Original Article

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

Mojtaba Samoudi [1*], Negar Omid Yeganeh [2], Hossein Shahbani Zahiri [1], Parvin Shariati [1], and Reza Hajhosseini [2]

1. Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2. Department of Biology, Payame-Noor University, Tehran, Iran

Abstract

Background: Coenzyme Q_{10} (CoQ_{10}) 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 CoQ_{10}-producing *Escherichia coli (E. coli)*.

Methods: Two CoQ_{10}-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 CoQ_{10} production only in recombinant *E. coli* Br although induction by arabinose resulted in partial reduction of CoQ_{10} 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 CoQ_{10} 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 CoQ_8 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 CoQ_{10} 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 CoQ_{10}.

*** Corresponding author:**
Mojtaba Samoudi, Ph.D.,
Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
Tel: +98 21 44787359
Fax: +98 21 22932451
E-mail:
m.samoudi@nigeb.ac.ir
Received: 31 Mar 2015
Accepted: 30 May 2015

Avicenna J Med Biotech 2015; 7(3): 113-120

Keywords: Coenzyme Q_{10}, Decaprenyl diphosphate synthase, Farnesyl diphosphate, Isoprenoid

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 CoQ_{10}, while *Escherichia coli (E. coli)* contains CoQ_8 which possesses eight isoprene units in its side chain [12-16]. The synthesis of the CoQ_{10} 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 CoQ_{10} in trans-

Copyright © 2015, Avicenna Journal of Medical Biotechnology. All rights reserved.

formed *E. coli* cells along with CoQ_8. Accordingly, several CoQ_{10}-producing *E. coli* strains have been devolved 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 CoQ_{10} 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 CoQ_{10} biosynthesis in engineered CoQ_{10}-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 CoQ_{10}-producing *E. coli* strains can control the biosynthesis of CoQ_{10}. 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 CoQ_{10} and CoQ_8 accumulation in engineered *E. coli* cells. Two CoQ_{10}-producing strains previously constructed by introducing Dds encoding gene from two different organisms [24] 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 cultures [25]. For CoQ_{10} 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 ℃. 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 OD_{600} of 0.1, and were then

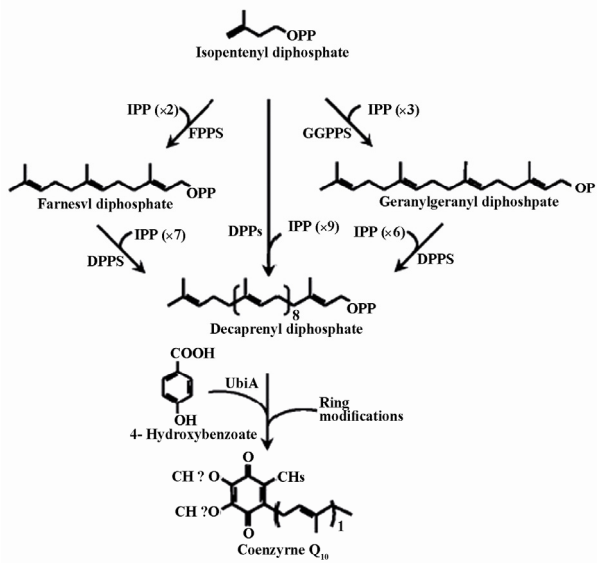

Figure 1. The tandem condensation reactions by decaprenyl diphosphate synthase result in the polymerization of isopentenyl diphosphate (IPP) molecules into decaprenyl diphosphate. The indicated enzymes are as follow: FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; DPPS,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

incubated at 30 ℃ 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). T_4DNA 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 elsewhere [23]. The construction of plasmids harboring dds gene either from *A. tumefaciens* (atdds) or *R. sphaeroide* (rsdds) were described previously [24]. 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 pBr1MCS2 plasmid which had previously been digest-

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

Name	Description	Reference
Strains		
***E. coli* DH5α**	F/endA1 hsdR17 (rk- mk+) gin V44 thi-1 RecA1 gyrA (Nal1) relA1Δ (lacZYA-arg U169 deoR5 (Ψ80dlacΔ (lacZ) M15)]	(Gibco BRL)
***E. coli* Ba**	*E. coli* DH5α harboring pBatdds	(24)
***E. coli* Br**	*E. coli* DH5α harboring pBrsdds	(24)
***E. coli* BaTi**	*E. coli* Ba harboring pTispA	(This study)
***E. coli* BrTi**	*E. coli* Br harboring pTispA	(This study)
***E. coli* BaDi**	*E. coli* Ba harboring pDispA	(This study)
***E. coli* BrDi**	*E. coli* Br harboring pDispA	(This study)
Plasmids		
pTrc99A	Ptrc expression vector, pBR322 origin, lac/q, Amp^r, high copy	(Amersham,Bioscience)
pBBR1MCS2	Plac cloning vector, pBBR1 origin, lacZα, Kan^r	(37)
pBAD24	P_{BAD} cloning vector, pBR322/ColE1 origin, araC, Amp^r	(38)
pBatdds	pBBR1MCS2 with dds gene from *A. tumefaciens*	(24)
pBrsdds	pBBR1MCS2 with dds gene from *R. sphaeroides*	(24)
pTispA	pTrc99A with ispA gene from *E. coli*	(This study)
pDispA	pBAD24 with ispA gene from *E. coli*	(This study)
Primers		
Atdds-3	5'-TCCCCCCGGGCACAGGAAACAGACCATGGAGTTCTTG-3' (XmaI)	
Atdds-4	5'-TCCCCCCGGGTTAGTTGAGACGCTCGATGCAGAAG-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 pBrsdds. FPPS encoding gene (ispA) had been isolated from the *E. coli* (GenBank: D00694.1) as reported previously [26]. 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 method [27].

CoQs extraction

CoQs content of cells was determined by extraction. Specifically, 500 *µl* of culture was centrifuged at 10,000 *xg* (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 *µl* 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 ℃ for 30 *min*. 900 *µl* of a hexane/propanol (5:3 *v/v*) mixture was added to lysed cells and centrifuged at 11,000 *xg* for 2 *min*. The hexane organic phase containing CoQs was transferred to a clean tube. Hexane (500 *µl*) was added to the aqueous phase and centrifuged at 11,000 *xg* 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 *µl* of absolute ethanol (HPLC grade, Merck, US).

Determination and quantification of CoQs

The CoQ content was determined from 10 *µl* 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, CoQ_9 and CoQ_{10}. Quantification of CoQs were performed with the aid of an appropriate standard curve.

Results

CoQs production in E. coli

As mentioned in our previous work [24], two type of dds genes, rsdds and atdds, were inserted into the pBr1MCS2 plasmid, and resulted in the formation of plasmids pBrsdds and pBatdds. The recent plasmids were transformed into *E. coli* DH5α, 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 CoQ_{10} together with CoQ_8. On average, *E. coli* Ba had CoQ_{10} 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 CoQ_8 content was also measured in CoQ_{10}-producing strains. Expression of dds led to a significant decrease in CoQ_8 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* DH5α [24]. An example of HPLC chromatogram for CQs identification in *E. coli* Ba is shown in figure 2. The recombinant E. coli strain produced CoQ_{10} and slight amounts of CoQ_9 in addition to naturally occurring CoQ_8. The CoQ_9 might be produced as a result of immature release of a fraction of the growing polyprenyl chains from the Dds [17].

Effect of ispA over-expression under the control of P_{BAD} promoter in CoQ_{10}-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 reactions [4]. 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 CoQ_{10} production in *E. coli* BaDi, with negligible change from 629±40 to 636±35 *µg/g* DCW (Figure 4), while relative increase was observed

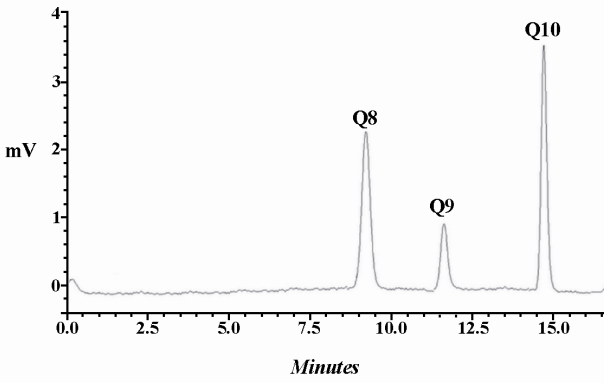

Figure 2. HPLC chromatogram for CQs identification in *E. coli* Ba. The recombinant *E. coli* produced CoQ_{10} and slight amounts of CoQ_9 in addition to naturally occurring CoQ_8.

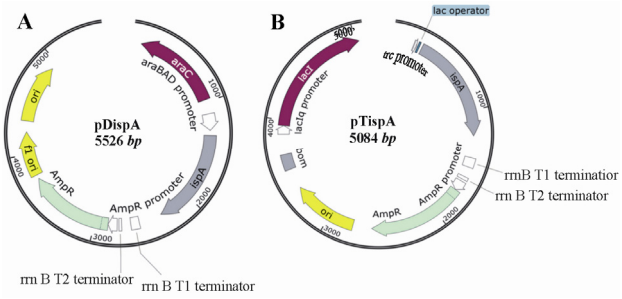

Figure 3. Schematic representation of recombinant pDispA plasmid, encoding for ispA gene under the control of P_{BAD} promoter (A) and pTispA plasmid, encoding for ispA gene under the control of trc promoter B). The genetic maps are generated by SnapGene software (from GSL Biotech; available at snapgene.com).

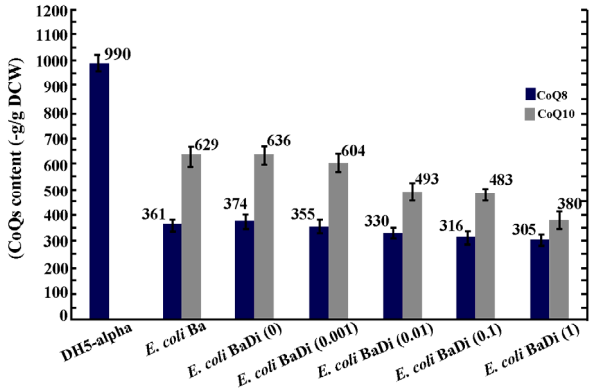

Figure 4. Plasmid pDispA was introduced into *E. coli* Ba, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BaDi. Numbers in brackets indicate arabinose mM concentration in cultures. Error bars indicate the standard error of the mean of three independent experiments.

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 CoQ_{10} production was obtained which was proportional to the concentration of arabinose in both strains. In the absence of induction, the CoQ_8 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 CoQ_8 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 CoQ_{10}-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 pTrc99A

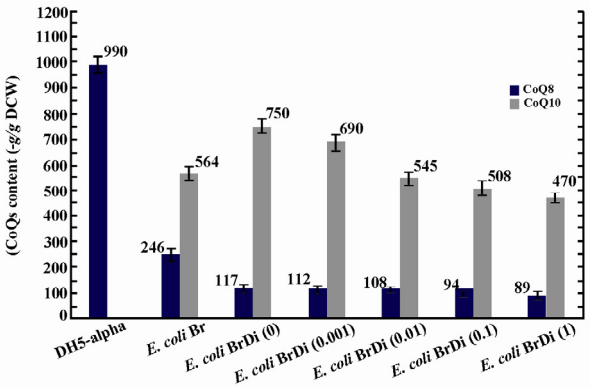

Figure 5. Plasmid pDispA was introduced into *E. coli* Br, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BrDi. Numbers in brackets indicate arabinose mM concentration in cultures. Error bars indicate the standard error of the mean of three independent experiments.

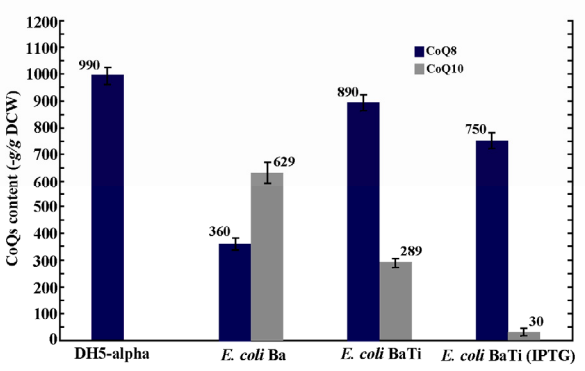

Figure 6. Plasmid pTispA was introduced into *E. coli* Ba, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BaTi. Error bars indicate the standard error of the mean of three independent experiments.

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 CoQ_{10} biosynthesis in both *E. coli* BaTi (Figure 6) and *E. coli* BrTi (Figure 7) and reduced CoQ_{10} 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 CoQ_{10} 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 CoQ_{10} 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 CoQ_{10} production in both *E. coli* BaTi and *E. coli* BrTi

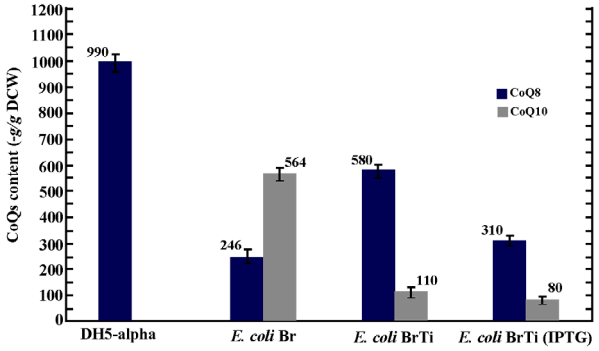

Figure 7. Plasmid pTispA was introduced into *E. coli* Br, and the coenzyme Qs content was quantified in the resulting strain, as referred to *E. coli* BrTi. Error bars indicate the standard error of the mean of three independent experiments.

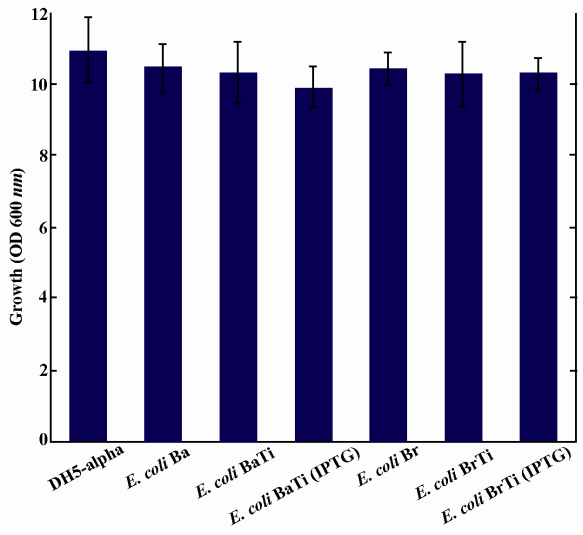

Figure 8. No significant changes were observed in growth associated with ispA over-expression. Error bars indicate the standard error of the mean of three independent experiments.

occurred simultaneously with an increase in CoQ_8 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 CoQ_8 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 CoQs [28-31]. Accordingly, various strategies have been used to improve CoQ_{10} 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 CoQ_{10}

accumulation, there has not yet been an effective method developed [17]. Specifically, it has been demonstrated that other intermediate metabolites alongside IPP contribute to the control of ubiquinone biosynthesis [36].

IPP, GPP, FPP and GGPP are potential substrates for the stepwise addition of IPP by Dds to make the long 50-carbon chain of CoQ_{10}. 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 CoQ_{10}-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 CoQ_{10} production in *E. coli* BrDi when no induction was applied. Induction by arabinose, which results in higher FPP level, led to reduction in CoQ_{10} accumulation in both strains. Thus, the higher FPP concentrations may have negative impact on CoQ_{10} accumulation. The over-expression of ispA caused a large decrease in CoQ_{10} 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 CoQ_{10} production, confirming the hypothesis that high concentration of FPP inhibits CoQ_{10} accumulation; probably through inhibition of Dds enzymatic activity.

When it comes to monitor the simultaneous CoQ_8 production, it was observed that the production of CoQ_{10} brings about a decline in CoQ_8 production and vice versa. It can be assumed that the introducing of dds gene leads the substrate flow to be conducted towards CoQ_{10} production, thus decreasing CoQ_8 biosynthesis in *E. coli* Ba and *E. coli* Br. In contrast, due to the negative effects of ispA gene expression on CoQ_{10} production in ispA-producing strains, the substrate flow might be conducted again towards CoQ_8 biosynthesis to some extent, because FPP can also function as a substrate for Ods as well as Dds [4]. However, higher level of FPP derived from induction by IPTG or higher level of arabinose reduced CoQ_8 production as well as that of CoQ_{10} 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 CoQ_8 biosynthesis. Growth was not significantly affected by the expression of ispA. It seems that the residual amount of CoQ_8 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 CoQ_{10} production (from 546 to 750 $\mu g/g$ DCW) while it had no significant effect on CoQ_{10} production in *E. coli* BaDi (from 629 to 636 $\mu g/g$ DCW). This may have resulted from the relatively low similarity between the enzymes, which is reflected in differences in their biochemical characteristics [23].

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 CoQ_{10} 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 CoQ_{10} production while using pBAD24 plasmid with an inducible weaker promoter has no severe, reducing effect on CoQ_{10} production and even relatively increased CoQ_{10} 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 rate [24]. 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 CQ_{10} 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.

Acknowledgement

We hereby thank Dr. Fatemeh Tabandeh and Dr. Sarah W. Harcum for their scientific editing of the work. This work was supported by National Institute of Genetic Engineering and Biotechnology (NIGEB).

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. Mekkriengkrai 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, Samoodi 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 nonmevalonate 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.