J. Microbiol. Biotechnol. (2006), **16**(6), 933–938

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Production of Coenzyme Q_{10} by Recombinant *E. coli* Harboring the Decaprenyl Diphosphate Synthase Gene from *Sinorhizobium meliloti*

SEO, MYUNG-JI[1], EUN-MI IM[1], JIN-HAENG HUR[1], JUNG-YEON NAM[1], CHANG-GU HYUN[1,†], YU-RYANG PYUN[2], AND SOON-OK KIM[1]*

[1]*Division of Bio·New Drug Development, Central Research Institute, Chem Tech Research Incorporation (C-TRI), Whasung 445-743, Korea*
[2]*Department of Biotechnology, Yonsei University, Seoul 120-749, Korea*
[†]*Present address: Jeju Institute. BioSpectrum, Inc., Jeju 690-121, Korea*

Received: November 7, 2005
Accepted: January 26, 2006

Abstract Decaprenyl diphosphate synthase (DPS) is the key enzyme for the production of coenzyme Q_{10} (CoQ_{10}). A *dps* gene from *Sinorhizobium meliloti* KCCM 11232 (IFO 14782) was isolated by PCR and then cloned in *Escherichia coli*. DNA sequencing analysis revealed an open reading frame of 1,017 bp encoding a 338-amino-acid protein. The protein was identical at the 98% level to the putative octaprenyl diphosphate synthase (IspB) of *S. meliloti* 1021. The deduced amino acid sequence included the DDxxD domains conserved in the majority of the prenyl diphosphate synthases. Heterologous expression in *E. coli* BL21(DE3) was carried out, and the CoQ_{10} produced was then analyzed by HPLC. *E. coli* BL21(DE3) harboring the *dps* gene from *S. meliloti* produced CoQ_{10} in addition to endogenous coenzyme Q_8 (CoQ_8), whereas wild-type *E. coli* BL21(DE3) host did not have the ability of producing CoQ_{10}. The results suggest that the putative *dps* from *S. meliloti* KCTC 2353 encoded the DPS.

Key words: Coenzyme Q_{10}, decaprenyl diphosphate synthase, *Sinorhizobium meliloti*

Coenzyme Q (CoQ), a natural compound widely distributed in living organisms, is a well-known component of the electron transfer system, and composed of a benzoquinone moiety and an isoprenoid side chain. Living organisms possess different species of CoQ, depending on the length of the isoprenoid side chain [6]. The CoQ extracted from humans and tobacco have mainly 10-unit isoprenoid side chains, CoQ_{10} (2-dimethyl-5-methyl-6-decaprenyl-1,4-benzoquinone), whereas *E. coli* and *Saccharomyces cerevisiae* produce CoQ_8 and CoQ_6, respectively [19].

*Corresponding author
Phone: 82-31-226-0541; Fax: 82-31-226-0542;
E-mail: sokim@c-tri.com

Among the CoQs, the CoQ_{10} is used as an antioxidant for cosmetics and pharmaceuticals [1], and it prevents cardiovascular disease and mitochondrial respiratory-chain diseases [16]. CoQ_{10} is embedded in the mitochondrial inner membranes of *Schizosaccharomyces pombe* [15] and in the cellular membranes of *Gluconobacter suboxydans* [14] and *Agrobacterium tumefaciens* [9], and it is synthesized from the *p*-hydroxybenzoic acid and isoprene decaprenyl diphosphate. Industrially, CoQ_{10} has been produced by isolating the coenzymes from plants such as tobacco and synthetically altering the side chain. In addition, the other promising process for producing CoQ_{10} is to extract the compound from the culture broth of the microorganism.

Decaprenyl diphosphate synthase (DPS), of which activity is essential for CoQ_{10} production, catalyzes the condensation reaction of isopentenyl diphosphate (IPP) with allylic diphosphate, and is a key enzyme to synthesize the decaprenyl in the tail in CoQ_{10} [13]. A gene encoding DPS has been recently identified in several strains, such as *Sz. pombe* [18], *G. suboxydans* [14], and *A. tumefaciens* [9]. Most polyprenyl diphosphate synthases (PPS), which synthesize long-chain isoprenoids, including DPS, has a 30–50% homology and the two conserved domains (DDxxD) that are binding sites for the substrates [3, 7]. To date, the genes encoding PPS, such as octaprenyl diphosphate synthase (IspB) from *E. coli* [2], DPS from *G. suboxydans* [14], and DPS from *A. tumefaciens* [9], have been cloned and characterized. However, there has been no study on PPS from *S. meliloti*, except the information of the deduced amino acid sequence of the putative *ispB* gene from *S. meliloti* 1021 (GenBank accession no. CAC45418). In this study, the *dps* gene from *S. meliloti* KCCM 11232 was isolated by PCR, and then cloned and expressed in *E. coli* BL21(DE3) to be identified as DPS.

MATERIALS AND METHODS

Microorganisms, Plasmids, and Cultivation

S. meliloti KCCM 11232, used as a source for the *dps* gene, was cultured at 28°C in an X medium containing 1% mannitol, 0.1% yeast extract, 0.05% K_2HPO_4, 0.02% NaCl, 0.02% $MgSO_4 \cdot 7H_2O$, and 4.88×10^{-4}% $FeCl_3$, pH 7.2. *E. coli* TOP10 and BL21(DE3) were used as host cells for gene cloning and expression, respectively. *E. coli* cells were cultured at 37°C in Luria-Bertani (LB) medium. When necessary, an appropriate amount of kanamycin and isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the medium. The cell growth was determined by spectrophotometically measuring optical density at 600 nm. Plasmids pGEM-T easy (Promega, U.S.A.) and pET28a vector (Novagen, Germany) were used as vectors for sequencing and subcloning for expression.

Cloning and Expression of *dps* Gene

The oligonucleotides, 5'-CAGGAGTCCGGCATATGGG-CGTAGT-3' as sense primer (F1, the NdeI site in underline) and 5'-CAAGGAAACATGAATTCGCTCAGCT-3' as antisense primer (R1, the EcoRI site in underline), were synthesized to amplify the *dps* gene from *S. meliloti* by polymerase chain reaction (PCR). The PCR-amplified product was ligated into the pGEM-T easy vector and then digested with NdeI and EcoRI (Takara, Japan). The digested DNA fragment was recloned into the NdeI/EcoRI-digested expression vector pET28a to yield a plasmid, designated as pMJS1. The plasmid pMJS1 was introduced into *E. coli* BL21(DE3). Transformants were cultured overnight at 37°C, and then inoculated into 50 ml of LB medium containing 50 μg/ml of kanamycin. The cells harboring the *dps* gene were induced with 1 mM IPTG and then further grown at 30°C for 5 h, when optical density at 600 nm of the culture broth reached 0.6. The DPS protein expressed in *E. coli* BL21(DE3)/pMJS1 was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the method of Laemmli [8] with 10% gels and Coomassie brilliant blue R-250 as a stain.

Extraction of CoQ_{10}

The cultured cells were harvested at 8,000 ×*g* for 15 min. The cell pellets were suspended in 5 ml (1 vol) of methanol and 0.3% NaCl mixture (10:1, v/v) containing 1% Triton X-100. The suspended cells were sonicated for 20 s at 20-s interval 15 times with a sonic dismembrator (Fisher Scientific, NH, U.S.A.) on an ice bath. The disrupted cells were extracted with 10 ml (2 vol) of a *n*-hexane and isopropanol mixture (5:3, v/v). After extraction, the upper phase was collected, and then evaporated. The yellow-colored residue was resuspended in ethanol for the assay of CoQ_{10}.

Partial Purification of CoQ_{10}

The upper organic phase solution after extraction for CoQ_{10} was applied to a column packed with silica gel (70–230 meshed, Merck, Germany) to partially purify CoQ_{10}. Then, the elution was carried out with a step gradient of a *n*-hexane and diethylether mixture in the following ratios (v/v): 30:1, 20:1, 15:1, 10:1, and 5:1. Fractions containing CoQ_{10} were combined and evaporated, and the concentrate was then dissolved in a small amount of ethanol for the assay of CoQ_{10}.

Assay of CoQ_{10}

The CoQ_{10} extracted was analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). TLC was carried out on a RP-18 F_{254} plate (Merck, Germany) with a mixture of ethanol and methanol (2:1, v/v). The TLC plate was visualized using 50% H_2SO_4 solution. The CoQ_{10} was also analyzed by HPLC system on a C18 reverse-phase column (Inertsil® ODS-2; 250×4.6 mm, GL Sciences, CA, U.S.A.) with 100% ethanol as a mobile phase at a flow rate of 1.0 ml/min. Detection was carried out at 275 nm. The molecular mass of CoQ_{10} was determined with liquid chromatography/mass spectrometry (LC/MS; Quattro LC Triple Quadrupole Tandem Mass Spectrometer, Waters, U.S.A.) using an analytical mode of atmospheric pressure chemical ionization (APCI).

Protein Database Search and Nucleotide Sequence Accession Number

The deduced amino acid sequence of the *dps* gene from *S. meliloti* was compared with related enzymes from various sources using the BLAST Network at the National Center for Biotechnology Information (NCBI). The multiple sequence alignment between the *S. meliloti* DPS and related enzymes was carried out with a CLUSTAL-W program [23]. The nucleotide sequence of the *dps* gene from *S. meliloti* KCCM 11232 has been deposited in the GenBank database under accession number DQ241792.

RESULTS AND DISCUSSION

Isolation of *dps* Gene from *S. meliloti*

The amino acid sequences deduced from the *dps* sequences from *A. tumefaciens* BNQ0605 (GenBank accession no. AAP56240), which has been known to produce CoQ_{10}, were compared with related enzymes from other sources using the BLAST search. The deduced amino acid sequence showed a homology with DPS from *G. oxidans* 621H (50% and 67% for identity and similarity, respectively, YP_192388), IspB from *E. coli* CFT073 (46% and 64%, NP_755811), IspB from *Mesorhizobium loti* MAFF303099 (*M. loti*, 73% and 86%, NP_107745), and especially, putative

IspB from *S. meliloti* 1021 (88% and 92%, CAC45418) (data not shown). However, until now, there has been no study on the putative IspB from *S. meliloti*, such as cloning and characterization, except for the information of the deduced amino acid sequence of putative IspB. Therefore, in the present study, *S. meliloti* KCCM 11232 was cultured, and CoQ was then extracted to investigate the species of CoQs produced and identify the function of PPS. Based on the deduced amino acid sequence of putative IspB from *S. meliloti* 1021, which was supposed to encode an octaprenyl diphosphate synthase, *S. meliloti* KCCM 11232 was expected to produce CoQ_8. However, against our expectation, *S. meliloti* KCCM 11232 was found to produce CoQ_{10} (data not shown).

To identify the function of PPS from *S. meliloti* KCCM 11232, PCR was carried out using primers F1 and R1 designed from IspB amino acid sequences from *S. meliloti* 1021. The N- and C-terminal sequences of the origin of IspB from *S. meliloti* 1021 was TTGGGCGTAGTGATACCG (F1 primer region underlined) and ATCGATCGGGTA-AGCTGA (R1 primer region underlined), respectively. The resulting 1.0-kb DNA fragment was introduced into pGEM-T easy vector and sequenced. The sequence contained a 1,017-bp open reading frame (ORF) encoding a putative 338-amino-acid protein (data not shown). A BLAST search identified a close relationship between the PCR product from *S. meliloti* and a subset of several PPSs. As shown in Fig. 1, the deduced amino acid sequence of the PCR product from *S. meliloti* KCCM 11232 (DQ241792) showed a homology with IspB from *S. meliloti* 1021 (98% and 98%, CAC45418), DPS from *A. tumefaciens* BNQ0605 (86% and 91%, AAP56240), IspB from *M. loti* MAFF303099 (72% and 86%, NP_107745), DPS from *Paracoccus zeaxanthinifaciens* R114 (*P. zeaxanthinifaciens*, 52% and 68%, CAD24417), DPS from *G. oxidans* 621H (50% and 67%, YP_192388), and IspB from *E. coli* CFT073 (47% and 64%, NP_755811). The multiple sequence alignment between the PCR product from *S. meliloti* KCCM 11232 and several PPSs showed numerous homologous sites scattered throughout the sequences. Among the homologous sites, the existence of two aspartate-rich domains (DDxxD), which are thought to be the binding sites for the diphosphate moieties of IPP and the allylic substrate [5, 17, 21], demonstrated that *S. meliloti* KCCM 11232 could produce

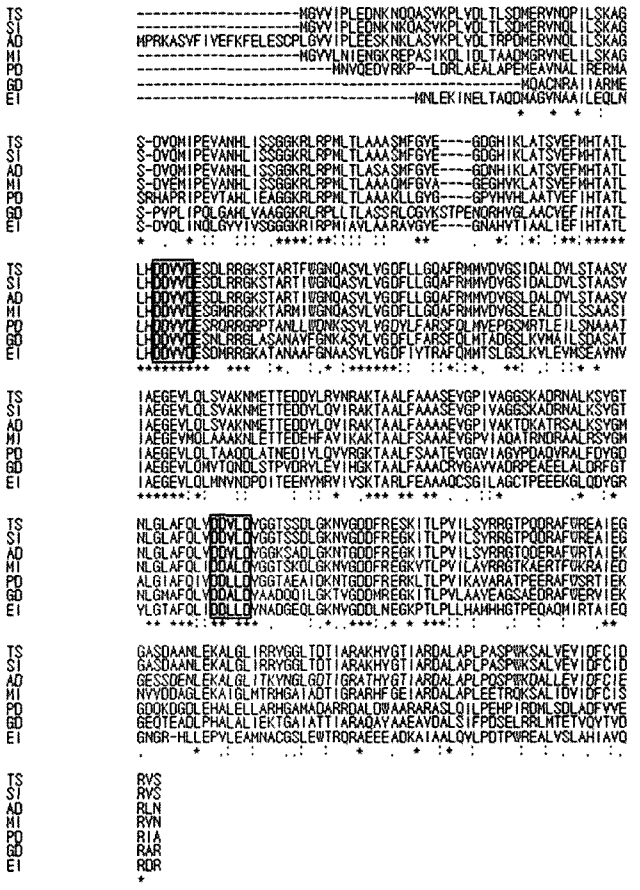

Fig. 1. Amino acid sequence alignment of various polyprenyl diphosphate synthases including DPS from *S. meliloti*.

The deduced amino acid sequences of DPS from *S. meliloti* KCCM 11232 in this study (TS; GenBank accession no. DQ241792), IspB from *S. meliloti* 1021 (SI; CAC45418), DPS from *A. tumefaciens* (AD; AAP56240), IspB from *M. loti* (MI; NP_107745), DPS from *P. zeaxanthinifaciens* (PD; CAD24417), DPS from *G. oxidans* (GD; YP_192388), and IspB from *E. coli* (EI; NP_755811) were aligned. Two aspartate-rich domains (DDxxD) were enclosed in the gray boxes.

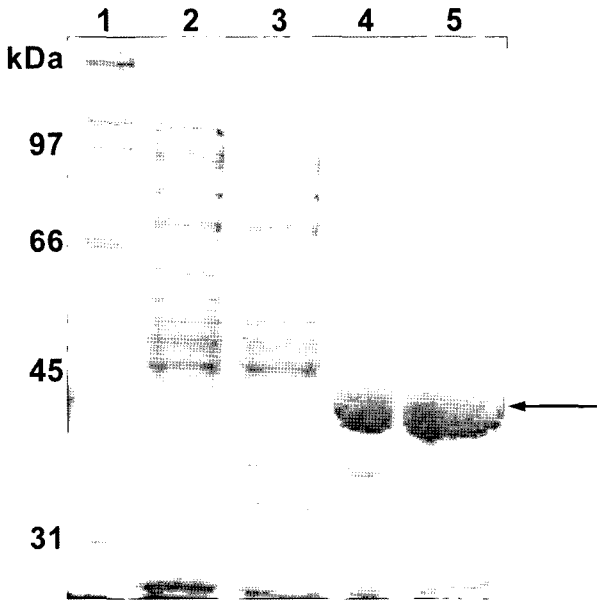

Fig. 2. SDS-PAGE analysis of the DPS protein expressed in *E. coli* BL21(DE3).

Lane 1, molecular weight marker; lane 2, cell-free extract of *E. coli* BL21(DE3); lane 3, cell-free extract of *E. coli* BL21(DE3)/pET28a after induction with IPTG; lane 4, total protein of cell extract of *E. coli* BL21(DE3)/pMJS1 after induction with IPTG; lane 5, cell-free extract of *E. coli* BL21(DE3)/pMJS1 after induction with IPTG.

CoQ. In addition, the amino acid residues located on the fifth position in front of the first DDxxD conserved domain that are thought be a chain length determination site, was alanine, which is typical of long-chain isoprenoid-producing PPSs [12, 22]. These results suggest that *S. meliloti* KCCM 11232 expresses DPS and is able to produce CoQ_{10}.

Heterologous Expression of *dps* Gene in *E. coli*

In order to express the DPS from *S. meliloti*, pMJS1 was constructed as mentioned above and then introduced into *E. coli*. The cell-free extract from the recombinant *E. coli* BL21(DE3) harboring pMJS1 was analyzed by SDS-PAGE, and a major band with the size of approximately 37 kDa was observed (Fig. 2). This protein was not produced in the wild-type *E. coli* and recombinant *E. coli* BL21(DE3)/pET28a.

To verify the functional expression of DPS in the recombinant *E. coli*, the CoQs from the harvested cells were extracted and analyzed using HPLC, as mentioned above. The CoQ_{10} was detected in the cultures of BL21(DE3)/pMJS1 in addition to endogenous CoQ_8, whereas only CoQ_8 was detected in the cultures of BL21(DE3) and BL21(DE3)/pET28a. In addition, the recombinant BL21(DE3)/pMJS1 produced CoQ_9, which was not detected in the cultures of BL21(DE3) and BL21(DE3)/pET28a (Fig. 3).

The wild-type *E. coli* synthesized only CoQ_8 [10], whereas the introduction of the *dps* gene from *S. meliloti* changed the distribution of total CoQs (100% of total distribution), such as CoQ_8 (44.6%), CoQ_9 (11.3%), and CoQ_{10} (44.1%), whose concentration was 0.72 mg/l. The CoQ_9 was not observed in the cases of *A. tumefaciens* and *P. denitrificans*; however, CoQs containing eight, nine, and ten prenyl tails were detected in *Sz. pombe* and *G. suboxydans* [9, 15, 20]. Such patterns of CoQ_{10} accumulation are probably due

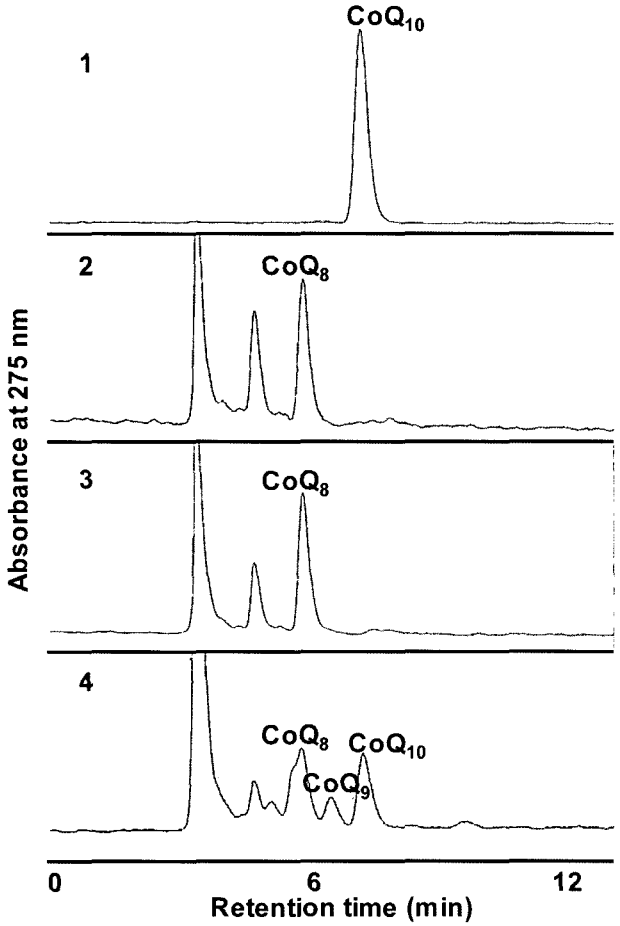

Fig. 3. HPLC analysis of ubiquinone species in recombinant *E. coli* expressing the *dps* gene.
1, Control CoQ_{10} from Sigma; 2, crude extract from *E. coli* BL21(DE3); 3, crude extract from *E. coli* BL21(DE3)/pET28a; 4, crude extract from *E. coli* BL21(DE3)/pMJS1.

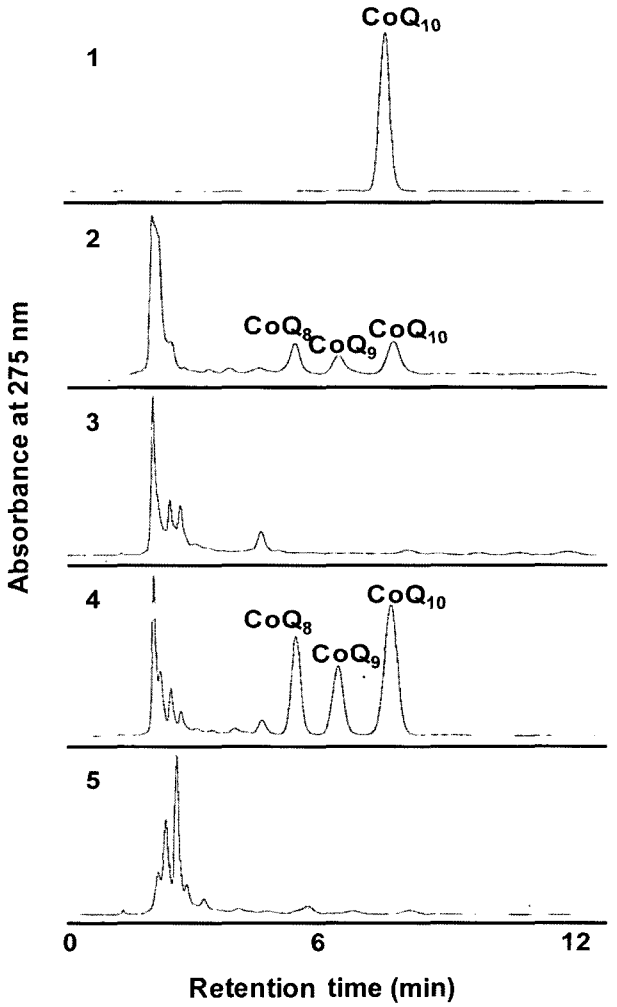

Fig. 4. HPLC analysis of partially purified ubiquinone species in recombinant *E. coli* expressing the *dps* gene.
1, Control CoQ_{10} from Sigma; 2, crude extract from *E. coli* BL21(DE3) harboring pMJS1; 3, eluted with 20:1; 4, eluted with 15:1; 5, eluted with 5:1.

to nonspecific action of *p*-hydroxybenzoate octaprenyl diphosphate transferase, which combines *p*-hydroxybenzoate and polyprenyl diphosphate [11]. It is, therefore, suggested that the *dps* gene from *S. meliloti* KCCM 11232 is functionally expressed in *E. coli*, and that it encodes a DPS with an essential role in determining the side chain of the CoQ_{10}.

Partial Purification of CoQ_{10}

The extracted CoQ_{10} from the recombinant *E. coli* BL21(DE3) was partially purified using a silica column, as mentioned above. The fraction containing CoQ_{10} was eluted with a mixture of *n*-hexane and diethylether (15:1, v/v), and CoQ_{10} was then identified by TLC and HPLC analyses (Fig. 4). The LC/MS analyses of a standard CoQ_{10} solution and the fraction eluted by a mixture of *n*-hexane and diethylether (15:1, v/v), gave identical MS peak at *m/z* 864.5(±0.5), which was 863.4 g/mol molecular weight (Fig. 5). In addition, LC/MS analysis of the HPLC fraction eluted by a mixture of *n*-hexane and diethylether (15:1, v/v) showed other MS peaks without CoQ_{10} decreased.

Recently, CoQ_{10} has become a therapeutically important medicine for heart disease [4] and widely used as a functional cosmetic compound. One of the most effective processes for producing CoQ_{10} is believed to extract the compound from the culture broth of microorganisms. However, the processes do not provide enough production, because of their low yield and complicated operation. To overcome these problems and effectively produce CoQ_{10}, it has been attempted to transfer the *dps* gene, which is the key gene participating in the biosynthesis of CoQ_{10}, into a microorganism such as *E. coli* and express the gene. Until now, the *dps* gene had been isolated from several microorganisms, including *Sz. Pombe* [18], *G. suboxydans* [14], and *A.*

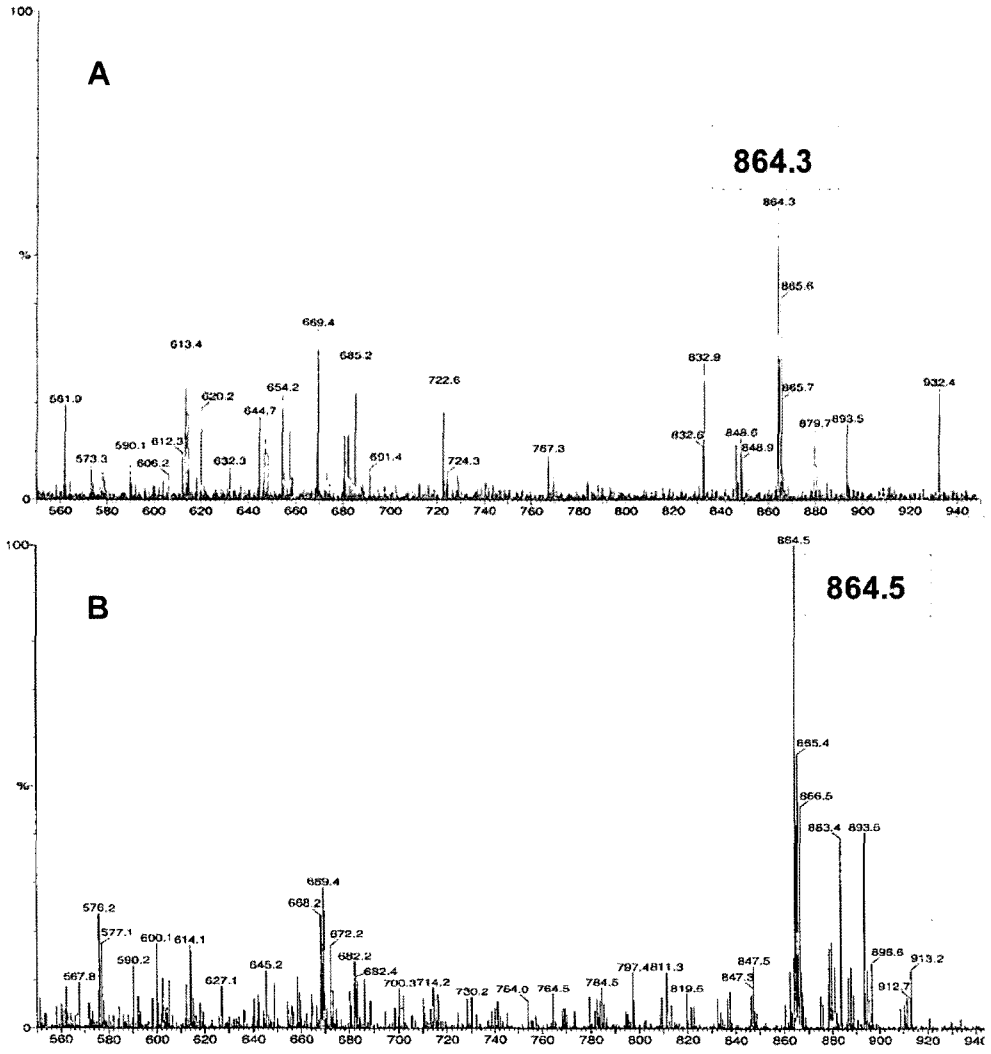

Fig. 5. LC/MS analysis of control CoQ_{10} from Sigma (**A**) and the fraction eluted by a mixture of *n*-hexane and diethylether (15:1, v/v) (**B**).

tumefaciens [9]. However, these microorganisms do not show enough productivity for CoQ_{10}. Therefore, it is highly desirable to isolate the *dps* gene from a microorganism having a high CoQ_{10} producing capability.

To the best of our knowledge, this study is the first study on the *dps* gene from *S. meliloti*, a strain with a high potential for industrial production of CoQ_{10}. The molecular cloning and functional expression of the *S. meliloti dps* gene in *E. coli* was carried out in this study: The *dps* gene from *S. meliloti* KCCM 11232 was isolated and introduced into *E. coli*, which does not produce CoQ_{10}. The specific DPS enzyme was synthesized and CoQ_{10} was then produced by *E. coli* harboring the *dps* gene. Further studies on recombinant *E. coli* harboring the *dps* gene are needed in order to produce CoQ_{10} with high productivity, and industrial production of CoQ_{10} would then be possible.

REFERENCES

1. Alleva, R., M. Tomasetti, L. Andera, N. Gellert, B. Borghi, C. Weber, M. P. Murphy, and J. Neuzil. 2001. Coenzyme Q blocks biochemical but not receptor-mediated apoptosis by increasing mitochondrial antioxidant protection. *FEBS Lett.* **503:** 46–50.
2. Asai, K., S. Fujisaki, Y. Nishimura, T. Nishino, K. Okada, T. Nakagawa, M. Kawamukai, and H. Matsuda. 1994. The identification of *Escherichia coli ispB* (*cel*) gene encoding the octaprenyl diphosphate synthase. *Biochem. Biophys. Res. Commun.* **202:** 340–345.
3. Chen, A., P. A. Kroon, and C. D. Poulter. 1994. Isoprenyl diphosphate synthases: Protein sequence comparisons, a phylogenetic tree, and predictions of secondary structure. *Protein Sci.* **3:** 600–607.
4. Ernster, L. and G. Dallner. 1995. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* **1271:** 195–204.
5. Joly, A. and P. A. Edwards. 1993. Effect of site-directed mutagenesis of conserved aspartate and arginine residues upon farnesyl diphosphate synthase activity. *J. Biol. Chem.* **268:** 26983–26989.
6. Kawamukai, M. 2002. Biosynthesis, bioproduction and novel roles of ubiquinone. *J. Biosci. Bioeng.* **94:** 511–517.
7. Koyama, T., S. Obata, M. Osabe, A. Takeshita, K. Yokoyama, M. Uchida, T. Nishino, and K. Ogura. 1993. Thermostable farnesyl diphosphate synthase of *Bacillus stearothermophilus*: Molecular cloning, sequence determination, overproduction, and purification. *J. Biochem. (Tokyo)* **113:** 355–363.
8. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
9. Lee, J. K., G. Her, S. Y. Kim, and J. H. Seo. 2004. Cloning and functional expression of the *dps* gene encoding decaprenyl diphosphate synthase from *Agrobacterium tumefaciens*. *Biotechnol. Prog.* **20:** 51–56.
10. Meganathan, R. 2001. Ubiquinone biosynthesis in microorganisms. *FEMS Microbiol. Lett.* **203:** 131–139.
11. Melzer, M. and L. Heide. 1994. Characterization of polyprenyldiphosphate: 4-Hydroxybenzoate polyprenyltransferase from *Escherichia coli*. *Biochim. Biophys. Acta* **1212:** 93–102.
12. Ohnuma, S., K. Narita, T. Nakazawa, C. Ishida, Y. Takeuchi, C. Ohto, and T. Nishino. 1996. A role of the amino acid residue located on the fifth position before the first aspartate-rich motif of farnesyl diphosphate synthase on determination of the final product. *J. Biol. Chem.* **271:** 30748–30754.
13. Okada, K., K. Suzuki, Y. Kamiya, X. Zhu, S. Fujisaki, Y. Nishimura, T. Nishino, T. Nakagawa, M. Kawamukai, and H. Matsuda. 1996. Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone. *Biochim. Biophys. Acta* **1302:** 217–223.
14. Okada, K., T. Kainou, K. Tanaka, T. Nakagawa, H. Matsuda, and M. Kawamukai. 1998. Molecular cloning and mutational analysis of the *ddsA* gene encoding decaprenyl diphosphate synthase from *Gluconobacter suboxydans*. *Eur. J. Biochem.* **255:** 52–59.
15. Saiki, R., A. Nagata, N. Uchida, T. Kainow, H. Matsuda, and M. Kawamukai. 2003. Fission yeast decaprenyl diphosphate synthase consists of Dps1 and the newly characterized Dlp1 protein in a novel heterotetrameric structure. *Eur. J. Biochem.* **270:** 4113–4121.
16. Sarter, B. 2002. Coenzyme Q_{10} and cardiovascular disease: A review. *J. Cardiovasc. Nurs.* **16:** 9–20.
17. Song, L. and C. D. Poulter. 1994. Yeast farnesyl-diphosphate synthase: Site-directed mutagenesis of residues in highly conserved prenyltransferase domains I and II. *Proc. Natl. Acad. Sci. USA* **91:** 3044–3048.
18. Suzuki, K., K. Okada, Y. Kamiya, X. F. Zhu, T. Nakagawa, M. Kawamukai, and H. Matsuda. 1997. Analysis of the decaprenyl diphosphate synthase (*dps*) gene in fission yeast suggests a role of ubiquinone as an antioxidant. *J. Biochem.* **121:** 496–505.
19. Takada, M., T. Yuzuriha, K. Katayama, K. Iwamoto, and J. Sunamoto. 1984. Increased lung uptake of liposomes coated with polysaccharides. *Biochim. Biophys. Acta* **802:** 237–244.
20. Takahashi, S., T. Nishino, and T. Koyam. 2003. Isolation and expression of *Paracoccus denitrificans* decaprenyl diphosphate synthase gene for production of ubiquinone-10 in *Escherichia coli*. *Biochem. Eng. J.* **16:** 183–190.
21. Tarshis, L. C., M. Yan, C. D. Poulter, and J. C. Sacchettini. 1994. Crystal structure of recombinant farnesyl diphosphate synthase at 2.6-A resolution. *Biochemistry* **3:** 10871–10877.
22. Tarshis, L. C., P. J. Proteau, B. A. Kellogg, J. C. Sacchettini, and C. D. Poulter. 1996. Regulation of product chain length by isoprenyl diphosphate synthases. *Proc. Natl. Acad. Sci. USA* **93:** 15018–15023.
23. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.