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Cloning of the *sdsA* Gene Encoding Solanesyl Diphosphate Synthase from *Rhodobacter capsulatus* and Its Functional Expression in *Escherichia coli* and *Saccharomyces cerevisiae*

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Different organisms produce different species of isoprenoid quinones, each with its own distinctive length. These differences in length are commonly exploited in microbial classification. The side chain length of quinone is determined by the nature of the polyprenyl diphosphate synthase that catalyzes the reaction. To determine if the side chain length of ubiquinone (UQ) has any distinct role to play in the metabolism of the cells in which it is found, we cloned the solanesyl diphosphate synthase gene (*sdsA*) from *Rhodobacter capsulatus* SB1003 and expressed it in *Escherichia coli* and *Saccharomyces cerevisiae*. Sequence analysis revealed that the *sdsA* gene encodes a 325-amino-acid protein which has similarity (27 to 40%) with other prenyl diphosphate synthases. Expression of the *sdsA* gene complemented a defect in the octaprenyl diphosphate synthase gene of *E. coli* and the nonrespiratory phenotype resulting from a defect in the hexaprenyl diphosphate synthase gene of *S. cerevisiae*. Both *E. coli* and *S. cerevisiae* expressing the *sdsA* gene mainly produced solanesyl diphosphate, which resulted in the synthesis of UQ-9 without any noticeable effect on the growth of the cells. Thus, it appears that UQ-9 can replace the function of UQ-8 in *E. coli* and UQ-6 in *S. cerevisiae*. Taken together with previous results, the results described here imply that the side chain length of UQ is not a critical factor for the survival of microorganisms.

Prenyl diphosphate synthase catalyzes the condensation of isopentenyl diphosphate with allylic diphosphate to give isoprenoids of defined length, which are used as precursors in the synthesis of steroids, carotenoids, dolichol, prenyl quinones, and prenylated proteins (7) (Fig. 1). Short-chain isoprenoids (C_{10} to C_{20}) are used as precursors for many different products, while long-chain isoprenoids (C_{30} to C_{50}) are only used for the side chains of isoprenoid quinones (Fig. 1). For this reason, polyprenyl diphosphate synthases which produce isoprenoid chains longer than C_{30} have not been studied well, compared to prenyl diphosphate synthases that produce short-chain isoprenoids (C_{10} to C_{20}). However, species of quinones, e.g., ubiquinone (UQ), menaquinone, and plastoquinone, and the chain lengths of quinones are important criteria for the taxonomic study of microorganisms (3).

It was shown that the side chain length of UQ is determined by polyprenyl diphosphate synthase (15, 16). So, the structure and function of individual prenyl diphosphate synthases provide useful information about the species of quinone produced in a given organism. Several genes for prenyl diphosphate synthase that synthesize long-chain isoprenoids from bacteria and yeasts have been cloned and characterized. These include the hexaprenyl diphosphate synthase (Coq1) gene from *Saccharomyces cerevisiae* (2), the heptaprenyl diphosphate synthase genes from *Bacillus subtilis* (28, 29) and *Bacillus stearothermophilus* (12), the octaprenyl diphosphate synthase (IspB) gene from *Escherichia coli* (1), and the decaprenyl diphosphate synthase (DPS) gene from *Schizosaccharomyces pombe* (22). The length of the ultimate product of each of these enzymes is precisely defined by each prenyl diphosphate synthase. Amino acid sequence comparisons of those polyprenyl diphosphate synthases revealed the existence of seven highly conserved regions containing two aspartate-rich domains (domain II and domain VI), which are thought to be the binding sites for the diphosphate moieties of the isopentenyl diphosphate (IPP) and the allylic substrate (10, 21, 24). Studies of farnesyl diphosphate synthase from avian sources (25) and from *B. stearothermophilus* (14) revealed that two amino acids provide the chain-length determinant of prenyl diphosphate synthases that synthesize short-length isoprenoids. However, the identities of the amino acids that function to determine the chain lengths of isoprenoids produced by long-chain-producing prenyl diphosphate synthases remain unknown.

To gain further insight into the structure and function of long-chain-producing prenyl diphosphate synthases, we isolated the *sdsA* gene encoding solanesyl diphosphate synthase from *Rhodobacter capsulatus* and expressed it in an *E. coli ispB* mutant and an *S. cerevisiae COQ1* mutant. The expressed *sdsA* gene complemented the function of the corresponding polyprenyl diphosphate synthases of *E. coli* and *S. cerevisiae*, and the cells produced mainly UQ-9. These results indicate that UQ-9 is tolerated by *E. coli* and *S. cerevisiae*, which normally produce UQ-8 and UQ-6, respectively, as components of their electron transfer systems. Our results raise the question of the importance of side chain length of UQ in microorganisms.

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MATERIALS AND METHODS

Materials. Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo Co., Ltd., and New England Biolabs, Inc. IPP, all-

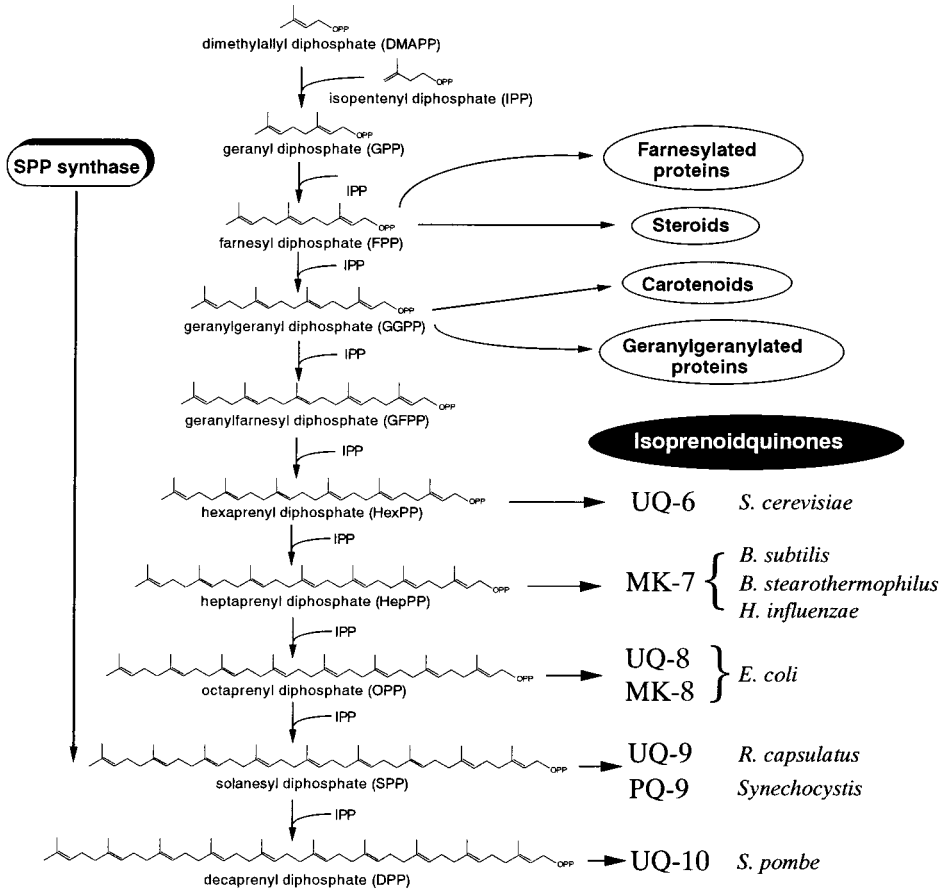

FIG. 1. Sequential condensation of polyprenyl diphosphate and its derivatives. *R. capsulatus* solanesyl diphosphate (SPP) synthase catalyzes the synthesis of SPP as the ultimate isoprenoid product, and the resulting SPP is used for the side chain of UQ-9 in this organism. The kinds of isoprenoid quinones produced by different organisms are indicated to the right. The microorganisms and quinones that correspond with the structure of the prenyl diphosphate synthase gene are indicated. Abbreviations for quinones: MK, menaquinone; PQ, plastoquinone.

E-farnesyl diphosphate (all-*E*-FPP), solanesol (all-*E*-nonaprenol), and polyprenols (C_{40} to C_{60}) from *Ailanthus altissima* were purchased from Sigma Chemical Co. [1-^{14}C]IPP (1.96 TBq/mol) was purchased from Amersham Co. Kiesel gel 60 F_{254} thin-layer chromatography plates were purchased from Merck. Reversed-phase LKC-18 thin-layer chromatography plates were purchased from Whatman Chemical Separation, Inc.

Strains and plasmids. *E. coli* JM109 was used for the general construction of plasmids (19). Plasmids pT7Blue-T, pUC118, and YEp13 M4 were used as vectors (17, 19). The strains and plasmids used in this study are listed in Table 1.

Genomic DNA preparation. *R. capsulatus* grown in 500 ml of minimal medium RCV (27) at 34°C under illumination was harvested in late log phase. Genomic DNA was prepared from *R. capsulatus* by the standard method described elsewhere (19) and was purified with a Qiagen Genomic-tip. The genomic DNA was partially digested with *Sau*3AI, and the resulting fragments were used as the template for the PCR (18).

Cloning of a conserved region of the polyprenyl diphosphate synthase gene by PCR. Three degenerated oligonucleotide primers (Table 2), DP1 (region II; sense primer), DP5 (region II; sense primer), and DP2 (region VI; antisense primer) were designed from the amino acid sequences of highly conserved regions of many prenyl diphosphate synthases. PCR was performed with Ex Taq polymerase (Takara) and with genomic DNA, which partially digested with *Sau*3AI. The PCR protocol used was 30 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C followed by extension at 72°C for 5 min (Perkin-Elmer thermal cycler). All DNA products of PCR were purified, subcloned into a pT7Blue T-vector, and sequenced by the dideoxy chain termination method (20).

Cloning of the full-length *sdsA* gene with cassettes and cassette primers. Genomic DNA walking was used to obtain the full length of the *sdsA* gene by PCR amplification using cassettes and the cassette primer method (8). The oligonucleotide cassette DNA for each *Eco*RI, *Hin*dIII, *Pst*I, *Sal*I, *Xba*I, and *Sau*3AI restriction site and cassette primers were purchased from Takara Shuzo Co. Each of the four oligonucleotide primers S1U, S2U, S1D, and S2D was designed to amplify upstream and downstream regions of partial fragments of the *sdsA* gene (Table 2). PCR-mediated genomic DNA walking was performed by the method of Isegawa et al. (8) with slight modification. Each PCR protocol entailed 30 cycles of 30 s at 94°C, 2 min at 55°C, and 1 min at 72°C (Perkin-Elmer thermal cycler). The resulting amplified products were extracted from the agarose gel and cloned into the pT7Blue-T vector. The sequence of the flanking regions of the partial *sdsA* gene fragment was determined as described below. To confirm the discrepancy of sequences of the PCR-amplified *sdsA* gene, two oligonucleotide primers, RCN (sense primer) and RCC (antisense primer), were designed and used for amplification of an open reading frame (ORF) of the *sdsA* gene. The amplified *sdsA* gene was recloned into pUC118 to yield pRC10. The sequences of several different clones were determined.

DNA sequence analysis. Deletion clones for sequencing were constructed with exonuclease III (19). Two oligonucleotide primers were designed to determine the sequences in the upstream direction (SE1) and downstream direction (SE2). Double-stranded DNA was sequenced in both directions by the dideoxy chain termination method (20) using an ABI Prism 377 DNA sequencer. Computer-assisted analysis and comparisons of DNA and protein sequences were performed by using the BLAST program in the NCBI network service.

Heterologous expression of the *sdsA* gene in *E. coli* and *S. cerevisiae*. To express the SdsA protein in *E. coli*, plasmid pRC10, which has the *lac* promoter upstream of the *sdsA* gene, was constructed as described above. Plasmid pRC10 was used to transform *E. coli* JM109. Transformants were grown at 37°C in LB (1% [wt/vol] polypeptone, 0.5% [wt/vol] yeast extract, 1% [wt/vol] NaCl) containing ampicillin at 50 μg/ml with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). To express the SdsA protein in yeast mitochondria, the *COQ1-sdsA* fused gene was constructed in plasmid pYD10. To express the *sdsA* gene under the control of the *COQ1* promoter, an RCN and RCC primer set was used to amplify the *sdsA* gene by PCR. The amplified fragment was digested with *Eco*RI-*Hin*dIII and was cloned into the same site of pSA1 (15), which has 53 amino acids of the Coq1 mitochondrial import signal, to yield pCD10. The 1.2-kb *Bam*HI-*Hin*dIII fragment from pCD10 was cloned into the same site of the yeast shuttle vector YEp13 M4 (17) to yield pYD10. Plasmid pYD10 was used to transform the yeast *COQ1*-defective strain YKK6. The transformation of yeast was performed according to the lithium acetate method of Ito et al. (9). Trans-

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)[a]	Source or reference
Strains		
E. coli JM109	*lacI*^{q} *lacZ*ΔM15	19
E. coli KO229	Cm^r Sp^r Δ*ispB*::*cat*; harbors pKA3	16
S. cerevisiae YKK6	*URA3*$^+$ Δ*COQ1*::*URA3*	15
R. capsulatus SB1003	Wild type	27
Plasmids		
pT7Blue-T	Ap^r *lacZ*	Novagen
pUC118	Ap^r *lacZ*	19
YEp13 M4	Ap^r *LEU*$^+$ 2μm	17
pKA3	Sp^r; 3-kb *Eco*RI fragment including *ispB* in pCL1920	16
pSA1	Ap^r; 0.3-kb *Bam*HI-*Eco*RI fragment of 5′-end *COQ1* in pBluescript KS$^+$	15
pCD10	Ap^r; 1.0-kb *Eco*RI-*Hin*dIII *sdsA* gene in pSA1	This study
pYD10	Ap^r *LEU*$^+$; 1.2-kb *Bam*HI-*Hin*dIII fragment from pCD10 in YEp13 M4	This study
pRC3	Ap^r; 0.4-kb PCR fragment of *sdsA* in pT7Blue-T	This study
pRCU	Ap^r; 1.4-kb upstream region of *sdsA* in pT7Blue-T	This study
pRCL	Ap^r; 2.2-kb downstream region of *sdsA* in pT7Blue-T	This study
pRC10	Ap^r; 1.0-kb *Eco*RI-*Hin*dIII *sdsA* in pUC118	This study

[a] Ap, ampicillin; Cm, chloramphenicol; Sp, spectinomycin.

formants were grown at 30°C in synthetic complete (SC) (0.67% [wt/vol] yeast nitrogen base, 2% [wt/vol] glucose or 3% [wt/vol] glycerol, and the appropriate amino acids)-Leu-Ura medium.

Preparation of crude enzyme from *E. coli* and *S. cerevisiae* expressing the *sdsA* gene. JM109 harboring pRC10 was grown to late log phase in LB medium containing ampicillin at 50 μg/ml and 1 mM IPTG for induction. The cells were ruptured by sonication with an ultrasonic disintegrator according to methods described previously (1).

Yeast cells were grown to mid- to late log phase in SC-Leu-Ura medium containing glucose or glycerol. The cells were washed and ruptured by shaking vigorously with glass beads and sonication with an ultrasonic disintegrator as described previously (15).

Prenyl diphosphate synthase assay and product analysis. Enzyme activity was measured by determining the amounts of [^{14}C]IPP incorporated into 1-butanol-extractable (for *E. coli*) or chloroform-extractable (for yeast) polyprenyl diphosphate according to methods described previously (1, 15). The concentration of $MgCl_2$ in the reaction mixture was 10 mM. The 1-butanol or chloroform extracts were dried up and hydrolyzed with acid phosphatase (Boehringer Mannheim) according to the method of Fujii et al. (6). The products of hydrolysis were extracted with hexane and analyzed by reversed-phase thin-layer chromatography with reference prenols. The reversed-phase chromatography was carried out on an LKC-18 plate (Whatman) with acetone-water (19:1, vol/vol). Radioactivity on the thin-layer chromatography plate was detected with the image analyzer BAS1500-Mac (Fuji Film Co.). The spots of the marker prenols were visualized by exposure of the plate to iodine vapor.

Complementation analysis. To test whether *sdsA* complements a defect in the *ispB* gene of *E. coli*, the chromosomal *ispB* disruption mutant KO229 harboring the *ispB* expression vector pKA3, was used for the plasmid-swapping experiment as described previously (16). Spectinomycin-sensitive and ampicillin-resistant strains which had only pRC10, and not pKA3, were selected. The yeast *COQ1*-defective strain YKK6 was grown on a glycerol nonfermentable carbon source to test for complementation with the *sdsA* gene as described previously (15). YKK6 was transformed with pYD10, and the resulting transformants were replicated on glycerol-containing SC-Leu-Ura agar medium. These transformants were then tested for the restoration of respiration deficiency.

UQ extraction and measurement. UQ was extracted by the methods of Wallace et al. (26). The extracted crude UQ was analyzed by normal-phase thin-layer chromatography using UQ-10 as a standard. Normal-phase thin-layer chromatography was carried out on a Kiesel gel 60 F_{254} plate (Merck) with benzene-acetone (93:7, vol/vol). The UV-visualized band containing UQ was collected from the thin-layer chromatography plate and extracted with chloroform-methanol (1:1, vol/vol). Samples were dried, and the precipitate was dissolved in ethanol. The purified ubiquinone was further analyzed by high-performance liquid chromatography with ethanol as the solvent phase (4).

Nucleotide sequence accession number. The nucleotide sequence of *sdsA* has been deposited in the DDBJ, GenBank, and EMBL databases (accession no. AB001997).

RESULTS

Isolation of the gene encoding polyprenyl diphosphate synthase. Prior to the start of our cloning experiment, the species of UQ produced by *R. capsulatus* SB1003 were examined. UQ-9 was the predominant form, with UQ-10 accounting for about 33% of the total (see Fig. 4A). This result suggests that this bacterium codes for a solanesyl diphosphate synthase gene. In order to obtain a fragment containing a conserved amino acid region of solanesyl diphosphate synthase, we synthesized three degenerated oligonucleotide primers designed

TABLE 2. Primers used for PCR and DNA sequencing

Primer	Sequence[a]	Application
DP1	5′-AAGGATCCTNYTNCAYGAYGAYGT-3′	PCR
DP2	5′-AAGGATCCTCRTCNACNARYTGRAA-3′	PCR
DP5	5′-AAGGATCCYTNYTNCAYGAYGA-3′	PCR
S1U	5′-GTCGTCTGTTATTTGGAAAGCA-3′	Genomic DNA walking
S2U	5′-TGTCCGGCAAAACGACGTAAAGGTTCACG-3′	Genomic DNA walking
S1D	5′-TTTATACATGATGATGTG-3′	Genomic DNA walking
S2D	5′-TGAGTCTGGCTTACGCCGTGGCAGA-3′	Genomic DNA walking
RCN	5′-CTGAATTCGATGGCCATCGATTTCAA-3′	PCR
RCC	5′-CCCAAGCTTTTATTGGATTCGGTCTA-3′	PCR
SE1	5′-CGCTGCAATACTGGCAGGCAAACCT-3′	Sequencing
SE2	5′-TTGCTGTTGGTCTGCCACGGCGTA-3′	Sequencing

[a] The letter R indicates A or G, the letter Y indicates C or T, and the letter N indicates A, C, G, or T.

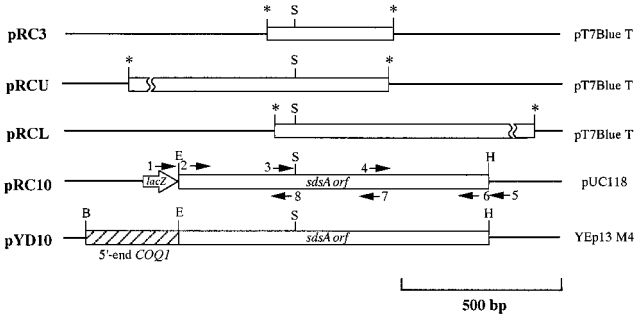

FIG. 2. Plasmid constructions used in this study. Asterisks indicate the sites of TA ligation with the T-tailed vector pT7Blue-T. pRC10 has the whole length of the *sdsA* gene under the control of the *lac* promoter, and the *sdsA* gene was expressed as a LacZ fusion protein. pYD10 has the mitochondrial import signal from the *COQ1* gene followed by the *sdsA* gene, and the *COQ1-sdsA* fused gene was expressed under the control of the native *COQ1* promoter. Numbered arrows indicate the primers for sequencing as follows: 1, M13 (forward); 2, RCN; 3, S1D; 4, SE1; 5, M13 (reverse); 6, RCC; 7, S1U; and 8, SE2. Abbreviations for restriction enzymes: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I.

from the amino acid sequences that are highly conserved in various prenyl diphosphate synthases (2, 13, 16). We obtained five kinds of products by PCR using the partially digested genomic DNA of *R. capsulatus* as the template. These products were directly cloned into a pT7Blue-T vector, and their nucleotide sequences were determined. Many clones were shown to be unrelated, but one clone, designated pRC3 (Fig. 2), which contained an approximately 400-bp insert, was found to have a sequence that resembled a typical prenyl diphosphate synthase motif, DDXXD (see Fig. 3). Then, we used PCR to clone the entire gene. As a result, a 1.4-kb fragment was amplified in the upstream direction with the *Pst*I cassette and a 2.2-kb fragment was amplified in the downstream direction with the *Hin*dIII cassette. The 1.4-kb fragment and the 2.2-kb fragment were cloned into the vector to yield pRCU and pRCL, respectively (Fig. 2). The sequences surrounding the gene were determined in both directions (data not shown). Three independent clones of the *sdsA* gene amplified by PCR were also sequenced to verify the correctness of the sequence. The sequence revealed the presence of an ORF consisting of 978 bp capable of encoding a 325-amino-acid protein. The protein encoded by this ORF contains the seven conserved regions found in typical prenyl diphosphate synthases (Fig. 3). Hence, we designated this gene *sdsA* (solanesyl diphosphate synthase). Southern blot analysis with a PCR fragment of pRC3 indicated the presence of 5-, 8-, and 15-kbp fragments in the hybridization pattern of the genomic DNA digested with *Pst*I, *Hin*dIII, and *Eco*RI, respectively (data not shown).

Comparison of the deduced *sdsA* amino acid sequences with the Swiss-Prot protein sequence database revealed 44.8% identity with *E. coli* octaprenyl diphosphate synthase (IspB), 43.3% identity with a *Haemophilus influenzae* HI0887 gene product which was recently found to encode heptaprenyl diphosphate synthase (16), 33.8% identity with *S. cerevisiae* hexaprenyl diphosphate synthase (Coq1), 36.5% identity with *Synechocystis* sp. strain PCC6803 solanesyl diphosphate synthase, 28.9% identity with *S. pombe* DPS, 30.1% identity with *B. subtilis* heptaprenyl diphosphate synthase (GerC3), and 27.0% identity with *B. stearothermophilus* heptaprenyl diphosphate synthase. These proteins represent all the known proteins that have been shown experimentally, and not just on the basis of sequence comparison, to possess the synthase function (Fig. 1).

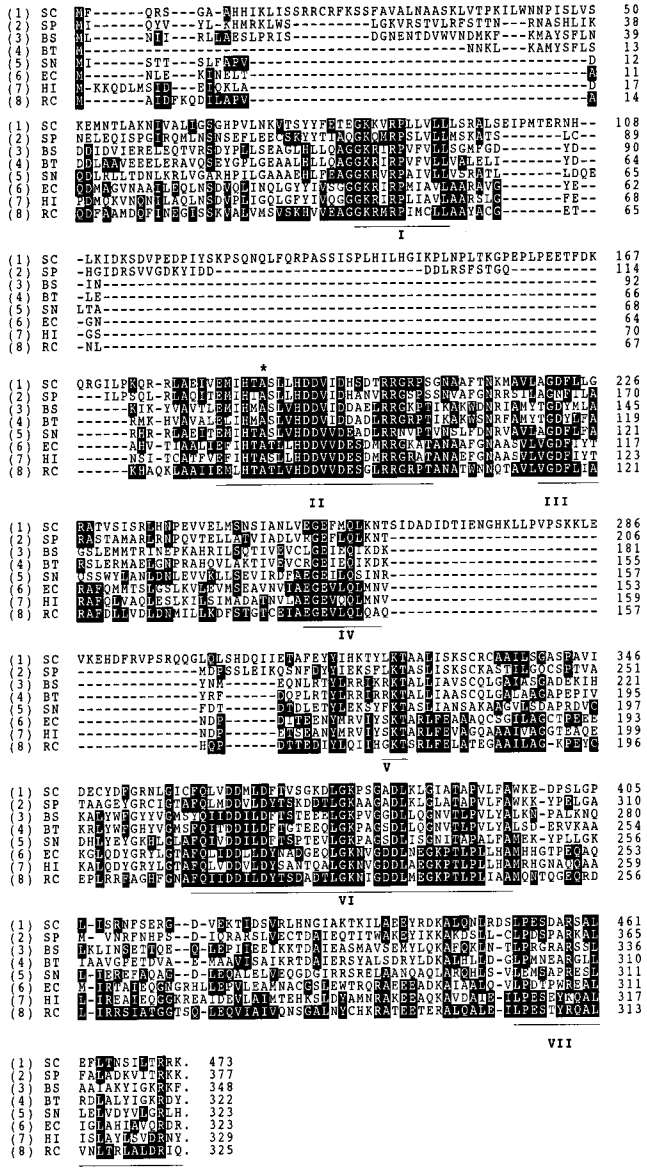

FIG. 3. Multiple alignment of eight prenyl diphosphate synthases that produce long-chain isoprenoids. Amino acids identical to those in the SdsA protein are shown within filled boxes. The seven highly conserved regions are underlined. The asterisk indicates the corresponding amino acid that is important for the chain length determination in short-chain prenyl diphosphate synthases. Sequences: (1) SC, hexaprenyl diphosphate synthase from *S. cerevisiae* (2); (2) SP, decaprenyl diphosphate synthase from *S. pombe* (22); (3) BS, heptaprenyl diphosphate (HepPP) synthase from *B. subtilis* (28); (4) BT, HepPP synthase from *B. stearothermophilus* (12); (5) SN, solanesyl diphosphate (SPP) synthase from *Synechocystis* sp. strain PCC6803 (11, 16); (6) EC, octaprenyl diphosphate synthase from *E. coli* (1); (7) HI, HepPP synthase from *H. influenzae* (5, 16); (8) RC, SPP synthase from *R. capsulatus*.

Expression of the *sdsA* gene in *E. coli* JM109. To verify the functional expression of the *sdsA* gene in *E. coli*, UQ was extracted from JM109 containing the *sdsA* expression vector. UQ was extracted from the cultures of JM109 harboring plasmid pRC10 which had the *sdsA* gene under the control of the *lac* promoter (Fig. 2). As shown in Fig. 4B, UQ-9 with 33% UQ-10 was detected in the cultures of JM109 harboring pRC10 in addition to endogenous UQ-8, while only UQ-8 was detected in cultures of JM109 (Fig. 4C). These results suggest

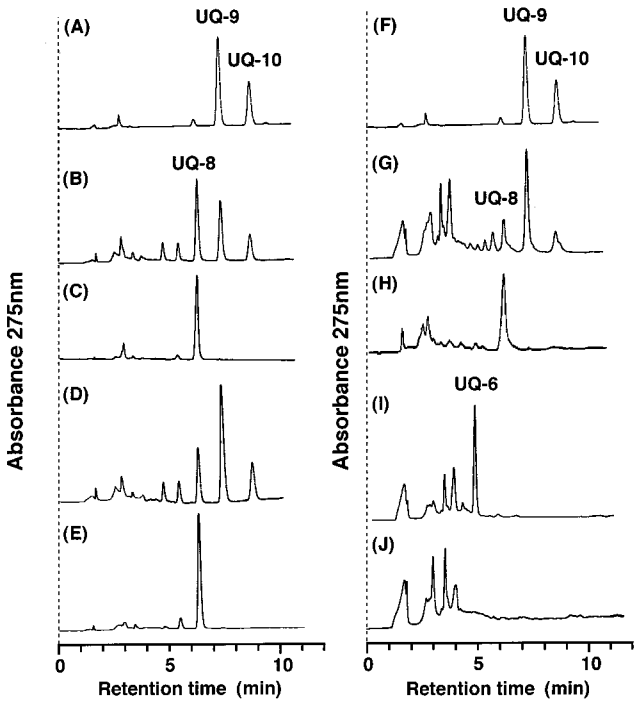

FIG. 4. Detection by high-performance liquid chromatography of UQ species in *R. capsulatus* and in *E. coli* and yeast expressing the *sdsA* gene. UQs were extracted from *R. capsulatus* SB1003 (A and F); JM109 harboring pRC10 (B); JM109 (C); KO229 harboring pRC10 (D); KO229 harboring pKA3 (E); YKK6 harboring pYD10, which has the *COQ1-sdsA* gene (G); YKK6 harboring pYE6, which has the *COQ1-ispB* gene (H) (15); YKK6 harboring YEpG3ΔSpH, which has the *COQ1* gene (I); and YKK6 (J). UQ-6, UQ-8, UQ-9, and UQ-10 are indicated.

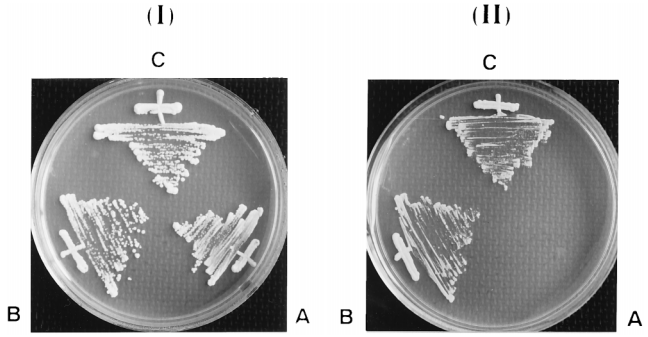

FIG. 5. Complementation of the *COQ1* disruptant by expression of the *COQ1-sdsA* fused gene. The YKK6 (*COQ1*::*URA3*) strain harboring YEp13 M4 (A); YEpG3ΔSpH (B), which carries the *COQ1* gene; and pYD10 (C), which carries the *COQ1-sdsA* gene; were grown on SC-Leu-Ura medium with glucose (I) or glycerol (II) for 7 days.

that the *sdsA* gene was functionally expressed in *E. coli* and that it encodes a solanesyl diphosphate synthase. These results also suggest that *E. coli* UbiA could transfer the solanesyl group to *para*-hydroxybenzoate.

Complementation of an *E. coli ispB* disruption mutant by *sdsA*. To investigate whether the *sdsA* gene can substitute for the function of *E. coli ispB*, the *sdsA* gene was expressed in the *E. coli ispB* disruption mutant KO229. *E. coli* KO229 survives only when it harbors the plasmid pKA3, which contains the *ispB* gene, because the *ispB* gene product is essential for the growth of *E. coli* (16). Experiments with plasmid swapping between pKA3 and pRC10 were carried out to see if the defect in the *ispB* gene could be complemented by the *sdsA* gene. KO229 harboring pKA3 was transformed with pRC10, and the resulting transformants were subcultured to cure pKA3 in nonselective media. KO229 harboring only pRC10 was successfully isolated, and then KO229/pRC10 was used to determine the nature of the UQ species and to measure prenyl diphosphate synthase activity. As shown in Fig. 4D, UQ-9 was detected in cultures of KO229/pRC10 as the main product, along with smaller amounts of UQ-8 and UQ-10, while only UQ-8 was detected in KO229/pKA3 (Fig. 4E). The growth of KO229 harboring pRC10 and that of KO229 harboring pKA3 did not differ significantly. Thus, the *sdsA* gene functionally complements a disruption of the *ispB* gene in *E. coli.*

Complementation of the respiratory deficiency of a *COQ1* mutant with the *COQ1-sdsA* fused gene. In *S. cerevisiae*, disruption of the *COQ1* gene, which encodes hexaprenyl diphosphate synthase, leads to the respiration deficiency (2, 15). To test whether the *sdsA* gene can function in an *S. cerevisiae* *COQ1* disruptant, the *S. cerevisiae COQ1* mutant YKK6 was transformed with pYD10, which has the *COQ1-sdsA* fused gene containing 53 amino acids of the Coq1 mitochondrial import signal (Fig. 2). The N-terminal 53-amino-acid sequence was previously shown to be important for the transfer of protein into mitochondria (15). Transformants were replicated on SC-Ura-Leu agar medium containing glycerol to test for the restoration of respiration deficiency. YKK6 harboring pYD10 could grow on SC-Ura-Leu agar medium containing glycerol, as could YKK6 harboring YEpG3ΔSpH (Fig. 5IIB and C), while YKK6 harboring the vector YEp13 M4 could not grow on such media (Fig. 5IIA). There was no significant growth rate difference between YKK6 harboring YEpG3ΔSpH and YKK6 harboring pYD10. Thus, the *sdsA* gene complemented for the loss of function of *COQ1* in *S. cerevisiae*. All three strains grew well when glucose was used as the fermentable carbon source (Fig. 5I). YKK6 harboring pYD10 was used to determine the nature of the UQ species and to measure prenyl diphosphate synthase activity. UQ-9 was mainly detected in YKK6 harboring pYD10 (Fig. 4G), UQ-8 was detected in YKK6 expressing the *ispB* gene (Fig. 4H), UQ-6 was detected in YKK6 expressing the *COQ1* gene (Fig. 4I), and no kind of UQ was detected in YKK6 alone (Fig. 4J).

Enzyme assay of prenyl diphosphate synthase. In order to show that the *sdsA* product catalyzes the condensation of IPP with the allylic substrate to give solanesyl diphosphate, prenyl diphosphate synthase activity in crude extracts from *E. coli* expressing the *sdsA* gene was measured. The result showed that two kinds of heterologous alcohols derived from the product were detected in addition to the octaprenol synthesized by the endogenous enzymes of JM109. One of the main products moved more slowly than authentic solanesol, and the other moved to the same position as that of authentic solanesol (Fig. 6, lane 2), while octaprenol was mainly detected in JM109 (Fig. 6, lane 1). These results indicate that the two alcohols are all-*E*-solanesol and all-*E*-decaprenol and that the *sdsA* gene is the structural gene for solanesyl diphosphate synthase. An enzyme assay using crude extracts from KO229 harboring pRC10 revealed that solanesol is the main product and that octaprenol and decaprenol are minor products of polyprenyl alcohol (Fig. 6, lane 4), while octaprenol was mainly detected in KO229 harboring pKA3 (Fig. 6, lane 3). These results of heterologous products basically reflect the final species of UQ (Fig. 4D).

Enzyme assays with crude extracts from YKK6/pYD10 revealed that solanesol was produced as the main alcohol (Fig. 6,

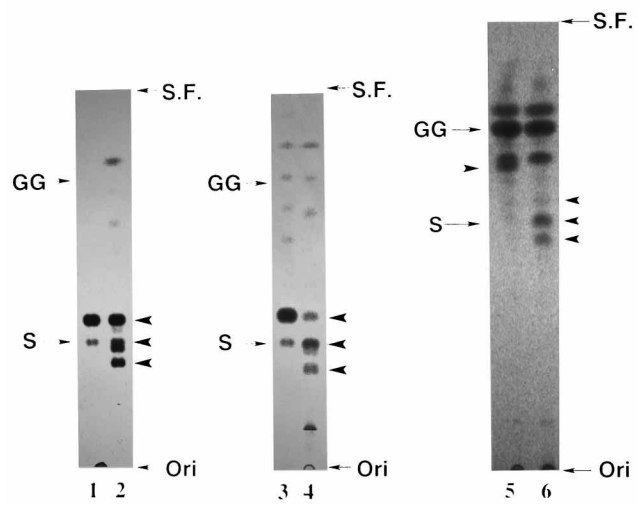

FIG. 6. Thin-layer chromatograms of alcohols derived from products of solanesyl diphosphate synthase. The enzyme reaction was carried out with [^{14}C]IPP and FPP as substrates and cell extracts as the enzyme source. The product was hydrolyzed with phosphatase acid, and the analysis of the resulting alcohol was carried out by reversed-phase thin-layer chromatography. Arrowheads indicate the positions of the synthesized isoprenoid alcohols. Arrows indicate the positions of the authentic alcohols: GG, all-*E*-geranylgeraniol; S, all-*E*-solanesol; Ori, origin; S.F., solvent front. The extracts analyzed in the different lanes are as follows: lane 1, *E. coli* JM109; lane 2, *E. coli* JM109/pRC10; lane 3, *E. coli* KO229/pKA3; lane 4, *E. coli* KO229/pRC10; lane 5, YKK6/YEpG3ΔSpH; and lane 6, YKK6/pYD10.

lane 6), while hexaprenol was detected in YKK6/YEpG3ΔSpH (Fig. 6, lane 5). These results indicate that the *COQ1-sdsA* fused gene produces mainly solanesol and that it can complement a defect of the *COQ1* gene in *S. cerevisiae*. In addition, the results suggest that UQ-9 can substitute for the function of UQ-6 in the respiratory chain of *S. cerevisiae.*

DISCUSSION

We have cloned the *R. capsulatus sdsA* gene, which encodes solanesyl diphosphate synthase, and functionally expressed it in *E. coli* and *S. cerevisiae*. The *sdsA* gene encodes a 325-amino-acid protein that has the typical structure of a long-chain-producing prenyl diphosphate synthase. Multiple alignment between SdsA and other polyprenyl diphosphate synthases shows the presence of seven highly conserved regions, and, in general, SdsA is quite similar to other polyprenyl diphosphate synthases (Fig. 3). So far, only one gene for solanesyl diphosphate synthase has been identified in *Synechocystis* sp. strain PCC6803 (11, 16). The amino acid sequence of SdsA from *R. capsulatus* shows more homology to IspB from *E. coli* than to solanesyl diphosphate synthase from *Synechocystis* sp. strain PCC6803, indicating that the sequence itself does not provide a true indication of enzymatic activity, even if there exists extensive homology. We previously showed this to be the case for the IspB protein of *E. coli* and the IspB homolog (HI10881) from *H. influenzae*, which both produce isoprenoids of different lengths even though they have a high level of amino acid identity (64%) (16).

Prenyl diphosphate synthases that synthesize short-chain isoprenoids, such as FPP synthase, have tyrosine or phenylalanine at the fifth amino acid position before the first aspartate-rich motif, which is thought to be a chain length determination site (14, 25). However, in the SdsA protein, the fifth amino acid before the first aspartate-rich motif is alanine, as is the case for other enzymes which synthesize long-chain (C_{30} to C_{50}) isoprenoids (Fig. 3). It is not known which regions or amino acids are involved in the determination of the final products of long-chain-producing prenyl diphosphate synthases.

We succeeded in expressing *sdsA* in both *E. coli* and *S. cerevisiae*. A number of genes encoding polyprenyl diphosphate synthase from bacteria have been successfully expressed in *E. coli* (16) and *S. cerevisiae* (15), but the expression of the *S. pombe dps* gene encoding DPS was not successful in either organism (22; our unpublished observation). Yeast enzymes and bacterial enzymes can be different in their requirements for ancillary factors. The SdsA protein is assumed to work as a homodimer, because it is functional without any other genes, unlike in the case of *B. subtilis* heptaprenyl diphosphate synthase (29).

We have recently shown that *E. coli* UbiA protein can transfer heptaprenyl and solanesyl groups to *para*-hydroxybenzoate to synthesize UQ-7 and UQ-9 in vivo (16). In this study, we have shown that the broad specificity of *E. coli* UbiA and *S. cerevisiae* Coq2 proteins with respect to their substrates is consistent with our previous results (15, 23); i.e., Coq2 can use substrates ranging from C_{30} to C_{50} polyprenyl diphosphate.

The *sdsA* gene expressed in *E. coli* produced mainly solanesyl diphosphate and, at lower levels, decaprenyl diphosphate and octaprenyl diphosphate. This result reflects quite well the bona fide activity of SdsA, as *R. capsulatus* SB1003 produces both UQ-9 and UQ-10. *R. capsulatus* is expected to produce UQ-10 as a main product according to the literature (3), but our strain mainly produced UQ-9. The heterologous distribution of UQ species has often been observed in certain bacteria. For example, although *Agrobacterium tumefaciens* and *Gluconobacter suboxydans* mainly produce UQ-10, they also produce UQ-9 and UQ-8 as minor products (3; our unpublished observation). *Acetobacter aceti* mainly produces UQ-9, with UQ-10 and UQ-8 as minor products (3). These bacteria probably produce different kinds of UQs because polyprenyl diphosphate synthase produces heterologous lengths of isoprenoids. In fact, heterologies in the lengths of products were found in the in vitro enzymatic assay for SdsA activity from *R. capsulatus*, reflecting the final product of UQ species. These results again support the idea that prenyl diphosphate synthase essentially determines the side chain length of UQs (15).

We previously showed that UQ-8 can replace the function of UQ-6 in *S. cerevisiae* (15). In this study, we have shown that an *S. cerevisiae COQ1* mutant expressing the *sdsA* gene mainly produces UQ-9 and that the strain grows normally. This indicates that UQ-9 can replace the function of UQ-6 in *S. cerevisiae*. The functional complementation of a defect in *E. coli ispB* and *S. cerevisiae COQ1* by the *sdsA* gene indicates that UQ-9 can be used as an electron transporter instead of the normal UQ-8 or UQ-6. These results lead to the interpretation that the length of the side chain of UQ in a given organism may not be a decisive factor for UQ activity. The type of UQ or other quinones is used as a criterion in the taxonomic study of microorganisms (3). Although this may be useful for classification, our results suggest that the biological significance of such differences is open to question and requires further clarification.

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