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ORIGINAL PAPER

Type 2 IDI performs better than type 1 for improving lycopene production in metabolically engineered *E. coli* strains

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Abstract In this study a comparison was made between type 1 and type 2 isopentenyl diphosphate isomerases (IDI) in improving lycopene production in *Escherichia coli*. The corresponding genes of *Bacillus licheniformis* and the host (i_{Bl} and i_{Ec}, respectively) were expressed in lycopene producing *E. coli* strains by pTlyci$_{Bl}$ and pTlyci$_{Ec}$ plasmids, under the control of *tac* promoter. The results showed that the overexpression of i_{Ec} improved the lycopene production from 33 ± 1 in *E. coli* Tlyc to 68 ± 3 mg/gDCW in *E. coli* Tlyci$_{Ec}$. In contrast, the expression of i_{Bl} increased the lycopene production more efficiently up to 80 ± 9 mg/gDCW in *E. coli* Tlyci$_{Bl}$. The introduction of a heterologous mevalonate pathway to elevate the IPP abundance resulted in a lycopene production up to 132 ± 5 mg/gDCW with i_{Ec} in *E. coli* Tlyci$_{Ec}$-mev and 181 ± 9 mg/gDCW with i_{Bl} in *E. coli* Tlyci$_{Bl}$-mev, that is, 4 and 5.6 times respectively. When fructose, mannose, arabinose, and acetate were each used as an auxiliary substrate with glycerol, lycopene production was inhibited by different extents. Among auxiliary substrates tested, only citrate was an improving one for lycopene production in all strains with a maximum of 198 ± 3 mg/gDCW in *E. coli* Tlyci$_{Bl}$-mev. It may be concluded that the type 2 IDI performs better than the type 1 in metabolic engineering attempts for isoprenoid production in *E. coli*. In addition, the metabolic engineering of citrate pathway seems a promising approach to have more isoprenoid accumulation in *E. coli*.

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Keywords *E. coli* · Metabolic engineering · Lycopene · Isopentenyl diphosphate isomerase (IDI)

Introduction

Carotenoids are fat-soluble isoprenoid pigments responsible for different colors such as yellow, orange and red in plants, fungi, algae and some bacteria. They play a critical role in photosynthetic process and protect cells from harmful oxygen radicals in both phototrophic and non phototrophic organisms (Krinsky 1989). There are more than 600 various carotenoids in nature but only a few of them are used industrially such as lycopene, β-carotene, canthaxanthin and astaxatnthin. They have received considerable attention in food industry, medicine and cosmetics because of their beneficial effects on human health (Kang et al. 2005). Carotenoids are mostly obtained by chemical synthesis, but the growing demand of natural additive has stimulated scientists to increase carotenoid production in carotenogenic sources and even produce them in non-carotenogenic bacteria such as *Escherichia coli* by metabolic engineering approaches (Rodríguez-Villalón et al. 2008). Microbial production of carotenoids is promising due to being environmental friendly and able to meet the growing demand of natural carotenoids (Das et al. 2007).

Lycopene is a linear carotenoid responsible for red color in some fruits and vegetables such as tomatoes and watermelon. It has 7 IPP (isopentenyl diphosphate) and 1 DMAPP (dimethylallyl diphosphate) in its structure. The color of a carotenoid is principally a consequence of the number of conjugated double bonds. As the number of double bonds increases, the carotenoid light absorbance

further to the red region of the spectrum (Garcia-Asua et al. 1998). Lycopene acts as an antioxidant and reduces risks of some human cancers and muscular disease (Hwang and Bowen 2002). It plays a primary role in carotenoid production and other carotenoids such as β-carotene and astaxanthin are derived from lycopene (Armstrong 1997; Ducrey Sanpietro and Kula 1998; Sandman 1991; Schmidt-Dannert et al. 2000).

Isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP), are common building blocks to isoprenoids. There are two different pathways to IPP and DMAPP biosynthesis including the mevalonate pathway (MVA), converting acetyl-CoA to IPP, and the 2-C-methyl-D-erythiol 4-phosphate (MEP) pathway converting pyruvate and glyceraldehyde-3-phosphate to IPP and DMAPP (Fig. 1) (Rohmer et al. 1993). IPP isomerase catalyzes an essential reaction in the mevalonate pathway by the reversible interconversion of IPP to DMAPP. In the MEP pathway, IPP and DMAPP are synthesized independently through the late steps of the pathway, that is, IPP isomerase seems to be non-essential for non-mevalonate organisms (Rodriguez-Concepción et al. 2000). Two types of IPP isomerase have been discovered that show no similarity in the amino acid sequence. Mammals utilize the mevalonate pathway and type 1 IPP isomerase, while some Gram-positive eubacteria and archaea have the mevalonate pathway and type 2 IPP isomerase. In contrast, some Gram-positive eubacteria and most Gram-negative eubacteria contain the non-mevalonate pathway and type 1 IPP isomerase. All higher plants seem to have both the mevalonate and non-mevalonate pathways and type 1 IPP isomerase (Kuzuyama and Seto 2003; Lange et al. 2000; Lee and Schmidt-Dannert 2002; Rohdich et al. 2002).

Escherichia coli is a non-carotenogenic bacterium that makes isoprenoids using non-mevalonate pathway. This bacterium has been transformed with *crt* genes of carotenogenic bacteria for the production of carotenoids like lycopene, β- carotene, zeaxanthin, and astaxanthin (Kim et al. 2006; Tao et al. 2006; Wang et al. 1999; Yoon et al. 2007). Various sophisticated approaches including manipulation of the native non-mevalonate pathway, introduction of a heterologous mevalonate pathway, generation of gene knockouts, and promoter replacement have been considered for improving the carotenoid production in *E. coli* (Alper et al. 2005a, b; Farmer and Liao 2001; Kim and Keasling 2001; Ruther et al. 1997; Vadali et al. 2005; Wang et al. 1999; Yoon et al. 2007, 2009, 2006; Yuan et al. 2006). Furthermore, there are reports on the critical role of IDI in the improvement of carotenoid biosynthesis in *E. coli* (Kajiwara et al. 1997; Schmidt-Dannert et al. 2000). In this study, the type 2 IPP isomerase of *Bacillus lichenformis* was evaluated in comparison with the native type 1 IPP isomerase of *E. coli* to improve lycopene production in metabolically engineered *E. coli* strains. The effect of some auxiliary carbon sources on the total lycopene accumulation by these strains was also studied in this evaluation.

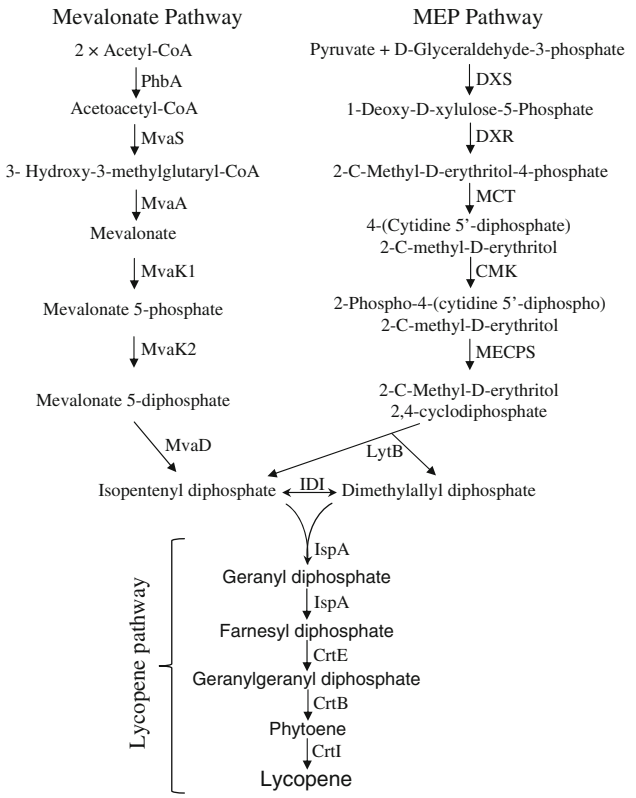

Fig. 1 The biosynthesis of lycopene in *E. coli*. Native MEP pathway consists of: DXS, deoxyxylulose 5-phosphate synthase; DXR, deoxyxylulose 5-phosphatereductoisomerase; MCT, 2-C-methylerythritol 4-phosphate cytidyl transferase; CMK, 4-(cytidine 5′-diphospho)-2-C-methylerythritol kinase; MECPS, 2-C-methylerythritol 2, 4-cyclodiphosphate synthase; LytB, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase. Engineered mevalonate pathway consists of: *PhbA* acetoacetyl-CoA thiolase, *MvaS* 3-hydroxy-3-methylglutaryl-CoA synthase, *MvaA* 3-hydroxy-3-methylglutaryl-CoA reductase, *MvaK1* mevalonate kinase, *MvaK2* phosphomevalonate kinase, and *MvaD* mevalonate 5-diphosphate decarboxylase, *IDI* isopentenyl diphosphate isomerase. Lycopene pathway consists of: *IspA* farnesyl diphosphate synthase, *CrtE* Geranylgeranyl diphosphate synthase, *CrtB* phytoene synthase, *CrtI* phytoene desaturase

Materials and methods

Bacterial strains and plasmid constructions

In this study, *E. coli* DH5α was used as a host for the recombinant plasmid construction and the lycopene production. The genes coding for geranylgeranyl diphosphate synthase (*crtE*), phytoene synthase (*crtB*) and phytoene desaturase (*crtI*) of lycopene production pathway had been isolated and inserted in pTrc99A in a previous study

(Zahiri et al. 2009). The resulting recombinant plasmid designated as pTcrtEIB contained the genes in the form of a synthetic operon under the control of *tac* promoter of pTrc99A. The genes coding for IDI were isolated from the chromosomes of *E. coli* and *B. licheniformis* using idi_{Ec} and idi_{Bl} specific primers and designated as i_{Ec} and i_{Bl} respectively (Table 1). The recognition sites of *Xba*I and *Hind*III were designed in the forward and reverse primers respectively by which the amplified *idi* genes could be restricted and inserted in pTcrtEIB just down stream of lycopene synthetic operon. The resulting plasmids were designated as $pTlyci_{Ec}$ and $pTlyci_{Bl}$ containing *crt EIB* and *idi* genes in a single operon under the control of the *trc* promoter of pTrc99A. The newly constructed plasmids, $pTlyci_{Ec}$ and $pTlyci_{Bl}$, as well as pTcrtEIB were used for the transformation of *E. coli* DH5α resulting in *E. coli* $Tlyci_{Ec}$, *E. coli* $Tlyci_{Bl}$, and *E. coli* Tlyc respectively. The recent strains were then transformed by pSSNSN containing six genes of *Streptococcus pneumonia* and *Ralstonia eutropha* in a synthetic operon coding for a mevalonate pathway (Zahiri et al. 2006). The resulting strains designated as *E. coli* $Tlyci_{Ec}$-mev, *E. coli* $Tlyci_{Bl}$-mev, and *E. coli* Tlyc-mev were each harboring two plasmids for the lycopene production and the elevated IPP biosynthesis (Table 1; Fig. 1).

Culture media and condition

Escherichia coli strains used in this study were cultured on Luria–Bertani (LB) medium at 37°C. The cultures were maintained at 4°C and renewed with subcultures from −70°C stocks. For lycopene production, a single colony of each strain was grown overnight on 2YT medium (1.6% tryptone, 1% yeast extract, 0.5% NaCl) in a shaking incubator at 37°C and 200 rpm. This culture was inoculated in 2YTG medium (2YT + 0.5% glycerol) as a main culture for lycopene production. Additional carbon sources including fructose, mannose, arabinose, citrate and acetate were sterilized separately and included in 2YTG at 0.2 and 0.5% concentrations (w/v). All main cultures were conducted in shake flasks (100 ml) containing 20 ml of culture broth. The cultures were almost synchronized using appropriate inoculation size to set the initial absorbance at 600 nm to 0.1.

The main cultures were incubated at 37°C and 200 rpm for 24 h. Ampicillin (100 μg/ml) and chloramphenicol (50 μg/ml) were included in culture media to maintain plasmid stability. Optical density, as a function of growth, was measured using a spectrophotometer at 600 nm and converted to dried cell weight using a standard curve.

Table 1 List of strains, plasmids and primers

Strains	Descriptions	
E. coli DH5α	[F′/*endA1 hsdR17* ($r_K^- m_K^+$) *glnV44 thi-1 recA1 gyrA* (Nal^r) *relA1 Δ*(*lacIZYA-argF*)*U169 deoR 5*(*φ*80*dlacΔ*(*lacZ*)*M15*)]	(Gibco BRL)
E. coli Tlyc	*E. coli* DH5α harboring pTcrtEIB	(Zahiri et al. 2009)
E. coli $Tlyci_{Ec}$	*E. coli* DH5α harboring $pTlyci_{Ec}$	(this study)
E. coli $Tlyci_{Bl}$	*E. coli* DH5α harboring $pTlyci_{Bl}$	(this study)
E. coli $Tlyci_{Ec}$-mev	*E. coli* DH5α harboring $pTlyci_{Ec}$ and pSSNSN	(this study)
E. coli $Tlyci_{Bl}$-mev	*E. coli* DH5α harboring $pTlyci_{Bl}$ and pSSNSN	(this study)
Plasmids	**Descriptions**	
pTrc99A	P*trc* expression vector, pBR322 origin, $lacI^q$, Amp^r, high copy	(Amersham Biosci.)
pTcrtEIB	pTrc99A with *crtE, crtI and crtB* genes from *E. herbicola*	(Zahiri et al. 2009)
$pTlyci_{Ec}$	pTcrtEIB with *idi* gene of *E. coli*	(this study)
$pTlyci_{Bl}$	pTcrtEIB with *idi* gene of *B. licheniformis*	(this study)
pSTV28	pACYC184 origin, P*lac* promoter, LacZα, Cm^r, low copy	(Takara Shuzo Co.)
pSSNSN	pSTV28 with *mvaA, mvaS, mvaK1, mvaD* and *mvaK2* genes of *S. pneumoniae*; *phbA* from *R. eutropha*	(Zahiri et al. 2006)

Primers	Sequences
idi_{Ec}-F:	5′-ATATCTAGAGGAGAGAAATTATGCAAACGG-3 ′(*XbaI*)
idi_{Ec}-R:	5′-ATAAAGCTTCAGGTCGACTCTAGTGCATGC-3′ (*HindIII*)
idi_{Bl}-F:	5′-ATATCTAGAGGAGATGATGGTGACGCGAG-3′ (*XbaI*)
idi_{Bl}-R:	5′-ATAAAGCTTCGGCTCAAGAGAAGCCGG-3′ (*HindIII*)

Quantification of lycopene content of cells

The lycopene content of recombinant *E. coli* strains were quantified as explained elsewhere (Zahiri et al. 2009). Briefly, cells in 1 ml of culture were harvested at 5,000 rpm for 5 min. The harvested cells were washed with water and then resuspended in 400 µl of methanol/chloroform (70:30 v/v) by vortexing for 5 min. This approach resulted in the extraction of lycopene by the solvent mixture. After centrifugation at 5,000 rpm for 5 min, the resulting pellet was extracted once more by 400 µl of fresh methanol/chloroform mixture. The two lycopene extracts were pooled together in a clean microfuge tube and the total volume was elevated up to 1 ml by the addition of fresh solvent mixture. The absorbance of the resulting extract was measured at 474 nm (OD_{474}) and converted to lycopene concentration (µg/ml) using a standard curve obtained by commercial lycopene (sigma).

Results

Evaluation of i_{Bl} for improving lycopene production in *E. coli*

Transformation of *E. coli* DH5α with pTcrtEIB, carrying *crt*E, *crt*B, and *crt*I genes of *Erwinia herbicola*, and the subsequent expression of the genes in transformed *E. coli* cells (*E. coli* Tlyc) resulted in lycopene production (Zahiri et al. 2009). As a result, the red colonies of *E. coli* Tlyc appeared on solid medium. *E. coli* Tlyc was cultured on 2YTG medium and lycopene production in this strain was studied. After 24 h, lycopene production was measured up to 143 mg/l with a maximum growth of 16.6 determined using $OD_{600\ nm}$.

The effect of the IDI of *B. licheniformis* on the lycopene production in *E. coli* was studied by the expression of the corresponding gene, i_{Bl}, in comparison with the overexpression of the native *idi* gene of *E. coli*, i_{Ec}, under the control of same promoter. A homology search for the *idi* genes in the genomes of *E. coli* and *B. licheniformis* resulted in the identification of two ORF of 549 and 1,050 bp long respectively. The genes were amplified from chromosomal DNA of these bacteria using specific primers and cloned in pTrc99A. The i_{Ec} had the same sequence as that previously submitted to GenBank (accession number: AF119715) (Wang et al. 1999). The sequence of the i_{Bl} was determined in this study and submitted to GenBank (accession number: HQ013323). The *idi* genes were then introduced in pTcrtEIB and the resulting plasmids designated as $pTlyci_{Ec}$ and $pTlyci_{Bl}$ were used for transformation of *E. coli* DH5α. In resulting lycopene producing *E. coli* $Tlyci_{Ec}$ and *E. coli* $Tlyci_{Bl}$ lycopene production was significantly improved to 288 and 352 mg/l respectively. Final growth of *E. coli* Tlyc, *E. coli* $Tlyci_{Ec}$ and *E. coli* $Tlyci_{Bc}$ were almost the same. The lycopene productions by these strains were calculated 33 ± 1, 68 ± 3, and 80 ± 9 mg/gDCW respectively (Fig. 2).

Effect of carbon metabolism on lycopene production by these strains was studied by adding various auxiliary carbons sources including fructose, mannose, arabinose, sodium citrate, and sodiumm acetate in 2YTG medium with glycerol. At 0.2% concentration, all carbon sources except citrate decreased lycopene production as well as growth of *E. coli* Tlyc. The lycopene production was decreased by fructose, mannose, arabinose, and sodium acetate to 8 ± 2, 22 ± 5, 24 ± 2, and 21 ± 1 mg/gDCW respectively. Growth (OD_{600}) was decreased by these carbon sources to 8.4 ± 1, 13.5 ± 1, 13.8 ± 0.2, 14 ± 0.2 respectively (Fig. 2). Citrate was the only carbon source which improved lycopene production to 47 ± 1 mg/gDCW. Growth of *E. coli* Tlyc was not significantly affected by the presence of citrate. In the case of *E. coli*

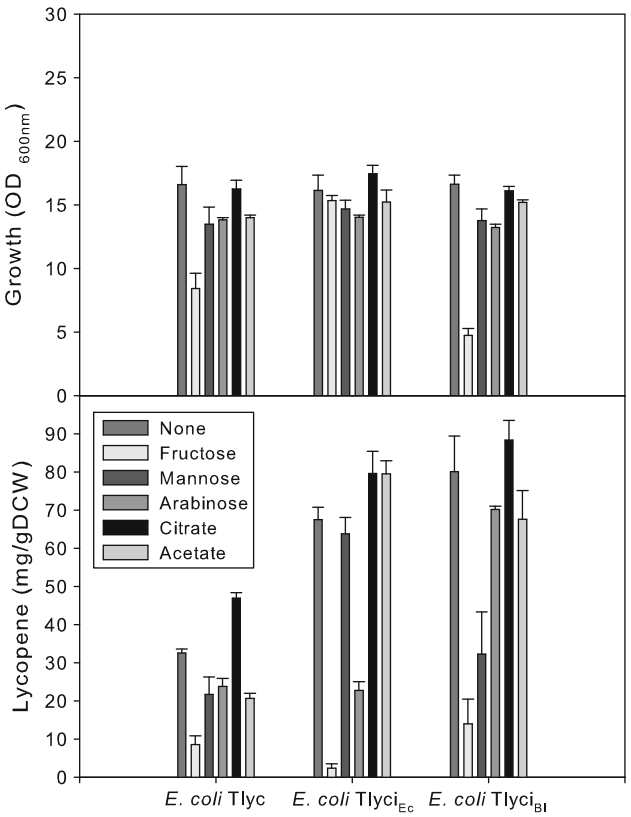

Fig. 2 Effects of the *idi* genes of *E. coli* (i_{Ec}) and *B. licheniformis* (i_{Bl}) on the lycopene production and growth of engineered *E. coli* strains. *E. coli* Tlyc harboring pTcrtEIB (pTrc99A with *crt* E, *crt* I, and *crt* B coding for lycopene production pathway), *E. coli* $Tlyci_{Ec}$ harboring $pTlyci_{Ec}$ (pTrc99A with *crt* E, *crt* I, *crt* B and i_{Ec}), and *E. coli* $Tlyci_{Bl}$ harboring $pTlyci_{Bl}$ (pTrc99A with *crt* E, *crt* I, *crt* B and i_{Bl}). The strains were grown on 2YTG medium supplemented with an auxiliary carbon source (0.2%)

Tlyci$_{Ec}$, lycopene production was severely inhibited by fructose and arabinose to 2 ± 1 and 23 ± 2 mg/gDCW respectively (Fig. 2). Using mannose, citrate, and acetate, the lycopene production level was up to 64 ± 4, 80 ± 6, and 79 ± 3 mg/gDCW respectively. Citrate showed a positive impact on both lycopene production and growth of *E. coli* Tlyci$_{Ec}$. An almost similar pattern was observed in the case of *E. coli* Tlyci$_{Bl}$. The inhibitory effect of fructose was severe, decreasing the lycopene production and growth (OD_{600}) to 14 ± 6 mg/gDCW and 4.7 ± 0.5 respectively (Fig. 2). Arabinose was less inhibiting on lycopene production of *E. coli* Tlyci$_{Bl}$ compared with that of *E. coli* Tlyci$_{Ec}$. Citrate improved the lycopene production up to 88 ± 0.5 mg/gDCW with no significant impact on growth.

Effects of all the carbon sources including fructose, mannose, arabinose, sodium citrate, and sodium acetate on lycopene production of *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$ were also studied at 0.5% concentration. The results were meaningfully corresponding with that at 0.2% concentration of the carbon sources (Fig. 3). Fructose at 0.5% concentration strongly inhibited the lycopene production in all strains. In the case of *E. coli* Tlyc, *E. coli* Tlyci$_{Bl}$ growth was also restricted at this concentration of fructose. However growth of *E. coli* Tlyci$_{Ec}$ was not affected. Mannose decreased the lycopene production by *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$ to 8 ± 2, 48 ± 2, and 18 ± 6 mg/gDCW respectively. Likewise, arabinose decreased the lycopene production by *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$ to 18 ± 2, 17 ± 1, and 50 ± 5 mg/gDCW respectively. Although acetate was a strong inhibitor of lycopene production in *E. coli* Tlyc, it was not a significant inhibitor in *E. coli* Tlyci$_{Bl}$ and even was improving the lycopene production in *E. coli* Tlyci$_{Ec}$. lycopene production in *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$ in the presence of 0.5% acetate was measured up to 9 ± 5, 91 ± 2, and 76 ± 10 mg/gDCW respectively. Citrate at 0.5% concentration increased the lycopene production in all the strains up to 45 ± 6, 94 ± 5, and 90 ± 2 mg/gDCW respectively (Fig. 3).

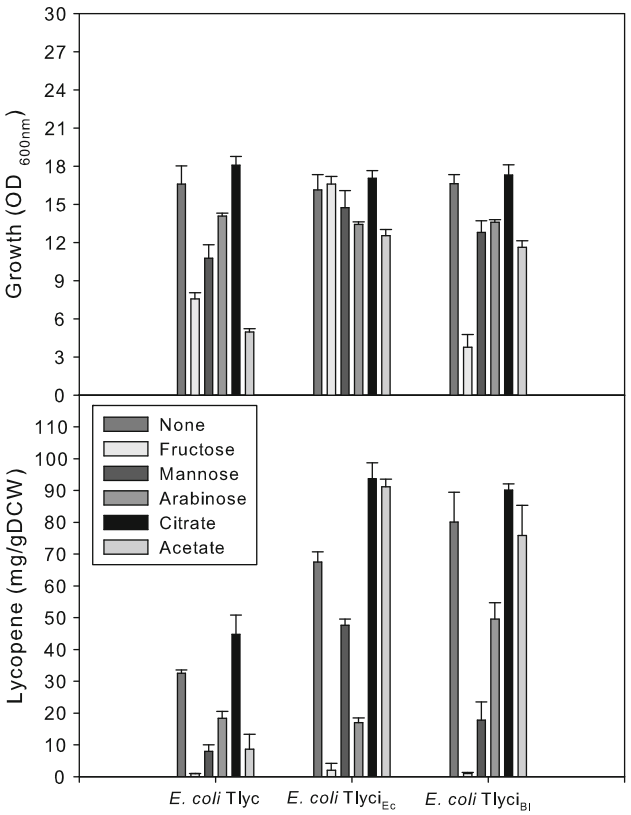

Fig. 3 Effects of auxiliary carbon sources on the lycopene production and growth of *E. coli* strains. Fructose, mannose, arabinose, citrate and acetate were each added at 0.5% concentration to 2YTG medium; and *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$ were grown on these media for lycopene production under otherwise the same condition

Evaluation of i_{Bl} for improving lycopene production in an *E. coli* strain with a heterologous mevalonate pathway

A heterologous mevalonate pathway was introduced in lycopene producing *E. coli* Tlyci$_{Ec}$ and *E. coli* Tlyci$_{Bl}$ strains using pSSNSN plasmid. The resulting strains, designated as *E. coli* Tlyci$_{Ec}$-mev and *E. coli* Tlyci$_{Bl}$-mev, were grown for lycopene production on 2YTG medium. The results showed that the *E. coli* Tlyci$_{Bl}$-mev produced more lycopene up to 181 ± 9 mg/gDCW while the lycopene production in *E. coli* Tlyci$_{Ec}$-mev was 132 ± 5 mg/gDCW (Fig. 4). Additional carbon sources including fructose, arabinose, citrate, and acetate were each used as an auxiliary carbon source in 2YTG medium to study the effect of carbon metabolism on lycopene production. At 0.2% concentration of sodium citrate, the lycopene production in *E. coli* Tlyci$_{Bl}$-mev and *E. coli* Tlyci$_{Ec}$-mev was increased up to 192 ± 5 and 140 ± 3 mg/gDCW respectively (Fig. 4). A similar effect of citrate was observed at 0.5% concentration resulting in a lycopene production of 198 ± 3 and 152 ± 3 mg/gDCW in *E. coli* Tlyci$_{Bl}$-mev and *E. coli* Tlyci$_{Ec}$-mev respectively (Fig. 5). In the case of *E. coli* Tlyci$_{Bl}$-mev, the addition of fructose, arabinose, and acetate at 0.2% concentration decreased lycopene production to 153 ± 10, 47 ± 9, and 67 ± 7 mg/gDCW respectively (Fig. 4). A similar trend was observed at 0.5% concentration of these carbon sources decreasing the lycopene production in *E. coli* Tlyci$_{BL}$-mev to 161 ± 17, 49 ± 3, and 55 ± 8 mg/gDCW respectively (Fig. 5). Fructose, arabinose, and acetate at 0.2% concentration decreased lycopene production in *E. coli* Tlyci$_{Ec}$-mev, to 138 ± 14, 4 ± 0.2, and 91 ± 1 mg/gDCW respectively (Fig. 4). At 5% concentration of these carbon sources, the

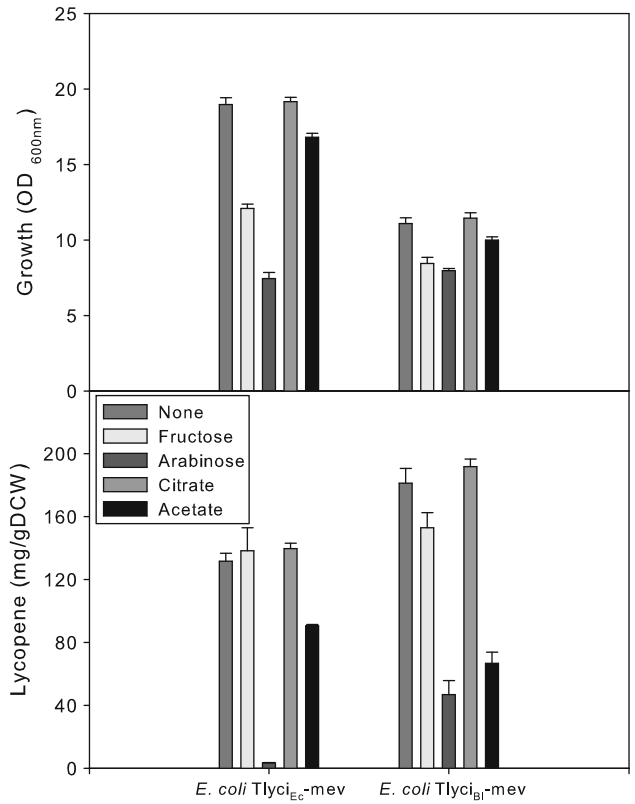

Fig. 4 Effects of i_{Ec} and i_{Bl} genes on the lycopene production and growth of engineered *E. coli* strains harboring heterologous lycopene and mevalonate pathways. Fructose, arabinose, citrate and acetate were each added at 0.2% concentration to 2YTG medium; and *E. coli* Tlyci$_{Ec}$-mev harboring both pTlyci$_{Ec}$ and pSSNSN, and *E. coli* Tlyci$_{Bl}$-mev harboring both pTlyci$_{Bl}$ and pSSNSN were grown on these media for lycopene production under otherwise the same condition

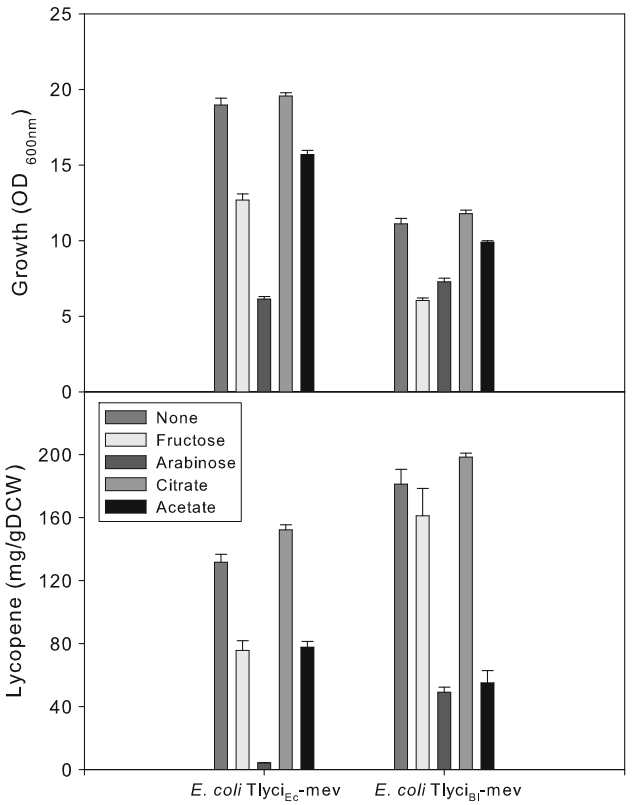

Fig. 5 Effects of i_{Ec} and i_{Bl} genes on the lycopene production and growth of engineered *E. coli* strains harboring heterologous lycopene and mevalonate pathways. Fructose, arabinose, citrate and acetate were each added at 0.5% concentration to 2YTG medium. *E. coli* Tlyci$_{Ec}$-mev harboring both pTlyci$_{Ec}$ and pSSNSN, and *E. coli* Tlyci$_{Bl}$-mev harboring both pTlyci$_{Bl}$ and pSSNSN were grown on these media for lycopene production under otherwise the same condition

lycopene production in *E. coli* Tlyci$_{Ec}$-mev decreased to 76 ± 6, 4 ± 0.1, and 78 ± 4 mg/gDCW respectively (Fig. 5).

Discussion

Escherichia coli is a non-carotenogenic bacterium that has been considered as a promising candidate for the production of carotenoids like lycopene and β-carotene. Because of some intrinsic advantages in terms of easy culture and known physiology as well as proneness to genetic manipulation, in several studies this bacterium has been used as a surrogate host for the expression of heterologous genes involved in isoprenoid biosynthesis (Cheng 2006; Ruther et al. 1997; Sandmann 2002; Yuan et al. 2006). Carotenogenic genes from bacteria, fungi, and plants may be functionally expressed in *E. coli*. In metabolic engineering attempts of researchers for isoprenoid production both the genes involved directly in the isoprenoid production and those involved in the IPP production have been tested. It is possible to introduce these genes simultaneously in *E. coli* using compatible plasmids as expression vectors.

In this study as a first report, the type 2 *idi* gene of *B. lichemiformis* coding for IPP isomerase was isolated and used for improving lycopene production in *E. coli*. The effect of the gene on lycopene accumulation in *E. coli* was evaluated in comparison with the overexpression of the native type 1 *idi* of *E. coli* under the control of the *trc* promoter of pTrc99A. Lycopene producing *E. coli* strains were created by transformation of this bacterium with *crt*E, *crt*I, and *crt*B genes directly involved in lycopene biosynthesis (*E. coli* Tlyc), *idi* involved in the interconversion of IPP to DMAPP (*E. coli* Tlyci$_{Ec}$ and *E. coli* Tlyci$_{Bl}$) and *mva*A, *mva*S, *mva*K1, *mva*D and *mva*K2, and *phb*A genes involved in IPP biosynthesis via mevalonate pathway (*E. coli* Tlyci$_{Ec}$-mev and *E. coli* Tlyci$_{Bl}$-mev).

It has been shown through a disruption experiment that *idi* is a nonessential gene in *E. coli* (Yoon et al. 2009). However, the overexpression of *idi* results in significant

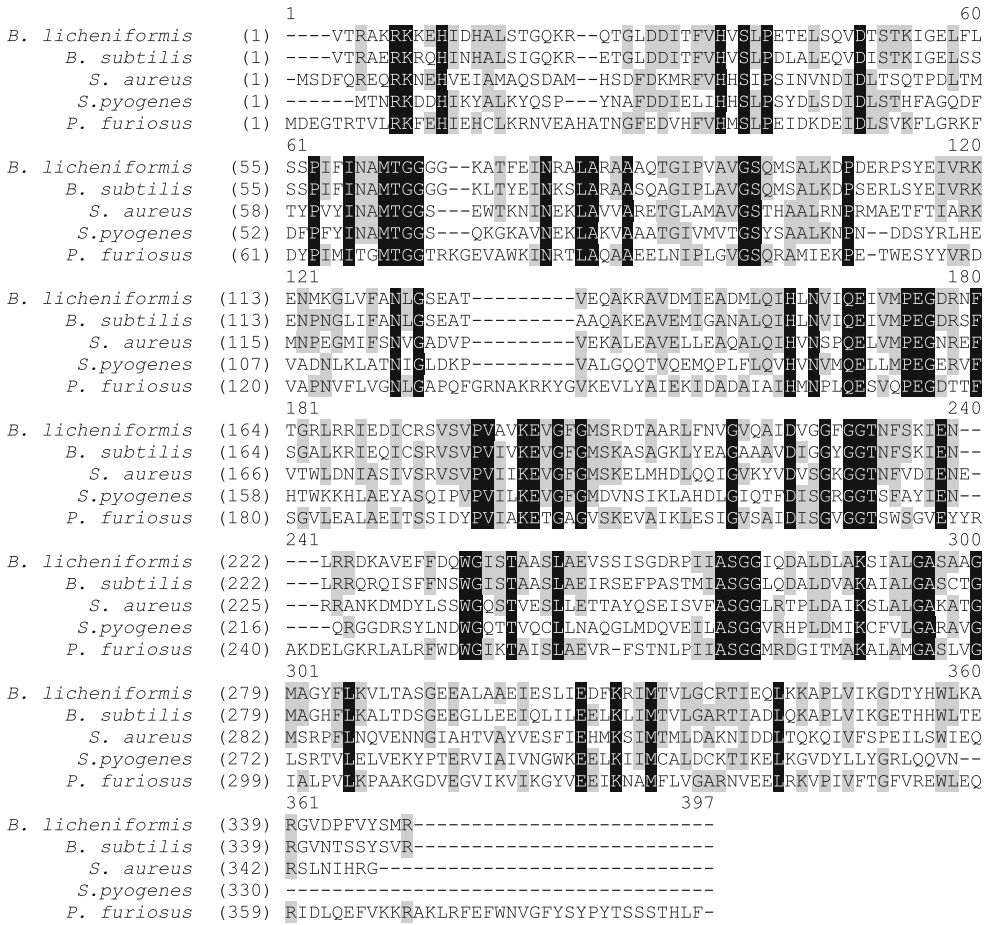

Fig. 6 Alignment of translated amino acid sequence of the *idi* of *B. licheniformis* with other type 2 *idi* genes of *B. subtilis, S. aureus*, *S. pyogenes*, and *P. furiosus*. Identical amino acids are shown in *black background* and the conserved residues are in *gray*

improvement of isoprenoid production in this bacterium (Kajiwara et al. 1997; Wang et al. 1999). Therefore, this gene has been considered as an essential player in several recent metabolic engineering attempts for isoprenoid production in *E. coli* (Hahn et al.1999; Yoon et al. 2007, 2006). Here, it was shown that both the overexpression of native *idi* and the expression of the orthologous *idi* of *B. lichemiformis* significantly increased lycopene production in transgenic *E. coli* Tlyci$_{Ec}$ and *E. coli* Tlyci$_{Bl}$ up to 2.1 and 2.5 times respectively. The *idi* gene of *E. coli* is 549 bp long and that of *B. lichemiformis* is 1,050 bp long. Similarity between the translated amino acid sequences of the genes was estimated around 6%. A genome search in databases revealed that the counterpart type 2 genes in *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pyrococcus furiosus* are 1,050, 1,050, 990 and 1,185 bp long. The multiple sequence alignment of the translated protein sequences of the genes with that of *B. lichemiformis* revealed similarities of 75%, 46%, 40%, and 34% respectively (Fig. 6). The conserved amino acids were characteristic of type 2 IDI enzymes (Dutoit et al. 2008). It had been suggested that a conserved cysteine in NxxCxHP consensus sequence and a conserved glutamate in Ex**E** consensus sequence make the active site of type 1 IDI (Hahn et al. 1996). As expected, these sequences are not conserved in *B. licheniformis, B. subtilis, S. aureus,* and *S. pyogenes.*

When different carbon sources including fructose, mannose, arabinose, citrate, and acetate were each used as an auxiliary substrate with glycerol interesting results were achieved. Just citrate was improving the lycopene production in *E. coli* Tlyc. Other carbon sources showed significant adverse impact on lycopene production and growth as well. However, the overexpression of the *idi* gene reversed the effect of acetate on lycopene production in *E. coli* Tlyci$_{Ec}$. In a similar way, the introduction of the *idi* gene in *E. coli* Tlyci$_{Bc}$ alleviated the inhibitory effect of acetate on lycopene production.

The introduction of a heterologous mevalonate pathway improved the lycopene production in both *E. coli* Tlyci$_{Bl}$-mev and *E. coli* Tlyci$_{Ec}$-mev up to 5.6 and 4 times, respectively, compared to the lycopene production in *E. coli* Tlyc. Except with citrate, feeding with other auxiliary carbon sources generally inhibited the lycopene production in both strains. Arabinose was the most severe inhibitor of lycopene production among all tested auxiliary carbon sources. Although fructose strongly inhibited the lycopene production in *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and

E. coli Tlyci$_{Bc}$, the inhibitory effect of this sugar was alleviated in both *E. coli* Tlyci$_{Ec}$-mev and *E. coli* Tlyci$_{Bl}$-mev.

Lee et al. studied the effects of glycerol and glucose as a sole carbon source on lycopene production (Lee et al. 2004). They showed that glucose imposed strong inhibition on the lycopene production in *E. coli*. Yoon et al. used glucose, galactose, xylose, maltose, or glycerol at 0.5% (w/v) as an auxiliary carbon source with 0.2% (w/v) arabinose in 2YT medium. All the carbon sources except glycerol inhibited β-carotene production in *E. coli* (Yoon et al. 2009). In this current study, fructose, mannose, arabinose, citrate, or acetate was added to culture medium with 0.5% glycerol (w/v). Fructose was found as the strongest inhibitor of lycopene production in *E. coli* Tlyc, *E. coli* Tlyci$_{Ec}$, and *E. coli* Tlyci$_{Bl}$. However, in the case of *E. coli* Tlyci$_{Ec}$-mev and *E. coli* Tlyci$_{Bl}$-mev harboring a heterologous mevalonate pathway, arabinose strongly inhibited the lycopene production. Accordingly, for metabolic engineering of *E. coli* for isoprenoid production it is advisable not to use the arabinose-inducible promoters. As mentioned by other researchers, the exact mechanism of carbon source inhibition has yet to be elucidated (Kim et al. 2010). In contrast, citrate was proved to be useful in increasing both the lycopene production and growth of all strains. It seems that manipulation of citrate cycle is an efficient approach to improve isoprenoid production in *E. coli*. By this study, it was shown that the type 2 IDI of *B. lichemiformis* is more efficient than the type 1 IDI of *E. coli*. In a previous study by Harada et al., the heterologous mevalonate pathway of *Streptomyces* sp. strain CL190 containing the type 2 IDI of this bacterium was used to improve lycopene production in *E. coli*. They suggested that the expression of the type 2 *idi* gene was very likely to be responsible for enhancing isoprenoid levels (Harada et al. 2009). In this current study we revisited this issue more carefully and it was confirmed that the type 2 IDI may perform more efficient than type 1 IDI for isoprenoid production.

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