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ORIGINAL RESEARCH PAPER

# Lycopene production in recombinant strains of *Escherichia coli* is improved by knockout of the central carbon metabolism gene coding for glucose-6-phosphate dehydrogenase

**Yan Zhou · Komi Nambou · Liujing Wei · Jingjing Cao · Tadayuki Imanaka · Qiang Hua**

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**Abstract** Genetic manipulation was undertaken in order to understand the mechanism involved in the heterologous synthesis of lycopene in *Escherichia coli*. Knockout of the central carbon metabolic gene *zwf* (glucose-6-phosphate dehydrogenase) resulted in the enhancement of lycopene production (above 130 % relative to control). The amplification and overexpression of rate-limiting steps encoded by *idi* (isopentenyl diphosphate isomerase), *dxs* (1-deoxyxylulose-5-phosphate synthase) and *ispDF* (4-diphosphocytidyl-2C-methyl-D-erythritol synthase and 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase) genes improved lycopene synthesis from 0.89 to 5.39 mg $g^{-1}$ DCW. The combination of central metabolic genes knockout with the amplification of MEP pathway genes yielded best amounts of lycopene (6.85–7.55 mg $g^{-1}$ DCW). Transcript profiling revealed that *idi* and *dxs* were up-regulated in the *zwf* knock-out strain, providing a plausible explanation for the increase in lycopene yield observed in this strain. An increase in precursor availability might also have contributed to the improved lycopene production.

**Keywords** *Escherichia coli* · Glucose-6-phosphate dehydrogenase · Lycopene · MEP pathway

## Introduction

Lycopene, a useful natural product that holds great commercial and health value, is widely used in cosmetic, food and pharmaceutical industries on account of its anti-oxidant, anti-inflammatory and anti-cancer properties (Mein et al. 2008; Bignotto et al. 2009; Erdman et al. 2009). Recent advances in biochemical engineering have provided a very promising route for the enhancement of the heterologous production of lycopene, even in non-carotenogenic microorganisms such as *Escherichia coli* which has been widely utilized as microbial cell factory for the synthesis of various carotenoids (Ye and Bhatia 2012; Albermann 2011). Lycopene building blocks, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), are naturally synthesized via MEP pathway in *E. coli* (Fig. 1) (Misawa et al. 1990). Among MEP pathway genes, *idi* (isopentenyl diphosphate isomerase), *dxs* (1-deoxyxylulose-5-phosphate synthase) *ispD* (4-diphosphocytidyl-2C-methyl-D-erythritol synthase) and *ispF* (2C-methyl-D-erythritol 2,4-cyclodiphosphate

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Y. Zhou · K. Nambou · L. Wei · J. Cao · T. Imanaka · Q. Hua (✉)
State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, People's Republic of China
e-mail: qhua@ecust.edu.cn

synthase) are key genes displaying a significant role in the isoprenoid flux (Yuan et al. 2006; Matthews and Wurtzel 2000; Kim and Keasling 2001). A number of works focused on the over-expression of various MEP pathway genes and aiming at improving isoprenoid accumulation (Kim and Keasling 2001; Martin et al. 2003; Matthews and Wurtzel 2000; Rodríguez-Villalón et al. 2008) was accomplished and synergistic or combinatorial effects were usually observed when two or more of these genes were over-expressed (Albrecht et al. 1999; Alper et al. 2005a, b; Reiling et al. 2004; Wang et al. 1999).

The metabolic engineering method of improving lycopene biosynthesis generally relies on the channelization of the carbon flux toward the targeted product. The central carbon metabolism supplies precursors such as glyceraldehyde-3-phosphate (G3P) and pyruvate to which is branched the isoprenoid pathway. The balance between these precursors was established by Farmer and Liao (2001) through the engineering of MEP pathway in order to efficiently redirect the carbon flux to lycopene production and high amount of lycopene was achieved (25 mg $g^{-1}$ DCW). Vadali et al. (2005), by inactivating many competing pathways originating from pyruvate and acetyl-coA nodes in their attempt of channeling the central carbon flux to lycopene, achieved lycopene content of 4.28 mg $g^{-1}$ DCW. Computational biology

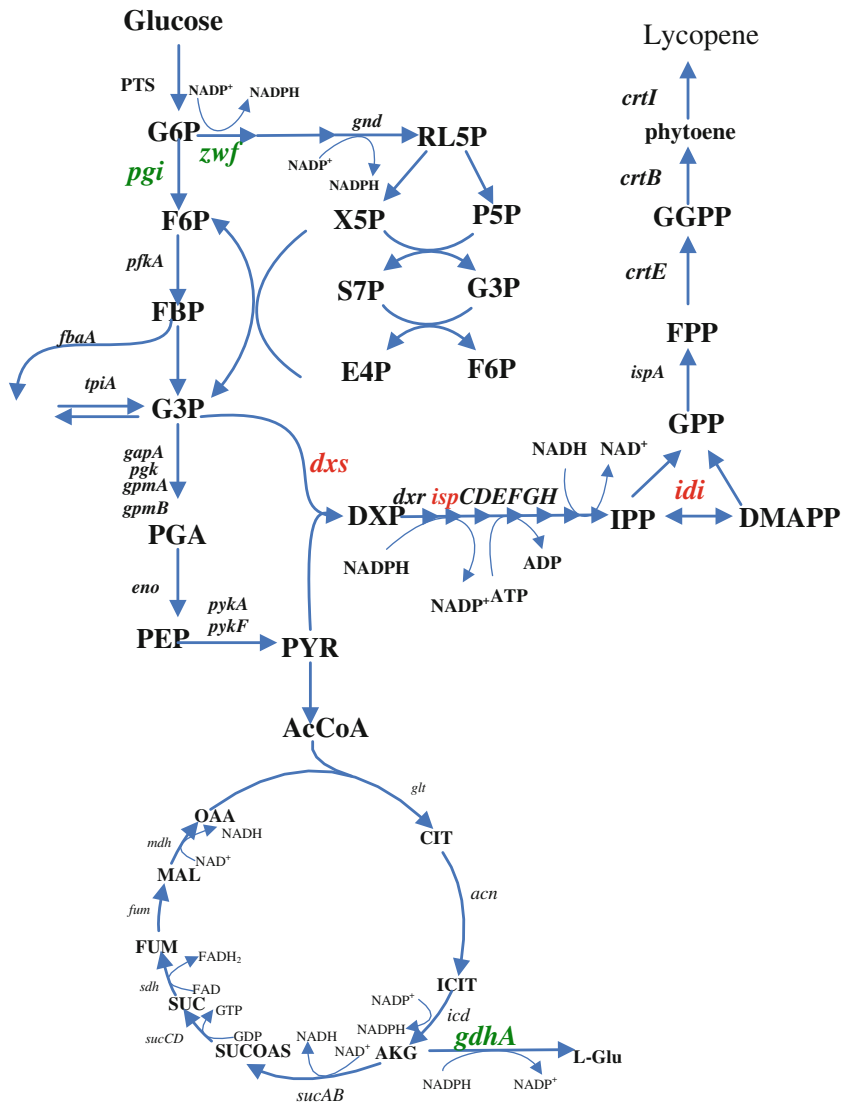


**Fig. 1** Simplified metabolic pathways of *E. coli* with the introduced lycopene synthesis pathway. For the lycopene pathway, the crt genes for lycopene production from FPP were provided on the low copy-number pAC-LYC plasmid. For the isoprenoid pathway, the *idi*, *dxs* and *ispDF* genes were amplified on pSE380-*idi-dxs-ispDF* plasmid. The *pgi*, *gdhA* and *zwf* genes were deficient, respectively

has also been shown to be a useful tool in gene targets identification. With strains obtained through the in silico identification of gene knockout targets and the gene amplification targets search, high amounts of lycopene content were attained (Alper et al. 2005a, b; Choi et al. 2010; Jin and Stephanopoulos 2007). In spite of this progress, a lot remains to be done because lycopene yields through the MEP pathway scarcely reach theoretical maxima predicted for the pathway (Rude and Schirmer 2009). Above all, the effects of central carbon metabolism modifications on lycopene production need to be further investigated.

Therefore, the present study is aimed at examining the effect of central carbon metabolism on lycopene production in *E. coli* by exploring the impact of the deficiency of central metabolic genes, namely *pgi* (glucosephosphate isomerase), *gdhA* (glutamate dehydrogenase) and *zwf* (glucose-6-phosphate dehydrogenase) and the overexpression of primary metabolic genes (*idi*, *dxs* and *ispDF*) on lycopene metabolism. Analogously, an insight of the transcriptional profile will be achieved by quantitative real-time polymerase chain reaction (qRT-PCR).

## Materials and methods

### Bacterial strains and plasmids construction

*Escherichia coli* BW25113 and its derivatives were employed as hosts for lycopene production. Mutant strains JW3985, JW1750 and JW1841 were, respectively, derived from strain BW25113 by the knockout of *pgi* (glucosephosphate isomerase), *gdhA* (glutamate dehydrogenase) or *zwf* (glucose-6-phosphate dehydrogenase) genes according to the protocol established by Datsenko and Wanner (2000). These strains were purchased from the Keio collection and were kanamycin resistant. All strains and plasmids used are listed in Supplementary Table 1. Mutations were confirmed by polymerase chain reaction (PCR) performed with confirmation primers (Supplementary Table 2) and the results are shown on Supplementary Fig 1. Plasmid pAC-LYC containing *crtE* (geranyl-geranyl pyrophosphate synthase), *crtB* (phytoene synthase) and *crtI* (phytoene desaturase) genes from *Erwinia herbicola* (Cunningham et al. 1994) was introduced into strains BW25113, JW3985, JW1750 and JW1841 to obtain strains BWL, BPL, BGL and BZL, respectively. The *idi* (isopentenyl diphosphate isomerase), *dxs* (1-deoxyxylulose-5-phosphate synthase) *ispD* (4-diphosphocytidyl-2C-methyl-D-erythritol synthase) and *ispF* (2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase) genes isolated from *E. coli* JM109 by PCR were cloned into vector pSE380 (Invitrogen) carrying the strong IPTG-inducible *trc* promoter. These genes were designed to have the SD sequences (AGGAG) and a spacer (8 bp) before ATG by engineering this sequence into the PCR primers. The *idi* gene was amplified by using primers *idi*-F and *idi*-R (Supplementary Fig. 2), JM109 genome and *pfu* DNA polymerase (Fermentas). The obtained PCR fragment was digested by *Xho* I and *Sal* I then isolated and ligated to plasmid pSE380 to form plasmid pSE380-*idi* (Genbank: KF361311). Similarly, plasmids pSE380-*idi-dxs* (Genbank: KF361312) and pSE380-*idi-dxs-ispDF* (Genbank: KF361313) were constructed by introducing successively *dxs* (using primers *dxs*-F and *dxs*-R) and *ispDF* (using primers *dxs ispDF*-F and *ispDF*-R) genes into plasmid pSE380-*idi*. PCR verification of plasmid pSE380-*idi-dxs-ispDF* is shown on Supplementary Fig. 2. These plasmids were sequenced after each gene ligation. Plasmids pSE380, pSE380-*idi*, pSE380-*idi-dxs* and pSE380-*idi-dxs-ispDF* were separately introduced into strain BWL and formed strains BWL-PA, BWL-PB, BWL-PC and BWL-PD, respectively. The transformation of pSE380-*idi-dxs-ispDF* into strains BPL, BGL and BZL gave respectively strains BPL-PD, BGL-PD and BZL-PD.

### Culture conditions and analytical methods

Lycopene fermentation was performed in triplicate in 250 ml flasks containing 50 ml of Luria–Bertani (LB) medium (10 g tryptone $l^{-1}$, 5 g yeast extract $l^{-1}$ and 10 g NaCl $l^{-1}$) inoculated with an overnight cultivated preculture (1 % v/v) under agitation conditions for 24 h. The culture medium was supplemented with appropriate antibiotics (100 mg ampicillin $l^{-1}$, 25 mg chloramphenicol $l^{-1}$ and 50 mg kanamycin $l^{-1}$). No inducer was added in the medium.

DCW was calculated by the method established by Sauer et al. (1999). Cell growth during the cultivations was monitored by measuring the optical density at 600 nm (OD600). For dry cell weight (DCW) determination in selected cases, a known volume of fermentation broth was centrifuged for 10 min in pre-weighed test tubes at 4 °C and 3,000×*g*, washed once with water, and dried for 24 h

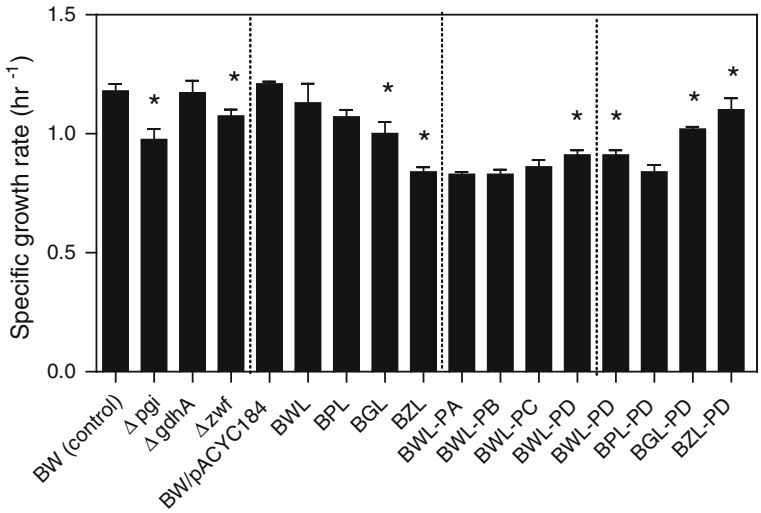


**Fig. 2** Specific growth rate of different strains. Recombinant *E. coli* was cultured at 37 °C, 220 rpm. BW 25113 was used as control. Data represent the average of three replicates and *error bars* represent standard deviation. *Asterisk* indicates statistically significant results ($p < 0.05$). BW, BW25113; Δ*pgi*, BW25113 Δ*pgi::kan*; Δ*gdhA*, BW25113 Δ*gdhA::kan*; Δ*zwf*, BW25113 Δ*zwf::kan*; BW/pACYC184, BW25113 containing plasmid pACYC184; BWL, BW25113 containing plasmid pACLYC; BPL, BW25113 Δ*pgi::kan* containing plasmid pACLYC; BGL, BW25113 Δ*gdhA::kan* containing plasmid pACLYC; BZL, BW25113 Δ*zwf::kan* containing plasmid pACLYC; BWL-PA, BW25113 containing plasmid pACLYC and plasmid pSE380; BWL-PB, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi*; BWL-PC, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi-dxs*; BWL-PD, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BPL-PD, BW25113 Δ*pgi::kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BGL-PD, BW25113 Δ*gdhA::-kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BZL-PD, BW25113 Δ*zwf::kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*

at 90 °C to a constant weight. The exponential growth phase was identified and the specific growth rate was determined as described by Sauer et al. (1999).

Lycopene was extracted from cells pellets (1 ml fermented broth) with acetone (0.5 ml) at 55 °C for 15 min under agitation conditions (Martinez et al. 2008), and its concentration was determined using a HPLC system (SHIMADZU) with a 5 μm column (Agilent) eluted with a solution of acetonitrile/methanol/isopropanol (80:15:5, by vol.) at 1 ml $min^{-1}$. The detection was realized at 472 nm (Kim and Keasling 2001). Lycopene (Sigma-Aldrich), dissolved in acetone, was used to generate the standard curve.

### Transcription profiles determined by quantitative real time polymerase chain reaction (qRT-PCR)

The qRT-PCR experiments were performed after 12 h of cultivation. Transcriptional expression levels of genes were detected by qRT-PCR of mRNA isolated from appropriate strains. Total RNA was extracted with a TRIzol reagent (Life Technologies) and the cDNA was amplified using a ReverTra Ace qPCR RT Kit (TOYOBO). The qRT-PCR experiment was carried out on a CFX96 system (Bio-Rad) using the FastStart Universal SYBR Green Master (Roche). The 16S rRNA gene was used as internal standard for the determination of the expression level of each gene from qRT-PCR values. The fold change in expression was calculated by $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

### Statistical analysis

All data were analyzed by two-way ANOVA with statistical significance fixed for $p < 0.05$ for cell growth and lycopene synthesis and for $p < 0.01$ for transcription analysis.

## Results and discussion

### Effect of central metabolic gene deletions on cell growth and lycopene production by mutant strains

To assess the effect of *pgi* (glucose phosphate isomerase), *gdhA* (glutamate dehydrogenase) and *zwf*

(glucose-6-phosphate dehydrogenase) knockouts on the cell growth, the specific growth rate was measured. Figure 2 shows that the *ΔgdhA* strain growth behavior was similar to that of the wild type strain while Δ*pgi* and Δ*zwf* strains exhibited significantly decreased specific growth rate with more pronounced effect for Δ*pgi* strain. The introduction of plasmid pAC-LYC into BW25113 and the above knockout strains adversely affected the specific growth rate with moderate effect for pAC-LYC introduction and relatively accentuated impact for *zwf*, *gdhA* and pgi knockouts in this decreasing order. Only *zwf* and *gdhA* knockouts showed significant effect compared to the control strain (BW25113 transformed by the empty vector pACYC184) which shared almost similar growth behavior with the wild type strain. The introduction of plasmid pAC-LYC was translated by a slight increase of the specific growth rate for Δ*pgi* strain while Δ*zwf* and *ΔgdhA* strains, transformed by this plasmid, showed further decrease in specific growth rate. This decrease in the specific growth rate can be attributed to either lycopene accumulation in cells membrane (Alper et al. 2005a, b; Kim et al. 2009) or genes' deletion as it was reported in the case of *zwf* (Lim et al. 2002; Zhao et al. 2004a, b).

As depicted on Fig. 3, the deletion of *zwf* (strain BZL) augmented lycopene content up to 2.52 mg $g^{-1}$ DCW (more than 130 % of enhancement) relative to strain BWL harboring only the pAC-LYC plasmid (1.09 mg $g^{-1}$ DCW).

GAPDH, encoded by *zwf,* controls the entry of carbon into the pentose phosphate pathway. The deletion of *zwf* does not significantly affect growth rates on glucose or pyruvate but flux analysis demonstrated that a change in cellular metabolism substrate uptake rates and $CO_2$ production were found to be higher on glucose but lower on pyruvate while the TCA pathway flux increases on glucose but decreases on pyruvate (Zhao et al. 2004b). The knockout of *zwf* results in higher substrate uptake rates and $CO_2$ production on glucose and lower of both parameters are found on acetate (Zhao et al. 2004a). Flux analysis demonstrated that this could be attributed to higher flux through TCA to replace anabolic reducing equivalents normally provided by oxidative pentose phosphate pathway. Growth on acetate shows lower TCA flux and increased glyoxylate shunt. The *zwf* knockout results in increased glycolytic flux given that more than 98.9 % of the total carbon flux go through

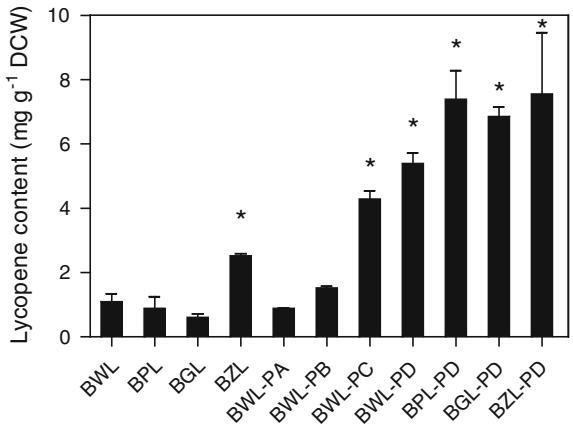


**Fig. 3** Lycopene production of different strains. Lycopene contents were measured after 24 h cultivation. Recombinant *E. coli* was cultured at 37 °C, 220 rpm. Data represent the average of three replicates and *error bars* represent standard deviation. *Asterisk* indicates statistically significant results ($p < 0.05$). BWL, BW25113 containing plasmid pACLYC; BPL, BW25113 Δ*pgi::kan* containing plasmid pACLYC; BGL, BW25113 Δ*gdhA::kan* containing plasmid pACLYC; BZL, BW25113 Δ*zwf::kan* containing plasmid pACLYC; BWL-PA, BW25113 containing plasmid pACLYC and plasmid pSE380; BWL-PB, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi*; BWL-PC, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi-dxs*; BWL-PD, BW25113 containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BPL-PD, BW25113 Δ*pgi::kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BGL-PD, BW25113 Δ*gdhA::-kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*; BZL-PD, BW25113 Δ*zwf::kan* containing plasmid pACLYC and plasmid pSE380-*idi-dxs-ispDF*

the EMP pathway (Zhao et al. 2004b). Hua et al. (2003) similarly revealed that the deficiency of *zwf* gene increased fluxes of the EMP pathway. In the present study, the improvement of lycopene content after *zwf* deletion is probably due to the channelization of the carbon flux toward the Embden–Meyerhof–Parnas (EMP) pathway, conducting possibly to the increase of lycopene indirect (glyceraldehyde-3-phosphate (G3P) and pyruvate) (Farmer and Liao 2001) and direct (isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)) precursors' amount. The *Zwf* protein is essential for the synthesis of the energy producing cofactor NADPH, but it was found that *zwf* is down regulated in lycopene overproducing strains of *E.coli* (Mickus 2009) suggesting that the deletion of *zwf* may be helpful for yielding high amount of lycopene and this is in agreement with our results.

Strains deprived of *pgi* or *gdhA* genes led to a decrease in lycopene content though these values were not significantly different from the amount produced by strain BWL. These results are explicable by the fact that *pgi* deficiency blocks in chain the carbon flux toward G3P and pyruvate accumulation whilst the deletion of *gdhA* seems to guide the flux toward the citric acid cycle, then limiting lycopene synthesis because of G3P and pyruvate limitation. Differences in lycopene content between strains are the result of central metabolism genes' knockouts, proving the existence of a direct connection between the central metabolism and the secondary metabolism of lycopene. This argument is in conformity with the work of Farmer and Liao (2001) who found that alterations in the central metabolism that allow G3P accumulation improve lycopene production, whereas shifts that direct carbon flux far-off the G3P pool deteriorate lycopene biosynthesis.

To further understand the regulation of above genes on lycopene production, expression profiles were determined by qRT-PCR. Figure 4a shows that the deletion of *pgi* and *gdhA* did not affect the expression of *idi* (isopentenyl diphosphate isomerase) and *dxs* (1-deoxyxylulose-5-phosphate synthase) genes while the deletion of *zwf* slightly increased the expression level of these two genes, relatively to strain BWL. On the contrary, the expression level of the secondary metabolic gene *crtE* (geranylgeranyl pyrophosphate synthase) almost remained invariant and seemed to be invulnerable to the knockout of the three genes. According to the obtained result, two antagonist but equally evocative arguments can be drawn. The first one stipulates that the deletion of the *zwf* gene strengthens the DXP pathway through the overexpression of the *idi* and *dxs* genes, conducting to an increase of lycopene content. The second one supports the idea that the knockout of either *pgi* or *gdhA* genes represses the expression of *idi* and *dxs* genes and subsequently results in the lessening of lycopene production. The results of the present work demonstrated the utmost role played by the DXP pathway and the effect of the *zwf* gene on its efficiency in lycopene production. In *E. coli*, the low expression levels of genes of this native pathway could probably be bottlenecks in the process of lycopene production. The reinforcement of the DXP pathway through genetic manipulation and the introduction of external

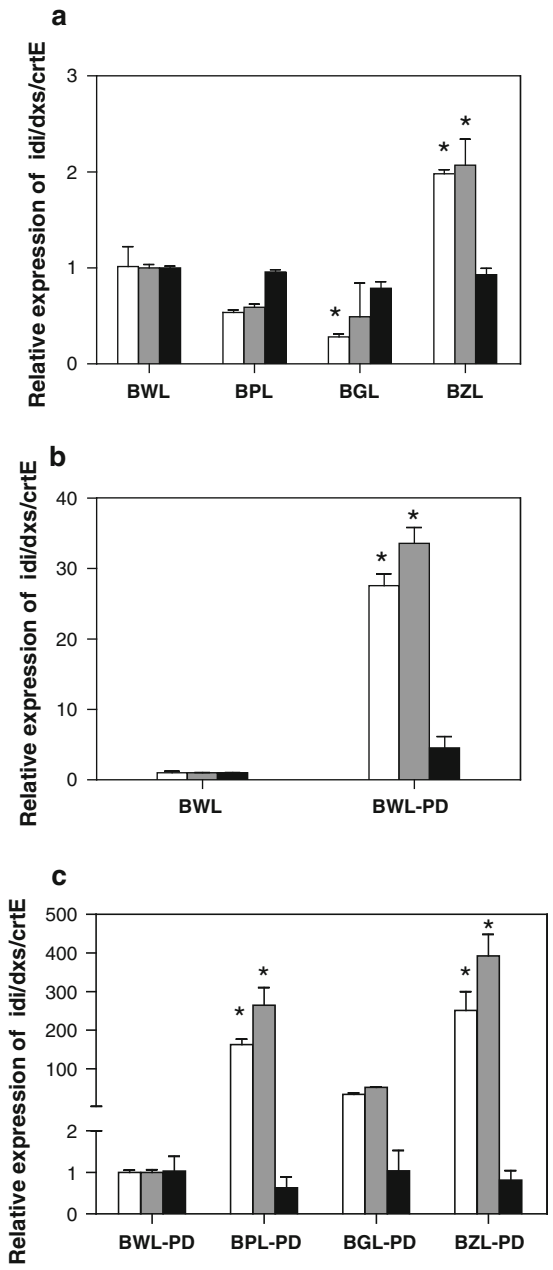


**Fig. 4** Relative expression levels of *idi*, *dxs* and *crtE* genes of different strains. *idi* (*white bar*), *dxs* (*gray bar*) and *crtE* (*black bar*). Gene relative expression levels were measured after 12 h cultivation. Recombinant *E. coli* was cultured at 37 °C, 220 rpm. Data represent the average of three replicates and *error bars* represent standard deviation. *Asterisk* indicates statistically significant results ($p < 0.01$). **a** The levels of *idi*, *dxs* and *crtE* genes in strain BWL were determined as absolute quantitative value (100 %). **b** The levels of *idi*, *dxs* and *crtE* genes in strain BWL were determined as absolute quantitative value (100 %). **c** The levels of *idi*, *dxs* and *crtE* genes in strain BWL-PD were determined as absolute quantitative value (100 %)

genes could increase the pool of lycopene precursors and ameliorate lycopene synthesis in *E. coli*.

## Effect of strain transformation by amplified DXP pathway genes on cell growth and lycopene production

In order to alleviate the limited expression levels of *idi*, *dxs*, *ispD* (4-diphosphocytidyl-2C-methyl-D-erythritol synthase) and *ispF* (2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase) genes in the process of lycopene synthesis, plasmids pSE380-*idi*, pSE380-*idi-dxs* and pSE380-*idi-dxs-ispDF* were engineered.

Figure 2 presents the cell growth behavior of transformants. The introduction of each new gene of the DXP pathway resulted in an increase of the specific growth rate. Only strain BWL-PD exhibited significant specific growth rate increase.

Lycopene production of engineered strains is illustrated on Fig. 3. Over-expression of the *idi* gene (strain BWL-PB) resulted in lycopene content of 1.52 mg $g^{-1}$ DCW, corresponding to an improvement of 70.8 % in comparison with strain BWL-PA. Likewise, a notable augmentation of lycopene production was observed when *idi* and *dxs* genes on one hand, and *idi*, *dxs* and *ispDF* genes on the other hand, were co-over-expressed in the cell. With plasmid pSE380-*idi-dxs* (strain BWL-PC), lycopene content of 4.29 mg $g^{-1}$ DCW (182.2 % of enhancement relatively to strain BWL-PB) was attained while plasmid pSE380-*idi-dxs-ispDF* (strain BWL-PD) produced 5.39 mg $g^{-1}$ DCW (25.6 % of enhancement comparatively to strain BWL-PC) of lycopene content. It is clear that each of these genes plays an important role in the lycopene production with cumulative effect. The two genes *idi* and *dxs* seem to be pivotal genes governing the synthesis of lycopene. The *ispDF* genes similarly contribute in the total amount of produced lycopene. It was reported that the coamplification of *idi* with other genes of the primary metabolism resulted in lycopene content of 9.98 mg $g^{-1}$ DCW (Choi et al. 2010). Kim and Keasling (2001) published that the introduction of *dxs* gene in arabinose or isopropyl-β-D-thiogalactopyranoside (IPTG) induced *E. coli* enhanced lycopene synthesis and some of the obtained values of lycopene content were close to those of our results although we did not use inducers. Parallel to this, lycopene content reached 45 mg $g^{-1}$ DCW when *dxs* gene was amplified simultaneously with *gps* (geranylgeranyl diphosphate (GGPP) synthase) mutant (Wang et al. 2000).

Figure 4b indicates that the transformation of cells by operon *idi-dxs-ispDF* was accompanied by the overexpression of *idi* and *dxs* (22.6- and 33.6-fold, respectively) which is probably at the origin of the enhanced production of lycopene, since IDI and DXS are the key rate-limiting steps of the DXP pathway (Sitthithaworn et al. 2010; Leonard et al. 2010). The overexpression of these genes could help to resolve the problem linked to their limited expression (Yuan et al. 2006) and subsequently permit lycopene overproduction. This idea is corroborated by a previous work claiming that the increase in plasmid copy number and promoter strengthening can modulate the expression of DXP pathway and lead to better taxadiene content (Ajikumar et al. 2010). In other studies, the overexpression of genes of the DXP pathway such as idi and dxs was found to be similarly increasing the isoprenoid production (Kajiwara et al. 1997; Matthews and Wurtzel 2000).

## Combination of operon *idi-dxs-ispDF* with different gene deletions

The growth characteristics and lycopene content of genes disrupted strains transformed with pSE380-*idi-dxs-ispDF* are illustrated on Fig. 2. Comparatively to strain BWL-PD, the specific growth rate of strains BGL-PD and BZL-PD were upgraded while the specific growth rate of BPL-PD slashed. Strain BZL-PD exhibited the best growth property (specific growth rate about 1.13 $h^{-1}$) and almost recovered the growth behavior of the parent strain BW25113 (1.18 $h^{-1}$). Lycopene content of strains BZL-PD (7.55 mg $g^{-1}$ DCW) and BPL-PD (7.39 mg $g^{-1}$ DCW) were slightly equal but were higher than that of BGL-PD (6.85 mg $g^{-1}$ DCW) and BWL-PD (5.39 mg $g^{-1}$ DCW). Considerable amount of lycopene was produced with more positive effect, even in strains BPL-PD and BGL-PD, instead of simply cumulating the positive and negative effects. The highest lycopene content (7.55 mg $g^{-1}$ DCW) was obtained with strain BZL-PD in which the knockout of *zwf*, along with the coamplification of *idi*, *dxs* and *ispDF* genes, may route the carbon flux into the pool of the DXP pathway to finally result in elevated amount of lycopene. Our result is consistent with preceding work reporting that the *gdhA* knockout together with the overexpression of

*idi*, *dxs* and *ispDF* genes increased lycopene production by 13 % (Alper et al. 2005a).

Figure 4c illustrates that after the combination of gene deletions with operon *idi-dxs-ispDF*, *idi* and *dxs* genes were exceedingly upregulated while *crtE* gene expression levels almost remained unchanged. In strains BPL-PD and BZL-PD, relative expression levels of *idi* and *dxs* were above 100-fold than that in strain BWL-PD. Relative expression levels of *idi* and *dxs* of strain BGL-PD were 33.54- and 51.87-fold respectively than that in strain BWL-PD.

## Conclusion

In this study, we demonstrated that through the knockout of central metabolic genes, and the overexpression of certain genes of the DXP pathway, the enhancive production of lycopene was attainable. Furthermore, we found that both central metabolic genes and primary metabolic genes are both governing the lycopene production either by individual effect or by a sequence of multiple metabolic interactions. The strain BZL-PD engineered in this study achieved the highest lycopene content and displayed an excellent growth behavior. These results are very promising and could enable more economical industrial production of lycopene. Concisely, comprehensive data describing the metabolism of lycopene production in *E. coli*, from genomic, transcriptional and metabolic point of view were achieved through the present study. We thank one of the reviewers for his/her valuable comments.

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