

Inhibition of Coenzyme Qs Accumulation in Engineered *Escherichia coli* by High Concentration of Farnesyl Diphosphate

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Abstract

Background: Coenzyme Q₁₀ (CoQ₁₀) is an isoprenoid component used widely in nutraceutical industries. Farnesyl diphosphate synthase (FPPS) is a responsible enzyme for biosynthesis of farnesyl diphosphate (FPP), a key precursor for CoQs production. This research involved investigating the effect of FPPS over-expression on CoQs production in engineered CoQ₁₀-producing *Escherichia coli* (*E. coli*).

Methods: Two CoQ₁₀-producing strains, as referred to *E. coli* Ba and *E. coli* Br, were transformed by the encoding gene for FPPS (*ispA*) under the control of either the *trc* or P_{BAD} promoters.

Results: Over-expression of *ispA* under the control of P_{BAD} promoter led to a relative increase in CoQ₁₀ production only in recombinant *E. coli* Br although induction by arabinose resulted in partial reduction of CoQ₁₀ production in both recombinant *E. coli* Ba and *E. coli* Br strains. Over-expression of *ispA* under the control of stronger *trc* promoter, however, led to a severe decrease in CoQ₁₀ production in both recombinant *E. coli* Ba and *E. coli* Br strains, as reflected by reductions from 629±40 to 30±13 and 564±28 to 80±14 µg/g Dried Cell Weight (DCW), respectively. The results showed high level of FPP reduces endogenous CoQ₈ production as well and that CoQs are produced in a complimentary manner, as the increase in production of one decreases the production of the other.

Conclusion: The reduction in CoQ₁₀ production can be a result of Dds inhibition by high FPP concentration. Therefore, more effort is needed to verify the role of intermediate metabolite concentration and to optimize production of CoQ₁₀.

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Introduction

Ubiquinone, also known as CoQ, is an isoprenoid component and lipid-soluble molecule which plays various roles in both eukaryotic and prokaryotic cells. The most important function of CoQ is in energy generation, where it is responsible for electron transfer in the respiratory chain. In addition, CoQ has also been found to have an antioxidant function and is involved in various biological processes including gene expression, formation of disulfide bonds in proteins and generation of cellular signals ¹⁻⁷. The lack of CoQ has been associated with various diseases ⁸.

Ubiquinone has been used as a medicine, but is now widely used as a nutritional supplement ^{9,10}. The effect of ubiquinone on the lifespan of humans is currently being studied extensively while studies on other roles

of ubiquinone are also underway ¹¹. Ubiquinone is generally composed of a benzene ring and an isoprenoid side chain, which is a homopolymer of Isopentenyl diphosphate (IPP), also known as the isoprene unit. The length of the side chain determines the type of CoQ in various organisms. For example, the CoQ side chain in human cells is comprised of ten isoprene units and forms CoQ₁₀, while *Escherichia coli* (*E. coli*) contains CoQ₈ which possesses eight isoprene units in its side chain ¹²⁻¹⁶. The synthesis of the CoQ₁₀ side chain is catalyzed by Dds, whereas Octaprenyl diphosphate synthase (Ods) is the enzyme responsible for biosynthesis of the corresponding side chain in *E. coli*. Therefore, the expression of the heterologous gene encoding for Dds can lead to the production of CoQ₁₀ in trans-

formed *E. coli* cells along with CoQ₈. Accordingly, several CoQ₁₀-producing *E. coli* strains have been developed by introducing Dds encoding gene from various sources either via plasmid-expression system or chromosomal integration^{4,15-18}. The initial step in biosynthesis of isoprenoid side chain involves a condensation reaction between an IPP and its allelic isomer, Dimethylallyl diphosphate (DMADP) by Geranyl diphosphate synthase (GPPS) which results in the formation of Geranyl diphosphate (GPP) with ten carbons. FPPS adds another IPP to GPP and produces Farnesyl diphosphate (FPP) with 15 carbons. Likewise, Geranylgeranyl diphosphate synthase (GGPPS) uses FPP as a substrate and adds another IPP to form Geranylgeranyl diphosphate (GGPP) with 20 carbons. Finally, the polyprenyl diphosphate synthases such as Dds can use the above mentioned molecules (IPP, GPP, FPP and GGPP) as substrates for addition of more IPP molecules to make corresponding isoprenoid side chain. Finally, attachment of the synthesized side chain to benzene ring and subsequent modifications of the ring result in CoQ production^{3,16,17,19,20}. Figure 1 illustrates the biosynthetic steps for CoQ₁₀ production.

Several Dds have been characterized and isolated from different sources^{7,21-23}. FPP is found to be the most preferred substrate for all types of Dds in reactions taking place *in vitro*⁴. Accordingly, FPP can be an attractive precursor to control CoQ₁₀ biosynthesis in engineered CoQ₁₀-producing *E. coli* strains. Indeed, this suggests a hypothesis that the over-expression of the heterologous gene encoding for FPPS (ispA) under the control of various promoters in CoQ₁₀-producing *E. coli* strains can control the biosynthesis of CoQ₁₀. This strategy allows whole biosynthetic pathway productivity to be controlled through the intermediate metabolites levels.

The current study was conducted to determine the effect of high FPP concentration on both CoQ₁₀ and CoQ₈ accumulation in engineered *E. coli* cells. Two CoQ₁₀-producing strains previously constructed by introducing Dds encoding gene from two different organisms²⁴ were used for concomitant expression of FPPS using either the *trc* or *P_{BAD}* promoter to control the level of expression.

Materials and Methods

Strain, media and culture condition

E. coli DH5α was selected as the host for construction of recombinant strains (Table 1). Luria-Bertani (LB) medium was used for cloning related cultures²⁵. For CoQ₁₀ production, cells were initially grown in 5 ml of 2YT pre-culture medium (1% yeast extract, 1.6% tryptone and 0.5% NaCl) at 37 °C. For production phase, seven ml of 2YTG medium (2YT medium supplemented with 0.5% glycerol and 0.01% 4-hydroxybenzoic acid) was used to carry out the cultivation in 25 ml tubes. The 2YTG media were inoculated with the pre-culture to an initial OD₆₀₀ of 0.1, and were then

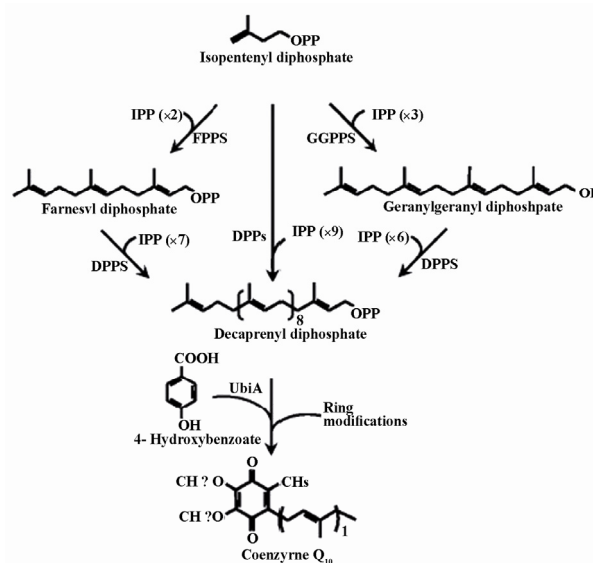


Figure 1. The tandem condensation reactions by decaprenyl diphosphate synthase result in the polymerization of isopentenyl diphosphate (IPP) molecules into decaprenyl diphosphate. The indicated enzymes are as follow: FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; DPPS, decaprenyl diphosphate synthase and UbiA, 4HB-polyprenyl transferase (Zahiri *et al*, 2009).

incubated at 30 °C for 48 hr in a rotary shaking incubator (GFL, UK) at 200 rpm. Ampicillin (100 µg/ml) and kanamycin (30 µg/ml) were added to the culture media as required. The growth of cells was then monitored by measuring optical density of the culture samples at 600 nm. The dry cell weight (g DCW/l) of the culture samples was calculated from a standard curve. CoQs quantification was determined in triplicate culture of recombinant strains.

Plasmid construction and transformation

All restriction enzymes were purchased from Fermentas (Germany). T₄DNA ligase was purchased from New England Biolab (UK). Plasmid isolation and PCR product purification kits were from QIAGEN (US). The Dds encoding gene (dds) had previously been isolated from *Agrobacterium tumefaciens* (*A. tumefaciens*) (ATCC 33970) and *Rhodobacter sphaeroide* (*R. sphaeroide*) 2.4.1 (ATCC 17023), as reported elsewhere²³. The construction of plasmids harboring dds gene either from *A. tumefaciens* (atdds) or *R. sphaeroide* (rsdds) were described previously²⁴. Briefly, atdds was obtained from the existing pTatdds plasmid and amplified by PCR using Atdds-3 and Atdds-4 primers (Table 1). The PCR product was then double digested by XmaI and ligated into the pBBR1MCS2 plasmid which had been digested by the same restriction enzyme, resulting in the formation of the pBatdds plasmid. Likewise, rsdds gene was amplified by PCR using existing pTrsdds plasmid as template and Rsdds-F and Rsdds-R primers (Table 1). Next, the PCR product was double digested with EcoRI and Sac I and inserted into the pBBR1MCS2 plasmid which had previously been digest-

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

Name	Description	Reference
Strains		
<i>E. coli</i> DH5 α	F/endA1 hsdR17 (rk- mk+) gin V44 thi-1 RecA1 gyrA (Nal ^I) relA1 Δ (lacZYA-arg U169 deoR5 (Ψ80dlac Δ (lacZ) M15)]	(Gibco BRL)
<i>E. coli</i> Ba	<i>E. coli</i> DH5 α harboring pBatdds	(24)
<i>E. coli</i> Br	<i>E. coli</i> DH5 α harboring pBrdsds	(24)
<i>E. coli</i> BaTi	<i>E. coli</i> Ba harboring pTispA	(This study)
<i>E. coli</i> BrTi	<i>E. coli</i> Br harboring pTispA	(This study)
<i>E. coli</i> BaDi	<i>E. coli</i> Ba harboring pDispA	(This study)
<i>E. coli</i> BrDi	<i>E. coli</i> Br harboring pDispA	(This study)
Plasmids		
pTrc99A	P _{trc} expression vector, pBR322 origin, lac/q, Amp ^r , high copy	(Amersham,Bioscience)
pBBR1MCS2	Plac cloning vector, pBBR1 origin, lacZ α , Kan ^r	(37)
pBAD24	P _{BAD} cloning vector, pBR322/ColE1 origin, araC, Amp ^r	(38)
pBatdds	pBBR1MCS2 with dds gene from <i>A. tumefaciens</i>	(24)
pBrdsds	pBBR1MCS2 with dds gene from <i>R. sphaeroides</i>	(24)
pTispA	pTrc99A with ispA gene from <i>E. coli</i>	(This study)
pDispA	pBAD24 with ispA gene from <i>E. coli</i>	(This study)
Primers		
Atdds-3	5'-TCCCCCGGGCACAGGAAACAGACCATGGAGTTCTTG-3' (XmaI)	
Atdds-4	5'-TCCCCCGGGTTAGTTGAGACGCTCGATGCAGAAG-3' (XmaI)	
Rsdds-F	5'-ATAGAATTCAGGAGGTCATCGGGATGGGATTGGACGAGGTTTC-3' (EcoRI)	
Rsdds-R	5'-TAAGAGCTCAAGGGATCAGGCGATGCGTTCGAC-3' (SacI)	
ispA-F	5'- ATAGAATTCAGGAGGAATGAGTAATGGAC-3' (EcoRI)	
ispA-R	5'-ATAGGATCCGGTACCTCATTATTTATTACGC-3' (BamHI)	

ed with the same enzymes, leading to the formation of pBrdsds. FPPS encoding gene (ispA) had been isolated from the *E. coli* (GenBank: D00694.1) as reported previously²⁶. The ispA gene was obtained from the existing pBatdds ispA ubi CABG plasmid by PCR using ispA-F and ispA-R primers (Table 1). The PCR product was subsequently double digested by EcoRI and BamHI and ligated to pTrc99A plasmid under the control of the trc promoter, leading to the formation of pTispA plasmid. The double digested ispA fragment was also ligated to pBAD24 plasmids under the control of P_{BAD} promoter, using the same enzymes, and led to the formation of pDispA plasmid. Both pTrc99A and pBAD24 plasmids had previously been digested by EcoRI and BamHI. The recombinant plasmids were introduced to *E. coli* cells using a modified chemical transformation method²⁷.

CoQs extraction

CoQs content of cells was determined by extraction. Specifically, 500 μ l of culture was centrifuged at 10,000 $\times$ g (Heraeus, Biofuge, UK). The pellet was washed with 1 ml of distilled water, then 1 ml of 20 mM Tris-HCl buffer (pH=7.6). 450 μ l of lysis buffer [8% sucrose, 5% Triton X-100, 50 mM Tris-HCl (pH=8), 50 mM EDTA (pH=8) and 1 mg/ml of lysozyme] was added to washed pellet and incubated at 37 °C for 30 min. 900 μ l of a hexane/propanol (5:3 v/v) mixture was added to lysed cells and centrifuged at

11,000 $\times$ g for 2 min. The hexane organic phase containing CoQs was transferred to a clean tube. Hexane (500 μ l) was added to the aqueous phase and centrifuged at 11,000 $\times$ g for 2 min. The second hexane phase was added to the first and dried in a vacuum evaporator (Speedvac®, AES 1010, US). The pellet was dissolved in 500 μ l of absolute ethanol (HPLC grade, Merck, US).

Determination and quantification of CoQs

The CoQ content was determined from 10 μ l injected sample by HPLC (Shimadzu 10A system, Japan) equipped with a Symmetry® C18 column (Waters, US). The mobile phase was the mixture of ethanol and methanol (70:30 v/v) with flow rate of 1 ml/min. CoQs were detected using a UV detector at 275 nm. The identification of corresponding peaks in the HPLC chromatograms of experimental samples was carried out using authentic standards, CoQ₉ and CoQ₁₀. Quantification of CoQs were performed with the aid of an appropriate standard curve.

Results

CoQs production in *E. coli*

As mentioned in our previous work²⁴, two type of dds genes, rsdds and atdds, were inserted into the pBr1MCS2 plasmid, and resulted in the formation of plasmids pBrdsds and pBatdds. The recent plasmids were transformed into *E. coli* DH5 α , and designated as

E. coli Br and *E. coli* Ba, respectively. The recombinant strains were grown in 2YTGH medium under the same experimental conditions. Their CoQs content was extracted and determined quantitatively by detection of the corresponding peaks in the HPLC chromatogram. The expression of rsdds or atdds in the transformed *E. coli* Ba and *E. coli* Br led to the production of CoQ₁₀ together with CoQ₈. On average, *E. coli* Ba had CoQ₁₀ content of 629±40 µg/g DCW while *E. coli* Br had 564±28 µg/g DCW under the described experimental conditions. *E. coli* naturally has an *Ods* encoding gene. So, the CoQ₈ content was also measured in CoQ₁₀-producing strains. Expression of dds led to a significant decrease in CoQ₈ production by both *E. coli* Ba (from 990±32 to 360±23 µg/g DCW) and *E. coli* Br (from 990±32 to 246±24 µg/g DCW), when compared to that in *E. coli* DH5α²⁴. An example of HPLC chromatogram for CQs identification in *E. coli* Ba is shown in figure 2. The recombinant *E. coli* strain produced CoQ₁₀ and slight amounts of CoQ₉ in addition to naturally occurring CoQ₈. The CoQ₉ might be produced as a result of immature release of a fraction of the growing polyprenyl chains from the Dds¹⁷.

Effect of *ispA* over-expression under the control of *P*_{BAD} promoter in CoQ₁₀-producing *E. coli*

The expression of *ispA* results in an increased level of FPP production. FPP is the most suitable substrate for all types of Dds in vitro reactions⁴. In order to study the effect of FPPs on CoQs production, the *ispA* gene was ligated into the pBAD24 plasmid, leading to the formation of pDispA plasmid (Figure 3A). This recombinant plasmid was used for transformation of *E. coli* Ba and *E. coli* Br. The resulting recombinant cells, designated as *E. coli* BaDi and *E. coli* BrDi, were cultured in 2YTG medium, and their CoQs were extracted and quantified by HPLC.

In the absence of inducer, the expression of *ispA* under the control of *P*_{BAD} promoter does not have significant effect on CoQ₁₀ production in *E. coli* BaDi, with negligible change from 629±40 to 636±35 µg/g DCW (Figure 4), while relative increase was observed

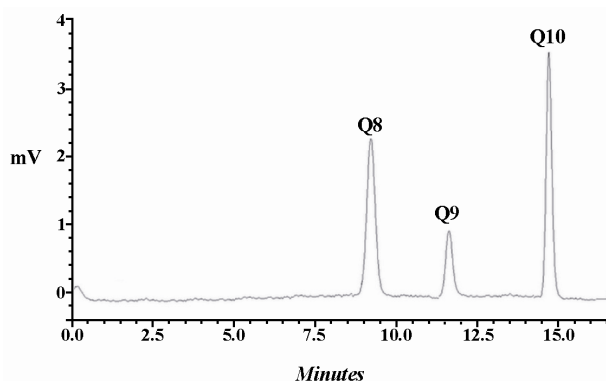


Figure 2. HPLC chromatogram for CQs identification in *E. coli* Ba. The recombinant *E. coli* produced CoQ₁₀ and slight amounts of CoQ₉ in addition to naturally occurring CoQ₈.

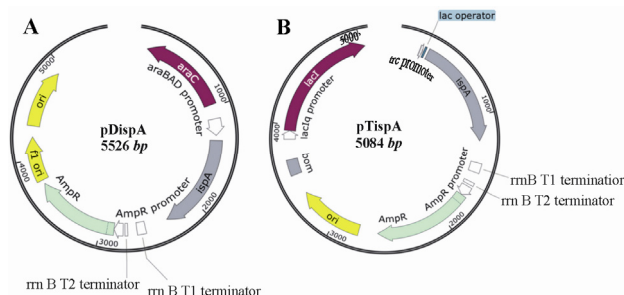


Figure 3. Schematic representation of recombinant pDispA plasmid, encoding for *ispA* gene under the control of *P*_{BAD} promoter (A) and pTispA plasmid, encoding for *ispA* gene under the control of *trc* promoter (B). The genetic maps are generated by SnapGene software (from GSL Biotech; available at snapgene.com).

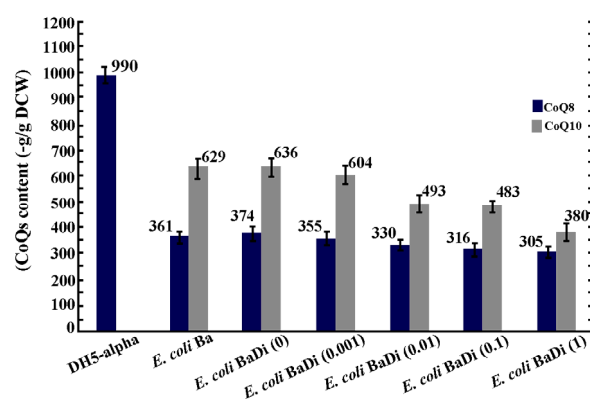


Figure 4. Plasmid pDispA was introduced into *E. coli* Ba, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BaDi. Numbers in brackets indicate arabinose mM concentration in cultures. Error bars indicate the standard error of the mean of three independent experiments.

in *E. coli* BrDi from 564±28 to 750±28 µg/g DCW (Figure 5). However, once induction by arabinose was applied, relative reduction in CoQ₁₀ production was obtained which was proportional to the concentration of arabinose in both strains. In the absence of induction, the CoQ₈ production demonstrated roughly a two-fold reduction in *E. coli* BrDi (from 246±24 to 117±13 µg/g DCW), while no significant change (from 361±23 to 373±28 µg/g DCW) was observed in *E. coli* BaDi. Insignificant reduction in CoQ₈ production was observed in both strains with increased arabinose concentration (Figures 4 and 5). As a whole, the results showed that CoQs production in both *ispA*-expressing strains is inhibited once the induction is applied.

Effect of *ispA* over-expression under the control of *trc* promoter in CoQ₁₀-producing *E. coli*

In order to confirm that the reduction in CoQs production is due to the inhibitory effect of high FPP concentration, *ispA* was expressed under the control of *trc* promoter which is a stronger promoter than *P*_{BAD} and regulated alternatively by adding IPTG to the culture media. So, the *ispA* gene was ligated into the pTrec99A

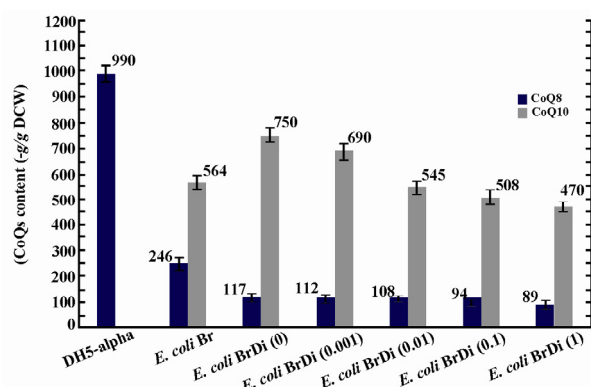


Figure 5. Plasmid pDispA was introduced into *E. coli* Br, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BrDi. Numbers in brackets indicate arabinose mM concentration in cultures. Error bars indicate the standard error of the mean of three independent experiments.

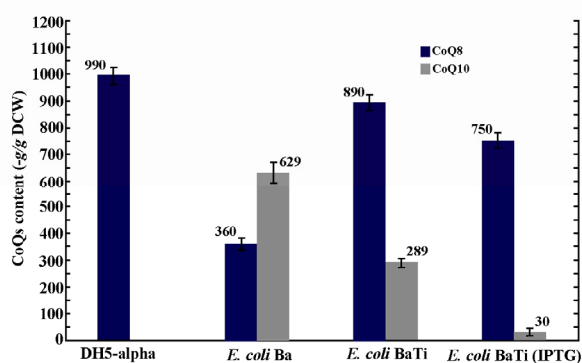


Figure 6. Plasmid pTispA was introduced into *E. coli* Ba, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BaTi. Error bars indicate the standard error of the mean of three independent experiments.

plasmid, leading to the formation of pTispA (Figure 3B). This recombinant plasmid was used for transformation of *E. coli* Ba and *E. coli* Br. The resulting recombinant cells, designated as *E. coli* BaTi and *E. coli* BrTi, were cultured in 2YTG medium under the same conditions, as described previously. Then, their CoQs were extracted and quantified by HPLC. The results of this study demonstrated that the over-expression of *ispA* was highly inhibiting for CoQ₁₀ biosynthesis in both *E. coli* BaTi (Figure 6) and *E. coli* BrTi (Figure 7) and reduced CoQ₁₀ production levels in the two strains from 629±40 to 289±16 µg/g DCW and from 564±28 to 110±20 µg/g DCW, respectively, even in the absence of an inducer. The presence of the inducer, IPTG (0.05 mM), led to a further decrease in CoQ₁₀ production to 30±13 µg/g DCW in *E. coli* BaTi and 80±14 µg/g DCW in *E. coli* BrTi. These results show that the inhibition of CoQ₁₀ production was more severe when compared to the *E. coli* BrDi and *E. coli* BaDi where the *ispA* gene is controlled by P_{BAD} promoter.

In addition, it was observed that the reduction of CoQ₁₀ production in both *E. coli* BaTi and *E. coli* BrTi

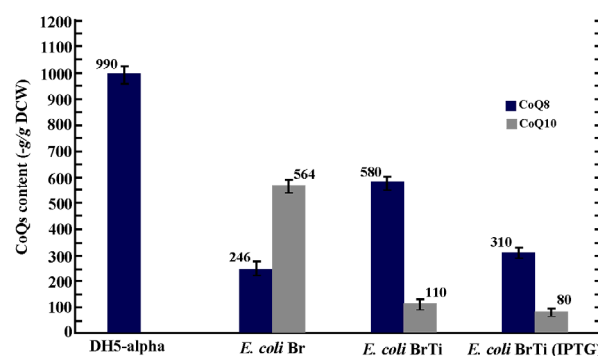


Figure 7. Plasmid pTispA was introduced into *E. coli* Br, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BrTi. Error bars indicate the standard error of the mean of three independent experiments.

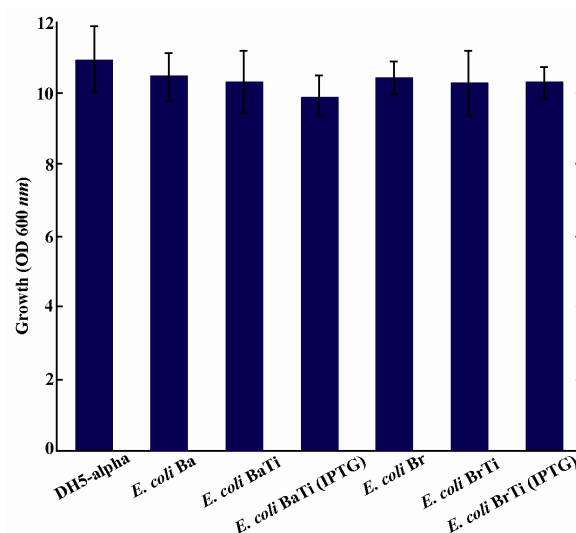


Figure 8. No significant changes were observed in growth associated with *ispA* over-expression. Error bars indicate the standard error of the mean of three independent experiments.

occurred simultaneously with an increase in CoQ₈ production when compared to *E. coli* Ba and *E. coli* Br (from 360 to 890 µg/g DCW in *E. coli* BaTi and 246 to 580 µg/g DCW in *E. coli* BrTi). However, induction by IPTG (0.05 mM) decreased the CoQ₈ biosynthesis as well in both *E. coli* BaTi (to 750 µg/g DCW) and *E. coli* BrTi (to 310 µg/g DCW) as shown in figures 6 and 7. The growth of recombinant strains was also studied and showed no significance changes by *ispA* over-expression (Figure 8).

Discussion

Previous isoprenoid research has implicated the critical role of IPP levels in the biosynthesis of isoprenoid including CoQs²⁸⁻³¹. Accordingly, various strategies have been used to improve CoQ₁₀ production through IPP availability by introducing genes that encode for mevalonate biosynthetic pathway enzymes^{28,32-35}. Although these efforts have led to an increase in CoQ₁₀

accumulation, there has not yet been an effective method developed¹⁷. Specifically, it has been demonstrated that other intermediate metabolites alongside IPP contribute to the control of ubiquinone biosynthesis³⁶.

IPP, GPP, FPP and GGPP are potential substrates for the stepwise addition of IPP by Dds to make the long 50-carbon chain of CoQ₁₀. Previous researches have indicated that FPP is the most suitable substrate for the Dds reactions *in vitro*⁴. In this study, FPPS encoding gene was introduced into two CoQ₁₀-producing strains under the control of either the *trc* or *P_{BAD}* promoters. The *ispA* gene was expressed under the control of *P_{BAD}* promoter by which expression can be modulated over a wide range of inducer (arabinose) concentrations. The results showed relative increase in CoQ₁₀ production in *E. coli* BrDi when no induction was applied. Induction by arabinose, which results in higher FPP level, led to reduction in CoQ₁₀ accumulation in both strains. Thus, the higher FPP concentrations may have negative impact on CoQ₁₀ accumulation. The over-expression of *ispA* caused a large decrease in CoQ₁₀ production when the *trc* promoter was employed (*E. coli* BaTi and *E. coli* BrTi). In fact, the higher FPP concentrations from high-copy plasmid with strong *trc* promoter led to more reduction in CoQ₁₀ production, confirming the hypothesis that high concentration of FPP inhibits CoQ₁₀ accumulation; probably through inhibition of Dds enzymatic activity.

When it comes to monitor the simultaneous CoQ₈ production, it was observed that the production of CoQ₁₀ brings about a decline in CoQ₈ production and vice versa. It can be assumed that the introducing of *dds* gene leads the substrate flow to be conducted towards CoQ₁₀ production, thus decreasing CoQ₈ biosynthesis in *E. coli* Ba and *E. coli* Br. In contrast, due to the negative effects of *ispA* gene expression on CoQ₁₀ production in *ispA*-producing strains, the substrate flow might be conducted again towards CoQ₈ biosynthesis to some extent, because FPP can also function as a substrate for Ods as well as Dds⁴. However, higher level of FPP derived from induction by IPTG or higher level of arabinose reduced CoQ₈ production as well as that of CoQ₁₀ accumulation. It seems that high concentrations of FPP may have an inhibitory effect on the *E. coli* Ods enzyme activity as well as Dds, thus causing a substantial decrease in CoQ₈ biosynthesis. Growth was not significantly affected by the expression of *ispA*. It seems that the residual amount of CoQ₈ produced in *ispA*-expressing strains is still sufficient for respiration and consequently supporting the growth (Figure 8).

It seems that biochemical properties of various Dds from different organisms also play role in determining their enzymatic activities. For example, introduction of *pDispA* in *E. coli* BrDi resulted in relative increase in CoQ₁₀ production (from 546 to 750 µg/g DCW) while it had no significant effect on CoQ₁₀ production in *E. coli* BaDi (from 629 to 636 µg/g DCW). This may have resulted from the relatively low similarity between the

enzymes, which is reflected in differences in their biochemical characteristics²³.

It seems that the biosynthesis of CoQs is controlled by intermediate metabolite concentration in a complex way that inhibition of polyprenyl diphosphate synthases by precursors is a major bottleneck and adjusting the precursor's concentrations is of great importance. Different strategies can be employed to achieve desirable precursor's concentration in engineered *E. coli*. For example, it is proposed that the type of expression vectors and the promoter strength are very important for CoQ₁₀ production in recombinant *E. coli*⁶. This idea was confirmed in the current study where *pTrc99A* plasmid, a high-copy number vector with strong promoter, was found to dramatically reduce CoQ₁₀ production while using *pBAD24* plasmid with an inducible weaker promoter has no severe, reducing effect on CoQ₁₀ production and even relatively increased CoQ₁₀ production in *E. coli* BrDi. It has also been observed that the co-production of other isoprenoid compounds which share same intermediate precursors may also contribute to control biosynthesis of CoQs through controlling metabolite flow rate²⁴. These findings suggest that the over-expression of the *dds* gene with a high-copy plasmid, which leads to higher Dds concentration, alongside the *ispA* may enhance the CoQ₁₀ production via quenching the inhibition caused by the high concentration level of FPP.

Conclusion

In summary, the regulation of the metabolite flow rate is a crucial step, and thus highly important from a biotechnological perspective. Therefore, more effort is needed to verify the role of intermediate metabolite concentration in the biotechnology strategy and to optimize the methods through which the production of CoQs can be elevated.

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Conflict of Interest

None of the authors has any conflicts of interest to declare.

References

1. Alleva R, Tomasetti M, Battino M, Curatola G, Littarru GP, Folkers K. The roles of coenzyme Q10 and vitamin E on the peroxidation of human low density lipoprotein subfractions. *Proc Natl Acad Sci USA* 1995;92(20):9388-9391.
2. Groneberg DA, Kindermann B, Althammer M, Klapper M, Vormann J, Littarru GP, et al. Coenzyme Q10 affects expression of genes involved in cell signalling, metabo-

- lism and transport in human CaCo-2 cells. *Int J Biochem Cell Biol* 2005;37(6):1208-1218.
3. Jeya M, Moon HJ, Lee JL, Kim IW, Lee JK. Current state of coenzyme Q(10) production and its applications. *Appl Microbiol Biotechnol* 2010;85(6):1653-1663.
 4. Kawamukai M. Biosynthesis, bioproduction and novel roles of ubiquinone. *J Biosci Bioeng* 2002;94(6):511-517.
 5. Kontush A, Hübner C, Finckh B, Kohlschütter A, Beisiegel U. Antioxidative activity of ubiquinol-10 at physiologic concentrations in human low density lipoprotein. *Biochim Biophys Acta* 1995;1258(2):177-187.
 6. Huang M, Wang Y, Liu J, Mao Z. Multiple strategies for metabolic engineering of *Escherichia coli* for efficient production of coenzyme Q10. *Chin J Chem Eng* 2011;19(2):316-326.
 7. Søballe B, Poole RK. Ubiquinone limits oxidative stress in *Escherichia coli*. *Microbiology* 2000;146 (Pt 4):787-796.
 8. Kumar A, Kaur H, Devi P, Mohan V. Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacol Ther* 2009;124(3):259-268.
 9. Damian MS, Ellenberg D, Gildemeister R, Lauermann J, Simonis G, Sauter W, et al. Coenzyme Q10 combined with mild hypothermia after cardiac arrest: a preliminary study. *Circulation* 2004;110(19):3011-3016.
 10. Quinzii CM, DiMauro S, Hirano M. Human coenzyme Q10 deficiency. *Neurochem Res* 2007;32(4-5):723-727.
 11. Ochoa JJ, Pamplona R, Ramirez-Tortosa MC, Granados-Principal S, Perez-Lopez P, Naudí A, et al. Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q10. *Free Radic Biol Med* 2011;50(9):1053-1064.
 12. Aguilaniu H, Durieux J, Dillin A. Metabolism, ubiquinone synthesis, and longevity. *Genes Dev* 2005;19(20):2399-2406.
 13. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1995;1271(1):195-204.
 14. Meganathan R. Biosynthesis of the isoprenoid quinones menaquinone (vitamin K2) and ubiquinone (coenzyme Q). *Escherichia coli and Salmonella: cellular and molecular biology* 1996;1:642-656.
 15. Okada K, Suzuki K, Kamiya Y, Zhu X, Fujisaki S, Nishimura Y, et al. Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone. *Biochim Biophys Acta* 1996;1302(3):217-223.
 16. Zhang D, Shrestha B, Li Z, Tan T. Ubiquinone-10 production using *Agrobacterium tumefaciens* dps gene in *Escherichia coli* by coexpression system. *Mol Biotechnol* 2007;35(1):1-14.
 17. Zahiri HS, Yoon SH, Keasling JD, Lee SH, Won Kim S, Yoon SC, et al. Coenzyme Q10 production in recombinant *Escherichia coli* strains engineered with a heterologous decaprenyl diphosphate synthase gene and foreign mevalonate pathway. *Metab Eng* 2006;8(5):406-416.
 18. Huang M, Yunyan C, Jianzhong L. Chromosomal Engineering of *Escherichia coli* for Efficient Production of Coenzyme Q10. *Chin J Chem Eng* 2014;22(5):559-569.
 19. Kaur D, Brennan PJ, Crick DC. Decaprenyl diphosphate synthesis in *Mycobacterium tuberculosis*. *J Bacteriol* 2004;186(22):7564-7570.
 20. Mekkiengkrai D, Sando T, Hirooka K, Sakdapipanich J, Tanaka Y, Fukusaki E, et al. Cloning and characterization of farnesyl diphosphate synthase from the rubber-producing mushroom *Lactarius chrysorrheus*. *Biosci Biotechnol Biochem* 2004;68(11):2360-2368.
 21. Suzuki K, Okada K, Kamiya Y, Zhu XF, Nakagawa T, Kawamukai M, et al. Analysis of the decaprenyl diphosphate synthase (dps) gene in fission yeast suggests a role of ubiquinone as an antioxidant. *J Biochem* 1997;121(3):496-505.
 22. Takahashi S, Nishino T, Koyama T. Isolation and expression of *Paracoccus denitrificans* decaprenyl diphosphate synthase gene for production of ubiquinone-10 in *Escherichia coli*. *Biochem Eng J* 2003;16(2):183-190.
 23. Zahiri HS, Noghabi KA, Shin YC. Biochemical characterization of the decaprenyl diphosphate synthase of *Rhodobacter sphaeroides* for coenzyme Q10 production. *Appl Microbiol Biotechnol* 2006;73(4):796-806.
 24. Zahiri HS, Noghabi KA, Samoudi M, Yeganeh NO, Rad SA, Safari A, et al. Effect of concomitant lycopene biosynthesis on CoQ10 accumulation in transformed *Escherichia coli* strains. *Iranian J Biotechnol* 2009;7(4):224-232.
 25. Sørensen HP, Mortensen KK. Advanced genetic strategies for recombinant protein expression in *Escherichia coli*. *J Biotechnol* 2005;115(2):113-128.
 26. Fujisaki S, Hara H, Nishimura Y, Horiuchi K, Nishino T. Cloning and nucleotide sequence of the ispA gene responsible for farnesyl diphosphate synthase activity in *Escherichia coli*. *J Biochem* 1990;108(6):995-1000.
 27. Casali N, Preston A. *E. coli* Plasmid Vectors. Methods and Applications. Totowa, N.J.: Humana Press;2003.
 28. Cluis CP, Burja AM, Martin VJ. Current prospects for the production of coenzyme Q10 in microbes. *Trends Biotechnol* 2007;25(11):514-521.
 29. Huang Q, Roessner CA, Croteau R, Scott AI. Engineering *Escherichia coli* for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. *Bioorg Med Chem* 2001;9(9):2237-2242.
 30. Kim SW, Keasling JD. Metabolic engineering of the non-mevalonate isopentenyl diphosphate synthesis pathway in *Escherichia coli* enhances lycopene production. *Biotechnol Bioeng* 2001;72(4):408-415.
 31. Sandmann G. Combinatorial biosynthesis of carotenoids in a heterologous host: a powerful approach for the biosynthesis of novel structures. *Chembiochem* 2002;3(7):629-635.
 32. Harker M, Bramley PM. Expression of prokaryotic 1-deoxy-D-xylulose-5-phosphatases in *Escherichia coli* increases carotenoid and ubiquinone biosynthesis. *FEBS Lett* 1999;448(1):115-119.
 33. Martin VJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in *Escherichia*

- coli for production of terpenoids. *Nat Biotechnol* 2003;21(7):796-802.
34. Matthews PD, Wurtzel ET. Metabolic engineering of carotenoid accumulation in *Escherichia coli* by modulation of the isoprenoid precursor pool with expression of deoxyxylulose phosphate synthase. *Appl Microbiol Biotechnol* 2000;53(4):396-400.
35. Rodríguez-Villalón A, Pérez-Gil J, Rodríguez-Concepción M. Carotenoid accumulation in bacteria with enhanced supply of isoprenoid precursors by upregulation of exogenous or endogenous pathways. *J Biotechnol* 2008;135(1):78-84.
36. Cluis CP, Ekins A, Narcross L, Jiang H, Gold ND, Burja AM, et al. Identification of bottlenecks in *Escherichia coli* engineered for the production of CoQ(10). *Metab Eng* 2011;13(6):733-744.
37. Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, et al. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 1995;166(1):175-176.
38. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 1995;177(14):4121-4130.