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Increase of CoQ_{10} Production Level by the Coexpression of Decaprenyl Diphosphate Synthase and 1-Deoxy-D-xylulose 5-Phosphate Synthase Isolated from *Rhizobium radiobacter* ATCC 4718 in Recombinant *Escherichia coli*

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Abstract Two genes, *dps* encoding decaprenyl diphosphate synthase and *dxs* encoding 1-deoxy-D-xylulose 5-phosphate synthase, were isolated from *Rhizobium radiobacter* ATCC 4718. DNA sequencing analysis of the *dps* and *dxs* genes revealed an open reading frame of 1,077 bp and 1,920 bp, respectively. The heterologous expression in *Escherichia coli* BL21(DE3) was carried out in order to identify their functions. Recombinant *E. coli* BL21(DE3) harboring the *dps* gene produced CoQ_{10} as well as CoQ_8 and CoQ_9, whereas *E. coli* harboring only the *dxs* gene produced more CoQ_8 compared with the wild-type *E. coli*. Additionally, the coexpression of *dps* and *dxs* genes in *E. coli* was carried out. The recombinant *E. coli* harboring only the *dps* gene produced 0.21±0.04 mg/l of CoQ_{10}, whereas the coexpressed *E. coli* with *dps* and *dxs* genes produced 0.37±0.07 mg/l of CoQ_{10}. HPLC analysis also showed that the CoQ_{10} fraction (100% of the total CoQs distribution) was increased from 15.86±0.66% (only *dps*) to 29.78±1.80% (*dps* and *dxs*).

Keywords: Coenzyme Q_{10}, decaprenyl diphosphate synthase, 1-deoxy-D-xylulose 5-phosphate synthase, *Rhizobium radiobacter*

Coenzyme Q_{10} (CoQ_{10}; 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone) is an essential component of the electron transfer system in the mitochondrial inner membrane of eukaryotes and in the plasma membranes of prokaryotes. It is composed of two major groups, in which the head group is a benzoquinone ring group derived from the shikimate pathway for the synthesis of aromatic amino acids through chorismate, and the tail group is the 10 U of the isoprenoid side chain group produced through the nonmevalonate pathway [10, 13], and is used as a therapeutically important medicine against heart disease and aging and as a functional cosmetic compound [1, 3].

The biosynthetic pathway of CoQ_{10} is typically composed of three elements, including quinonoid ring synthesis, decaprenyl diphosphate synthesis, and quinonoid ring modification [2, 8]. Among these elements, decaprenyl diphosphate synthesis plays a crucial role in synthesizing the 10 U of the isoprenoid side chain of CoQ_{10}. The isoprenoid side chain is produced through a nonmevalonate pathway of which the first reaction is mediated by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), which catalyzes the condensation reaction of the D-glyceraldehyde 3-phosphate and pyruvate, which synthesizes isopentenyl diphosphate (IPP), is a five-carbon building block in CoQ biosynthesis [13]. Decaprenyl diphosphate synthase (DPS), a key enzyme of CoQ_{10} biosynthesis, catalyzes the condensation reaction between IPP and dimethylallyl diphosphate (DMAPP) to synthesize the decaprenyl tail of CoQ_{10}. Previously, we constructed the expression system of the recombinant *E. coli* harboring the *dps* gene from *Sinorhizobium meliloti* producing CoQ_{10} [14]. Additionally, a number of studies on the *dps* gene of *G. suboxydans* and *A. tumefaciens* have reported CoQ_{10} production by the heterologous expression of the *dps* gene in a recombinant *E. coli* system [7, 11]. Further studies have also reported that the amplification of the *dxs* level increased lycopene production and, similarly, amplification of the *dxs* level of *Pseudomonas aeruginosa* increased the production of CoQ_{10}, using IPP as a precursor as in lycopene [4, 5].

In this study, the *dps* and *dxs* genes were isolated from genomic DNA of *Rhizobium radiobacter* ATCC 4718 and amplified using primers designed from AGR_C_1125p (GenBank Accession Number AAK86441) and AGR_C_1351p (AAK86554) sequences, respectively, in the genome sequence of *A. tumefaciens* str. C58 having the

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ability to produce CoQ_{10}. Oligonucleotides, specifically 5'-TTGCCGCACAAGGCATCAGTTTTT-3' as a sense primer and 5'-TCAGTTGAGACGCTCGATGCAGAA-3' as an antisense primer, were used to amplify the *dps* gene, and 5'-AACGATTGACCGGAATGCCACA-3' (sense primer) and 5'-ATCTTGTCAGCCGGCGAAACC-3' (antisense primer) were used for amplification of the *dxs* gene. The deduced amino acid sequence of the amplified *dps* gene with an open reading frame (ORF) of 1,077 bp showed homology with DPS44 from *A. tumefaciens* BNQ0605 (99% identity and 99% similarity, AAP56240), DPS from *A. tumefaciens* LBA 4404 (96% and 98%, AAY82368), and DPS from *S. meliloti* KCCM 11232 (86% and 91%, ABB72030). A sequence analysis of the amplified *dxs* gene revealed an ORF of 1,920 bp and the deduced amino acid sequence had homology with DXS11 from *A. tumefaciens* BNQ0605 (99% and 99%, AAP56243), DXS from *R. etli* CFN 42 (85% and 93%, ABC89724), and DXS from *S. meliloti* 1021 (86% and 91%, CAC45452). The amplified genes were deposited in the GenBank database under the accession numbers DQ865262 for *dps* and DQ865263 for *dxs*.

Two plasmids were constructed for heterologous expression of *dps* and *dxs* in *E. coli* BL21(DE3). The amplified *dps* gene using a sense primer, 5'-CGCGGATCCTTGCCG-CAC-AAGGCATC-3' (BamHI site underlined), and an antisense primer, 5'-CCCAAGCTTTCAG-TTGAGACG-CTCG-3' (HindIII site underlined), by PCR was ligated into the BamHI/HindIII-digested pET-28a (Novagen, Germany) to yield a plasmid, designated as pMJS5. In the same manner, the *dxs* gene was amplified with 5'-CGCGGAT-CCTTGACCG-GAATGCCAC-3' (sense primer, BamHI site underlined) and 5'-CCCAAGCTTTCAGCCGGCGA-AACC-3' (antisense primer, HindIII site underlined) and then cloned into pET-32a (Novagen, Germany) digested with BamHI and HindIII to generate a pMJS6 plasmid. Each of the constructed plasmids was introduced into *E. coli* BL21(DE3) for a heterologous expression. In order to investigate the effect of the amplification of the *dxs* gene on the increase of CoQ_{10} production, a coexpression of *dps* and *dxs* in *E. coli* BL21(DE3) was carried out. The above constructed plasmid pMJS6 was transferred into the recombinant *E. coli* BL21(DE3) harboring pMJS5, and the recombinant *E. coli* BL21(DE3) harboring the two genes was cultured in a LB medium containing 50 µg/ml each of kanamycin and ampicillin at 37°C. Although the two plasmids, pMJS5 and pMJS6, carried the same replication origin, we used the incompatible plasmids carrying different antibiotic resistance genes, kanamycin and ampicillin [15]. When the cell density reached OD_{600}=0.6, IPTG (isopropyl-β-D-thiogalactopyranoside) was added to a final concentration of 1 mM to induce the recombinant protein. Further cultivation was then carried out at 30°C for 5 h after IPTG induction. The expressed proteins in *E. coli* BL21(DE3) were analyzed by SDS-PAGE as described by Laemmli [6] (Fig. 1). The recombinant DPS (rDPS) from *E. coli* BL21(DE3) harboring pMJS5 was observed with an approximate size of 40 kDa, whereas the recombinant DXS (rDXS) from *E. coli* BL21(DE3)/pMJS6 was approximately detected with a molecular mass of 90 kDa. The recombinant *E. coli* BL21(DE3) harboring both pMJS5 and pMJS6 produced two major bands with sizes of 40 kDa and 90 kDa, suggesting that the rDPS was coexpressed with the rDXS in *E. coli* BL21(DE3) in the presence of the two antibiotics although the two plasmids were compatible.

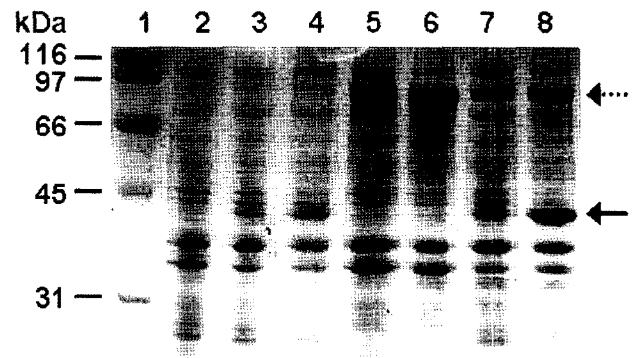

Fig. 1. SDS-PAGE analysis of rDPS and rDXS expressed in *E. coli* BL21(DE3).

Recombinant *E. coli* BL21(DE3) cells were harvested and then disrupted by sonication. Lane 1, molecular mass marker; lane 2, total protein (TP) of wild-type *E. coli*; lane 3, TP of *E. coli*/pMJS5; lane 4, cell-extract (CE) of *E. coli*/pMJS5; lane 5, TP of *E. coli*/pMJS6; lane 6, CE of *E. coli*/pMJS6; lane 7, TP of *E. coli*/pMJS5+pMJS6; lane 8, CE of *E. coli*/pMJS5+pMJS6. The solid and dashed lines indicate the expressed rDPS and rDXS, respectively.

To examine the effects of rDPS and rDXS on the change of the CoQs pattern produced from recombinant *E. coli* BL21(DE3), CoQs were extracted from the harvested cells and analyzed using an HPLC system. The extraction of CoQ_{10} and the HPLC analysis were carried out using the procedures described in our previous report [14]. As shown in Fig. 2, the *E. coli* BL21(DE3) produced only CoQ_8, whereas the recombinant *E. coli* BL21(DE3) harboring pMJS5 produced CoQ_8, CoQ_9, and CoQ_{10}. However, CoQ_8 was mainly produced and the ratio of CoQ_{10} to total CoQs (15.86±0.66%) was lower than that of CoQ_8, which is likely due to the use of plasmid pMJS5 with a high-copy number, as reported in a similar work, in which the lowlevel of CoQ_{10} production in recombinant *E. coli* may be due to a toxic effect of the high-copy expressed *dps* gene that is involved in the cellular membrane biosynthesis of the host cells [12]. Additionally, the detection of CoQ_9 as well as CoQ_8 and CoQ_{10} in the *E. coli* BL21(DE3)/pMJS5 was in all probability due to the nonspecific combination of ρ-hydroxybenzoate (PHB) and polyprenyl diphosphate by ρ-hydroxybenzoate octaprenyl diphosphate transferase [9]. CoQ_{10} was not detected in the extracts of the recombinant

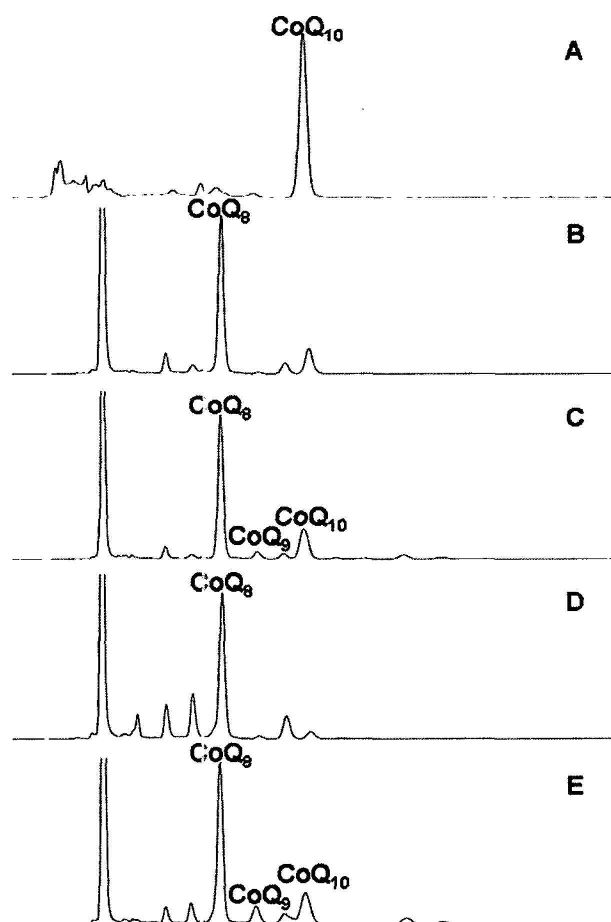

Fig. 2. HPLC analysis of CoQs extracted from recombinant *E. coli* BL21(DE3).
A. Authentic CoQ_{10}; **B.** Wild-type *E. coli*; **C.** *E. coli*/pMJS5; **D.** *E. coli*/pMJS6; **E.** *E. coli*/pMJS5+pMJS6.

E. coli BL21(DE3)/pMJS6 cells, which produced a greater amount of CoQ_8 than the *E. coli* BL21(DE3) (Fig. 2D). These results suggest that the rDXS in *E. coli* BL21(DE3)/pMJS6 increased the IPP level, an elemental precursor for CoQs, which subsequently increased the level of CoQ_8. On the other hand, the rDPS catalyzed the condensation between IPP and DMAPP to produce the decaprenyl

Table 1. Effect of gene expression on the CoQ_{10} production in recombinant *E. coli* BL21(DE3)[a].

Gene	Dry cell mass (g/l)[b]	CoQ_{10} production		
		Concentration (mg/l)	Content (mg/g)	Fraction (%)[c]
dps	1.69±0.03	0.21±0.04	0.13±0.02	15.86±0.66
dps+*dxs*	1.72±0.07	0.37±0.07	0.22±0.03	29.78±1.80

[a]All values are averages of three replications and standard errors are shown.
[b]Dry cell mass is determined according to the optical density of the culture broth at 600 nm using a predetermined standard curve.
[c]CoQ_{10} fraction is calculated by the ratio of CoQ_{10} to total CoQs.

diphosphate, and the CoQ_{10} was produced by the introduction of the *dps* gene into *E. coli* BL21(DE3), which did not have the capability of CoQ_{10} synthesis.

In order to examine the effect of the rDXS overexpression on the increase in CoQ_{10} production in the recombinant *E. coli* BL21(DE3)/pMJS5 producing CoQ_{10}, a coexpression of rDPS and rDXS in *E. coli* BL21(DE3) was carried out and the distribution of CoQs produced from the recombinant *E. coli* BL21(DE3)/pMJS5 harboring pMJS6 was analyzed (Table 1). The dry cell mass of the *E. coli* BL21(DE3)/pMJS5+pMJS6 was 1.72±0.07 g/l, which was similar to that of the *E. coli* BL21(DE3)/pMJS5 (1.69±0.08 g/l). However, both the CoQ_{10} concentration and the content were increased by more than 1.7-fold with a maximum CoQ_{10} concentration of 0.37±0.07 mg/l and content of 0.22±0.03 mg/g by the coexpression of rDXS with rDPS, as compared with the exclusive expression of rDPS. In addition, the CoQ_{10} fraction was increased from 15.86±0.66% to 29.78±1.80% (over 1.8-fold). The results would be supported by data of another research group, who found that the CoQ_{10} relative content was increased over two-fold by the coexpression of the *ddsA* gene from *G. suboxydans* and the *dxs* gene from *P. aeruginosa* with a CoQ_{10} content of 0.94±0.12 mg/g, which was increased over 2.1-fold compared with 0.44±0.08 mg/g for the expression of the *ddsA* gene alone [4]. The low CoQ_{10} level in this study compared with that in Kim's study is most likely due to the expression system, in which plasmids with an inducible T7 promoter were used in this study, whereas Kim's group used a constitutive *lac* promoter [4]. The increment of CoQ_{10} productivity by the introduction of the *dxs* gene into genetically engineered *E. coli* synthesizing the CoQ_{10} may be due to the restoration of the intracellular IPP responsible for production of the CoQ_{10} via the expression of the *dps* gene.

Thus far, studies regarding the CoQ_{10} production from the recombinant *E. coli* have mostly focused on the *dps* gene's expression directly related with production of the 10 U of the isoprenoid side chain [7, 11, 14]. However, the expression of the *dps* gene only causes the depletion of the IPP level, followed by a limited CoQ_{10} production in the recombinant *E. coli* system. Therefore, a coexpression of the *dps* and *dxs* genes in *E. coli* was attempted and the productivity of the CoQ_{10} was increased in this study. Finally, it turned out that the enhancement of CoQ_{10} concentration and content were significantly increased by the coexpression of the *dps* and *dxs* genes in the recombinant *E. coli* system, resulting in the restoration of the consumed IPP pool, a backbone essential for the synthesis of the isoprenoid side chain.

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