Biotechnol Lett
DOI 10.1007/s10529-015-1922-1

ORIGINAL RESEARCH PAPER

Putative carotenoid genes expressed under the regulation of Shine–Dalgarno regions in *Escherichia coli* for efficient lycopene production

Weiyue Jin · Xian Xu · Ling Jiang · Zhidong Zhang · Shuang Li · He Huang

Received: 11 June 2015 / Accepted: 13 July 2015

Abstract
Objectives Putative genes *crtE*, *crtB*, and *crtI* from *Deinococcus wulumiqiensis* R12, a novel species, were identified by genome mining and were co-expressed using the optimized Shine-Dalgarno (SD) regions to improve lycopene yield.
Results A lycopene biosynthesis pathway was constructed by co-expressing these three genes in *Escherichia coli*. After optimizing the upstream SD regions and the culture medium, the recombinant strain EDW11 produced 88 mg lycopene g^{-1} dry cell wt (780 mg lycopene l^{-1}) after 40 h fermentation without IPTG induction, while the strain EDW without optimized SD regions only produced 49 mg lycopene g^{-1} dry cell wt (417 mg lycopene l^{-1}).
Conclusion Based on the optimization of the upstream SD regions and culture medium, the yield of the strain EDW11 reached a high level during microbial lycopene production until now.

Keywords Carotenoid genes · *Deinococcus wulumiqiensis* R12 · *Escherichia coli* · Lycopene · Regulation · Shine-Dalgarno regions

Weiyue Jin and Xian Xu have contributed to the work equally and should be regarded as co-first authors.

Electronic supplementary material The online version of this article (doi:10.1007/s10529-015-1922-1) contains supplementary material, which is available to authorized users.

W. Jin · X. Xu · S. Li · H. Huang (✉)
State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, People's Republic of China
e-mail: biotech@njtech.edu.cn

W. Jin
e-mail: aileenfish2013@njtech.edu.cn

X. Xu
e-mail: xuxian@njtech.edu.cn

S. Li
e-mail: lishuang@njtech.edu.cn

L. Jiang
College of Food Science and Light Industry, Nanjing Tech University, Nanjing, People's Republic of China
e-mail: jiangling@njtech.edu.cn

Z. Zhang
Institute of Microbiology, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang Uigur Autonomous Region, People's Republic of China
e-mail: zhangzheedong@sohu.com

H. Huang
School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing, People's Republic of China

Published online: 08 August 2015

Introduction

Lycopene is a C_{40} carotenoid with efficient antioxidant properties that is widely used in the food, pharmaceutical, nutraceutical, and cosmetic industries (Kong et al. 2010). In nature, there are two lycopene biosynthesis pathways: the mevalonate (MVA) pathway, and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Zhao et al. 2013). In the MEP pathway, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are converted to farnesyl pyrophosphate (FPP) by FPP synthase, which is encoded by *ispA* in *Escherichia coli*. Lycopene is then synthesized from FPP by three key enzymes geranylgeranyl diphosphate synthase, phytoene synthase, and phytoene desaturase, which are encoded by *crtE*, *crtB*, and *crtI*, respectively (Fig. 1).

Considerable efforts have been made to improve lycopene production in non-carotenogenic bacteria and yeasts through metabolic engineering using heterologous functional genes cloned from pigmented bacteria (Zhou et al. 2013; Sun et al. 2014). Lycopene biosynthesis genes from microorganisms, such as *Pantoea ananatis*, *Pantoea agglomerans*, and *Brevibacterium linens,* have been co-expressed in recombinant strains and good lycopene yields were obtained (Kim et al. 2010; Yoon et al. 2007; Zhao et al. 2013). Investigation of the promoter regions upstream of the genes in plasmids may also help increase the yield of lycopene (Bahieldin et al. 2014). With the increasing numbers of microorganisms being discovered, there are greater opportunities for investigating organisms with special carotenoid biosynthesis capacities, and to identify novel carotenoid biosynthesis genes.

In this study, three putative carotenoid genes were identified by genome mining of a red-pigmented strain, *Deinococcus wulumuqiensis* R12, which was characterized as a new member of the genus *Deinococcus*. This strain has extremely powerful antioxidative capabilities, which are provided by its specific and major carotenoid, deinoxanthin (Wang et al. 2010; Xu et al. 2013; Tian et al. 2007). A polycistronic plasmid harboring the putative lycopene biosynthesis genes (*crtEBI*) was constructed to produce lycopene. To improve lycopene production, optimized combinations of the Shine-Dalgarno (SD) sequence and the aligned spacing (AS) sequence, which is located between the SD sequence and the initial translation codon, were incorporated upstream of the lycopene biosynthesis genes. IPTG induction dosages and culture media were also experimentally investigated.

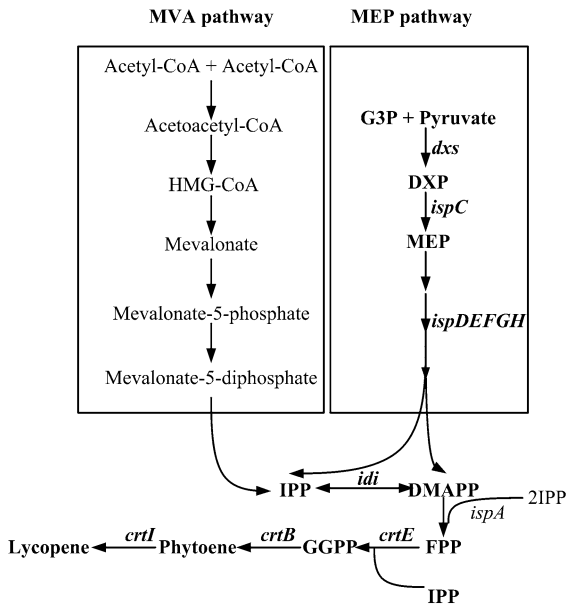

Fig. 1 Two lycopene biosynthesis pathways in natural environment. The MVA pathway exists in all eukaryotic cells, the cytoplasm and mitochondria of plants. The MEP pathway exists in bacteria, other prokaryotes and plastids in plants. G3P: glyceraldehyde 3-phosphate, DXP: 1-deoxy-D-xylulose 5-phosphate, MEP: 2C-methyl-D-erythritol 4-phosphate, IPP: isopentenyl diphosphate, DMAPP: dimethylallyl diphosphate, FPP: farnesyl diphosphate, GGPP geranylgeranyl diphosphate

Materials and methods

Genome mining and bioinformatic analysis of carotenoid genes from *D. wulumuqiensis* R12

The genome of *D. wulumuqiensis* R12 was examined by BLAST analysis in GenBank using carotenoid genes from the strain *Deinococcus radiodurans* R1 (Pennacchio et al. 2008) to identify possible homologs in the *D. wulumuqiensis* R12 genome. Multiple sequence alignment was conducted using CLUSTALX (Chenna et al. 2003). The theoretical isoelectric point and molecular weight were calculated for each corresponding protein using the Compute pI/Mw tool (http://us.expasy.org/tools/pi_tool.html). SignalP (http://www.cbs.dtu.dk/services/SignalP-1.1/) was used to predict the signal peptide for each of the three

enzymes. The transmembrane prediction program TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) was used to identify transmembrane regions.

Accession numbers of nucleotide sequences

The nucleotide sequences of the three genes were deposited in GenBank as follows orf01490 (*crtE*), GenBank accession no. KP319019; orf00123 (*crtB*), GenBank accession no. KP319020; and orf00124 (*crtI*), GenBank accession no. KP319021.

Bacterial strains, plasmids, and growth conditions

Bacterial strains and plasmids used in this study are listed in Supplementary Table 1. For lycopene production, a single colony was used to inoculate 50 ml LB medium in a 250 ml flask, which was then incubated at 37 °C and 200 rpm for 16 h. Three ml of pre-culture was then inoculated into 50 ml LB medium and incubated at 37 °C and 200 rpm for 3 h. The cultures were then transferred to 30 °C and 200 rpm for 50 h with or without IPTG (0–1 mM). 100 mg ampicillin l^{-1} was added to maintain plasmids. Cultivation was in the dark. To determine dry cell weight (DCW), 1 ml of sample was centrifuged (13,000×*g*, 5 min), washed with double-distilled water, centrifuged again, and dried at 100 °C until a constant weight was achieved.

DNA manipulation and plasmid construction

The genomic DNA of *D. wulumuqiensis* R12 was isolated using a genomic DNA extraction kit (Takara, China). Fragments of *crtE* (orf01490), *crtB* (orf00123), *crtB*1, *crtB*2, *crtI* (orf00124), *crtI*1, and *crtI*2 were individually amplified from the genomic DNA of *D. wulumuqiensis* R12 using the primers listed in Supplementary Table 2. *crtB*1 and *crtB*2, and *crtI*1 and *crtI*2, were identical to *crtB* and *crtI*, respectively; however, they contained manipulated SD and AS regions that were varied using different primers. Expression plasmid pET-EBI was constructed as shown in Fig. 2a, and plasmids pET-EB1I1, pET-EB1I2, pET-EB2I1, and pET-EB2I2 were constructed in a similar manner. In addition, plasmids pET-I1 and pET-I2 were constructed by digesting the *crtI*1 and *crtI*2 fragments with *Hind*III and *Xho*I and then ligating the products into pET-22b, and plasmids pET-B1 and pET-B2 were constructed by digesting the *crtB*1 and *crtB*2 fragments with *EcoR*I and *Hind*III and then ligating the products into pET-22b. Plasmids pET-22b, pET-I1, pET-I2, pET-B1, pET-B2, pET-EBI, pET-EB1I1, pET-EB1I2, pET-EB2I1, and pET-EB2I2 were transformed into chemically competent *E. coli* BL21 (DE3), resulting in the strains EDWe, EDW-I1, EDW-I2, EDW-B1, EDW-B2, EDW, EDW11, EDW12, EDW21, and EDW22, respectively (Fig. 2b).

Isolation and analytical methods

Following cultivation, 10 ml broth was collected and centrifuged (13,000×*g*, 4 °C, 5 min). Cell pellets were washed with double-distilled water, re-suspended in 5 ml acetone and then incubated at 55 °C for 15 min. The samples were centrifuged (13,000×*g*, 25 °C, 10 min) and the supernatants were used for HPLC analysis. All extraction steps were conducted in the dark (Sun et al. 2014).

For HPLC analysis, 20 µl of each supernatant were analyzed using a Venusil XBP C18 column (4.6 × 150 mm, 5 µm; Agela Technologies) at 472 nm and eluted with acetonitrile/methanol/isopropanol (80:15:5, by vol) at 1 ml min^{-1} for 40 min. Commercial lycopene was dissolved in acetone and used to generate a standard curve using the above-mentioned method. The results represent the mean ± standard deviation of three independent experiments.

Results and discussion

Identification of putative carotenoid genes in the genome of *D. wulumuqiensis* R12

The 16S rRNA gene sequence of *D. wulumuqiensis* R12 shows 97 % similarity to that of *D. radiodurans* R1, indicating that *D. wulumuqiensis* R12 is a novel species of the genus *Deinococcus* (Wang et al. 2010). Lycopene genes identified from the genome of *D. radiodurans* R1 (GenBank accession no. AE000513), DR1395 (*crtE*), DR0862 (*crtB*), and DR0861 (*crtI*), were used to search the genome of *D. wulumuqiensis* R12 for putative lycopene biosynthesis genes. Three corresponding regions, orf01490, orf00123, and orf00124, showed 86, 88, and 87 % sequence identity to DR1395, DR0862, and DR0861, respectively,

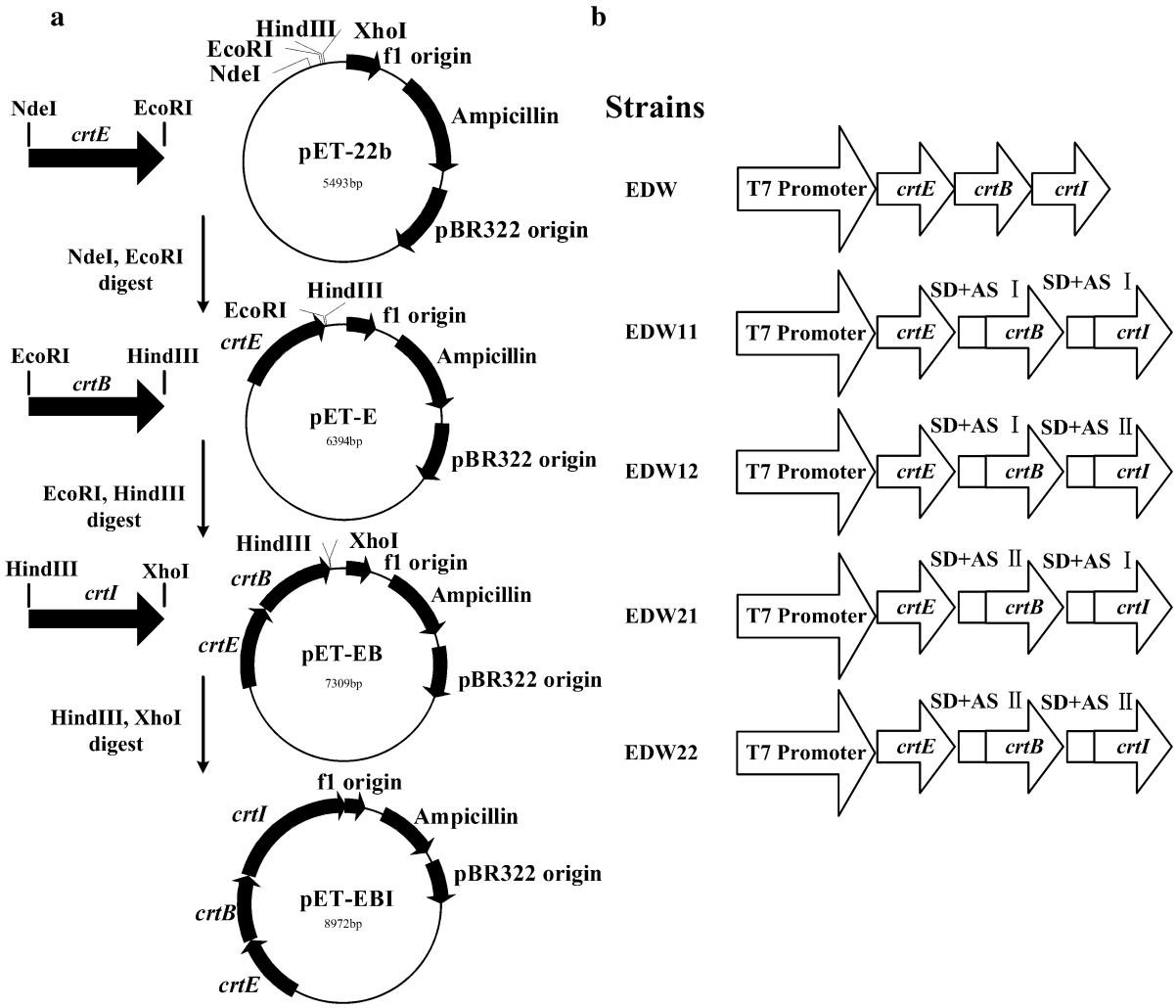

Fig. 2 Schematic presentation of polycistronic plasmids. **a** The *crtE* PCR fragment was digested with *Nde*I and *Eco*RI, purified and then ligated into plasmid pET-22b to construct plasmid pET-E. Plasmid pET-EB was constructed by digesting fragment *crtB* with *Eco*RI and *Hin*dIII, purifying and then ligating into plasmid pET-E, and fragment *crtI* was digested with *Hin*dIII and *Xho*I, purified and ligated into plasmid pET-EB to construct plasmid pET-EBI. **b** Presentation of polycistronic plasmids and the recombinant strains, promoters and genes are indicated by closed arrows, SD + AS sequences (SD + AS I and SD + AS II) are indicated by closed boxes

indicating that orf1490, orf00123, and orf00124 may be the respective lycopene biosynthesis genes *crtE*, *crtB*, and *crtI*. Moreover, the corresponding proteins had no signal peptide or transmembrane domains, suggesting that they were intracellular enzymes.

Lycopene production in *E.coli* EDW using carotenoid genes from *D. wulumuqiensis* R12

To understand the function of the *crtE*, *crtB*, and *crtI* genes and their enzymatic products, the product was isolated and detected by co-expression of the three genes from *D. wulumuqiensis* R12. The acetone supernatants from the expression strains EDWe, EDW, EDW11, EDW12, EDW21, and EDW22 were detected at 30 min by HPLC, and a specific peak corresponding to lycopene was found in all strains except EDWe by comparison with the commercial lycopene (Supplementary Fig. 1).

To ensure optimal conditions for lycopene production in the *E.coli* expression system, the lycopene content and cell biomass of the strain EDW were

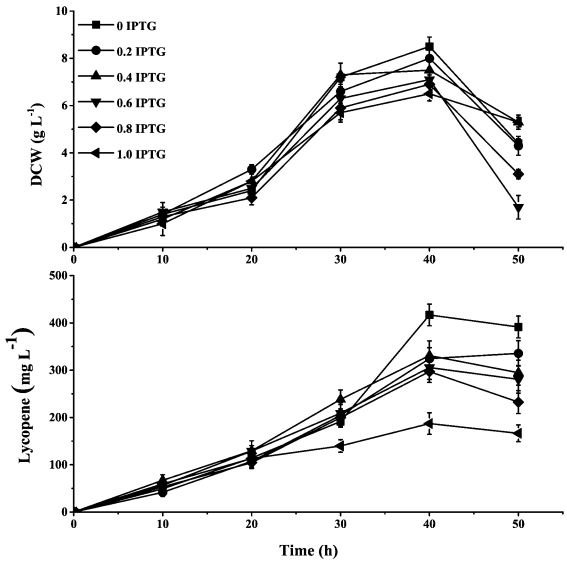

Fig. 3 Cell growth and lycopene production of the strain EDW in LB medium with the addition of 0–1 mM IPTG at 30 °C for 50 h. The data are means of three independent experiments. *Error bars* represent standard deviations

examined under different IPTG concentrations (Fig. 3). The highest concentration of lycopene was achieved at 40 h with maximum biomass obtained at the same time. However, as nutrients were consumed, toxic metabolites accumulated in the cultures, and the highly-hydrophobic lycopene which can only be stored in the membrane may exert metabolic stress on the cells (Verwaal et al. 2010). Because of this, the biomass decreased after 40 h cultivation and the lycopene content also dropped between 40 to 50 h, possibly as a result of cells lysis and the instability of itself after a long period of fermentation time. An increase in IPTG concentration (0.2–1 mM) caused decreases in both biomass and lycopene production. The highest yield of lycopene was 49 mg lycopene g^{-1} DCW (417 mg lycopene l^{-1}) at 40 h with no IPTG induction. IPTG is a non-utilizable inducer and is toxic to cells, which might cause the decrease in biomass and lead to a low lycopene yield.

The finding that no IPTG induction in recombinant *E. coli* produced a better yield than with IPTG induction agrees with previous studies (Yoon et al. 2007; Thakker et al. 2011; Ji et al. 2015). There are several possible explanations for this phenomenon: (1) leaky expression of the plasmids in LB medium might be sufficient for lycopene biosynthesis (Yoon et al. 2007); (2) the protein burden caused by the expression of a large amount of unneeded protein (Thakker et al. 2011; Ji et al. 2015); (3) insufficient FPP for vital metabolic reactions in cells when lycopene genes are overexpressed under IPTG induction (Bahieldin et al. 2014). However, there is no definite explanation for this phenomenon, and it should be studied.

Optimization of SD + AS combinations upstream of the *crtB* and *crtI* genes

Heterologous gene expression levels are influenced by several regulatory elements, including promoters, ribosome binding sites, and sequences upstream of the initial codon (Kosuri et al. 2013). We therefore examined regulatory elements of gene expression to improve lycopene production. The four strains EDW11, EDW12, EDW21, and EDW22 changed to varying shades of red after 40 h cultivation (see Supplementary Fig. 2), which indicated different degrees of lycopene accumulation. The lycopene yield EDW11 was 70 mg g^{-1} DCW (690 mg lycopene l^{-1}), which was the highest of the four strains, and more than twice as much as that of EDW21 (29 mg g^{-1} DCW, 252 mg lycopene l^{-1}) (Fig. 4).

Growth analyses showed that the strains EDW12 and EDW21 had relatively long lag phases and it took a long time for them to reach the stationary phase. The rapid growth phase of the strain EDW11 was the shortest while that of the strain EDW21 was the longest. The final cell biomass weights of strains EDW11 and EDW22 were greater than those of the strains EDW12 and EDW21, leading to the low lycopene yields of the strains EDW12 and EDW21. Therefore, the different combinations of the SD + AS sequences (SD + AS I and SD + AS I, SD + AS I and SD + AS II, SD + AS II and SD + AS I or SD + AS II and SD + AS II) upstream of the *crtB* and *crtI* genes had different effects on cell growth. The combination of different SD + AS sequences (i.e. SD + AS I and SD + AS II, or SD + AS II and SD + AS I) may cause growth inhibition, while the combination of the same SD + AS sequences (i.e. SD + AS I and SD + AS I, or SD + AS II and SD + AS II) may enhance lycopene production.

To compare the effects of SD + AS I and SD + AS II, the expression of phytoene dehydrogenase (encoded by *crtI*) and phytoene synthase (encoded by *crtB*), along with the growth of the strains EDW-I1, EDW-I2, EDW-B1, and EDW-B2, were

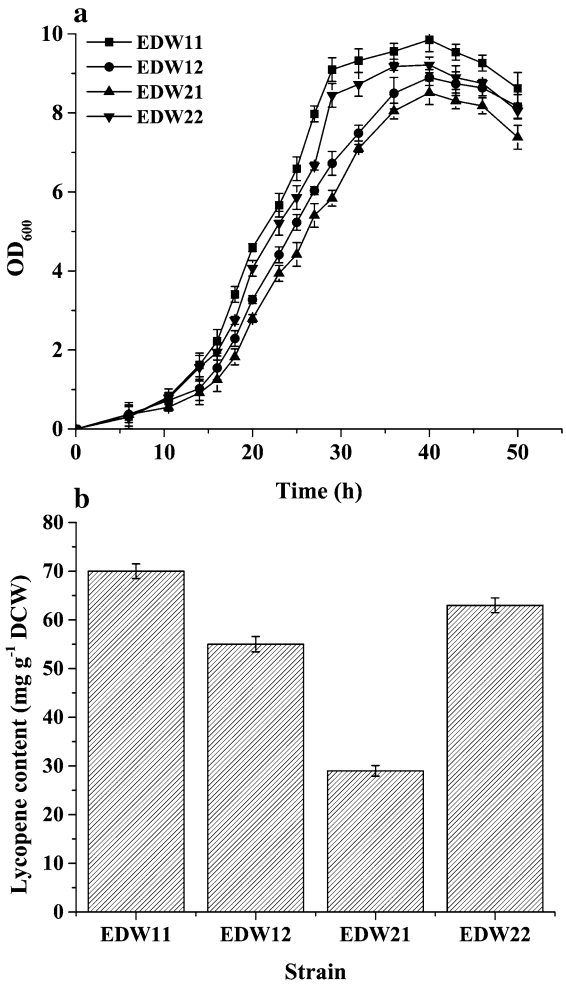

Fig. 4 **a** Cell growth of the strains EDW11, EDW12, EDW21, and EDW22 in LB medium with no IPTG at 30 °C for 50 h. **b** Specific lycopene content of the strains EDW11, EDW12, EDW21, and EDW22 in LB medium with no IPTG at 30 °C for 40 h. The *data* are means of three independent experiments. *Error bars* represent standard deviations

determined (see Supplementary Fig. 3 and Supplementary Fig. 4). Phytoene dehydrogenase expression from pET-I2 was much higher than that from pET-I1, which indicating that SD + AS II is much more efficient than SD + AS I at the translational level. However, growth analyses of the two strains showed that the strain EDW-I1 had a short lag phase and rapidly transitioned into logarithmic phase, while the lag and logarithmic phase of the strain EDW-I2 were relatively long. In addition, the cell density of the strain EDW-I1 was greater than that of the strain EDW-I2 at 40 h post-inoculation. In addition, there were no major differences between the strains EDW-I1 and EDW-I2 and the strains EDW-B1 and EDW-B2 in the expression of protein or in the growth profiles.

The observation that high protein levels led to a low cell biomass might be a result of "protein burden" (Novick and Weiner 1957). Cellular protein synthesis has a high energy requirement; thus unneeded protein places a considerable burden on the cell (Koch 1983). The yields from the four strains demonstrated that the combination of SD + AS I upstream of the *crtB* and *crtI* genes was optimal amongst the SD + AS combinations tested. However, it is interesting that the strains EDW12 and EDW21 showed relatively low lycopene yields even though they contained the SD + AS I combination.

Lycopene synthesis in vivo is a complex metabolic process catalyzed by multiple enzymes. Multi-enzyme reactions are complex and unpredictable, as they require genes to be expressed appropriately to avoid over-accumulation of intermediates or bottlenecks, which may lead to inhibition of cell growth (Pfleger et al. 2006). Some efforts have been made to overcome the shortcomings in multi-enzymatic biosynthetic pathways such as imbalanced pathway flux, formation of side products and accumulation of toxic intermediates that can inhibit host cell growth (Ajikumar et al. 2010; Zhao et al. 2013). Promoters of different strengths may be more favorable than strong promoters for gene expression, meaning that regulatory elements must be compatible with each other for gene expression (Chen et al. 2014). Therefore, in the current lycopene synthesis system, the differing yields of the four strains might be a result of several factors: protein burden, unbalanced expression of the multiple genes, uneven energy distribution, or incompatibility between the SD + AS I and SD + AS II combinations, which may cause a pressure on cell itself, leading to slow growth and low levels of lycopene production. The compatibility of regulatory elements in multiple gene expression systems is the focus of ongoing research.

Optimization of culture medium for lycopene accumulation

Heterologous gene expression systems are also influenced by the external environment. To determine the optimal culture medium, three media were tested: LB, 2 × YT + G, and SM. Given the catabolite

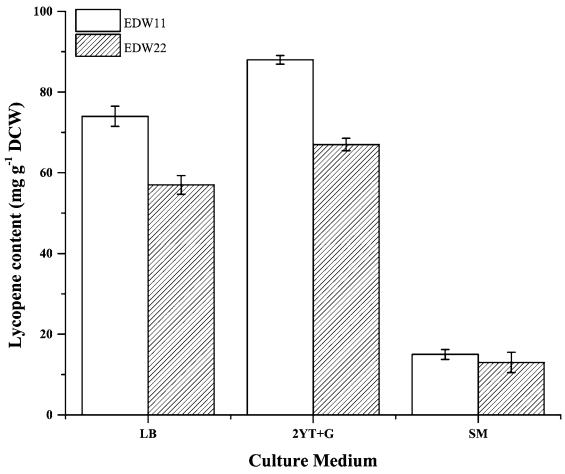

Fig. 5 Specific lycopene content of the strains EDW11 and EDW22 in LB, 2 × YT + G, and SM medium with no IPTG at 30 °C for 40 h. The *data* are means of three independent experiments. *Error bars* represent standard deviations

repression effect of glucose in the culture medium (Kim et al. 2011), glycerol was used as an additional carbon source in the 2 × YT complex medium. The 2 × YT + G medium was optimal for lycopene production (without IPTG induction) by the strains EDW11 and EDW22 (Fig. 5). Lycopene reached 88 mg g^{-1} DCW (780 mg lycopene l^{-1}) in the stain EDW11 culture in 2 × YT + G medium and 74 mg g^{-1} DCW (670 mg lycopene l^{-1}) in LB medium, but a yield of only 15 mg g^{-1} DCW (80 mg lycopene l^{-1}) was obtained in the SM medium. For EDW22, the lycopene yield was 67 mg g^{-1} DCW (600 mg lycopene l^{-1}) in 2 × YT + G medium, 57 mg g^{-1} DCW (540 mg lycopene l^{-1}) in LB medium, and 13 mg g^{-1} DCW (72 mg lycopene l^{-1}) in SM medium. In addition, in the 2 × YT + G medium, the combination of SD + AS I upstream of the *crtB* and *crtI* genes was more beneficial for increasing the accumulation of lycopene in *E. coli*. Although a previous report showed that SM medium was beneficial for lycopene production (Kim et al. 2011), our studies showed that lycopene production in 2 × YT + G medium was better than in LB or SM media.

In conclusion, lycopene biosynthesis genes were identified from the genome of newly identified strain *D. wulumuqiensis* R12, and polycistronic plasmids harboring these genes with different SD and AS combinations were constructed. Following optimization of the SD and AS regions and culture medium, the recombinant *E. coli* strain EDW11 produced 88 mg lycopene g^{-1} DCW (780 mg lycopene l^{-1}) in the absence of IPTG induction, which corresponded to a 79 % increase in specific lycopene content over the strain EDW (49 mg lycopene g^{-1} DCW), and a 87 % increase in lycopene production (417 mg lycopene l^{-1}). This represents the highest level of lycopene production by a recombinant *E. coli* strain to date.

Acknowledgments This work was supported by the National Science Foundation for Distinguished Young Scholars of China (21225626), the National High Technology Research and Development Program of China (2012AA021705, 2012AA022101), the National Natural Science Foundation of China for Young Scholars (21406111), and the Natural Science Foundation of Jiangsu Province, China (BK20131406).

Supporting information Supplementary Table 1—Bacterial strains and plasmids used in this work.

Supplementary Table 2—Primers used in this work.

Supplementary Fig. 1—HPLC profiles of the isolated carotenoids from the strain EDWe, EDW, EDW11, EDW12, EDW21, EDW22, and the commercial lycopene.

Supplementary Fig. 2—The color of the cell pellets of the strains EDW11, EDW12, EDW21, and EDW22.

Supplementary Fig. 3—Comparison of the phytoene dehydrogenase expression and the growth of the recombinant strains *E. coli* EDW-I1 and EDW-I2.

Supplementary Fig. 4—Comparison of the phytoene synthase expression and the growth of the recombinant strains *E. coli* EDW-B1 and EDW-B2.

References

Ajikumar PK, Xiao WH, Tyo KEJ et al (2010) Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli*. Science 330:70–74

Bahieldin A, Gadalla NO, Al-Garni SM et al (2014) Efficient production of lycopene in *Saccharomyces cerevisiae* by expression of synthetic *crt* genes from a plasmid harboring the ADH2 promoter. Plasmid 72:18–28

Chen J, Zhu X, Tan Z et al (2014) Activating C_4-dicarboxylate transporters DcuB and DcuC for improving succinate production. Appl Microbiol Biotechnol 98:2197–2205

Chenna R, Sugawara H, Koike T et al (2003) Multiple sequence alignment with the Clustal series of programs. Nucl Acid Res 31:3497–3500

Ji X, Liu L, Shen M et al (2015) Constructing a synthetic metabolic pathway in *Escherichia Coli* to produce the enantiomerically pure (R, R)-2,3-butanediol. Biotechnol Bioeng 112:1056–1059

Kim SH, Park YH, Schmidt-Dannert C, Lee PC (2010) Redesign, reconstruction, and directed extension of the *Brevibacterium linens* C_{40} carotenoid pathway in *Escherichia coli*. Appl Environ Microb 76:5199–5206

Kim Y, Lee J, Kim N et al (2011) Increase of lycopene production by supplementing auxiliary carbon sources in metabolically engineered *Escherichia coli*. Appl Microbiol Biot 90:489–497

Koch AL (1983) The protein burden of *lac* operon products. J Mol Evol 19:455–462

Kong K, Khoo H, Prasad KN et al (2010) Revealing the power of the natural red pigment lycopene. Molecules 15:959–987

Kosuri S, Goodman DB, Cambray G et al (2013) Composability of regulatory sequences controlling transcription and translation in *Escherichia coli*. Proc Natl Acad Sci USA 110:14024–14029

Novick A, Weiner M (1957) Enzyme induction as an all-or-none phenomenon. Proc Natl Acad Sci USA 43:553

Pennacchio A, Pucci B, Secundo F et al (2008) Purification and characterization of a novel recombinant highly enantioselective short-chain NAD(H)-dependent alcohol dehydrogenase from *Thermus thermophilus*. Appl Environ Microbiol 74:3949–3958

Pfleger BF, Pitera DJ, Smolke CD et al (2006) Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes. Nat Biotechnol 24:1027–1032

Sun T, Miao L, Li Q et al (2014) Production of lycopene by metabolically-engineered *Escherichia coli*. Biotechnol Lett 36:1515–1522

Thakker C, Zhu J, San K, Bennett G (2011) Heterologous *pyc* gene expression under various natural and engineered promoters in *Escherichia coli* for improved succinate production. J Biotechnol 155:236–243

Tian B, Xu Z, Sun Z et al (2007) Evaluation of the antioxidant effects of carotenoids from *Deinococcus radiodurans* through targeted mutagenesis, chemiluminescence, and DNA damage analyses. Biochim Biophys Acta 1770:902–911

Verwaal R, Jiang Y, Wang J et al (2010) Heterologous carotenoid production in *Saccharomyces cerevisiae* induces the pleiotropic drug resistance stress response. Yeast 27:983–998

Wang W, Mao J, Zhang Z et al (2010) *Deinococcus wulumuqiensis* sp. nov., and *Deinococcus xibeiensis* sp. nov., isolated from radiation-polluted soil. Int J Syst Evol Micr 60:2006–2010

Xu X, Jiang L, Zhang Z et al (2013) Genome sequence of a gamma- and UV-ray-resistant strain, *Deinococcus wulumuqiensis* R12. Genome Announc 1:e206–e213

Yoon S, Kim J, Lee S et al (2007) Engineering the lycopene synthetic pathway in *E. coli* by comparison of the carotenoid genes of *Pantoea agglomerans* and *Pantoea ananatis*. Appl Microbiol Biotechnol 74:131–139

Zhao J, Li Q, Sun T et al (2013) Engineering central metabolic modules of *Escherichia coli* for improving β-carotene production. Metab Eng 17:42–50

Zhou Y, Nambou K, Wei L et al (2013) Lycopene production in recombinant strains of *Escherichia coli* is improved by knockout of the central carbon metabolism gene coding for glucose-6-phosphate dehydrogenase. Biotechnol Lett 35:2137–2145