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

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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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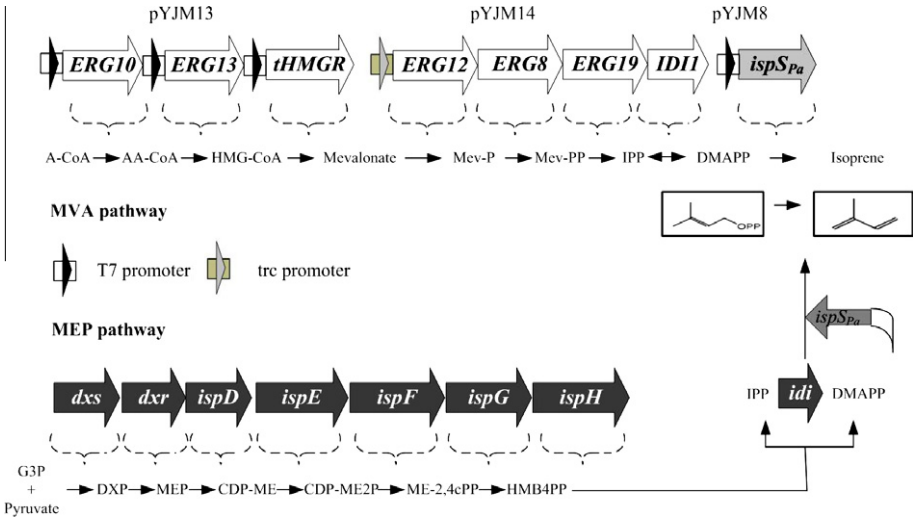


**Fig. 1.** Production of isoprene via the DXP or MVA pathways used in this study. Gene symbols and the enzymes they encode (all genes marked with white arrows were isolated from *S. cerevisiae*, the gene marked with light gray arrows derived from *P. alba* and all genes marked with gray arrows were 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