 was grown up in LB broth overnight at 37 °C. The culture was used to inoculate the same medium (1:100 dilution) and grown at 37 °C until the culture reached an OD_{600} of 0.6. IPTG was added to final concentration of 0.5 mM, and culture was further incubated at 30 °C for 5 h. The harvested cells were suspended in 20 mM sodium phosphate, and disrupted by sonication (3 s pulse on, 3 s pulse off on ice, processed for 1.5 min). The lysate was centrifuged (38,000*g*, 30 min) and the supernatant obtained was purified by Ni–NTA purification system (Invitrogen). Protein concentration in supernatant was determined using the Bradford Protein Assay Kit (Tiangen, China). Proteins were separated in 4–12% acrylamide gels (NuPAGE Gel System; Invitrogen, La Jolla, CA) and visualized with Coomassie brilliant blue R250. Isoprene synthase activity was assayed as described by Lehning et al. (1999) except that samples were incubated for 2 h at 40 °C, which is the optimum temperature of $IspS_{Pa}$ (Sasaki et al., 2005).

2.4. MVA quantification

The MVA production was determined by GC analysis as described (Pfleger et al., 2007). The strain YJM11 was inoculated in the 50 ml LB broth and incubated at 37 °C. When OD_{600} of bacterial culture reached 0.6, IPTG was added to final concentration of 0.5 mM, and culture was further incubated at 30 °C for 24 h. After centrifugation, the supernatant was adjusted to pH 2.0 with 3 M HCl and incubated at 45 °C for 1 h to convert mevalonate to mevalonic acid lactone. Then this solution was saturated with Na_2SO_4, and MVA was extracted with ethyl acetate. The ethyl acetate phase was transferred to a clean glass vial and dried under a stream of nitrogen. The residues were re-dissolved in 1 ml of ethyl acetate and analyzed by GC.

GC analysis was performed on an Agilent 7890A equipped with a flame ionization detector (FID) and a HP-AL/S column (25 m × 320 μm × 8 μm). N_2 was used as carrier gas with a linear velocity of 1 ml/min. The column temperature profile was 75 °C for 0.5 min, 25 °C/min to 150 °C, 15 °C/min to 200 °C, 30 °C/min to 250 °C, and 250 °C for 5 min. The product was characterized by direct comparison with an external standard method (Sigma–Aldrich, USA).

2.5. Shake-flask cultures

A single colony of *E. coli* BL21 (DE3) harboring different plasmids was grown up in LB broth overnight at 37 °C. The overnight cultures were used to inoculate 250 ml sealed shake-flasks containing 50 ml of LB broth and grew at 37 °C with shaking until the cultures reached an OD_{600} of 0.6. IPTG was added to final concentration of 0.5 mM, and culture was further incubated at 30 °C for 24 h. 1 ml of gas sample from the headspace of the sealed cultures was analyzed as described earlier (Julsing et al., 2007) using a GC (Agilent 7890A, America) equipped with a flame ionization detector (FID) and a HP-AL/S column (25 m × 320 μm × 8 μm). The product was characterized by direct comparison with standard isoprene (TCI-EP, Tokyo, Japan). The peak area was converted to isoprene concentration by comparing with a standard curve plotted with a set of known concentration of isoprene.

2.6. Fed-batch fermentation

The strain grew up overnight at 37 °C in 100 ml of M9 minimal media (Glazyrina et al., 2010). The overnight culture was used to inoculate a 5-l fermentor (BIOSTAT Bplus MO5L, Sartorius, Germany) containing 3 l fermentation medium. The temperature was controlled at 30 °C, the pH was maintained at 7.0 by adding ammonia, and Antifoam 204 was used for foam control. The expression of plasmid-borne exogenous gene(s) for isoprene production was initiated at an OD_{600} of 12 by adding IPTG to the final concentration of 0.5 mM and inducer was added every 8 h. The residual glucose was measured using a glucose analyzer (SBA-40D, China) and was maintained below 0.5 g/l by adding glucose solution during the course of fermentation. Then isoprene accumulation was measured at a series of time points by GC as described (Julsing et al., 2007). At the same time, the growth of the bacterial culture was determined by measuring the OD_{600} with a spectrophotometer (Cary 50 UV–vis, Varian).

3. Results and discussion

3.1. Evaluation of isoprene synthases

Isoprene synthase converts DMAPP, derived from the MEP pathway or MVA pathway, to isoprene. Despite of the fact that *E. coli* has produced DMAPP with MEP pathway, it is unable to synthesize isoprene due to the absence of isoprene synthase. The level of isoprene production of an engineered *E. coli* strain should reflect the activity of exogenous isoprene synthase accurately. Three isoprene synthase genes, $ispS_{Pa}$ from *P. alba*, $ispS_{Pn}$ from *P. nigra*, and $ispS_{Pp}$ from *P. alba* × *P. tremula*, were optimized according to the preferred codon usage of *E. coli*, and cloned into the vector pACYCDuet-1, resulting in recombinant plasmids pYJM8, pYJM9, and pYJM10, respectively. Three strains YJM8, YJM9, YJM10 carrying pYJM8, pYJM9 and pYJM10 respectively grew up in 100 ml shake-flask. When the culture reached an OD_{600} of 0.6, expression of isoprene synthase was induced by IPTG, and the culture was further incubated for 24 h. A significant difference in isoprene production was observed (Fig. 2). The *E. coli* strain carrying pYJM8 (named as YJM8) produced 0.86 mg isoprene per liter of bacteria culture, which is 7- and 1.3-fold higher than those carrying pYJM9 (YJM9) (0.12 mg/l) and pYJM10 (YJM10) (0.65 mg/l), whereas the *E. coli* BL21(DE3) strain carrying empty vector pACYCDuet-1 did not generate isoprene. This result demonstrated that the expression of exogenous *ispS* gene contributed to the isoprene production, and the enzyme activity of $IspS_{Pa}$ is the highest among these three enzymes. Noticeable difference of cell growth was not found.

The activity of purified $IspS_{Pa}$ protein was also determined *in vitro*. The recombinant $IspS_{Pa}$ protein without the 37-amino acid chloroplast transit peptide carrying a six-histidine tag at N-terminal, was purified from *E. coli* BL21(DE3), and detected at

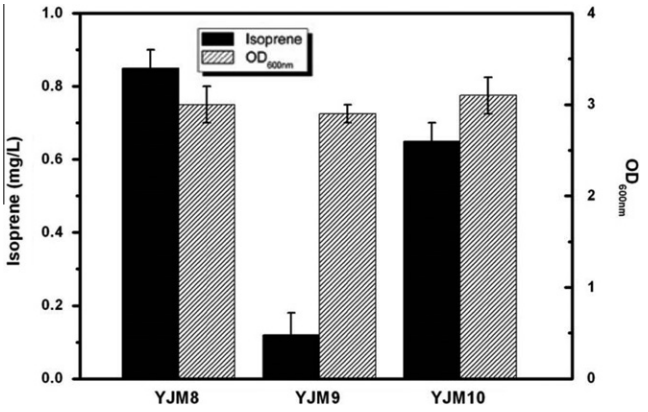

Fig. 2. Comparison of IspS activity. Isoprene production and cell growth in different genetic strain. When OD_{600} reaches 0.6–0.9, cultures was induced at 30 °C for 24 h using 0.5 mM IPTG. The experiment was performed in triplicate.

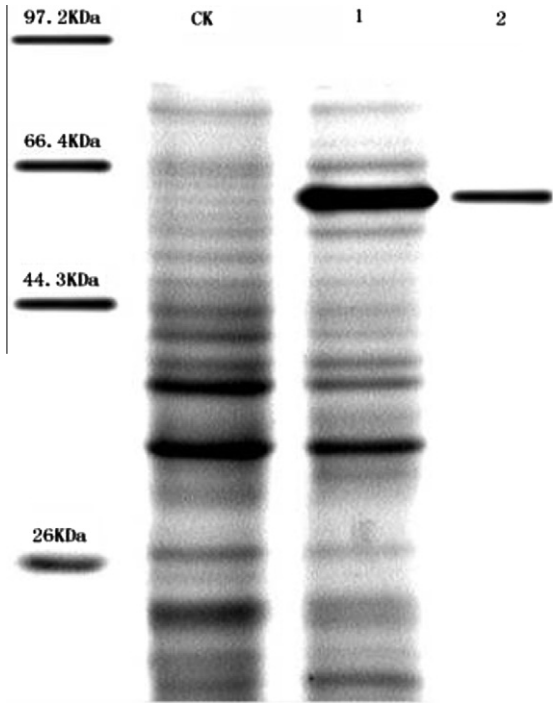

Fig. 3. SDS–PAGE analysis of $IspS_{Pa}$. CK: cell lysate from BL21(DE3) containing pACYDuet-1. (1) crude cell extracts from YJM8. (2) purified $IspS_{Pa}$. Briefly, $IspS_{Pa}$ was expressed in *E. coli* BL21 (DE3) harboring pYJM8, induced by 0.5 mM IPTG when an OD_{600} of 0.6 was reached. After an additional 4–5 h, cells were harvested and centrifuged. Cells were broken by sonication. The supernatants were collected by centrifugation and purified by Ni–NTA purification system (Invitrogen) for SDS–PAGE analysis.

the expected molecular mass of 64 kDa in SDS–PAGE (Fig. 3). 40 μg of purified enzyme was incubated with 0.67 μM DMAPP in the presence of 50 mM Tris–HCl (pH 8.0), 12 mM $MgCl_2$, 5% glycerol, and 2 mM dithiothreitol in an airtight vial at 40 °C for 2 h. The volatile compounds in the headspace of the reaction mixture were directly used for GC analysis. An isoprene-specific peak was detected (Supplementary Fig. 1), indicating that this isolated recombinant protein has isoprene synthase activity.

3.2. Fermentation study of *E. coli* strain harboring $ispS_{Pa}$

The YJM8 strain containing pYJM8 accumulated 0.86 mg/l isoprene when growing in a 100-ml shake-flask using LB broth. To further investigate its isoprene producing ability, fed-batch fermentation was performed at a 3-l scale using fermentation medium supplemented with glucose as the carbon source. When OD_{600} of culture reached 12, expression of isoprene synthase was induced by IPTG, and the culture was further incubated for 34 h. The YJM8 strain accumulated 110 mg/l isoprene, whereas the *E. coli* BL21(DE3) carrying empty vector produced little isoprene (data not shown). The isoprene production was much higher than that in shake-flask fermentation. Despite of this, the amount is still far from enough to be applied to industry.

E. coli employs the native MEP pathway to produce DMAPP, which is converted to isoprene by isoprene synthase. One of the key methods elevating the isoprene productivity is to increase the cellular concentration of its building block molecule DMAPP. Recently, several groups have reported their works on the MEP pathway to improve the production of DMAPP in *E. coli* (Zhao et al., 2011), *B. subtilis* strain DSM10 (Xue and Ahring, 2011) and *Cyanobacterium synechocystis* (Lindberg et al., 2010). Despite all these efforts, the native regulatory mechanisms of MEP pathway in these hosts restrict the isoprene production (Martin et al., 2003).

3.3. Establishing MVA pathway in *E. coli*

DMAPP is also produced by the MVA pathway, mainly existing in eukaryotes, archaebacteria, and cytosols of higher plants, but absent in *E. coli*. Since the MEP pathway may be bound to some physiological control elements in *E. coli*, attempt has been taken to circumvent it by introducing the MVA pathway of the *S. cerevisiae* into *E. coli*.

The genes coding the MVA pathway from *S. cerevisiae* were assembled into operons and expressed in *E. coli*. To simplify the task of engineering a seven-gene pathway, they were separated into two operons and cloned into two compatible plasmids. The operon made up of genes *ERG10*, *ERG13* and *tHMGR*, catalyzing acetyl-CoA to MVA, was referred to as 'upper' pathway and cloned into pCOLADuet-1. The resulting plasmid was named as pYJM13. The other operon composed of *ERG8*, *ERG12*, *ERG9*, and *IDI1*, which converts MVA to DMAPP, was referred to as 'down' pathway and cloned into pTrcHis2B. The resulting plasmid was named as pYJM14.

To test the functionality of the heterologous 'upper' pathway, samples prepared from the flask cultures of the *E. coli* BL21(DE3) bearing pYJM13 were analyzed by GC. An MVA-specific peak in the sample was identified by comparing with an authentic standard (Supplementary Fig. 2). The *E. coli* BL21 (DE3) carrying empty vector could not produce MVA (data not shown). The results proved that the synthetic operon of the 'upper' pathway is effective and capable of producing MVA in *E. coli*.

The 'down' pathway engineered in *E. coli* was also verified. Due to the difficulty in DMAPP detection and quantification, the plasmids pYJM14 and vector pTrcHis2B were transformed into *E. coli* BL21(DE3) strain carrying pYJM8, respectively. Therefore, the function of the "down" pathway could be indirectly testified through determining the isoprene production. The strain carrying pYJM8 and vector pTrcHis2B showed similar isoprene production with or without 2.5 mM MVA supplemented in medium, which indicates that this strain cannot utilize MVA and the isoprene is derived from MEP pathway. When growing in medium supplemented with 2.5 mM MVA, the isoprene production of the strain carrying pYJM8 and pYJM14 is about 60-fold higher than that without MVA supplementation, which is similar to the isoprene production of strain carrying pYJM8 and pTrcHis2B (Table 1). This result indicated that the synthetic operon of the 'down' pathway is functional and able to convert MVA to DMAPP, which is further converted to isoprene by $IspS_{Pa}$.

Table 1
Isoprene production by different strains with or without MVA under flask conditions. The experiment was done in triplicate.

Plasmids	*E. coli* BL21(DE3)/pYJM8	
	+MVA	−MVA
pTrcHis2B (mg/l)	0.83 ± 0.05	0.87 ± 0.06
pYJM14 (mg/l)	52 ± 4	0.78 ± 0.045

3.4. Microbial isoprene production using MVA pathway

DMAPP can be synthesized by both MVA pathway and MEP pathway. The MVA pathway has been used for producing several isoprenoids in both yeast and bacteria, such as β-carotene (Yoon et al., 2009), amorphadiene (Martin et al., 2003), and artemisinic acid (Ro et al., 2006). There are also a few examples of isoprenoid production via the MEP pathway by overexpression of one or more enzymes in the pathway; however, the productivity required in commercial application has not been achieved (Farmer and Liao, 2001; Kim and Keasling, 2001), probably because some unknown control mechanisms of the MEP pathway in *E. coli* limit the production of DMAPP.

To achieve high-level production of isoprene, an original biosynthesis pathway of isoprene was constructed by assembling the MVA pathway from *S. cerevisiae* and $ispS_{Pa}$ gene in *E. coli*. The plasmids pYJM13 and pYJM14 were transformed into YJM8 strain simultaneously, resulting in strain YJM13. Growing in 100 ml shake-flask, the isoprene production of strain YJM13 reached 2.48 mg/l, which was 2.9-fold higher than that of strain YJM8 (Fig. 4A). To further confirm the effectiveness of MVA pathway in increasing isoprene production, fed-batch fermentations were performed. The strain YJM13 accumulated 532 mg/l isoprene, which is about five-fold increase over that of strain YJM8 (Fig. 4B). The isoprene productivity achieved by using MVA pathway in *E. coli* is believed to be higher than those previously reported. During the fermentation, cell growth was also monitored by measuring OD_{600}. As shown in Fig. 5, the isoprene production was closely related to the growth of cells.

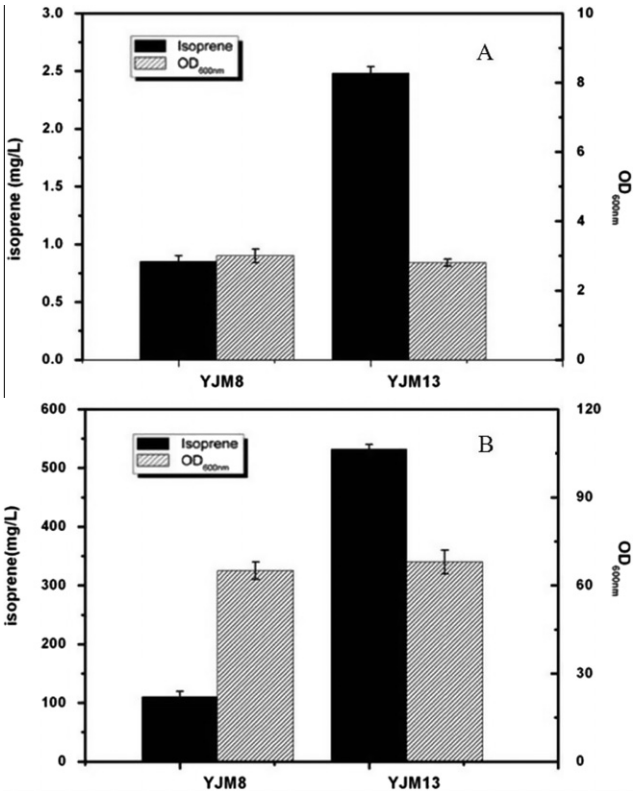

Fig. 4. Comparison of isoprene production from native MEP and heterologous MVA pathway. (A) Isoprene production of engineered *E. coli* under flask conditions. (B) Isoprene production of engineered *E. coli*. The batch fermentation was performed under the conditions described in Section 2.6. The experiment was performed in triplicate.

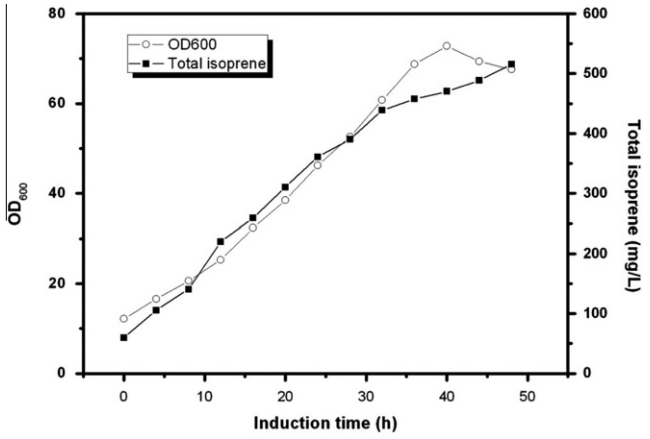

Fig. 5. The time course of isoprene production by YJM13. Isoprene accumulation (■) and cell growth (○) in YJM13, Induction was carried out at the time point of 12 h. Other experiment conditions were described in Section 2.6.

4. Conclusions

In this paper, a novel strategy was employed to biosynthesize isoprene. To eliminate the influence of native control mechanisms, the heterologous MVA pathway from *S. cerevisiae* rather than the MEP pathway, was introduced into *E. coli* to synthesize DMAPP, the precursor of isoprene. With co-expression of isoprene synthase $IspS_{Pa}$ and MVA pathway, the engineered strain accumulated 532 mg/l isoprene in fed-batch fermentation, which proves to be the highest isoprene productivity reported so far. This study established a reliable method of isoprene biosynthesis, which may play an important role in industrial isoprene production in the near future.

Acknowledgements

This work was financially supported by CAS 100 Talents Program (No. KGCXZ-YW-801), and National Science Foundation of China (20872075). I give my sincere thanks to Qingjuan Nie for refining the paper of English language.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.10.042.

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