Effect of concomitant lycopene biosynthesis on CoQ_{10} accumulation in transformed *Escherichia coli* strains

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Abstract

CoQ_{10} and lycopene are isoprenoid compounds with nutraceutical and pharmaceutical benefits. In this study, the effect of concomitant lycopene biosynthesis on CoQ_{10} accumulation in transformed *Escherichia coli* DH5α was studied. A lycopene production pathway including geranylgeranyl diphosphate synthase (*crtE*), phytoene synthase (*crtB*), and phytoene desaturase (*crtI*) from *Erwinia herbicola* was constructed in two CoQ_{10}-producing *E. coli* strains. *E. coli* Ba and *E. coli* Br containing *dds* orthologs encoding for decaprenyl diphosphate synthase (Dds), respectively from *Agrobacterium tumefaciens* and *Rhodobacter sphaeroides* were transformed by the lycopene pathway resulting in *E. coli* Ba-lyc and *E. coli* Br-lyc. The lycopene pathway in *E. coli* Br-lyc interestingly resulted in a significant increase in CoQ_{10} production from 564 ± 28 to 989 ± 22 μg /g DCW. To confirm that the improvement of CoQ_{10} production in *E. coli* Br-lyc was due to lycopene biosynthesis and not just geranylgeranyl diphosphate formation in the lycopene pathway, *crtE* was only introduced into *E. coli* Ba and *E. coli* Br strains. Surprisingly, *crtE* expression had adverse effects on CoQ_{10} production in both strains. The results shed light on the Dds-catalyzed reaction as a bottleneck controlled by precursors; and the efficiency of a parallel lycopene pathway to streamline the flow of metabolites.

Key words**:** Coenzyme Q_{10}; Decaprenyl diphosphate synthase; *Escherichia coli*; Lycopene; Metabolic engineering

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INTRODUCTION

Ubiquinone or coenzyme Q (CoQ) is a lipid-soluble molecule of the electron transport chain located in the mitochondrial inner membrane of human cells as well as the cytoplasmic membrane of bacteria. This coenzyme is essential in energy generation and some other processes including formation of disulfide bonds in proteins, regulation of gene expression and detoxification of reactive oxygen species (ROS) (Groneberg *et al*., 2005; Søballe and Poole, 2000; Alleva *et al*., 1995; Ernster *et al*., 1995; Kontush *et al*., 1995).

CoQ is generally composed of a benzene ring and an isoprenoid side chain comprised of isoprene units (isopentenyl diphosphate, IPP) (Fig. 1). The structure of the ring is common; however, the length of the side chain is a characteristic of various species of CoQs in different organisms. For example, the human isoprenoid side chain is comprised of 10 isoprene units resulting in the formation of CoQ_{10}. Other organisms may use different kinds of CoQs as electron and proton transporters in their own respiratory chains. For example, *Escherichia coli* contains CoQ_8 while *Saccharomyces cerevisiae* has CoQ_6 (Okada *et al*., 1998; Suzuki *et al*., 1997; Meganathan, 1996; Ernster, 1995; Grunler, 1994). The synthesis of the side chain of CoQ_{10} is catalyzed by decaprenyl diphosphate synthase; while in the case of CoQ_8, the octaprenyl diphosphate synthase is the responsible enzyme. Accordingly, all that is required for the production of a given ubiquinone in a living cell is the expression of a heterologous gene coding for the corresponding

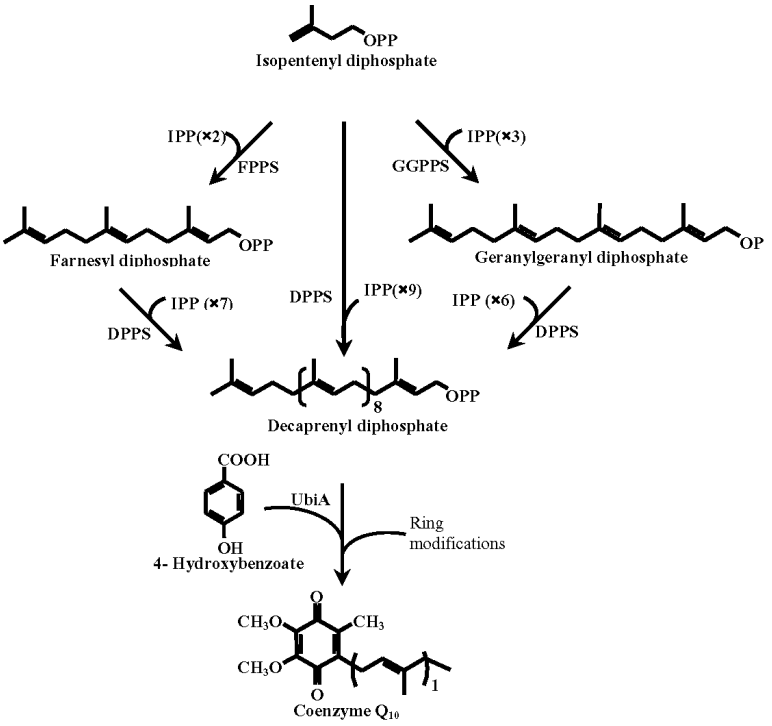

Figure 1. Schematic representation of the CoQ_{10} production pathway. The tandem condensation reactions, by decaprenyl diphosphate synthase (Dds), result in the polymerization of isopentenyl diphosphate (IPP) molecules into decaprenyl diphosphate.

polyprenyl diphosphate synthase (Kawamukai, 2002; Okada *et al*., 1996).

The attachment of decaprenyl diphosphate to a benzene ring and some subsequent ring modifications result in CoQ_{10} production. Dds may use IPP, farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) as substrates for addition of more IPP molecules to make decaprenyl diphosphate.

A condensation reaction between an isopentenyl diphosphate (IPP) and its allelic isomer, dimethylalyl diphosphate, results in the formation of geranyl diphosphate (GPP) with 10 carbons. The addition of a third IPP to GPP results in the formation of farnesyl diphosphate (FPP) with 15 carbons. Likewise, one more IPP may be added to FPP to make a 20-carbon molecule called geranylgeranyl diphosphate (GGPP). The latter two reactions are catalyzed by FPP synthase and GGPP synthase, respectively. IPP, GPP, FPP and GGPP are potential substrates for subsequent additions of IPP units by decaprenyl diphosphate synthase during CoQ_{10} biosynthesis (Fig. 1). Several decaprenyl diphosphate synthases have been purified and characterized from various organisms (Takahashi *et al*., 2003; Søballe and Poole, 2000; Suzuki *et al*., 1997). FPP and GGPP have been shown to be first and second most appropriate substrates for all the enzymes (Kawamukai, 2002).

Lycopene is a natural pigment which is biosynthesized from isoprene units as is CoQ_{10}. Two GGPP molecules are condensed head to head by phytoene synthase to make a 40-carbon molecule. The resulting phytoene molecule is then converted to lycopene via two tandem reactions catalyzed by an enzyme called phytoene desaturase (Sandmann, 2002) (Fig. 2).

Both CoQ_{10} and lycopene are known as natural antioxidants beneficial in keeping health and prevention of aging and ailments such as cardiovascular and neurodegenerative diseases and cancer. The separate production of CoQ_{10} and lycopene in *E. coli* has been investigated through several studies; but, there is very limited, if any, study on co-production of both materials in the same cell. The strategy of co-production of two metabolites in the same cell is indeed of importance from a biotechnology view point. However, the co-production of lycopene and CoQ_{10} was not the main target of this study. Instead, the current study was conducted to reveal the effect of concomitant lycopene biosynthesis on CoQ_{10} accumulation in transformed *E.*

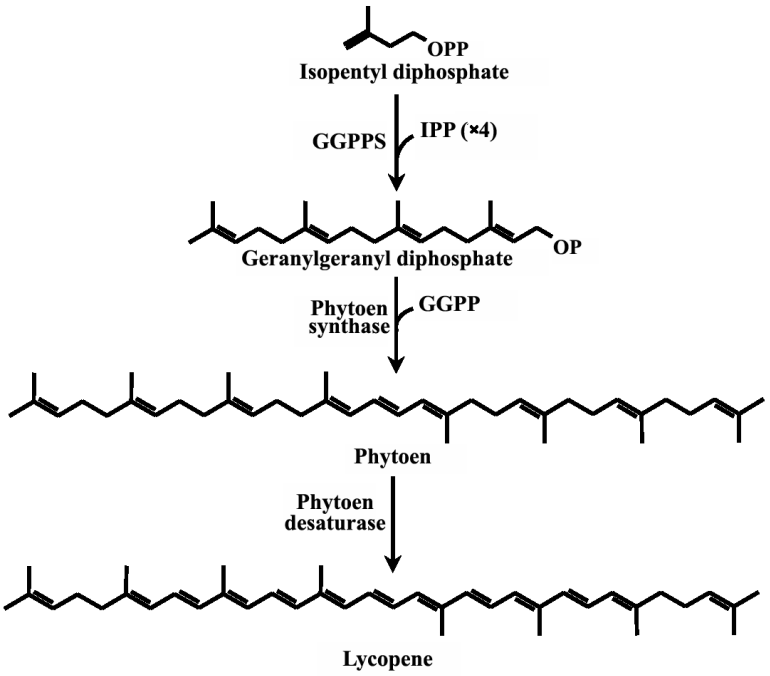

Figure 2. lycopene biosynthesis pathway comprised of geranylgeranyl diphosphate synthase (Ggds), phytoene synthase and phytoene desaturase. Geranylgeranyl diphosphate (GGPP) is a 20 carbone molecule composed of 4 isopentenyl diphosphate units (IPP). Head to head combination of two GGPP molecules by phytoene synthase and subsequent desaturations by phytoene desaturase result in the formation of lycopene.

coli. To this end, two CoQ_{10}-producing *E. coli* strains containing orthologs of decaprenyl diphosphate synthase from *Agrobacterium tumefaciens* and *Rhodobacter sphaeroides* were used for concomitant lycopene production.

MATERIALS AND METHODS

Media and culture conditions: *Escherichia coli* DH5α was grown on Luria-Bertani (LB) medium. In CoQ_{10} production experiments, 5ml of 2YT medium (1% yeast extract, 1.6% tryptone, 0.5% NaCl) was used for overnight precultivation of *E. coli* strains in a test tube at 37ºC. The main culture growth was conducted by using the same medium (7 ml) supplemented with 0.5% glycerol and 0.01% 4-hydroxybenzoic acid (sodium salt, Sigma, USA) (2YTGH), in 25 ml tubes Ampicillin (100 µg/ml) and kanamycin (30 µg/ml) were added to the culture media as required. Main cultures were inoculated to an initial OD_{600} of 0.1 and incubated at 30°C for 24 h in a rotary shaking incubator at 200 rpm (GFL, 3033). Cell growth was monitored by measuring turbidity at 600 nm, followed by conversion to dry cell weight (g DCW/l) using a standard curve.

Plasmids construction: *E. coli* DH5α [F′/endA1 hsdR17 (r_{K-} m_{K+}) glnV44 thi-1 recA1 gyrA (Nalr) relA1 Δ (lacIZYA-argF) U169 deoR 5(φ80dlac Δ (lacZ) M15] was used as a host for plasmid construction and CoQ_{10} production (Table 1). Restriction enzymes were purchased from Fermentas (Germany). T4 DNA ligase was purchased from New England Biolabs. *Pfu* DNA polymerase was purchased from Promega. Plasmid preparation, gel extraction and PCR purification kits were procured from Qiagen.

The genes encoding for decaprenyl diphosphate synthase (*dds*) were isolated from *Rhodobacter sphaeroides* 2.4.1 (ATCC 17023) and *Agrobacterium tumefaciens* (ATCC 33970) as reported elsewhere (Zahiri *et al*., 2006a). The *dds* gene of *Rhodobacter sphaeroides* 2.4.1 (*rsdds*) was obtained by double digestion of pTrsdds with *Eco*RI and *Sac*I (Table 1). The resulting fragment was inserted into a pBBR1MCS2 plasmid which was previously linearized by the same enzymes, thus giving rise to pBrsdds (Fig. 3). The *atdds* gene, coding for

decaprenyl diphosphate synthase of *A. tumefaciens*, was isolated from pTatdds by PCR using *pfu* DNA polymerase and oligonucleotides atdds-3 and atdds-4 as forward and reverse primers, respectively (Table 1). In the design of atdds-F a silent mutation was introduced to remove an inside *Eco*RI site from *atdds*. The PCR product was double digested by *Xma*I and inserted into pBBR1MCS2 to generate pBatdds (Fig. 3 and Table 1). The gene encoding for geranylgeranyl diphosphate synthase (*crtE*) was isolated from the *Erwinia herbicola* (ATCC 21434) genome using a PCR with crtE-F and crtE-R primers (Hundle *et al*., 1994). The PCR product was double digested with *Eco*RI and *Xba*I enzymes and ligated into pTrc99A linearized with the same enzymes. The resulting plasmid designated as pTcrtE was subsequently used in the construction of an artificial operon for lycopene production. The overlapping genes encoding for phytoene synthase (*crtB*) and phytoene desaturase (*crtI*) were isolated from *E. herbicola* as a single DNA fragment by a PCR using crtIB-F and crtIB-R primers (Hundle *et al*., 1994). The fragment was then inserted into pTcrtE after both the insert and vector had been restricted with *Xba*I and *Hin*dIII. The resulting plasmid was designated as pTcrtEIB. The recombinant plasmids were then introduced into *E. coli* cells using a modified chemical transformation method (Casali and Preston, 2003).

Determination and quantification of ubiquinones: The production of various coenzyme Qs in *E. coli* strains was analyzed by high performance liquid chro-

Table 1. Strains, plasmids and primers used in this study.

Name	Description	Referance
Strains		
E. coli DH5α	[F´/endA1 hsdR17 (rK^- mK^+) glnV44 thi-1 recA1 gyrA (Nal^r) relA1 Δ (lacIZYA-argF) U169 deoR 5 (φ80dlac Δ (lacZ)M15)]	(Gibco BRL)
E. coli Ba	*E. coli* DH5α harboring pBatdds	(This study)
E. coli Br	*E. coli* DH5α harboring pBrsdds	(This study)
E. coli Ba-crtE	*E. coli* Ba harboring pcrtE	(This study)
E. coli Br-crtE	*E. coli* Br harboring pcrtE	(This study)
E. coli Ba-lyc	*E. coli* Ba harboring pTcrtEIB	(This study)
E. coli Br-lyc	*E. coli* Br harboring pTcrtEIB	(This study)
E. coli Tlyc	*E. coli* DH5α harboring pTcrtEIB	(This study)
Plasmids		
pTrc99A	P*trc* expression vector, pBR322 origin, *lacI*^{q}, Amp^r, high copy	(Amersham Biosci.)
pBBR1MCS2	P*lac* cloning vector, pBBR1 origin, *LacZ*α, Km^r	(Kovach *et al*.,1995)
pTatdds	pTrc99A with *dds* gene from *A. tumefaciens*	(Zahiri *et al*., 2006b)
pTrsdds	pTrc99A with *dds* gene from *R. sphaeroides*	(Zahiri *et al*., 2006b)
pBatdds	pBBR1MCS2 with *dds* gene from *A. tumefaciens*	(This study)
pBrsdds	pBBR1MCS2 with *dds* gene from *R. sphaeroides*	(This study)
pTcrtE	pTrc99A with *crt*E gene from *E. herbicola*	(This study)
pTcrtEIB	pTrc99A with *crt*E, *crt*I and *crt*B genes from *E. herbicola*	(This study)
Primers		
Rsdds-F2	5'-ATAGAATTCAGGAGGTCATCGGGATGGGATTGGACGAGGTTTC-3' (*Eco*RI)	
Rsdds-R1	5'-TAAGAGCTCAAGGGATCAGGCGATGCGTTCGAC-3' (*Sac*I)	
atdds-3	5'-TCCCCCCGGGCACAGGAAACAGACCATGGAGTTCTTG-3' (*Xma*I)	
atdds-4	5'-TCCCCCCGGGTTAGTTGAGACGCTCGATGCAGAAG-3' (*Xma*I)	
crtE-F	5'-ATAGAATTCAGGAGGAATCATTCATGGTGAGTGGCAGTAAAGCG-3' (*Eco*RI)	
crtE-R	5'-ATATCTAGATTATCAGGCGATTTTCATGAC-3' (*Xba*I)	
crtIB-F	5'-ATATCTAGAGGAGGTATAAAGGATGAAAAAAACCGTTGTGATTG-3' (*Xba*I)	
crtIB-R	5'-ATAAAGCTTCTAAACGGGACGCTGCCAAAG-3' (*Hind*III)	

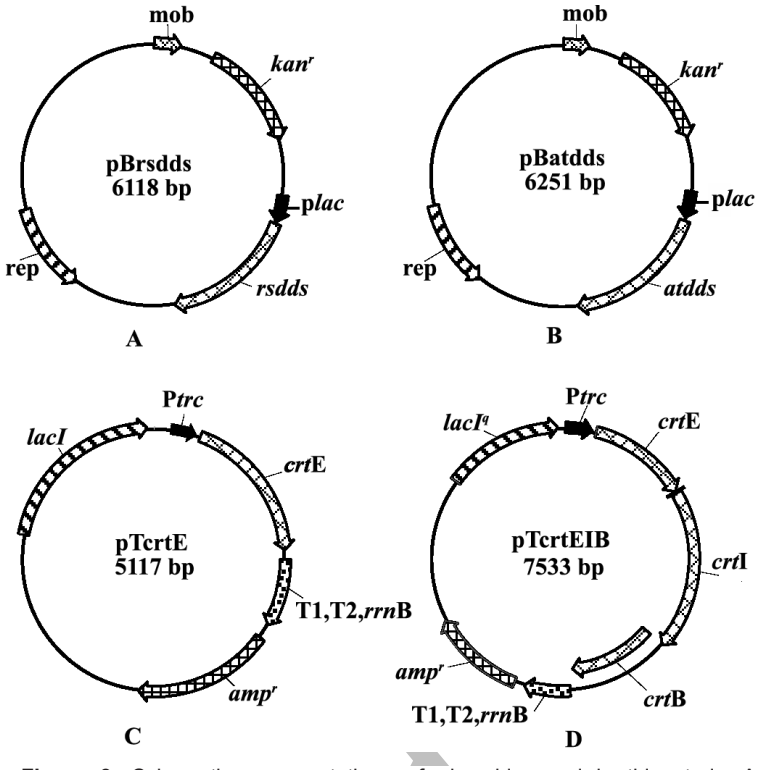

Figure 3. Schematic representations of plasmids used in this study. A: pBBR1MCS2 with the *rsdds* gene (decaprenyl diphosphate synthase) from *R. sphaeroides*. B: pBBR1MCS2 with *atdds* gene (decaprenyl diphosphate synthase) from *A. tumefaciens*. C: pTrc99A with *crtE* gene (geranyleranyl diphosphate synthase) from *E. herbicola*. D: pTrc99A with *crtE*, *crtI* (phytoene desaturase) and *crtB* (phytoene synthase) from *E. herbicola*.

matography (HPLC). CoQs were extracted from *E. coli* cells with hexane as explained elsewhere with some modifications. Briefly, a 500 µl sample of each culture was centrifuged at 13000 rpm (Heraeus, Biofuge) and the resulting pellet was washed once with 1 ml of distilled water and then with 1 ml of 20mM Tris-HCl, pH 7.6. The washed cells were lysed by being resuspended in lysis buffer [8% sucrose, 5% Triton X-100, 50 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0) and 1 mg/ml of lysozyme] and incubated at 37°C for 30 min. CoQs were extracted from the cell lysate with 900 µl of a hexane/propanol (5:3 v/v) mixture and then with 500 µl of hexane. Following each extraction, the hexane organic phase containing CoQ_{10} was separated from the aqueous phase by centrifugation at 14,000 rpm and pooled in a clean tube. The hexane extract was dried in a vacuum evaporator (speedvac®, AES 1010). The remaining pellet was dissolved in 500 µl of absolute ethanol (HPLC grade, Fisher Scientific); whereof 10 µl was injected into a HPLC machine (Shimadzu 10A system) equipped with a Symmetry® C18 column (Waters). The chromatography was run at room temperature using a ethanol and methanol mixture (70:30 v/v, HPLC grade, Fisher Scientific) as a mobile phase at a rate of 1 ml/min. The detection and quantification of CoQs were performed using a UV detector at 275 nm. The corresponding peaks of CoQ_8, CoQ_9 and CoQ_{10} in the HPLC chromatograms were identified using authentic standards (Sigma).

Quantification of lycopene: Lycopene production in transformed *E. coli* strains was quantified through extraction by a mixture of methanol/chloroform (70:30 v/v) and determination of the optical density of the extracts at 474 nm (OD_{474}). Cells were harvested by centrifugation of 1 ml of culture broth at 5000 rpm for 3 min. The pellet was then washed with water and resuspended in 400 µl of the methanol/chloroform mixture by vortexing for 5 min. After centrifugation, the supernatant was transferred to a clean tube and the extraction procedure was repeated once more. The supernatants were pooled together and the final volume of lycopene extract was raised up to one ml by adding more

solvent. The cell debris remained as a white pellet at the end of extraction. The optical density of the extract was converted to lycopene concentration by using a standard curve.

RESULTS

Coenzyme Q_{10} production in *E. coli*: Decaprenyl diphosphate synthase-encoding genes of *A. tumefaciens* (*atdds*) and *R. sphaeroides* (*rsdds*) were used for transformation of *E. coli* into a CoQ_{10} producing organism. The heterologous genes were cloned in pBBR1MCS2. The resulting plasmids, pBatdds and pBrsdds, were used for transformation of *E. coli* DH5α. *E. coli* is naturally a CoQ_8 producing organism. As a result of functional expression of the heterologous genes, the transformed *E. coli* DH5α cells, designated as *E. coli* Ba and *E. coli* Br, could produce CoQ_{10} in addition to CoQ_8. All strains were grown in 2YTGH medium under the same experimental conditions, as described above. The CoQs, including CoQ_8 and CoQ_{10}, were extracted using hexane and determined quantitatively by HPLC. The expression of the heterologous genes in *E. coli* Ba and *E. coli* Br led to a significant decrease in CoQ_8 production as compared to that of *E. coli* DH5α (Fig. 4). The CoQ_{10} production of *E. coli* Ba was reproducibly higher than that of *E. coli* Br. On average, the CoQ_{10} content of *E. coli* Ba was approximately 629 ± 40 μg/gDCW, while that of *E. coli* Br approximately 564 ± 28 μg/gDCW under the described experimental conditions.

Expression of a heterologous lycopene pathway in CoQ_{10} producing *E. coli*: An artificial operon representing the lycopene production pathway was constructed in the pTrc99A plasmid. This operon was comprised of geranylgeranyl diphosphate synthase (*crtE*), phytoene synthase (*crtB*) and phytoene desaturase (*crtI*) encoding genes. The resulting plasmid, designated as pTcrtEIB, was used for transformation of *E. coli* DH5α resulting in *E. coli* Tlyc (Fig. 3). The genome of *E. coli* lacks a counterpart gene of *crtE* encoding for geranylgeranyl diphosphate (GGPP) synthase. As a result of the expression of *crtE*, 20-carbon GGPP molecules are synthesized from isopentenyl diphosphate precursors. Two GGPP molecules, in turn, are combined head to head by phytoene synthase resulting in the formation of a 40-carbon phytoene. Phytoene molecules undergo tandem steps of modification catalyzed by phytoene desaturase resulting in the formation of lycopene (Fig. 2).

The expression of the heterologous pathway in *E. coli*

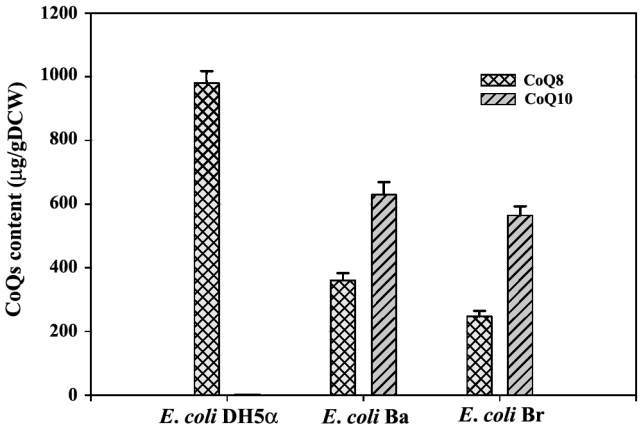

Figure 4. The effects of *atdds* and *rsdds* expression on CoQ_8 and CoQ_{10} production in *E. coli* DH5α. *E. coli* Ba and *E. coli* Br respectively harbor *atdds* and *rsdds* genes, encoding decaprenyl diphosphate synthase. Expression of the genes partially diverts ubiquinone biosynthesis from CoQ_8 to CoQ_{10} production. Error bars indicate the standard error of the mean of three independent experiments.

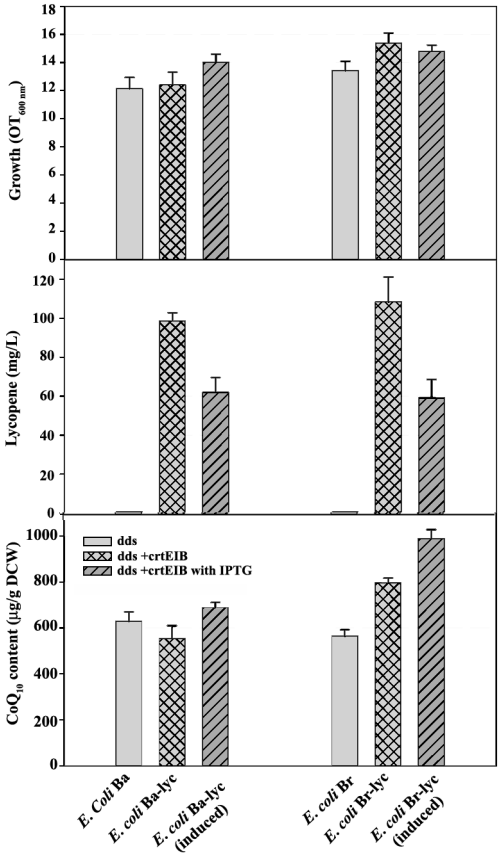

Figure 5. Effects of concomitant lycopene production on CoQ_{10} accumulation and growth of transformed *E. coli* strains. A lycopene pathway was introduced in *E. coli* Ba and *E. coli* Br, producing CoQ_{10} due to the expression of *dds* genes, respectively from *A. tumefaciens* and *R. sphaeroides*. The resulting strains, *E. coli* Ba-lyc and *E. coli* Br-lyc, were cultured in 2YTG medium for CoQ_{10} production. Error bars indicate the standard error of the mean of three independent experiments.

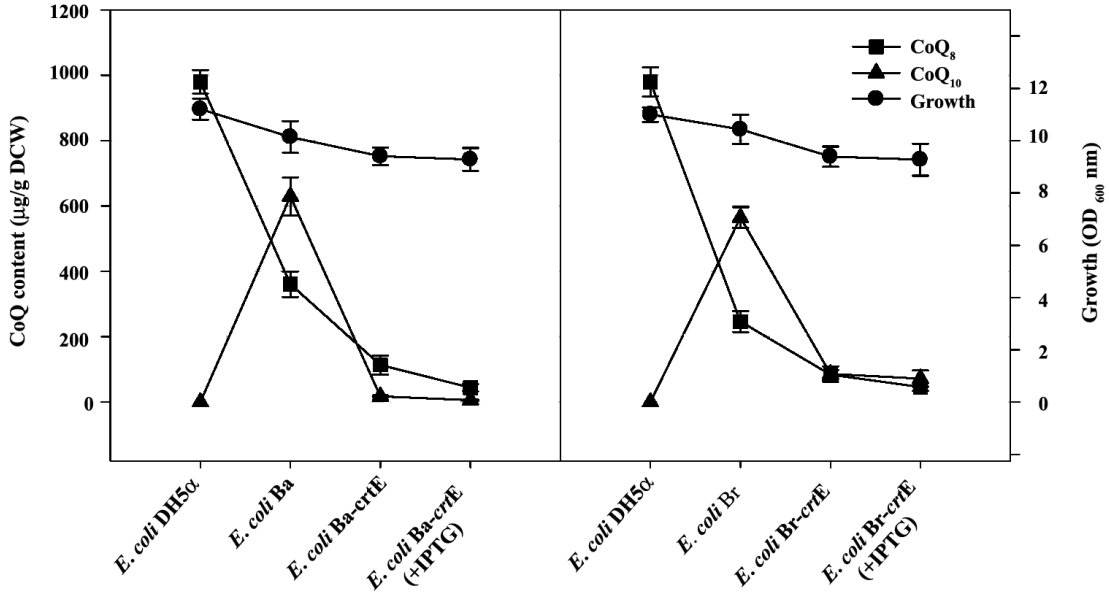

Figure 6. Effects of *crtE* expression on ubiquinone biosynthesis in CoQ_{10}-producing *E. coli* strains. A heterologous *crtE* gene encoding for geranylgeranyl diphosphate synthase from *E. herbicola* was introduced by the pTcrtE plasmid into *E. coli* Ba and *E. coli* Br. In resulting strains, *E. coli* Ba-crtE and *E. coli* Br-crtE, the expression of *crtE* under the control of *tac* promoter led to severe decreases in both CoQ_8 and CoQ_{10} production. Error bars indicate the standard error of the mean of three independent experiments.

Tlyc was confirmed by the formation of red colonies by the harboring cells. Lycopene production in *E. coli* Tlyc was approximately 140 ± 17 mg/l. This construct was then used for transformation of *E. coli* Ba and *E. coli* Br. The resulting strains were designated as *E. coli* Ba-lyc and *E. coli* Br-lyc, respectively. The latter strains were cultured for the purpose of CoQ_{10} production in 2YTG medium under the same conditions, as described previously. Lycopene production in *E. coli* Ba-lyc did not significantly affect CoQ_{10} production. However, in the case of *E. coli* Br-lyc, CoQ_{10} production was significantly improved from approximately 564 ± 28 to 796 ± 38 µg/g DCW. Induction of the cells by IPTG (0.05 mM) elevated CoQ_{10} production even further to approximately 989 ± 22 µg/g DCW (Fig. 5). *E. coli* Ba-lyc concomitantly produced lycopene up to 98 ± 4 mg/l, which was decreased to 62 ± 8 mg/l after induction by 0.05 mM IPTG. Lycopene production in *E. coli* Br-lyc was quantified as 108 ± 13 mg/l, which was decreased to 59 ± 10 mg/l after induction by 0.05 mM IPTG (Fig. 5). Growth was not significantly affected in both strains under concomitant lycopene production conditions (Fig. 5).

The effect of *crtE* expression in *E. coli* Ba and *E. coli* Br: The expression of *crtE* results in the formation of GGPP as an intermediate metabolite in the lycopene pathway. GGPP is the essential metabolite in lycopene production as well as the second most suitable substrate after FPP for CoQ_{10} production (Zahiri *et al*., 2006b; Takahashi *et al*., 2003). *E. coli* contains an *ispA* gene for FPP biosynthesis but does not have a counterpart for *crtE*. One may presume that the improvement of CoQ_{10} production in *E. coli* Br-lyc is due to GGPP formation as a result of *crtE* expression and does not have a direct relation with lycopene biosynthesis as a whole pathway.

To reveal the effect of *crtE* alone on CoQ_{10} production, the genes *crtI* and *crtB* were removed from pTcrtEIB. The resulting plasmid, pTcrtE, was used for transformation of *E. coli* Ba and *E. coli* Br. The resulting strains, *E. coli* Ba-crtE and *E. coli* Br-crtE, were cultured for coenzyme Q production under the same conditions, as described previously. Ubiquinones, including CoQ_8 and CoQ_{10}, were extracted and quantified by HPLC. The results showed that the expression of *crtE* was highly inhibiting for both CoQ_8 and CoQ_{10} production in *E. coli* Ba-crtE and *E. coli* Br-crtE, even in the absence of the inducer (Fig. 6). However, the inhibition of CoQ_{10} production was more severe in *E. coli* Ba-crtE. Growth of both strains was not significantly affected by *crtE* expression (Fig. 6). It seemed that in *E. coli* Ba-crtE, respiration was mainly supported by the residual amount of CoQ_8 production that is sufficient for supporting growth.

DISCUSSION

The IPP concentration has been suggested as a source of limitation in the biosynthesis of isoprenoids (Sandmann, 2002; Huang *et al*., 2001). Various metabolic engineering strategies have so far been attempted by researchers to improve isoprenoid production in *E. coli*. These attempts mainly focus on increasing IPP availability through manipulation of the native MEP pathway or introduction of a heterologous mevalonate pathway (Rodrígues-Villalthe ón *et al*., 2008; Kim and Keasling, 2003; Martin *et al*., 2003; Matthews and Wurtzel, 2000; Harker and Bramley, 1999). In the case of CoQ_{10}, manipulation of the MEP pathway through overexpression of DXS has been found to result in a slight increase in CoQ_{10} production by up to 940 µg/g DCW (Kim, 2006). In contrast, researchers have found that a heterologous mevalonate pathway results in significant, but not yet satisfactory, improvements in CoQ_{10} production by up to 2400 µg /g DCW (Zahiri *et al*., 2006b). When compared with amorphadiene production by engineering a heterologous mevalonate pathway in *E. coli*, the similar strategy has not been as efficient for CoQ_{10} production as expected (Newman, 2006). This probably is an indication of other bottlenecks than IPP availability in the pathways leading to CoQ_{10} biosynthesis (Cluis, 2007).

In contrast to IPP biosynthesis, little attention has so far been paid to the consumption rate of IPP in the cell. It is well known that the production and consumption rates of precursors are critical to achieve the maximum productivity of a biosynthetic pathway. Especially that accumulation of precursors may pose new bottlenecks via the inhibition of some enzymes of the pathway.

In this study the effect of concomitant lycopene production was studied on CoQ_{10} accumulation in *E. coli*. Isopentenyl diphosphate (IPP) is a common precursor for the biosynthesis of ubiquinones as well as lycopene. Two, three and four IPP molecules may combine together by specific enzymes to form GPP, FPP and GGPP, respectively. All the four molecules are potential substrates for the addition of more IPP molecules by decaprenyl diphosphate synthase in a step wise manner, in order to make the long 50-carbon chain of CoQ_{10} (Fig. 1). Experiments have revealed that FPP and GGPP are respectively the preferred substrates of decaprenyl diphosphate synthase, resulting in maximum *in vitro* reaction rates of the enzyme (Zahiri, 2006a; Lee *et al*., 2004; Takahashi et al., 2003). On the other hand, GGPP is the essential substrate for the biosynthesis of lycopene. Therefore, ubiquinone and lycopene biosynthesis pathways share the same pool of precursors.

In the current work, a heterologous lycopene production pathway was engineered in two CoQ_{10}-producing *E. coli* strains. The concomitant lycopene production did not significantly affect CoQ_{10} production in *E. coli* Ba-lyc carrying decaprenyl diphosphate synthase of *A. tumefaciens*. In contrast, CoQ_{10} production in *E. coli* Br-lyc harboring decaprenyl diphosphate synthase of *R. sphaeroides* was remarkably improved as a result of the concomitant production of lycopene. The main difference between these two strains is the biochemical characteristics of decaprenyl diphosphate synthases being used for CoQ_{10} production. The Dds enzymes of *A. tumefaciens* and *R. sphaeroides* have 56% similarity according to amino acid alignments (Zahiri *et al*., 2006a). The relatively low similarity between the enzymes may reflect in the difference in their biochemical characteristics. In both strains, lycopene production did not harmfully affect growth. It may be concluded that the net result of lycopene biosynthesis is synergistic for CoQ_{10} production in *E. coli* Br-lyc.

It is important to confirm that improved CoQ_{10} production in *E. coli* Br-lyc is due to lycopene production and not GGPP formation as an intermediate molecule of the lycopene pathway, which is a suitable substrate for CoQ_{10} production as well.

The expression of *crtE* in CoQ_{10} producing *E. coli* strains resulted in significant decreases in both CoQ_8 and CoQ_{10} production. However, growth was not significantly affected by the expression of *crtE*. As the gene codes for geranylgeranyl diphosphate synthase, it seems that the decrease in ubiquinone biosynthesis occurs as a result of the inhibition of both octa- and deca-prenyl diphosphate synthases by GGPP. This is the first report of the *in vivo* inhibition of prenyl diphosphate synthases by GGPP. To make sure of such an inhibitory effect of substrates on prenyl diphosphate synthases, *crtE* in pTcrtE was replaced with *ispA* encoding for farnesyl diphosphate synthase. The resulting plasmid, pTispA, was used for transformation of *E. coli* Ba and *E. coli* Br. The same results as with crtE were achieved with *ispA*. Ubiquinone biosynthesis was strongly inhibited as a result of *ispA* expression (data not shown). Here again, growth was not significantly affected.

The results may show that the inhibition of decaprenyl diphosphate synthase by the precursors is a major bottle neck affecting CoQ_{10} accumulation. The Dds of *R. sphaeroides* is less sensitive to inhibition by GGPP. Therefore, GGPP at tolerated levels may be used by this enzyme as a substrate for CoQ_{10} production. *R. sphaeroides* is a photosynthetic organism that

produces high levels of CoQ_{10} in addition to isoprenoid red pigments. It can be concluded that to take example of natural systems may be an efficient approach along with rational and computational approaches in designing metabolic engineering strategies. The concomitant production of biomaterials in the same cell is important from a biotechnological viewpoint. This strategy may result in higher accumulation of important products, which has applications in the pharmaceutical, food and feed industries.

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