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Effects of lipopolysaccharide structure on lycopene production in *Escherichia coli*

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ABSTRACT

Lipopolysaccharides, the major molecules in the outer membrane of *Escherichia coli*, affect the behavior of bacteria including outer membrane permeability, but its influence on lycopene production in *E. coli* has never been reported. In this study, the effects of lipopolysaccharides with different structures on lycopene biosynthesis were investigated. Firstly, the heterogenous *crtEBI* operon were overexpressed in 10 LPS mutant strains of *E. coli* W3110 (Δ*waaC*, Δ*waaF*, Δ*waaY*, Δ*waaG*, Δ*waaR*, Δ*waaO*, Δ*waaU*, Δ*waaP*, Δ*waaY* and Δ*waaB*), and their ability to produce lycopene were compared. Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI* and Δ*waaY*/pWSK29-*crtEBI* produced 4.19, 4.20, and 3.81 mg/g lycopene, respectively, while the control W3110/pWSK29-*crtEBI* produced 3.71 mg/g lycopene; the other strains produced less lycopene than the control. In order to enhance lycopene production, genes *dxr*, *dxr*, *ispA*, and *idi* were overexpressed in Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI* individually or in combination, and the lycopene production in each strain was analyzed. The maximum yield of 5.39 mg/g was achieved in Δ*waaC*/pWSK29-*crtEBI-SRA*, which is 142% higher than that in W3110/pWSK29-*crtEBI*. The results indicate that the length of lipopolysaccharide affects lycopene biosynthesis in *E. coli*, and the shorter lipopolysaccharide and higher outer membrane permeability might be beneficial to lycopene biosynthesis.

1. Introduction

Escherichia coli is an important platform for protein expressing and various product biosynthesis [1], however, the limited permeability of its outer membrane hinders the performance of *E. coli* as whole-cell biocatalyst [2]. The expression of SARS coronavirus small envelope protein [2] and deletion of *lpp* encoding lipoprotein in *E. coli* [3] were effective for increasing the outer membrane permeability. We have constructed *E. coli* mutants that could synthesize different length of lipopolysaccharides (LPS), the major molecules in the outer membrane, and found that the structure of LPS is closely relevant to the outer membrane permeability [4].

Lycopene is a bright red carotenoid pigment with 11 conjugated double bonds, and is an efficient singlet oxygen quencher. Recent advance in metabolic engineering has provided a very promising route for the heterologous production of lycopene within various microorganisms. *E. coli* has been widely utilized as microbial cell factory for the synthesis of various carotenoids [5]. *E. coli* can naturally synthesize (2E, 6E)-farnesyl diphosphate (FPP) from 3-phospho-D-glycerate and pyruvate through a series of catalytic reactions; expressing heterologous *crtE*, *crtB* and *crtI* in *E. coli* can convert FPP into lycopene (Fig. 1). Different types of plasmids have been used to carry the genes *crtE*, *crtB* and *crtI* to synthesize lycopene in *E. coli* [6]. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), are synthesized via MEP pathway in *E. coli* [7]. In the MEP pathway, isopentenyl diphosphate isomerase encoded by *idi*, 1-deoxyxylulose-5-phosphate synthase encoded by *dxs*, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase encoded by *ispD*, and 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase encoded by *ispF* play important roles; therefore, over-expressing these genes has been used to elevate isoprenoid accumulation [5,8,9]. For example, overexpressing *idi* could significantly stimulate carotenoid synthesis [10–13]. Enhancing the gene expression by chromosomal promoter replacement [14] or introduction of a heterologous mevalonate pathway to increase IPP and DMAPP supply [15] have also been used for carotenoid synthesis.

In this study, a series of *E. coli* LPS mutants strains [4] were used to

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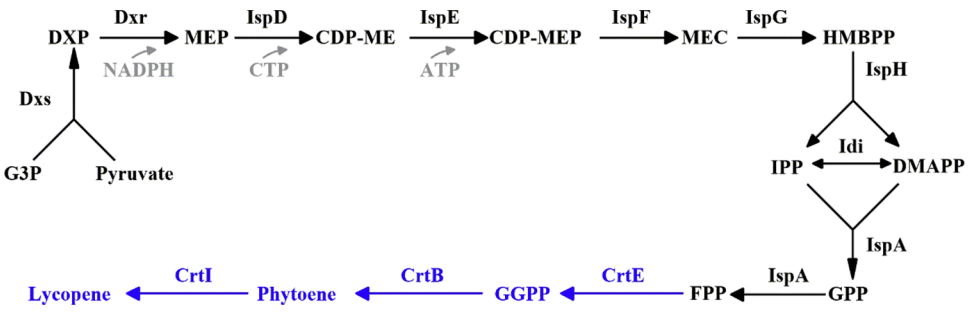


Fig. 1. The biosynthetic pathway of lycopene in the recombinant *E. coli*. The last three reactions do not exist in *E. coli* but was introduced into *E. coli* in this study. Dxs, 1-deoxy-D-xylulose-5-phosphate synthase; Dxr, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; IspD, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase; IspE, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase ; IspG, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; IspH, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase; IDI, isopentenyl-diphosphate Δ-isomerase; IspA, geranyl diphosphate farnesyl diphosphate synthase; CrtE, geranylgeranyl pyrophosphate synthase subunit; CrtB, phytoene synthase ; CrtI, phytoene dehydrogenase ; G3P, 3-phospho-D-glycerate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; MEC, 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; HMBPP, 4-hydroxy-3-methylbut-2-enyl diphosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; GPP, geranyl diphosphate; FPP, (2E,6E)-farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

Table 1
Bacterial strains and plasmids used in this study.

Strains or Plasmids	Description	Source
Strains		
W3110	Wild type *E. coli*, F−, λ−	Novagen
ATCC14067	Wild type *Corynebacterium glutamicum*	ATCC
Δ*waaC*	W3110 Δ*waaC*	[4]
Δ*waaF*	W3110 Δ*waaF*	[4]
Δ*waaG*	W3110 Δ*waaG*	[4]
Δ*waaO*	W3110 Δ*waaO*	[4]
Δ*waaR*	W3110 Δ*waaR*	[4]
Δ*waaU*	W3110 Δ*waaU*	[4]
Δ*waaP*	W3110 Δ*waaP*	[4]
Δ*waaQ*	W3110 Δ*waaQ*	[4]
Δ*waaY*	W3110 Δ*waaY*	[4]
Δ*waaB*	W3110 Δ*waaB*	[4]
Δ*waaC*/pWSK29-*crtEBI*	Δ*waaC* harboring pWSK29-*crtEBI*	This work
Δ*waaF*/pWSK29-*crtEBI*	Δ*waaF* harboring pWSK29-*crtEBI*	This work
Δ*waaG*/pWSK29-*crtEBI*	Δ*waaG* harboring WSK29-*crtEBI*	This work
Δ*waaO*/pWSK29-*crtEBI*	Δ*waaO* harboring pWSK29-*crtEBI*	This work
Δ*waaR*/pWSK29-*crtEBI*	Δ*waaR* harboring pWSK29-*crtEBI*	This work
Δ*waaU*/pWSK29-*crtEBI*	Δ*waaU* harboring pWSK29-*crtEBI*	This work
Δ*waaP*/pWSK29-*crtEBI*	Δ*waaP* harboring pWSK29-*crtEBI*	This work
Δ*waaQ*/pWSK29-*crtEBI*	Δ*waaQ* harboring pWSK29-*crtEBI*	This work
Δ*waaY*/pWSK29-*crtEBI*	Δ*waaY* harboring pWSK29-*crtEBI*	This work
Δ*waaB*/pWSK29-*crtEBI*	Δ*waaB* harboring pWSK29-*crtEBI*	This work
Δ*waaC*/pWSK29-*crtEBI-ispA*	Δ*waaC* harboring pWSK29-*crtEBI-ispA*	This work
Δ*waaF*/pWSK29-*crtEBI-ispA*	Δ*waaF* harboring pWSK29-*crtEBI-ispA*	This work
Δ*waaC*/pWSK29-*crtEBI-dxs*	Δ*waaC* harboring pWSK29-*crtEBI-dxs*	This work
Δ*waaF*/pWSK29-*crtEBI-dxs*	Δ*waaF* harboring pWSK29-*crtEBI-dxs*	This work
Δ*waaC*/pWSK29-*crtEBI-dxr*	Δ*waaC* harboring pWSK29-*crtEBI-dxr*	This work
Δ*waaF*/pWSK29-*crtEBI-dxr*	Δ*waaF* harboring pWSK29-*crtEBI-dxr*	This work
Δ*waaC*/pWSK29-*crtEBI-SR*	Δ*waaC* harboring pWSK29-*crtEBI-SR*	This work
Δ*waaF*/pWSK29-*crtEBI-SR*	Δ*waaF* harboring pWSK29-*crtEBI-SR*	This work
Δ*waaC*/pWSK29-*crtEBI-SRA*	Δ*waaC* harboring pWSK29-*crtEBI- SRA*	This work
Δ*waaF*/pWSK29-*crtEBI- SRA*	Δ*waaF* harboring pWSK29-*crtEBI- SRA*	This work
Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRA*	Δ*waaC* harboring pWSK29-*crtEBI* and pACYC184-*SRA*	This work
Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRA*	Δ*waaF* harbor the pWSK29-*crtEBI* and pACYC184-*SRA*	This work
Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRAI*	Δ*waaC* harbor the pWSK29-*crtEBI* and pACYC184-*SRAI*	This work
Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRAI*	Δ*waaF* harbor the pWSK29-*crtEBI* and pACYC184-*SRAI*	This work
Plasmids		
pWSK29	Expression vector	[26]
pACYC184	Expression vector	[27]
pBlueScript II SK+	Cloning vector, ColE1, *lacZ*, Amp^R	Stratagene
pWSK29-*crtEBI*	pWSK29 harboring the *crtEBI* operon from *C. glutamicum* ATCC14067	This work
pWSK29-*crtEBI-ispA*	pWSK29 harboring the *crtEBI* operon from *C. glutamicum* ATCC14067, and *ispA* from *E. coli* W3110	This work
pWSK29-*crtEBI-dxs*	pWSK29 harboring the *crtEBI* operon from ATCC14067 and *dxs* from W3110	This work
pWSK29-*crtEBI-dxr*	pWSK29 harboring the *crtEBI* operon from ATCC14067 and *dxr* from W3110	This work
pWSK29-*crtEBI-SR*	pWSK29 harboring the *crtEBI* operon from ATCC14067, and *dxs* and *dxr* from W3110	This work
pWSK29-*crtEBI-SRA*	pWSK29 harboring the *crtEBI* operon from ATCC14067, and *ispA*, *dxs* and *dxr* from W3110	This work
pACYC184-*SRA*	pACYC184 harboring the genes *ispA*, *dxs* and *dxr* from W3110	This work
pACYC184-*SRAI*	pACYC184 harboring the genes *ispA*, *dxs*, *dxr* and *idi* from W3110	This work

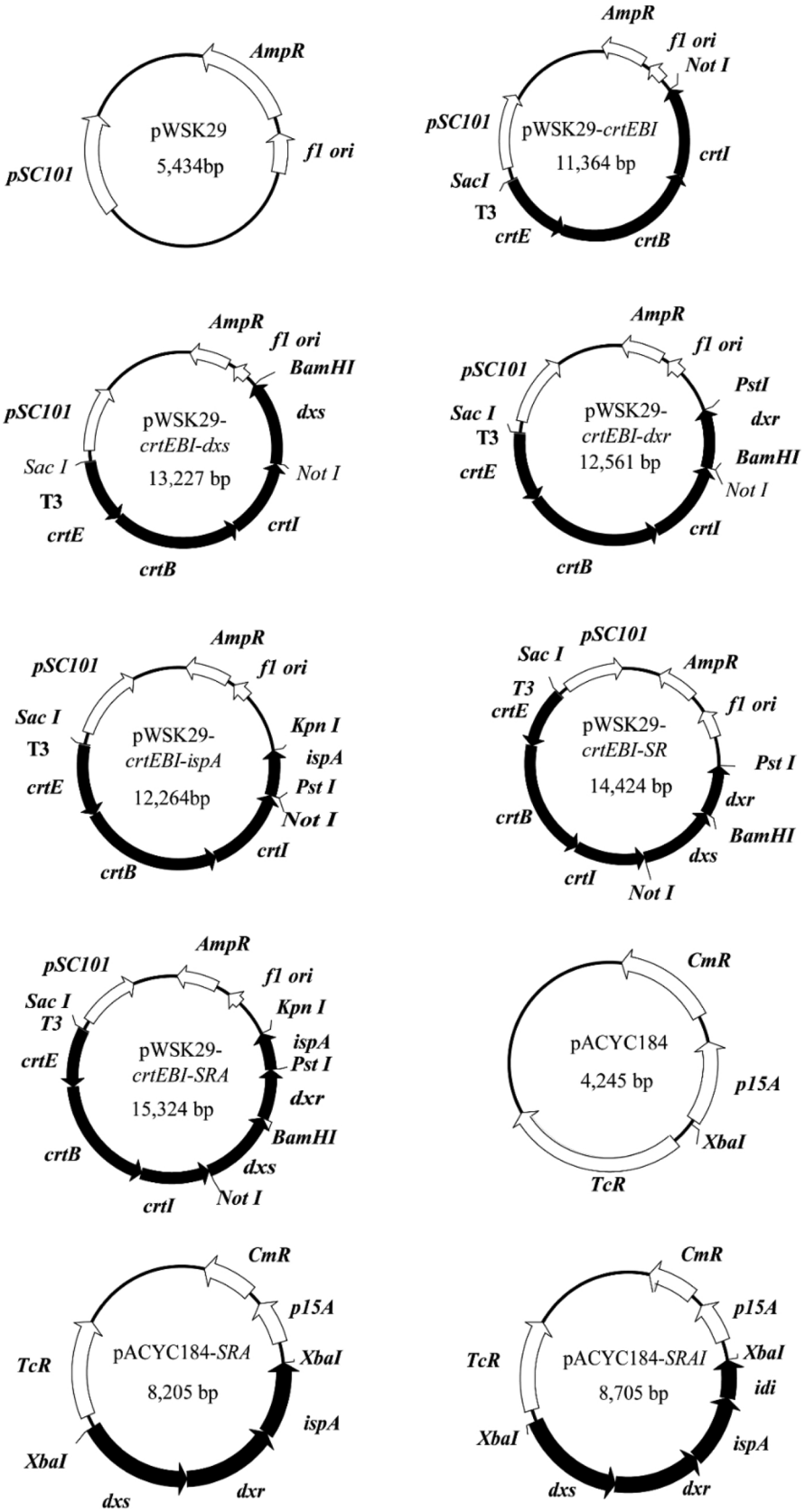


Fig. 2. Maps of different plasmids used in this study. The genes *crtE*, *crtI*, and *crtB* were amplified from the genome of *Corynebacterium glutamicum* ATCC14067.

Table 2
Primers used in this study.

Primer	Sequences (5′→3′)	Restriction site
crtEBI-29-F	ATGGGAGCTCAGGAGGTATATACCATGGACAATGGCATGACAAT	*SacI*
crtEBI-29-R	GGAGCGGCCGCTTAATGATCGTATGAGGTC	*NotI*
dxs-29-F	GAGGCGGCCGCAGGAGGTATATACCATGAGTTTTGATATTGCCAA	*NotI*
dxs-29-R	AACGGATCCTTATGCCAGCCAGGCCTTGAT	*BamHI*
dxr-29-F	AACGGATCCAGGAGGTATATACCATGAAGCAACTCACCATTCT	*BamHI*
dxr-29-R	AAGCTGCAGTCAGCTTGCGAGACGCATCAC	*PstI*
ispA-29-F	AACCTGCAGAGGAGGATTATAGGATGGACTTTCCGCAGCAACT	*PstI*
ispA-29-R	GCCGGTACCTTATTTATTACGCTGGATGATG	*KpnI*
idi-29-F	AACGGTACCAGGAGGATTATAGGATGCAAACGGAACACGTCATT	*KpnI*
idi-29-R	GGACTCGAGTTATTTAAGCTGGGTAAATGC	*XhoI*
184-srA-F	GGCTCTAGTAGGAGGTATATACCATGAGTTTTGATATTGCCAA	*XbaI*
184-srA-F	GCGTCTAGT TTATTTATTACGCTGGATGATG	*XbaI*
184-srAi-F	GGCTCTAGTAGGAGGTATATACCATGAGTTTTGATATTGCCAA	*XbaI*
184-srAi-R	GCGTCTAGTTTATTTAAGCTGGGTAAATGC	*XbaI*

synthesize lycopene by overexpressing the genes *crtE, crtB, crtI, dxr*, *dxs* and *ispA*, and the effects of different structures of LPS on lycopene biosynthesis were investigated.

2. Material and methods

2.1. Strains, media and growth conditions

All *E. coli* strains used in this study were listed in Table 1. *E. coli* strains were grown in LB medium (10 g/L trypton, 5 g/L yeast extract, 10 g/L NaCl) at 37 °C or 30 °C at 200 rpm. LB medium or 2 × YT + G medium (16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, 1 g/L glycerol), was used for shaking flask. When necessary, the medium was supplemented with ampicillin (100 μg/mL), kanamycin (30 μg/mL) or chloramphenicol (30 μg/mL) for plasmid maintenance or strain selection. Isopropyl-β-dthiogalactoside (IPTG) or arabinose were used as the inducer.

2.2. Construction of expression plasmids and recombinant strains

The maps of different plasmids carrying the genes *crtE*, *crtB*, *crtI*, *dxr*, *dxs* or *ispA* individually or in combination are shown in Fig. 2. Restriction enzymes, shrimp alkaline phosphatase, T4 DNA ligase and DNA ladder were purchased from Sangon (Shanghai, China). Plasmid DNA was prepared by using the EZ-10 spin column plasmid mini-preps kit from Bio Basic Inc. (Markham, Canada). PCR reaction mixtures (50 μL) contained: 5 μL 10 × *Ex Taq* buffer, 4 μL dNTP mixture (2.5 mM each), 1 μL plasmid template (100 ng/μL), 1 μL forward primer (20 μM), 1 μL reverse primer (20 μM) and 0.5 μL TaKaRa *Ex Taq* DNA polymerase. The PCR reaction was first heated to 94 °C and maintained for 10 min, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (30 s at 55 °C or 58 °C) and extension (72 °C for 3 min). At the end, an additional 10 min incubation at 72 °C was used. PCR products were purified by using the TIANgel midi purification kit from Tiangen (Beijing, China). Primer synthesis and DNA sequencing were performed by Sangon. The sequences of primers used in this study are listed in Table 2.

Fragments *crtE, crtB* and *crtI* were amplified from the genomic DNA of *Corynebacterium glutamicum* ATCC14067. Fragments *ispA*, *dxs*, *dxr* and *idi* were amplified from the genomic DNA of *E. coli* W3110, using different primer pairs listed in Table 2. PCR products were digested with the corresponding restriction enzymes and ligated with the vector pWSK29 or pACYC184 similarly digested, resulting in the plasmids pWSK29-*crtEBI*, pWSK29-*crtEBI-ispA*, pWSK29-*crtEBI-dxs*, pWSK29-*crtEBI-dxr*, pWSK29-*crtEBI-SR*, pWSK29-*crtEBI-SRA*, pACYC184-*SRA*, and pACYC184-*SRAI,* respectively.

The plasmid pWSK29-*crtEBI* was transformed into *E. coli* W3110 and its mutant strains Δ*waaC*, Δ*waaF*, Δ*waaG*, Δ*waaO*, Δ*waaR*, Δ*waaU*, Δ*waaP*, Δ*waaQ*, Δ*waaY*, and Δ*waaB*, resulting in the strains Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI*, Δ*waaG*/pWSK29-*crtEBI*, Δ*waaO*/pWSK29-*crtEBI*, Δ*waaR*/pWSK29-*crtEBI*, Δ*waaU*/pWSK29-*crtEBI*, Δ*waaP*/pWSK29-*crtEBI*, Δ*waaQ*/pWSK29-*crtEBI*, Δ*waaY*/pWSK29-*crtEBI*, and Δ*waaB*/pWSK29-*crtEBI*. The plasmids pWSK29-*crtEBI*, pWSK29-*crtEBI-ispA*, pWSK29-*crtEBI-dxs,* pWSK29-*crtEBI-dxr*, pWSK29-*crtEBI-SR* and pWSK29-*crtEBI-SRA* was transformed into Δ*waaC* and Δ*waaF,* respectively, resulting in the strains Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI*, Δ*waaC*/pWSK29-*crtEBI-ispA*, Δ*waaF*/pWSK29-*crtEBI-ispA*, Δ*waaC*/pWSK29-*crtEBI-dxs*, Δ*waaF*/pWSK29-*crtEBI-dxs*, Δ*waaC*/pWSK29-*crtEBI-dxr*, Δ*waaF*/pWSK29-*crtEBI-dxr*, Δ*waaC*/pWSK29-*crtEBI-SR*, Δ*waaF*/pWSK29-*crtEBI-SR,* Δ*waaC*/pWSK29-*crtEBI-SRA*, and Δ*waaF*/pWSK29-*crtEBI-SRA*. The plasmids pACYC184-*SRA*, pACYC184-*SRAI* was transformed into Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI,* resulting in the strains Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRA*, Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRA*, Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRAI*, and Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRAI*. Transformation of *E. coli* was performed according to the published protocol [16].

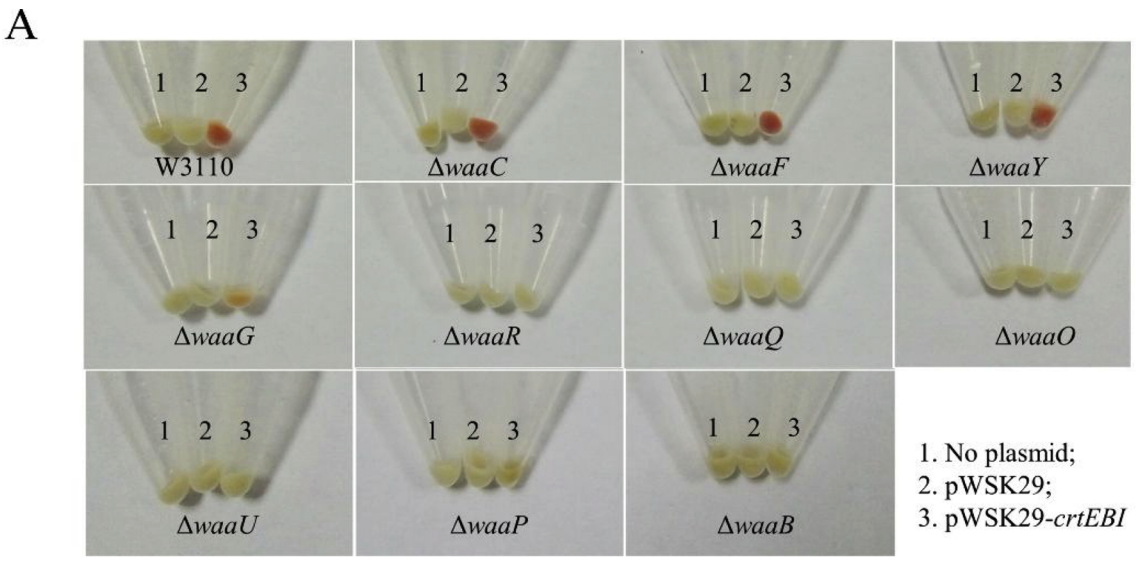
2.3. Analytical methods

Cell growth during the cultivations was monitored by measuring the optical density at 600 nm (OD_{600}). For dry cell weight (DCW) determination, a known volume of fermentation broth was centrifuged for 10 min in pre-weighed test tubes at 4 °C and 4000 rpm, washed once with water, and dried for 24 h at 90 °C to a constant weight.

To extract lycopene, the *E. coli* cells were grown in a 500-ml shaking flask at 37 °C and 200 rpm, harvested by centrifugation at 4000 rpm and 4 °C for 10 min, and rinsed twice with deionized water. Then the cells were suspended in 1 ml of acetone and incubated at 55 °C for 15 min in the dark. The samples were centrifuged at 4000 rpm for 10 min, and the acetone supernatant containing lycopene was transferred to a clean tube. The lycopene content of the extracts was measured according to the previous reported method [8]. Lycopene (purchased from Sigma) dissolved in acetone was used as the standard. The results were the mean from three independent determinations, and the standard deviations were in the range of ± 10% of the means.

2.4. Outer membrane permeability assay

To determined outer membrane permeability of *E. coli*, the fluorescent probe 1-*N*-phenyl-1-naphthylamine (NPN) assay was used [17]. *E. coli* strains were cultivated in LB broth at 37 °C, harvested by centrifugation at 4000 rpm for 10 min, washed and resuspended in phosphate buffer (10 mM, pH = 7.4), The value of OD_{600} was adjusted to 0.5 in the final cell suspension. Then 1.92 mL of cell suspension was mixed with 80 μL NPN (1 mM) into quartz cuvette, immediately. Fluorescence



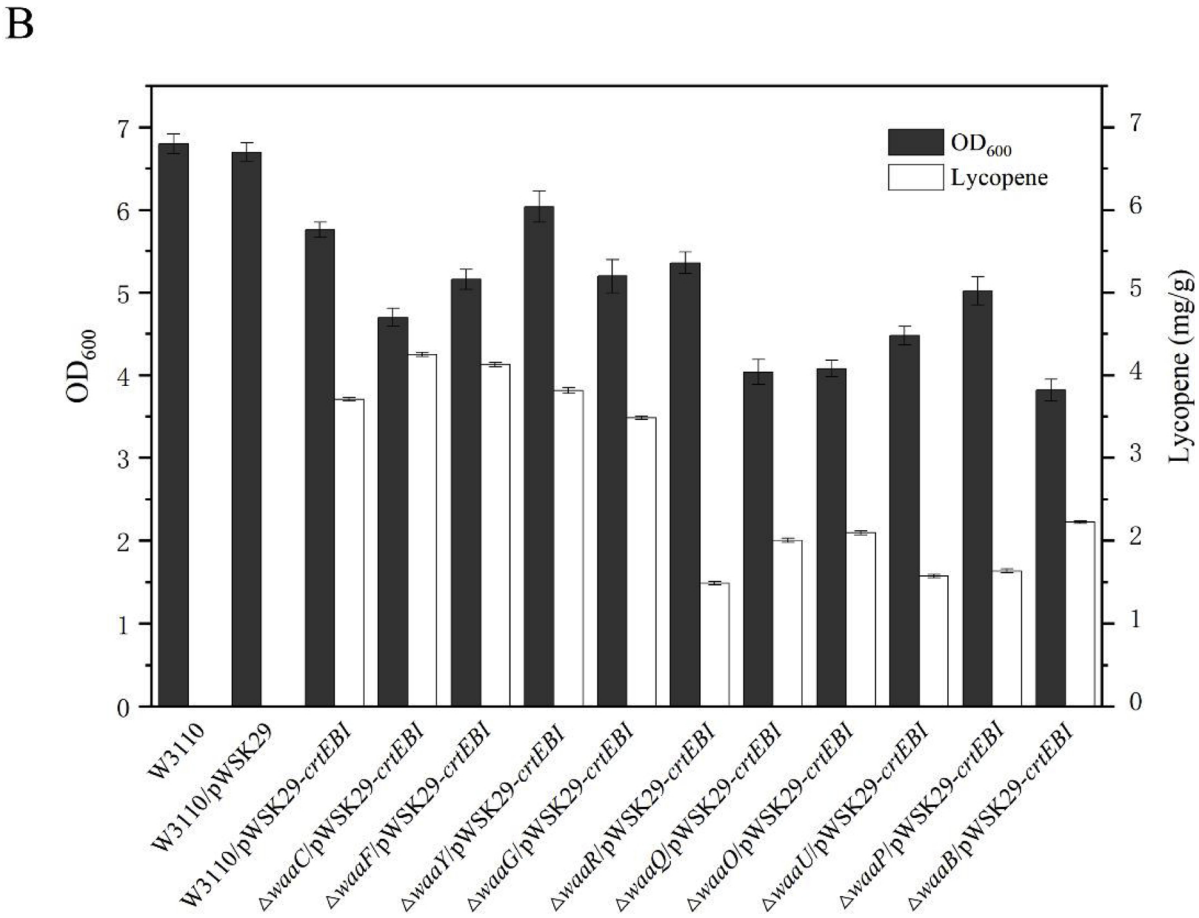


Fig. 3. A. Color comparison of different *E. coli* cells. B. Comparison of cell growth and lycopene yield in different *E. coli* cells. Data represent the average of three experiments and the error bars represent the standard deviation.

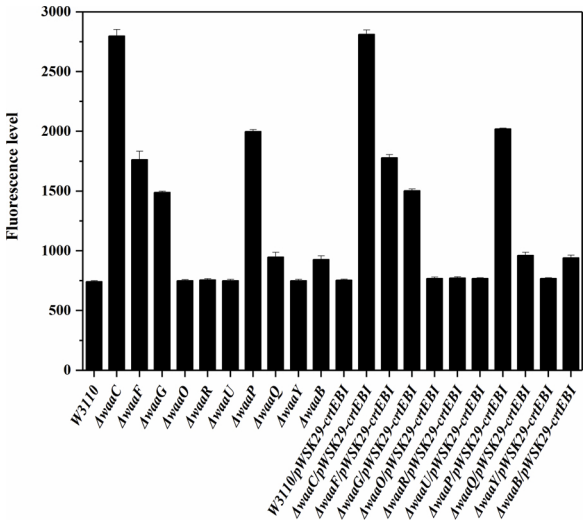


Fig. 4. Comparison of cell outer membrane permeability of different *E. coli* cells. Data represent the average of three experiments and the error bars represent the standard deviation.

was measured using a Fluorescence Spectrophotometer (650-60, Hitachi, Japan), using a slit width of 5 nm, an excitation wavelength of 350 nm and an emission wavelength of 420 nm.

3. Results

3.1. Lycopene biosynthesis in E. coli W3110 overexpressing the genes crtE, crtB, and crtI from C. glutamicum is affected by LPS structure

LPS mutants Δ*waaC*, Δ*waaF*, Δ*waaG*, Δ*waaO*, Δ*waaR*, Δ*waaU*, Δ*waaP*, Δ*waaQ*, Δ*waaY* and Δ*waaB* from *E. coli* W3110 synthesized different length of LPS and showed different outer membrane permeability [4]. In order to effect of LPS structure on lycopene biosynthesis, pWSK29-*crtEBI* and pWSK29 were transformed into these strains and their lycopene productions were investigated [19].

As shown in Fig. 3A, the color of the bacterial cells can be used to determine the amount of lycopene. W3110/pWSK29-*crtEBI* cells were red, but not W3110 and W3110/pWSK29 cells, suggesting that lycopene was produced only when the genes *crtE, crtB, and crtI* from *C. glutamicum* were overexpressed. Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI* and Δ*waaY*/pWSK29-*crtEBI* cells were also red, Δ*waaG*/pWSK29-*crtEBI* cells were light red, suggesting lycopene was synthesized in these cells. These results indicate that LPS structure affects lycopene synthesis in *E. coli*. These cells were broken and lycopene was extracted to quantify. As shown in Fig. 3B, all the strains harboring pWSK29-*crtEBI* can synthesize lycopene, but more lycopene was produced in Δ*waaC*/pWSK29-*crtEBI* (4.19 mg/g), Δ*waaF*/pWSK29-*crtEBI* (4.20 mg/g), Δ*waaY*/pWSK29-*crtEBI* (3.81 mg/g). This quantification is consistent with the red shades of the cells (Fig. 3A vs B). Based on their OD_{600} value, the cell growth was retarded when pWSK29-*crtEBI* was introduced in *E. coli*. By comparison, Δ*waaC*/pWSK29-*crtEBI* and Δ*waaF*/pWSK29-*crtEBI* grew better and produced more lycopene than other strains, therefore, they were used in further study.

The outer membrane permeability of the 10 *E. coli* LPS mutants Δ*waaC*, Δ*waaF*, Δ*waaG*, Δ*waaO*, Δ*waaR*, Δ*waaU*, Δ*waaP*, Δ*waaQ*, Δ*waaY* and Δ*waaB* with or without pWSK29-*crtEBI* were analyzed, using the wild type W3110 as the control (Fig. 4). Similar outer membrane permeabilities were observe for the same strain with or without pWSK29-*crtEBI*, suggesting that the lycopene produced in the strains Δ*waaC*/pWSK29-*crtEBI*, Δ*waaF*/pWSK29-*crtEBI*, Δ*waaG*/pWSK29-*crtEBI*, Δ*waaO*/pWSK29-*crtEBI*, Δ*waaR*/pWSK29-*crtEBI*, Δ*waaU*/pWSK29-*crtEBI*, Δ*waaP*/pWSK29-*crtEBI*, Δ*waaQ*/pWSK29-*crtEBI*, Δ*waaY*/pWSK29-*crtEBI* and Δ*waaB*/pWSK29-*crtEBI* does not affect the outer membrane permeability and might locate in the inner membranes. Δ*waaC*/pWSK29-*crtEBI* showed high outer membrane permeability and produced a large amount of lycopene, but the outer membrane permeability and the lycopene production were not proportionally increased for other strains such as Δ*waaY*/pWSK29-*crtEBI* and Δ*waaP*/pWSK29-*crtEBI*. The results suggested that outer membrane permeability is not the only factor affecting lycopene production in *E. coli*.

3.2. More lycopene was produced in ΔwaaC/pWSK29-crtEBI and ΔwaaF/pWSK29-crtEBI after enhancing the MEP pathway

To further improve lycopene yield, the key genes *dxs*, *dxr, ispA* and *idi* in the MEP pathway were overexpressed, individually or in combination, in Δ*waaC*/pWSK29-*crtEBI* and Δ*waaF*/pWSK29-*crtEBI*; the cell growth and lycopene production in these recombinant strains were investigated (Fig. 5).

Δ*waaC*/pWSK29-*crtEBI-ispA*, Δ*waaC*/pWSK29-*crtEBI-dxs*, and Δ*waaC*/pWSK29-*crtEBI-dxr* produced 4.53, 4.81 and 4.61 mg/g lycopene, respectively; Δ*waaC*/pWSK29-*crtEBI-SR*, Δ*waaC*/pWSK29-*crtEBI-SRA*, Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRA* and Δ*waaC*/pWSK29-*crtEBI*/pACYC184-*SRAI* produced 5.17, 5.39, 5.08 and 5.14 mg/g lycopene, respectively (Fig. 5). Compared to Δ*waaC*/pWSK29-*crtEBI,* these strains grew better and produced more lycopene, suggesting that enhancing the MEP pathway facilitates lycopene synthesis in *E. coli*.

Δ*waaF*/pWSK29-*crtEBI-ispA*, Δ*waaF*/pWSK29-*crtEBI-dxs*, and Δ*waaF*/pWSK29-*crtEBI-dxr* produced 4.35, 4.29 and 4.22 mg/g lycopene, respectively; Δ*waaF*/pWSK29-*crtEBI-SR*, Δ*waaF*/pWSK29-*crtEBI-SRA*, Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRA*, and Δ*waaF*/pWSK29-*crtEBI*/pACYC184-*SRAI* produced 4.95, 5.28, 4.87 and 5.08 mg/g lycopene, respectively. Compared to Δ*waaF*/pWSK29-*crtEBI,* these strains produced more lycopene, suggesting again that enhancing the MEP pathway facilitates lycopene synthesis in *E. coli*.

4. Discussion

Biocatalysis employing the whole cell had been increasingly developed as a green technology in the synthesis of various valuable products, while low permeability of cell membrane always lead to the low productivities [3]. The outer membrane of gram-negative bacteria provided the cell with an effective permeability barrier against external noxious agents [18], and at the same time to allow the influx of nutrient molecules [19]. In the previous publications, *lpp* deletion was developed as a general permeabilization method. The *lpp* mutant had higher permeability of outer membrane and higher ability to synthesize L-carnitine [3]. Besides, expression of SCVE viroporins in *E. coli* also improved the diffusivity of small molecules across outer membrane through introducing additional pores within the outer membrane. As expected, the biocatalysts efficiency of *E. coli* was enhanced [2].

LPS, as the major molecule in outer membrane, played important roles on membrane behavior. In *E. coli*, LPS typically consists of a hydrophobic domain known as lipid A, a nonrepeating core oligosaccharide, and a distal polysaccharide known as *O*-antigen repeats. The core oligosaccharide is assembled on lipid A via sequential glycosyl transfer from nucleotide sugar precursors. In *E. coli*, the chromosomal *waa* locus encodes enzymes required for biosynthesis of the core oligosaccharide [20]. Mutations in LPS could alter outer membrane stability, giving rise to pleiotropic phenotype [21,22]. Among 10 W3110 Δ*waa* mutant strains, W3110 Δ*waaC* has the simplest LPS structure. In compared to W3110, W3110 Δ*waaC* had four-fold higher membrane permeability, and this likely benefited for lycopene accumulation. Comparative transcriptome showed that mRNA levels of *dxr* and *ispA* in Δ*waaC* and Δ*waaF* were up-regulated, compared to W3110 (data not shown). The over-expression of the *dxr* gene provided more IPP precursors in the MEP pathway in tobacco, which consequently stimulated synthesis efficiency of isoprenoid downstream [23]. IPP and DMAPP

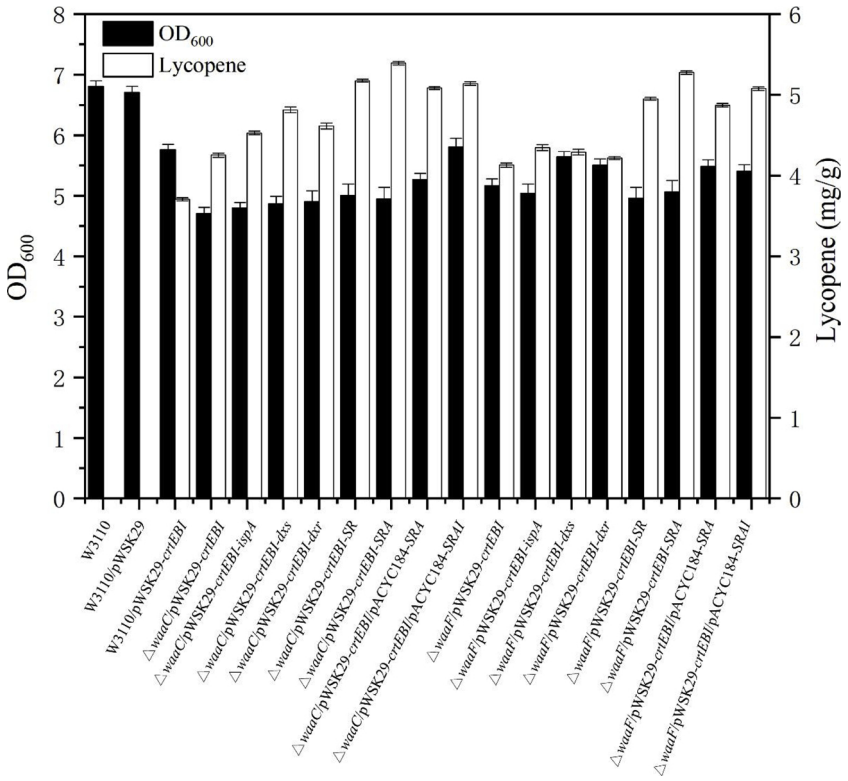


Fig. 5. Comparison of cell growth and lycopene yield in different *E. coli* cells. Data represent the average of three experiments and the error bars represent the standard deviation.

supply likely limited lycopene biosynthesis [9]. Besides, the accumulation of metabolites in the MEP pathway stimulated the transcription of *dxs* and *dxr* in *Arabidopsis* cell culture [24]. In the current study, therefore, the genes *dxs*, *dxr* and *idi* were overexpressed individually or in combination, to increase these precursors supply. As expected, the overexpression of genes in MEP pathway stimulated lycopene synthesis [25].

A recombinant *E. coli* strain overexpressing the genes *crtE*, *crtB*, and *crtI* from *Deinococcus radiodurans* R12 and optimizing the Shine Dalgarno regions and aligned spacing sequence could produce 88 mg/g lycopene after 40 h fermentation [28]. *E. coli* K12f could efficiently uptake fructose; overexpressing the *crtEBI* operon from *Pantoea ananatis*. in K12f could produce 192 mg/g lycopene when grown on LB medium containing 10 g/L fructose [29]. Therefore, overexpressing the *crtEBI* operon from other bacteria in Δ*waaC* or Δ*waaF* might increase lycopene production

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