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Cloning and characterization of decaprenyl diphosphate synthase from three different fungi.

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Abstract

Coenzyme Q (CoQ) is composed of a benzoquinone moiety and an isoprenoid side chain of varying lengths. The length of the side chain is controlled by polyprenyl diphosphate synthase. In this study, *dps1* genes encoding decaprenyl diphosphate synthase were cloned from three fungi: *Bulleromyces albus, Saitoella complicata,* and *Rhodotorula minuta.* The predicted Dps1 proteins contained seven conserved domains found in typical polyprenyl diphosphate synthases, and were 528, 440, and 537 amino acids in length in *B. albus, S. complicata,* and *R. minuta,* respectively. *E. coli* expressing the fungal *dps1* genes produced CoQ_{10} in addition to endogenous CoQ_8. Two of the three fungal *dps1* genes (from *S. complicata* and *R. minuta*) were able to replace the function of *ispB* in an *E. coli* mutant strain. *In vitro* enzymatic activities were also detected in recombinant strains. The three *dps1* genes were able to complement an *S. pombe dps1, dlp1* double mutant. Recombinant *S. pombe* produced mainly CoQ_{10}, indicating that the introduced genes were independently functional and did not require *dlp1*. The cloning of *dps1* genes from various fungi has the potential to enhance production of CoQ_{10} in other organisms.

Introduction

CoQ (Coenzyme Q, or ubiquinone), a natural compound present in almost all living organisms, localizes primarily to the plasma membrane in prokaryotes and to the mitochondrial inner membrane in eukaryotes. CoQ is essential for aerobic growth and oxidative phosphorylation in the electron transport system (Vinothkumar et al. 2014), antioxidation (Quinzii et al. 2014), disulfide formation (Bader et al. 1999), sulfide oxidation (Saiki et al. 2003b), and *de novo* UMP synthesis (López-Martín et al. 2007; Matsuo et al. 2013). The biochemical properties and the ongoing discovery of novel functions of CoQ have prompted substantial research interest. For example, one area of research focused on the role of human-type CoQ (CoQ_{10}) in cardiovascular disease and its use in clinical therapies and nutrition (Ayer et al. 2015).

CoQ is composed of a benzoquinone moiety and an isoprenoid side chain of varying lengths. Although the CoQ biosynthetic pathway in *E. coli* has been almost entirely characterized, additional factors remain to be determined in eukaryotes (Allan et al. 2015; Aussel et al. 2014; Kawamukai 2016). In *E. coli*, generation of the isoprenoid side chain is catalyzed by polyprenyl diphosphate synthase (poly-PDS). The isoprenoid side chain is condensed with *p*-hydroxybenzoate (PHB) or *p*-aminobenzoic acid (pABA) (in *S. cerevisiae*)

by PHB-polyprenyl diphosphate transferase (Fig. 1). A series of modification reactions of the benzoquinone ring, including methylations, decarboxylation, and hydroxylations, complete CoQ processing (Kawamukai 2016). CoQ biosynthetic genes in complex eukaryotes are thought to be similar to those found in *S. cerevisiae*, with the exception of those involved in isoprenoid side chain synthesis (Kawamukai 2009).

CoQ side chain length varies between species. For example, *S. cerevisiae* has six isoprene units in its side chain, *Candida utilis* has seven units, *E. coli* has eight units, mice and *Arabidopsis thaliana* have nine units, and *S. pombe* and humans have ten units (Kawamukai 2002; Okada et al. 2004; Saiki et al. 2005; Zhu et al. 1995). The isoprenoid side chain length of CoQ is defined by the product generated by poly-PDS (Okada et al. 1998a; Okada et al. 1996; Suzuki et al. 1994), but not by the substrate specificity of PHB-polyprenyl diphosphate transferases (Okada et al. 2004; Suzuki et al. 1994). *E. coli* ordinarily produces CoQ_8, but exogenous expression of heptaprenyl, solanesyl, or decaprenyl diphosphate synthase (deca-PDS) genes from *Haemophilus influenzae*, *Rhodobacter capsulatus*, or *Gluconobacter suboxydans*, respectively, allows production of CoQ_7, CoQ_9, or CoQ_{10}, respectively (Okada et al. 1998b; Okada et al. 1997a; Okada et al. 1997b; Park et al. 2005). Similarly, an *S. cerevisiae*

COQ1 disruptant that expressed various poly-PDS genes from different organisms produced the CoQ type of the donor organism (e.g., CoQ_5, CoQ_6, CoQ_7, CoQ_8, CoQ_9, or CoQ_{10}) (Okada et al. 1998a). Furthermore, when genetic engineering was used to enable deca-PDS production by rice mitochondria, the rice produced CoQ_{10} instead of the originally-synthesized CoQ_9 (Takahashi et al. 2006).

Long-chain PDSs for the production of CoQ side chains were previously characterized in many organisms and these are classified into the chain length they produce, sources of species and compositions of proteins (Table 1). Long-chain PDSs are classified into homodimer (e.g., IspB in *E. coli*), and heterotetramer (e.g., Dps1 and Dlp1 in *S. pombe*) types based on the pattern of their components. Homodimeric enzymes and Dps1 contain seven conserved regions (domains I–VII) and two aspartate-rich motifs DDXXD (domains II and VI). The first DDXXD motif is responsible for binding with FPP, and the second is responsible for binding with IPP. The Dlp1 protein is weakly similar in the sequence to Dps1, but lacks the conserved regions of domains II and VI (Saiki et al. 2003a). Solanesyl- and deca-PDSs from mice and humans were found to be heterotetramer types (Saiki et al. 2005).

To date, only heteromeric deca-PDSs have been identified in eukaryotes.

Therefore, cloning and expression of eukaryotic homomeric deca-PDS may be desirable for production of CoQ_{10} in various species. In this study, decaprenyl diphosphate synthase (*dps1*) genes were cloned from the fungi *Saitoella complicata, Bulleromyces albus,* and *Rhodotorula minuta* and characterized in *E. coli* and *S. pombe.*

Materials and methods

Materials

Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo Co., Ltd., and New England Biolabs, Inc. IPP, (*E*)-farnesyl diphosphate (all-(*E*)-FPP), geranylgeraniol, and solanesol (all-(*E*)-nonaprenol) were purchased from Sigma. [1-^{14}C]IPP (1.96 TBq/mol) was purchased from GE Healthcare UK Ltd. Kieselgel 60 F_{254} TLC plates were purchased from Merck. Reversed-phase LKC-18 TLC plates were purchased from Whatman.

Strains, Media and Plasmids

Fungal strains *Saitoella complicata* NBRC 10748*, Bulleromyces albus* NBRC 1192*,* and *Rhodotorula minuta* NBRC 0387 were purchased from the NITE Biological Resource Center (NBRC). Fungi were grown in YM medium (1%

glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract). *E. coli* strain DH5α was used for general plasmid construction and gene expression. KO229 (Cm^r, Sp^r, *ispB::cat*), an *ispB*-defective mutant of *E. coli,* harboring plasmid pKA3 (*ispB*) (Okada et al. 1997b), was used as a host strain to express fungal *dps1* genes for CoQ synthesis and complementation analysis. *E. coli* strains were cultured in LB medium (1% bactotryptone, 0.5% yeast extract, and 1% NaCl; pH 7.0). Plasmids pT7Blue T-Vector (Novagen, Madison, WI, USA) and pUCNT were used as vectors. pUCNT, which contained an *Nde*I restriction site, was modified from pUC19 (Ikenaka 2003). *S. pombe* wild-type strain PR110 (Saiki et al. 2005) and a double-deletion mutant of *dps1* and *dlp1*, LA1 (*Δdlp1::ura4::ADE2*, *Δdps1::kanMx6*) (Zhang et al. 2008), were used to express fungal *dps1* genes for CoQ synthesis and complementation analysis. *S. pombe* strains were grown in PM medium (0.3% potassium hydrogen phthalate, 0.56% sodium phosphate, 0.5% ammonium chloride, 2% glucose, vitamins, minerals, and salts). Plasmid pREP1 was used as the vector.

Cloning of *dps1* from *B. albus*

Genomic DNA was prepared from *B. albus* as described previously (Hoffman and Winston 1987). Total RNA was prepared from *B. albus* using an RNeasy

mini kit (Qiagen, Valencia, CA), and mRNA was isolated using an Oligotex-dT30 <Super> mRNA purification kit (Takara Shuzo Co., Ltd.). Primers DPS-1 and DPS-1 1AS (Table 2) were used for PCR amplification, and the resulting 220 bp amplicon was cloned into pT7Blue T-Vector to yield pT7-B1DPS. The inserted amplicon was sequenced. To obtain the 3′ region of the *dps1* gene, RT-PCR and PCR were performed using a PrimeScript High Fidelity RT-PCR Kit (Takara Shuzo Co., Ltd.), with primer B1S, whose sequence was in pT7-B1DPS, and mRNA as a template. An 850 bp fragment was obtained and cloned into pT7Blue T-Vector to yield pT7-B2DPS. To obtain the 5′ region of the *dps1* gene, RT-PCR was performed using a 5′-Full RACE Core Set (Takara Shuzo Co., Ltd.), with primer B7ASP (phosphorylated at the 5′ end), whose sequence was in pT7-B2DPS, and mRNA as a template. The resultant amplified cDNA fragment was circularized, and used a template for PCR with primers B5S and B4AS, whose sequences were in pT7-B2DPS. The resultant amplified fragment was used as a template for PCR with nested primers B6S and B3AS. The resultant 950 bp fragment was cloned into pT7Blue T-Vector to yield pT7-B3DPS. To obtain the full-length *dps1* gene, the 5′ region was amplified using BN1 and B3AS primers with pT7-B3DPS as a template, and the 3′ region was amplified using B1S and BCH primers with

pT7-B2DPS as a template. The resulting fragments (910 bp and 750 bp) were mixed and the full-length duplex was formed by denaturation, annealing, and extension with DNA polymerase. The full-length *dps1* gene was then amplified using BN1 and BCH primers. The 1.6 kb DNA fragment was digested with *Nde*I and *Hin*dIII and cloned into similarly-digested pUCNT to yield plasmid pNTB1-1. pREP1-B1dps1 was constructed using the gap-repair cloning (GRC) method (Matsuo et al. 2010). A fragment containing the *dps1* gene and flanking *nmt1* promoter and terminator sequences was amplified from pNTB1-1 using primers GRC_B1dps1-Fw and GRC_B1dps1-Rv. *S. pombe* PR110 was co-transformed with the *dps1* gene and a *Bam*HI/*Sma*I-digested pREP1 vector. Transformants were screened with PM plates containing uracil and pREP1-B1dps1 was extracted.

Cloning of *dps1* from *S. complicata*

Genomic DNA was prepared from *S. complicata* as described previously (Hoffman and Winston 1987). Primers DPS-1 and DPS-1 1AS were used for PCR amplification, and the resulting 220 bp amplicon was cloned into pT7Blue T-Vector to yield pT7-SaDPS. The inserted amplicon was sequenced. *S. complicata* genomic DNA was digested with *Eco*RI, and Southern analysis was

performed using standard methods (Sambrook et al. 1989). Southern blots were probed with a 145 bp DNA fragment amplified using primers Sa-1S and Sa-2AS, and labeled with an ECL direct nucleic acid labeling and detection system (GE Healthcare). Hybridization and detection of the labeled probe were performed according to the manufacturer's instructions. Southern analysis identified a 10 kb fragment, which was then excised from the gel and packaged using a Lambda DASH II phage kit (Agilent technology). Phage libraries were propagated in *E. coli* strain XL1-Blue MRA (P2). Plaque hybridization was performed using the same probe, and six positive phages were isolated. DNA from positive phage libraries was digested with *Sal*I or *Sac*I, and electrophoresed. Southern analysis of the digested DNA identified 4.5 kb and 3.5 kb genomic fragments in the *Sal*I and *Sac*I digested DNA, respectively. Another 3.0 kb fragment was sequenced for the full length of *dps1*. The full-length *S. complicata dps1* gene was amplified using primers Sa-N1 and Sa-C, digested with *Nde*I and *Eco*RI, and cloned into similarly-digested pUCNT to yield pNTSa1-1. pREP1-Sa1dps1 was constructed using the GRC method. The *dps1* gene was amplified from pNTSa1-1 using primers GRC_Sa1dps1-Fw and GRC_Sa1dps1-Rv. *S. pombe* PR110 was then co-transformed with the *dps1* gene and a *Bam*HI/*Sma*I-digested pREP1 vector.

Transformants were screened on PM plates containing uracil, and pREP1-Sa1dps1 was extracted.

Cloning of *dps1* from *R. minuta*

Genomic DNA was prepared from *R. minuta* as described previously (Hoffman and Winston 1987). Primers DPS-1 and DPS-1 1AS were used for PCR amplification, and the resulting 220 bp amplicon was cloned into pT7Blue T-Vector to yield pT7-RmDPS. The inserted amplicon was sequenced. *R. minuta* genomic DNA was digested with *Eco*RI, and Southern analysis was performed using standard methods (Sambrook et al. 1989). Southern blots were probed with a 144 bp DNA fragment amplified using primers Rm-1S and Rm-2AS, and labeled with an ECL direct nucleic acid labeling and detection system (GE Healthcare). Hybridization and detection of the labeled probe were performed according to the manufacturer's instructions. Southern analysis identified a 5.5 kb fragment, which was then excised from the gel and packaged using a Lambda DASH II phage kit (Agilent technology). Phage libraries were propagated in *E. coli* strain XL1-Blue MRF'. Plaque hybridization was performed using the same probe, and seven positive phages were isolated. Positive phagemid DNA was prepared by *in vivo* excision using the Lambda

DASH II phage kit (Agilent technology). Approximately 1.6 kb of insert was sequenced, and this contained the full-length *dps1* gene. The full-length *R. minuta dps1* gene was amplified using primers RM-1 and Rm-CE2, digested using *Nde*I and *Eco*RI, and then cloned into similarly-digested pUCNT to yield pNTRm2-1. For construction of shortened *R. minuta dps1*, a 1.2 kb fragment was amplified using primers Rm-4 and Rm-CE2 and digested with *Nde*I and *Nhe*I to produce a 600 bp fragment. This shortened fragment was used to replace the *Nde*I and *Nhe*I fragment (1.0 kb) in pNTRm2-1 to yield pNTRmSsp. A 70 bp fragment was amplified using primers RM-1 and RM-6R, digested with *Nde*I and *Ssp*I, and cloned into similarly-digested pNTRmSsp to yield pNTRm6-1. pREP1-Rm2dps1 and pREP1-Rm6dps1 were constructed using the GRC method. The *dps1* genes were amplified from pNTRm2-1 and pNTRm6-1 using primers GRC_Rmdps1-Fw and GRC_Rmdps1-Rv. *S. pombe* PR110 was then co-transformed with the *dps1* gene and a *Bam*HI/*Sma*I-digested pREP1 vector. Transformants were screened on PM plates containing uracil, and then pREP1-Rm2dps1 and pREP1-Rm6dps1 were extracted.

Complementation of *ispB* in an *E. coli ispB* disruption mutant with fungal *dps1*

E. coli KO229 (*ispB::cat*) harboring pKA3 (Okada et al. 1997b) was

transformed with plasmid containing a fungal *dps1* gene to produce transformants resistant to spectinomycin and ampicillin. Transformants were subcultured five times in LB medium containing 50 μg/ml ampicillin and were plated on LB agar medium containing ampicillin. The resulting colonies were then replicated on LB medium containing ampicillin or spectinomycin. Transformants that were both spectinomycin-sensitive and ampicillin-resistant contained the fungal *dps1* plasmid but not pKA3, and were selected for further analysis.

CoQ extraction and analysis

Recombinant *E. coli* strains were incubated in LB liquid medium with appropriate antibiotics to the mid-to-late log phase, then cells were collected by centrifugation at 3,500 rpm. For yeast strains, minimum medium with appropriate supplements were used for incubation. CoQ was extracted as described previously (Okada et al. 1998b; Saiki et al. 2005). The crude CoQ extract was analyzed by normal-phase TLC with CoQ_{10} as the standard. Normal-phase TLC was carried out on a Kieselgel 60 F_{254} plate with benzene. The UV-visualized band containing CoQ was collected from the TLC plate and extracted with chloroform-methanol (1:1, vol/vol). The solution was

evaporated to dryness and the residue was re-dissolved in ethanol. The purified CoQ was further analyzed by high-performance liquid chromatography (HPLC) using ethanol as a solvent. The CoQ_{10} quantification was performed by using CoQ_6 (Avanti Lipids Polar, Inc.) as an internal standard.

Prenyl diphosphate synthase assay and product analysis

PDS activity was assayed as described previously (Saiki et al. 2005). Cultures were grown to the mid-to-late log phase in the appropriate medium and then harvested by centrifugation. All subsequent steps were carried out at 4°C. Cells were re-suspended in a buffer containing 100 mM potassium phosphate (pH 7.4), 5 mM EDTA, and 1 mM 2-mercaptoethanol, and ruptured by vigorous shaking with glass beads 14 times for 30 s at 60 s intervals on ice. The homogenate was centrifuged at 1500 × g for 10 min, and the resulting supernatant was used as a crude enzyme extract. The incubation mixture contained 2 mM $MgCl_2$, 0.2% (w/v) Triton X-100, 50 mM potassium phosphate buffer (pH 7.4), 5 mM KF, 10 mM iodoacetamide, 20 μM [1-^{14}C]IPP (specific activity 0.92 MBq mol^{-1}), 100 μM FPP, and 1.5 mg.mL^{-1} of the enzyme in a final volume of 0.5 mL. Sample mixtures were incubated for 60 min at 30°C.

Prenyl diphosphates were extracted with 1-butanol-saturated water and

hydrolyzed with acid phosphatase. The hydrolysis products were extracted with hexane and analyzed by reverse-phase TLC with acetone/water (19:1, v/v). Radioactivity on the plate was detected with a BAS1500-Mac imaging analyzer (Fuji Film Co.). The spots of the marker prenols were visualized by exposure of the plate to iodine vapor.

Nucleotide sequence accession number

The sequences reported herein have been deposited in the GenBank database (*B. albus* Dps1, BD182059; *R. minuta* Dps1, BD170286; *S. complicate* Dps1, BD093645).

Results

Cloning of the fungal *dps1* genes from *B. albus, S. complicata*, and *R. minuta*

Fungi that produced CoQ_{10} were identified in order to find target deca-PDS genes for cloning. CoQ species from three fungi, *Bulleromyces albus, Saitoella complicata,* and *Rhodotorula minuta*, were examined, and all strains were found to produce CoQ_{10} (Fig. 2). The gene encoding deca-PDS was cloned from all three fungal species. PCR-mediated amplification methods were used to obtain fragments of the deca-PDS gene, which were then used to isolate the full-length gene.

Genomic DNAs were prepared from *B. albus, S. complicata*, and *R. minuta*.

DNA fragments were amplified by PCR using primers DPS-1 and DPS-1 1AS, which corresponded to a conserved region in typical prenyl diphosphate synthases. DNA fragments of approximately 220 bp were obtained from each genomic DNA and cloned into the pT7BlueT-Vector. Fragments were sequenced, and translated amino acid sequences revealed a conserved motif, GDFLLXRA, known to be conserved in typical prenyl diphosphate synthases. For *B. albus dps1* gene cloning, mRNA was prepared and used as template to amplify the 3′ end of the cDNA by RT-PCR, because we predicted that *B. albus dps1 gene* had introns based on the result of southern hybridization. Amplification used primer B1S, which corresponded to part of the 220 bp amplified genomic region, and produced a fragment of 850 bp. Sequence from the 3′ cDNA was used to design primer B7ASP, which was used to amplify a 950 bp 5′ region. Together, the 3′ and 5′ fragments constituted the full-length *dps1* cDNA from *B. albus*.

A different approach was taken to clone deca-PDSs from *S. complicata* and *R. minuta*. The 220 bp fragments amplified from genomic DNA were used as probes for Southern hybridization of *Eco*RI-digested genomic DNA. DNA fragments of 10 kb and 5.5 kb were identified from *S. complicata* and *R. minuta*, respectively. The 10 kb and 5.5 kb genomic fragments were then extracted from

the gel, cloned into phage DASH II, and packaged in phage using an *in vitro* packaging kit. *E. coli* strains XL1-Blue MRA (P2) or MRF' were infected with phage and plated on NZY medium. Phage were transferred to N-bond filter, then denatured and neutralized. Nine filters were hybridized using the 220 bp probe, and a small number of plaques were positively identified. Positive phagemid DNAs were then sequenced to determine the full-length *dps1* sequence from *S. complicata* and *R. minuta*. Ultimately, *dps1* was successfully cloned from *B. albus, S. complicata*, and *R. minuta*.

Amino acid sequences were predicted from the *dps1* gene sequences (Fig. 3). The *B. albus, S. complicata,* and *R. minuta dps1* genes were predicted to encode 528, 440, and 537 amino acid proteins, respectively, and these exhibited 50%, 51%, and 46% sequence similarity to *S. pombe* Dps1. Alignment of other prenyl diphosphate synthases indicated that the predicted Dps1 proteins from *B. albus, S. complicata*, and *R. minuta* were long-chain prenyl diphosphate synthases.

Expression of fungal *dps1* genes in *E. coli*

The *dps1* genes from *B. albus, S. complicata,* and *R. minuta* were expressed in wild-type *E. coli* strain DH5α, which naturally produces CoQ_8. CoQ species were tested from *E coli* harboring pNTB1-1, which expressed *B. albus dps1*,

pNTSa1-1, which expressed *S. complicata dps1*, pNTRm2-1, which expressed *R. minuta dps1,* or pNTRm6-1, which expressed a shorter version of *R. minuta dps1* (Fig. 4A). Because there is the serine-rich sequence, which does not align well with other Dps1s and might be a part of intron, in the N-terminal domain, a shorter version of Dps1 which lacks this region was made. Transformants all produced CoQ_{10}, albeit to varying degrees, in addition to CoQ_8 (Fig. 5). These results indicated that the cloned *dps1* genes encoded deca-PDSs and that they could be expressed in *E. coli.*

Complementation of an *E. coli ispB* mutant by fungal *dps1* genes

Long-chain prenyl diphosphate synthases are classified into two types: homomeric and heteromeric (Kawamukai 2009). Homomeric enzymes do not require additional subunits in order to be functional, and would therefore be able to complement an *E. coli* mutant lacking the homologous gene (*ispB*). Complementation was examined by expressing fungal *dps1* genes in an *E. coli ispB* disruptant (KO229). Because *ispB* is essential for growth in *E. coli* (Okada et al. 1997b), KO229 harboring pKA3, which expresses *ispB*, was used. KO229 cells harboring both plasmids were grown for a few days in LB medium containing ampicillin. Cells containing pNTSa1-1 (or pNTRm2-1 or

pNTRm6-1) but not pKA3 were isolated by selecting strains that were ampicillin-resistant and spectinomycin-sensitive. CoQ species from transformant strains were analyzed by HPLC (Fig. 6). *E. coli* KO229 harboring pKA3 synthesized only CoQ_8 (Fig. 6b), *E. coli* KO229 harboring pNTSa1-1 predominantly synthesized CoQ_{10} (Fig. 6c), and *E. coli* KO229 harboring pNTRm2-1 or pNTRm6-1 produced mainly CoQ_{10}, with small amounts of CoQ_9 and CoQ_8 (Fig. 6d, 6e). The *ispB* gene is essential for *E. coli* growth and is responsible for CoQ side chain length determination (Okada et al. 1997b), and these results clearly indicate that Dps1 proteins from *S. complicata* and *R. minuta* have deca-PDS activity and can act alone in *E. coli* to complement *ispB* (see also Fig. 7).

Attempts were made to exchange pKA3 with pNTB1-1, which encoded *B. albus* Dps1, but these were unsuccessful. In this case, Dps1 activity level may be insufficient to support growth.

Enzymatic activity of fungal Dps1

To determine whether the recombinant proteins exhibited any specific enzymatic activity, *in vitro* assays were performed with IPP and FPP as allylic substrates. Enzymatic activity was measured by determination of the amount of

[1-^{14}C]IPP incorporated into polyprenyl diphosphates. The product generated in the reaction was hydrolyzed by acid phosphatase and separated by reverse-phase TLC. As shown in Figure 7, the major product of wild-type *E. coli* DH5α was octaprenol. *E. coli* KO229 harboring pNTSa1-1, pNTRm2-1, or pNTRm6-1 instead produced solanesol and decaprenol. Decaprenol was detected in all three recombinants, verifying that the fungal Dps1 proteins were active in *E. coli* and had deca-PDS activity.

Complementation of a fission yeast *dps1* and *dlp1* disruptant with fungal *dps1* genes

CoQ biosynthesis in *S. pombe* is performed by a heterotetrameric deca-PDS composed of Dps1 and Dlp1 (Saiki et al. 2003a). Disruption of either the *dps1* or the *dlp1* gene causes a severe growth delay when *S. pombe* is grown on minimal medium. The phenotype can be recovered by introducing a complementary gene such as *ddsA* from *G. suboxydans,* which encodes deca-PDS (Saiki et al. 2003a). To examine the ability of *dps1* genes from *B. albus, S. complicata*, and *R. minuta* to complement deca-PDS deficiency in fission yeast, Dps1 proteins were expressed in CoQ-deficient mutant LA1 (*Δdps1*, *Δdlp1*). Four plasmids were constructed and introduced into LA1

(*Δdps1*, *Δdlp1*): pREP1-B1dps1, which expressed *B. albus dps1*; pREP1-Sa1dps1, which expressed *S. complicata dps1*; pREP1-Rm2dps1, which expressed *R. minuta dps1*; and pREP1-Rm6dps1, which expressed shortened *R. minuta dps1* (Fig. 4B). The growth delay phenotype of LA1 on minimal medium was rescued by all three *dps1* genes. Transformant growth was at levels close to that of LA1 harboring pREP1-dps1-dlp1, which expressed *S. pombe dps1* and *dlp1* (Fig. 8A). CoQ was extracted from transformants and analyzed by HPLC. LA1 harboring pREP1-B1dps1, pREP1-Sa1dps1, pREP1-Rm2dps1, or pREP1-Rm6dps1 all produced CoQ_{10} as the major product, although amounts of CoQ_{10} varied between transformants (Fig. 8B). The observation that the *dps1* genes from *B. albus, S. complicata*, and *R. minuta* could complement *S. pombe* LA1 (*Δdps1*, *Δdlp1*) and restore production of CoQ_{10} indicated that Dps1 from these species functioned as homomeric rather than as heteromeric enzymes.

CoQ_{10} productivity of *S. pombe* expressing fungal *dps1* genes

S. pombe naturally produces CoQ_{10}, and biosynthesis of CoQ_{10} in this species is a subject of increasing recent research interest. (Hayashi et al. 2014). We wished to determine whether CoQ_{10} production in *S. pombe* could be increased

by expressing *dps1* genes from other fungi. Four plasmids were used to introduce the *dps1* genes into fission yeast: pREP1-B1dps1, pREP1-Sa1dps1, pREP1-Rm2dps1 and pREP1-Rm6dps1. Plasmids were introduced into wild-type *S. pombe* PR110 and CoQ production was quantified by using CoQ_6 as an internal standard. The production of CoQ was evaluated per culture volume and dry cell weight (DCW) (Fig. 9). No increase or decrease in CoQ_{10} production was seen statistically with any of the *dps1* plasmids .

Discussion

Deca-PDSs are responsible for the synthesis of the 10-unit isoprene side chain in CoQ_{10}. CoQ_{10} is a commercially popular food supplement, and identifying and isolating biosynthetic genes from CoQ_{10}-producing microorganisms is valuable for enhancing future CoQ_{10} production. In this study, the genes encoding deca-PDSs in three CoQ_{10} producing fungi, *B. albus, S. complicata* and *R. minuta,* were cloned and characterized.

Long-chain trans-prenyl diphosphate synthases such as deca-PDS are classified into homodimer and heterotetramer types. IspB from *E. coli* (Kainou et al. 2001) and SPS1 from *Arabidopsis* (Jun et al. 2004) are homodimers.

Heterotetramers include the deca-PDSs Dps1 and Dlp1 from *S. pombe* (Saiki et al. 2003a) and humans (Saiki et al. 2005). Predicted amino acid sequences for the fungal Dps1 proteins obtained in this study are highly similar to *E. coli* IspB, *S. pombe* Dps1, and human DPS1. The sequence similarities of *B. albus* Dps1, *S. complicata* Dps1 and *R. minuta* Dps1 with *S. pombe* Dps1 were 50%, 51%, and 46%, respectively. The three fungal Dps1 proteins contained seven conserved regions (domains I–VII) typically found in long-chain trans-prenyl diphosphate synthases, and two of the proteins exhibited common DDXXD motifs for FPP and IPP recognition. An unusually long stretch of amino acid residues was seen between domains I and II in *B. albus* Dps1. Divergence between the proteins was substantial at the N terminus. This region is absent in *E. coli* IspB, and is thought to contain signal sequences responsible for subcellular localization (likely for mitochondria). CoQ is synthesized in mitochondria in *S. cerevisiae* (Tran and Clarke 2007) and *S. pombe* (Hayashi et al. 2014), and it is reasonable to suggest the same synthesis location in other fungi. As *B. albus* and *S. complicata* genome sequences are now available (Nishida et al. 2011), we searched for the sequence of *dps1* and found there is an almost identical gene (only 3 nucleotide differences) in *B. albus* and a

completely identical gene in *S. complicata*, but we did not find *dlp1* like genes in both species.

The fungal *dps1* genes were expressed in wild-type *E. coli*, and all the resultant transformants produced CoQ_{10} in addition to CoQ_8. These findings indicated that the Dps1 enzymes possessed deca-PDS activity and functioned as homomeric proteins. This was confirmed by expressing the *S. complicata* and *R. minuta dps1* genes in *E. coli* KO229, which lacks the chromosomal *ispB* gene. *E. coli* KO229 cannot survive without a plasmid carrying *ispB* or an episomal counterpart gene. The *ispB*-carrying plasmid was successfully replaced with plasmids carrying *dps1* from *S. complicata* or *R. minuta,* but not from *B. albus*. This suggested that the enzyme activity produced by *B. albus* Dps1 was insufficient to replace the activity of endogenous *ispB*. Enzymatic activities producing decaprenyl diphosphate were detected in KO229 expressing *S. complicata* and *R. minuta dps1*. Solanesyl diphosphate was produced in an *in vitro* reaction and CoQ_9 was produced in KO229 expressing *R. minuta*. *R. minuta* produces CoQ_{10}, and it is likely that a heterologous expression system alters the enzymatic reaction. Expression of *B. albus, S. complicata,* or *R. minuta dps1* in *S. pombe* CoQ-deficient mutant LA1 (*Δdps1*, *Δdlp1*), fully abrogated a growth delay phenotype on minimal medium, and

CoQ_{10} was the major product in these cells. The data from *E. coli* and *S. pombe* consistently show that the three fungal *dps1* genes cloned in this study produce proteins that are independently active in *E. coli* and *S. pombe* and have deca-PDS activity. In some cases, heterologous expression of Dps1 produced CoQ_9 in addition to the main CoQ_{10} product. We suggest that the enzyme activity of Dps1 was insufficient in the heterologous system, leading to release of a shorter length of isoprenyl diphosphate and thereby producing CoQ with a shorter tail length.

Recent studies explored CoQ_{10} biosynthesis in *S. pombe* (Hayashi et al. 2014), and CoQ_{10} productivity was increased (Moriyama et al. 2015). However, expression of endogenous *dps1* did not lead to an increase CoQ_{10} production, probably as a result of cell growth inhibition from localization of large amounts of autologous protein at the mitochondria (Moriyama et al. 2015). Here, we expressed exogeneous *dps1* genes from *B. albus*, *S. complicata,* and *R. minuta* in *S. pombe*; however, while no growth inhibition was seen, no increase in CoQ yield was observed. It is possible that exogeneous Dps1 interferes with endogenous *S. pombe* Dps1 or Dlp1. Heterologous protein interactions were observed previously between *S. cerevisiae* Coq1 and *S. pombe* Dps1 (Zhang et al. 2008) and between *E. coli* IspB and *S. pombe* Dps1 (Cui et al. 2010) and,

although these were positive interactions, it can be assumed that negative interactions are also possible. If exogeneous Dps1 interfered with endogenous *S. pombe* Dps1 or Dlp1, production of CoQ_{10} would not increase even though exogenous Dps1 is active by itself or may decrease.

In summary, *dps1* genes from the fungi *B. albus*, *S. complicata,* and *R. minuta* were successfully cloned. The genes were expressed in *E. coli* and *S. pombe*, and the Dps1 proteins possessed deca-PDS activity and functioned as homomeric enzymes. Those cloned *dps1* genes can be used to enhance efficient production of CoQ_{10} in a range of species.

Compliance with Ethical Standards

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Conflict of Interest:

All authors declare that they have no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals

performed by any of the authors.

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Figure legend

Figure 1. Proposed coenzyme Q (CoQ) biosynthetic pathway.

The biosynthetic pathway that converts PHB into CoQ consists of eight steps in prokaryotes (represented by *E. coli*) and eukaryotes (represented by *S. cerevisiae*). Starting from the condensation of PHB with *trans*-polyprenyl diphosphate, seven modifications of the aromatic ring are then needed to produce CoQ. In *S. cerevisiae*, pABA is also used as a substrate in addition to PHB. IspB (in *E. coli*) and Coq1 (in *S. cerevisiae*) convert FPP (farnesyl diphosphate) into PPP (polyprenyl diphosphate). UbiA (in *E. coli*) and Coq2 (in *S. cerevisiae*) catalyze the condensation of PHB and PPP.

Figure 2. Detection of CoQ species in three fungi.

CoQ was extracted from three fungi and analyzed by high-performance liquid chromatography (HPLC). Standard coenzyme Q_{10} (CoQ_{10}) (a) and CoQ extracted from *B. albus* (b), *S. complicata* (c), and *R. minuta* (d) are shown.

Figure 3. Alignment of predicted amino acid sequences of deca-PDSs and octaprenyl diphosphate synthase.

Amino acid residues that are identical in four or more sequences are indicated

by black boxes. Hyphens indicate the absence of corresponding amino acid residues at those positions. Seven regions (I–VII) that are highly conserved in long-chain poly-PDSs are underlined. Two aspartate-rich motifs in domains II and VI, which are considered to be substrate binding sites in poly-PDSs, are denoted by 'DDXXD'. Sequences were aligned using GENETYX software. Genbank accession numbers as are follows: *S. pombe* Dps1, O43091; *Homo sapiens* DPS1, Q5T2R2; *E. coli* IspB, P0AD57; *B. albus* Dps1, BD182059; *R. minuta* Dps1, BD170286; *S. complicate* Dps1, BD093645.

Figure 4. Plasmid construction.

(A) The cloning vector pUCNT, which contains an *Nde*I site, was modified from pUC19. Plasmids pNTB1-1, pNTSa1-1, and pNTRm2-1 contain full-length *dps1* genes from *B. albus*, *R. minuta,* and *S. complicata*, respectively, cloned into pUCNT. Plasmid pNTRm6-1 contains a truncated *R. minuta dps1* gene with a partial deletion in the 5′ region (see Materials and Methods). (B) Genes encoding deca-PDS were inserted into the pREP1 vector under the control of the *nmt1* promoter for expression in *S. pombe*. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nde*I; S, *Sma*I.

Figure 5. Detection of CoQ in *E. coli* DH5α expressing fungal *dps1* genes.

The *dps1* genes from *B. albus*, *S. complicata*, and *R. minuta* were expressed in *E. coli* DH5α. CoQ was extracted and analyzed using HPLC. Standard CoQ_{10} (a), and CoQ from DH5α (b), DH5α harboring pNTB1-1 (c), DH5α harboring pNTSa1-1 (d), DH5α harboring pNTRm2-1 (e), and DH5α harboring pNTRm6-1 (f) are shown.

Figure 6. Detection of CoQ in *E. coli* KO229 expressing fungal *dps1* genes.

The *dps1* genes from *B. albus*, *S. complicata*, and *R. minuta* were expressed in *E. coli* KO229, an *ispB*-defective mutant. CoQ was extracted and analyzed by HPLC. Standard CoQ_{10} (a), and CoQ from KO229 harboring pKA3 (*ispB*) (b), KO229 harboring pNTSa1-1 (c), KO229 harboring pNTRm2-1 (d), and KO229 harboring pNTRm6-1 (e) are shown.

Figure 7. Reversed-phase thin-layer chromatogram of products from recombinant *E. coli* KO229 strains.

Cell extracts were obtained from *E. coli* KO229 expressing fungal *dps1* genes and used for enzyme assays with [1-^{14}C]IPP and FPP as substrates. Reaction products were hydrolyzed by acid phosphatase, and the resulting alcohols were

analyzed by reversed-phase TLC. Arrowheads indicate positions of C_{40} (octaprenol) and C_{50} (decaprenol). Lane 1, KO229 harboring pKA3 (*ispB*); lane 2, KO229 harboring pNTRm2-1; lane 3, KO229 harboring pNTRm6-1; and lane 4, KO229 harboring pNTSa1-1. Ori., origin; S. F., solvent front.

Figure 8. Complementation of *S. pombe* LA1 (*Δdps1*, *Δdlp1*) with fungal *dps1* genes.

(A) Growth of LA1 (*Δdps1*, *Δdlp1*) on minimal medium was observed. LA1 harboring the indicated plasmids was grown for 4 days at 30°C on PM minimal medium containing uracil. (B) CoQ species in fission yeast LA1 expressing fungal *dps1* genes derived from *B. albus*, *S. complicata,* and *R. minuta* was analyzed by HPLC. CoQ was extracted from LA1 harboring plasmid pREP1 (a), pREP1-dps1-dlp1 (b), pREP1-B1dps1 (c), pREP1-Sa1dps1 (d), pREP1-Rm2dps1 (e), and pREP1-Rm6dps1 (f).

Figure 9. Effect of fungal *dps1* gene expression on CoQ_{10} production in fission yeast.

CoQ productivity in strains expressing fungal *dps1* genes was compared to that in reference strain PR110 (pREP1). Strains were grown at 30°C in PM minimal

medium containing uracil. Cultures were initiated at 1×10^5 cells/ml and harvested after 48 h growth. Production of CoQ_{10} was measured by HPLC. Gray bars represent CoQ_{10} content per volume (%), white bars represent CoQ_9 content per volume (%) and open diamonds represent total CoQ (CoQ_{10} + CoQ_9) content per dry cell weight (DCW) (%). Error bars represent the standard deviation of three measurements of total CoQ.

Table 1 Classification of polyprenyl diphosphate synthases

Species	Structure	Products	References
Saccharomyces cerevisiae	Homomer	C_{30}	Ashby 1990; Ayer et al. 2015; Zhang et al. 2008
Sulfolobus solfataricus	Homodimer	C_{30}	Sun et al. 1990
Escherichia coli	Homodimer	C_{40}	Asai et al. 1994; Kainou et al. 2001
Plasmodium falciparum	Homomer	C_{40}	Tonhosolo et al. 2005
Arabidopsis thaliana	Homodimer	C_{45}	Hirooka et al. 2003; Jun et al. 2004
Rhodobacter capsulatus	Homodimer	C_{45}	Okada et al. 1997a
Trypanosoma cruzi	Homodimer	C_{45}	Ferella et al. 2006
Gluconobacter suboxydans	Homodimer	C_{50}	Okada et al. 1998b; Lee et al. 2004
Mycobacterium tuberculosis	Homodimer	C_{50}	Chan et al. 2014
Rhodobacter sphaeroides	Homodimer	C_{50}	Seo et al. 2006; Zahiri et al. 2006
Sinorhizobium meliloti	Homomer	C_{50}	Cluis et al. 2007
Micrococcus luteus	Heterodimer	C_{30}	Shimizu et al. 1998
Mus musculus	Heterotetramer	C_{45}	Saiki et al. 2005
Aphis gossypii	Heteromer	C_{50}	Zhang 2013
Homo sapiens	Heterotetramer	C_{50}	Saiki et al. 2005
Schizosaccharomyces pombe	Heterotetramer	C_{50}	Saiki et al. 2003a; Suzuki et al. 1997

Table 2 Primers used in this study

Primer	Sequence
DPS-1	5′-AAGGATCCTNYTNCAYGAYGAYGT-3′
DPS-1 1AS	5′-ARYTGNADRAAYTCNCC-3′
B1S	5′-TCGGCATTACGGCGGAACCTG-3′
B7ASP	5′-CACACCATCAGACTC-3′
B5S	5′-TGGGTCAAGGATGTGGCGTA-3′
B4AS	5′-CAGATTGGCGATGACCGTCGC-3′
B6S	5′-GTTCGCCTACAAGCAGAACCC-3′
B3AS	5′-TTGAGCCAAAGGCGGATGGG-3′
BN1	5′-AAGGATCCATATGTTTCGTTCGGCGCGG-3′
BCH	5′-CCAAGCTTCTACTTCACTCTTCCAC-3′
Sa-1S	5′-GAGACCAGACGAAACGCACCA-3′
Sa-2AS	5′-TAACAGTAGCCAAAAGCTCAATCA-3′
Sa-N1	5′-AACATATGGCCTCACCAGCACTGCGG-3′
Sa-C	5′-AAGAATTCCTATCTTGACCTAGTCAACAC-3′
Rm-1S	5′-GCCATGAGGAGAGCACAAGCG-3′
Rm-2AS	5′-CACGGAGGCTACTAGCTCGAC-3′
RM-1	5′-ATCATATGATGCACCGACAAGCT-3′
Rm-CE2	5′-AAGAATTCCTACTTTGTTCGGTTGAGCACAG-3′
Rm-4	5′-ATCATATGAATATTCGACCCACTCCAACT-3′
RM-6R	5′-ACAATATTGTATTGAGGGCATTCGGGCGACTGC-3′
GRC_B1dps1-Fw	5′-GACTTATAGTCGCTTTGTTAAATCATATGTCGACTCTAGAGGATCCAATGTTTCGTTCGG-3′
GRC_B1dps1-Rv	5′-AAAAACCCTAGCAGTACTGGCAAGGGAGACATTCCTTTTACCCGGGCTACTTCACTCTTT-3′
GRC_Sa1dps1-Fw	5′-GACTTATAGTCGCTTTGTTAAATCATATGTCGACTCTAGAGGATCCAATGGCCTCACCAG-3′
GRC_Sa1dps1-Rv	5′-AAAAACCCTAGCAGTACTGGCAAGGGAGACATTCCTTTTACCCGGGCTATCTTGACCTAG-3′
GRC_Rmdps1-Fw	5′-GACTTATAGTCGCTTTGTTAAATCATATGTCGACTCTAGAGGATCCAATGATGCACCGAC-3′
GRC_Rmdps1-Rv	5′-CCTAGCAGTACTGGCAAGGGAGACATTCCTTTTACCCGGGCTACTTTGTTCGGTTGAGCA-3′

TOP

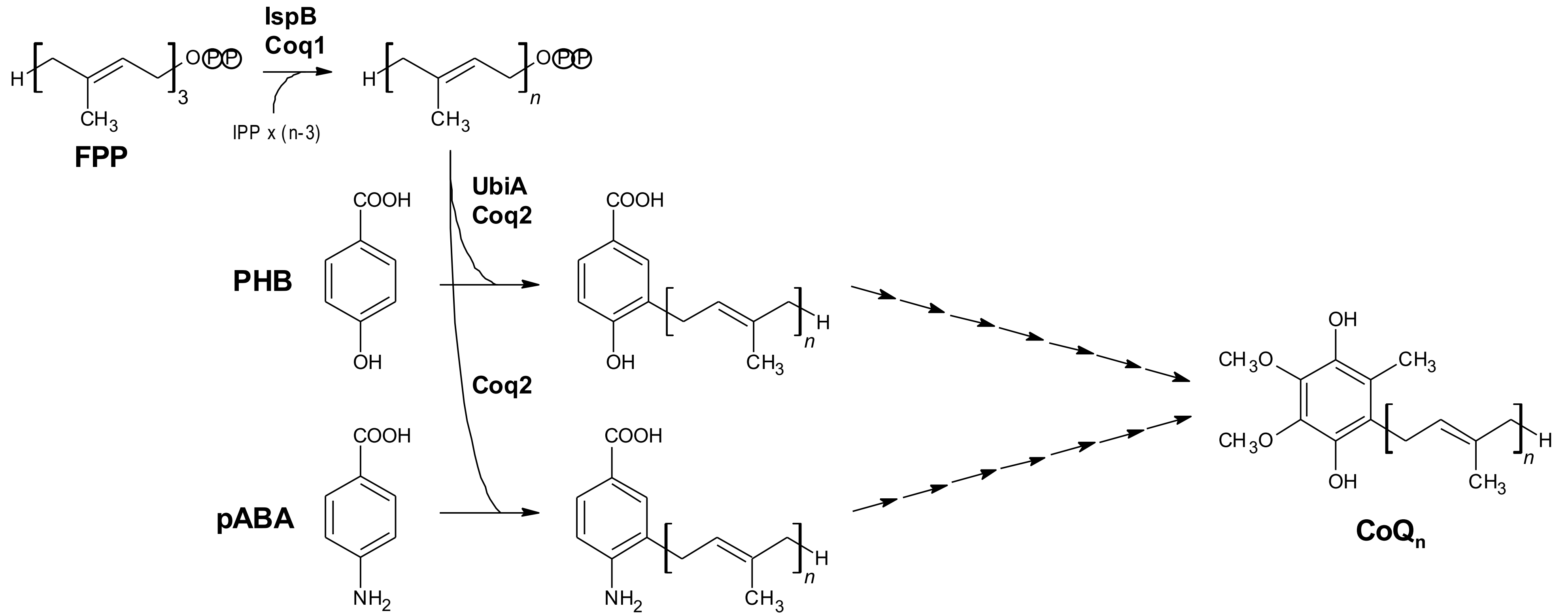

Fig. 1 Moriyama et al.

TOP

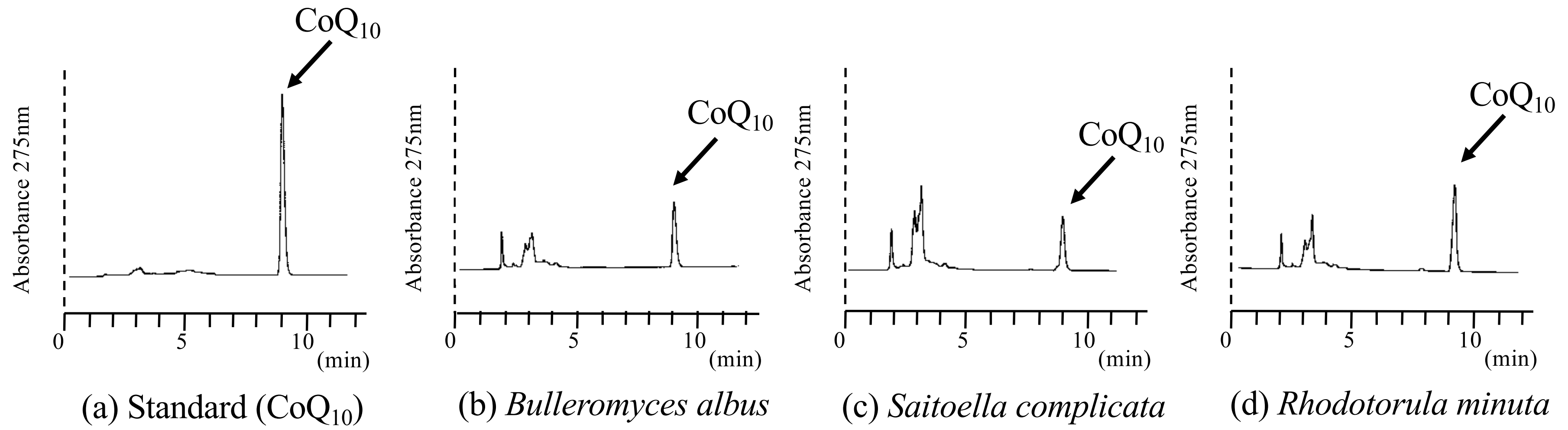

Fig. 2 Moriyama et al.

TOP

```
S. pombe Dps1                    1                                                                                     ·MIQYVYLKHMRKLWS 15
H. sapiens DPS1                  1                       MASRWWRWRRGCSWKPAARSPGPGSPGRAGPLGPSAAAEVRAQVHRRKGLDLSQIPYINLVKHLTSACPNVCRISRF 77
B. albus Dps1                    1                                      ·MFRSARAATRAARRAAGTRSSLIKSTPSPASDVANPSLAQRSIRSISTTRTSSSPQTAWAEATE 64
S. complicata Dps1               1                                     ·MASPALRIRSISSRSIASLRSVTLRTASAPSLRLR--------------CTPTSRPSSSWAAAVS 51
R. minuta Dps1                   1 MMHRQAACRVCSHTCSRPNALLAGIYGPSSASSSTTTTTSTSRSNHNNSVRFKHSLSNTSKPAARSTSTSAPALSPSSSTSDPQSSSSPSSSSSSSSILPDFLRSPLSSSSSSSSTSSSSS 121
R. minuta Dps1(shorter ver.)     1 MMHRQAACRVCSHTCSRPNAL---------------------------------------------------------------------------------------------------- 21

S. pombe Dps1                   16 LGKVRSTVLRFSTTNRNASHLIKNELEQISPGIRQMLNSNSEFLEECSKYYTIAQGKQMRPSLVLLMSKATSLCHGIDRSVVGDKYIDDDDLRSFSTG---------------------- 113
E. coli IspB                     1             MNLEKINELTAQDMAGVNAAILEQLNSDVQLINQLGYYIVSGGGKRIRPMIAVLAARAVGYEGNA------------------------------------------------ 65
H. sapiens DPS1                78 HHTTPDSKTHSGEKYTDPFKLGWRDLKGLYEDIRKELLISTSELKEMSEYYFDGKGKAFRPIIVALMARACNIHHNNSR------------------------------------------ 156
B. albus Dps1                   65 SAHSILTPPTANSPSQDPLEAIYSEISTLRSSLFSMLGSSHPSLDKVAKYYFQAEGKHLRPLLVLLLSQATNGLAGSDSWERARHEAQRRNVDDSLTSRGGVLNDWNPEQMGKEDQQSNPG 185
S. complicata Dps1              52 SASRLVEP-DPNQPLINPLNLVGPEMSNLTSNIRSLLGSGHPSLDTVAKYYVQSEGKHIRPLMVLLMAQATEVAPKVQGWEKVVEVPVN-----EGLAPPEVLNDKNPDMMN--------- 157
R. minuta Dps1                 122 SSSSNRSKNTNSNTIFDPLQLVGNELSSLRSNVQALLGSGHPALDTIAKYYFQAEGKHIRPMIVLLMSQATNGLAPG-FEERSKLELSGRKQTDPSRSINDPLEVKADEILN--------- 232
R. minuta Dps1(shorter ver.)    22 ------------NTIFDPLQLVGNELSSLRSNVQALLGSGHPALDTIAKYYFQAEGKHIRPMIVLLMSQATNGLAPG-FEERSKLELSGRKQTDPSRSINDPLEVKADEILN--------- 120
                                                                                      I

                                                                                                                     DDXXD
S. pombe Dps1                  114 ----------------------------------------------------------------------------QILPSQLRLAQITEMIHIASLLHDDVIDHANVRRGSPSSNVAFGNRRS 161
E. coli IspB                    66 -------------------------------------------------------------------------------HVTIAALIEFIHTATLLHDDVVDESDMRRGKATANAAFGNAAS 108
H. sapiens DPS1               157 ----------------------------------------------------------------------------HVQASQRAIALIAEMIHTASLVHDDVIDDASSRRGKHTVNKIWGEKKA 204
B. albus Dps1                  186 AVFANPFSITPSNRSTPSSTSSTASTEAPSNASSSLSDIFNSMLPSSSSYTVPLPPSLQSSPLASLYSSPGTPEILSTQRRLASITEMIHVASLLHDDVIDNSALRRNLPSAPSAFGSKLS 306
S. complicata Dps1             158 ------------MRSGPLTKDGEIEGQTSN----------------------------------------------ILASQRRLAEITEMIHAASLLHDDVIDASETRRNAPSGNQAFGNKMA 222
R. minuta Dps1                 233 -------------DSNPSSFAASSSSPLDSMPSTSN----------------------------------------VLPSQRRLAEITEMIHVASLLHDDVIDGSAMRRAQASAPAAFGNKIS 302
R. minuta Dps1(shorter ver.)   121 -------------DSNPSSFAASSSSPLDSMPSTSN----------------------------------------VLPSQRRLAEITEMIHVASLLHDDVIDGSAMRRAQASAPAAFGNKIS 190
                                                                                                                          II

S. pombe Dps1                  162 ILAGNFILARASTAMARLRNPQVTELLATVIADLVRGEFLQLKN---------TMDPSSLEIKQSNFDYYIEKSFLKTASLISKSCKASTILG--------QCSPTVATAAGEYGRCIGTA 265
E. coli IspB                   109 VLVGDFIYTRAFQMMTSLGSLKVLEVMSEAVNVIAEGEVLQLMN---------------VNDPDITEENYMRVIYSKTARLFEAAAQCSGILAG--------CTPEEEKGLQDYGRYLGTA 206
H. sapiens DPS1               205 VLAGDLILSAASIALARIGNTTVISILTQVIEDLVRGEFLQLGS---------------KENENERFAHYLEKTFKKTASLIANSCKAVSVLG--------CPDPVVHEIAYQYGKNVGIA 302
B. albus Dps1                  307 ILSGDFLLGRASVALSRLGSNEVVELLATVIANLVEGEVLQLR---------ATSSNESTIEWDRMFEEYMRKTYLKTASLMSKSCRAAVILGGCGRD---IESEWVKDVAYGYGRNLGIA 415
S. complicata Dps1             223 ILAGDFLLGRASVALARLRNPEVIELLATVIANLVEGEFMQLKN--------TVDDAIEATATQETFDYYLQKTYLKTASLIAKSCRASALLGG--------ATPEVADAAYAYGRNLGLA 327
R. minuta Dps1                 303 VLGGDFLLARASLYLSRLGSNEVVELVASVLANLVEGEVMQIKGNAPESNASGSKEVAVHRLTPEIFEHYMKKTYLKTASLIAKSTRATTILGGAGEKQGWIEGERIKDIAYSYGRNLGIA 423
R. minuta Dps1(shorter ver.)   191 VLGGDFLLARASLYLSRLGSNEVVELVASVLANLVEGEVMQIKGNAPESNASGSKEVAVHRLTPEIFEHYMKKTYLKTASLIAKSTRATTILGGAGEKQGWIEGERIKDIAYSYGRNLGIA 311
                                         III                  IV                         V

                                     DDXXD
S. pombe Dps1                  266 FQLMDDVLDYTSKDDTLGKAA-GADLKLGLATAPVLFAWKKYPELGAMIVNRFNHPSD----IQRARSLVECTDAIEQTITWAKEYIKKAKDSLLCLPDSPARKALFALADKVITRKK 378
E. coli IspB                   207 FQLIDDLLDYNADGEQLGKNV-GDDLNEGKPTLPLLHAMHHGTPEQAQMIRTAIEQGNGRHLLEPVLEAMNACGSLEWTRQRAEEEADKAIAALQVLPDTPWREALIGLAHIAVQRDR 323
H. sapiens DPS1               303 FQLIDDVLDFTSCSDQMGKPT-SADLKLGLATGPVLFACQQFPEMNAMIMRRFSLPGD----VDRARQYVLQSDGVQQTTYLAQQYCHEAIREISKLRPSPERDALIQLSEIVLTRDK 415
B. albus Dps1                  416 FQLVDDALDFIPASDMGKPSD-GADLSLGLATAPALFAYKQNPALGPLILRKFEGEGD----VQAAKKMIMESDGVKETINLAKKFANSAKDLLELLPESEARGALVGLTKKVVERVK 528
S. complicata Dps1             328 FQIVDDMLDYTVSATDLGKPA-GADLQLGLATAPALFAWKHHAELGPMIKRKFSDPGD----VERARELVEKSDGLEKTRALAEEYAQKALDAIRTFPESPARKALEQLTDKVLTRSR 440
R. minuta Dps1                 424 FQLVDDLLDFTATDAQFGKPSQGADLKLGLATAPALYAWEEFPEMGQMILRKFENEGD----VETARNLVRKSAGPEKTVKLAEKHAALAMEALQGLPESDAREALEGLTKTVLNRTK 537
R. minuta Dps1(shorter ver.)   312 FQLVDDLLDFTATDAQFGKPSQGADLKLGLATAPALYAWEEFPEMGQMILRKFENEGD----VETARNLVRKSAGPEKTVKLAEKHAALAMEALQGLPESDAREALEGLTKTVLNRTK 425
                                                   VI                                                                                VII
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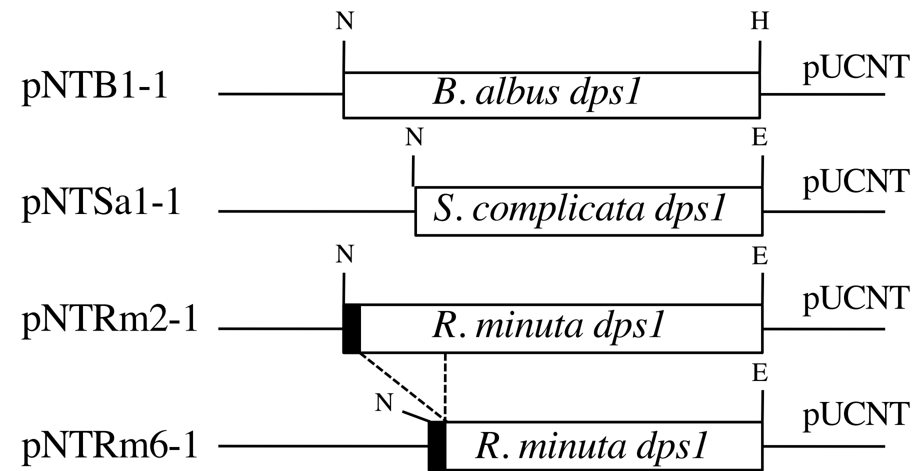
Fig. 3 Moriyama et al.

TOP



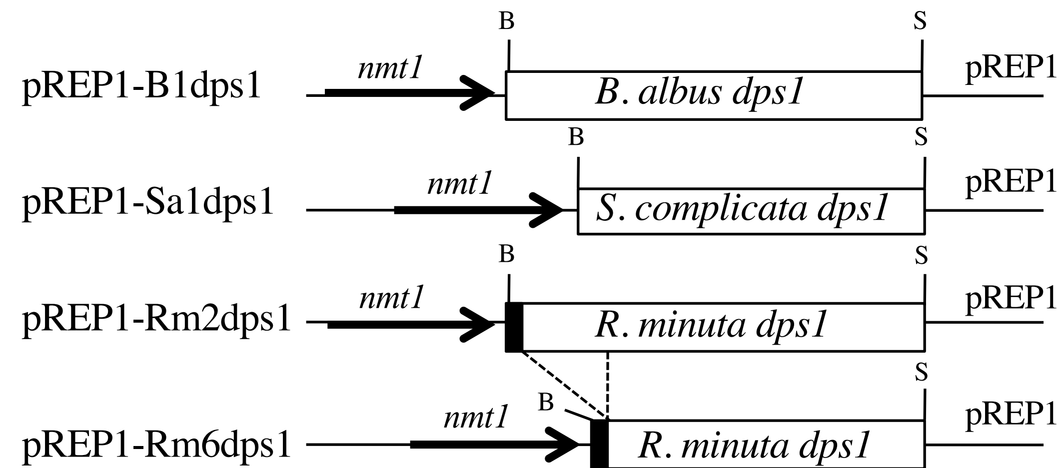


Fig. 4 Moriyama et al.

TOP

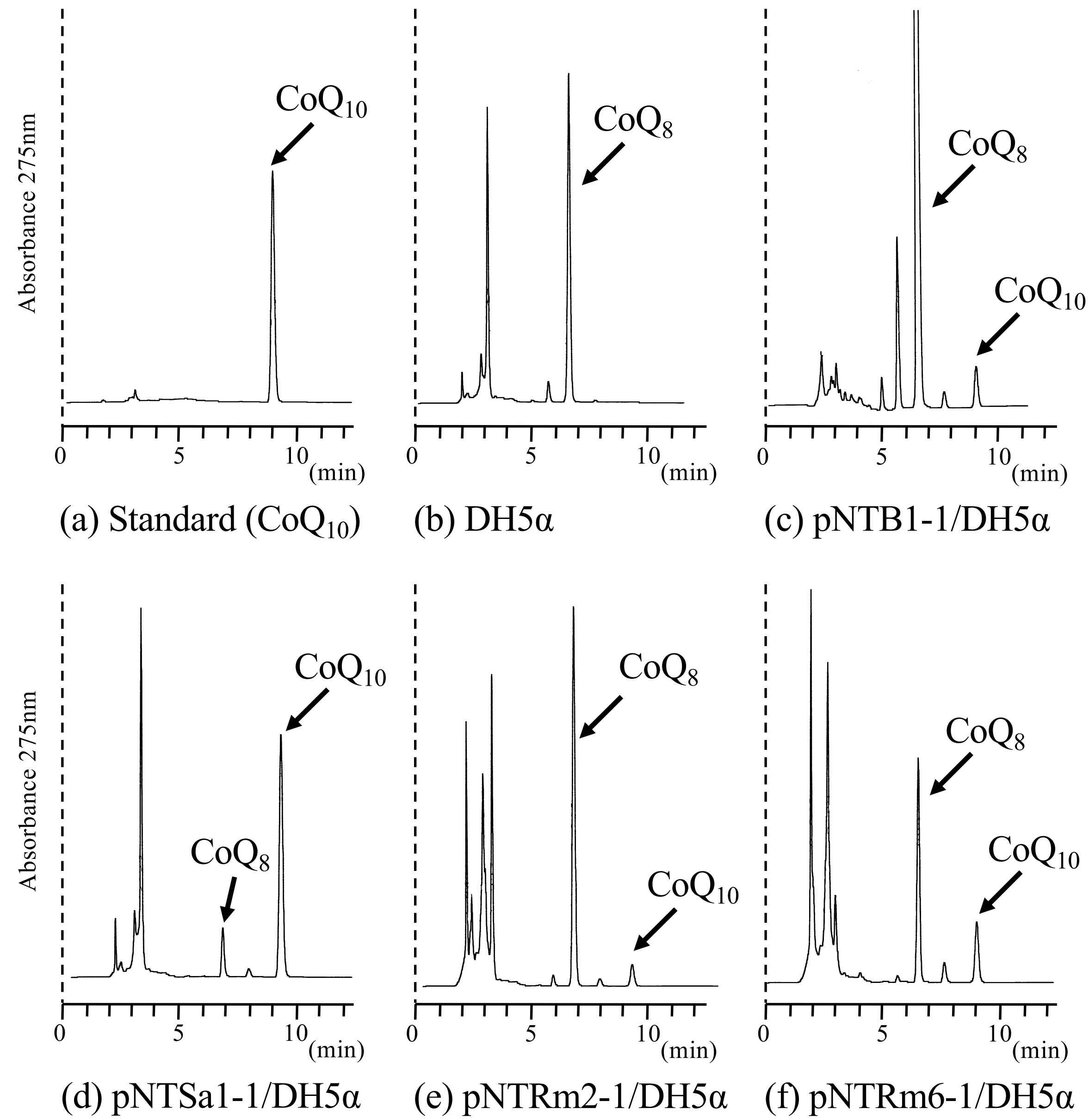


Fig. 5 Moriyama et al.

TOP

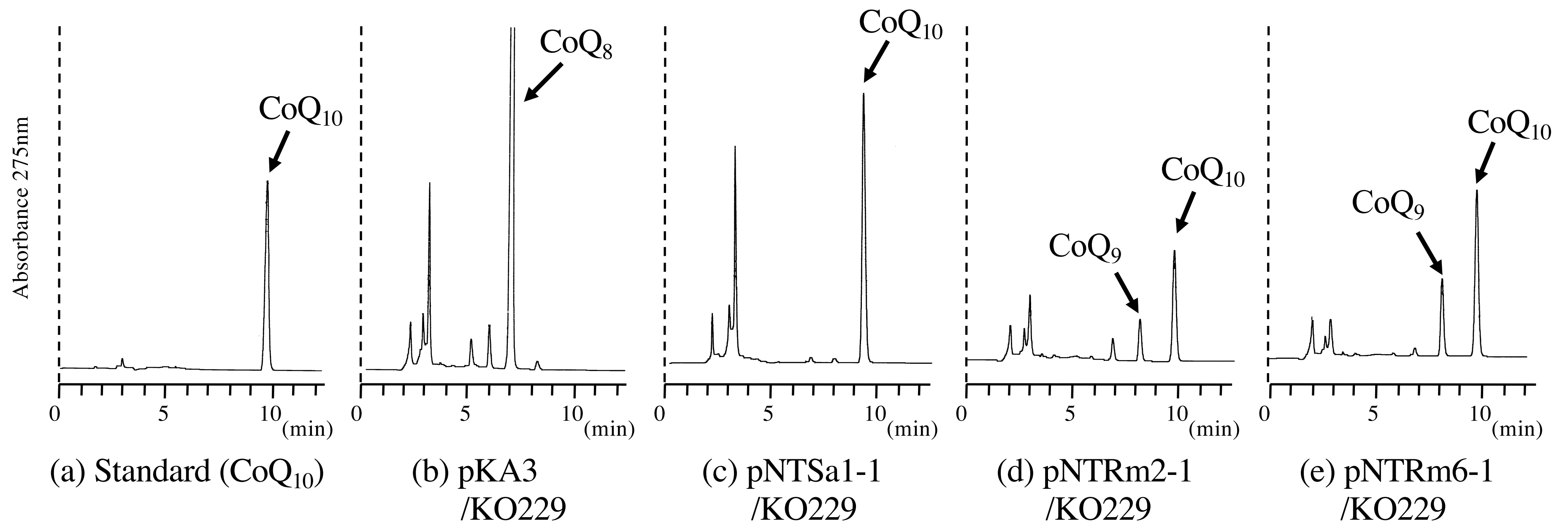


Fig. 6 Moriyama et al.

TOP

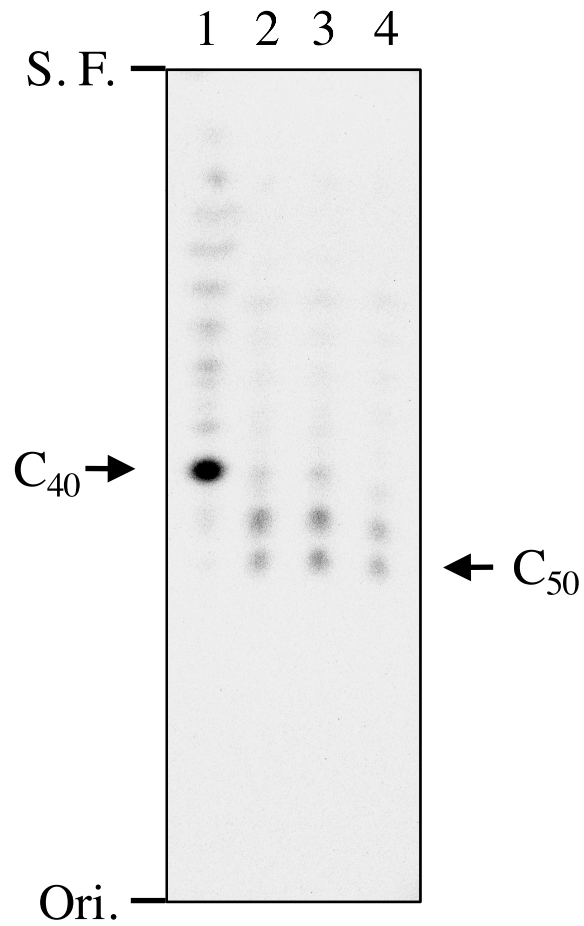
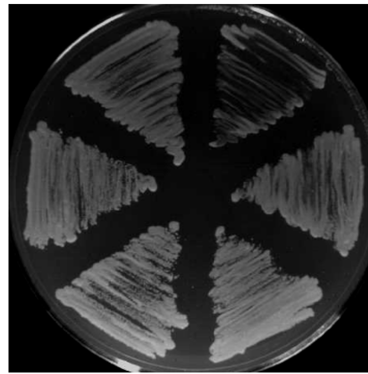


Fig. 7 Moriyama et al.

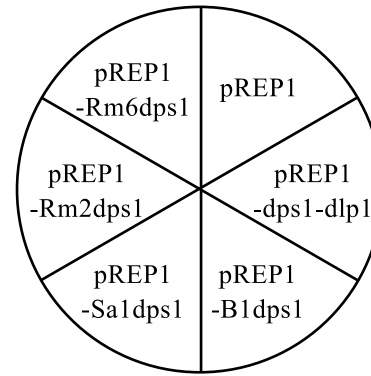
TOP

A

PM + ura



S. pombe LA1
(*Δdps1*, *Δdlp1*)



B

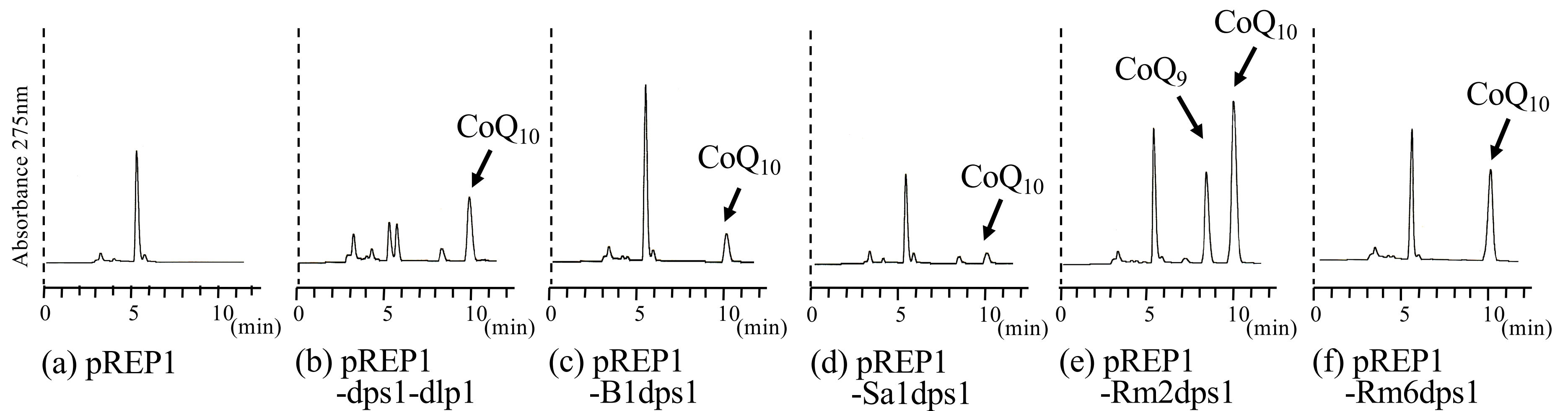


Fig. 8 Moriyama et al.

TOP

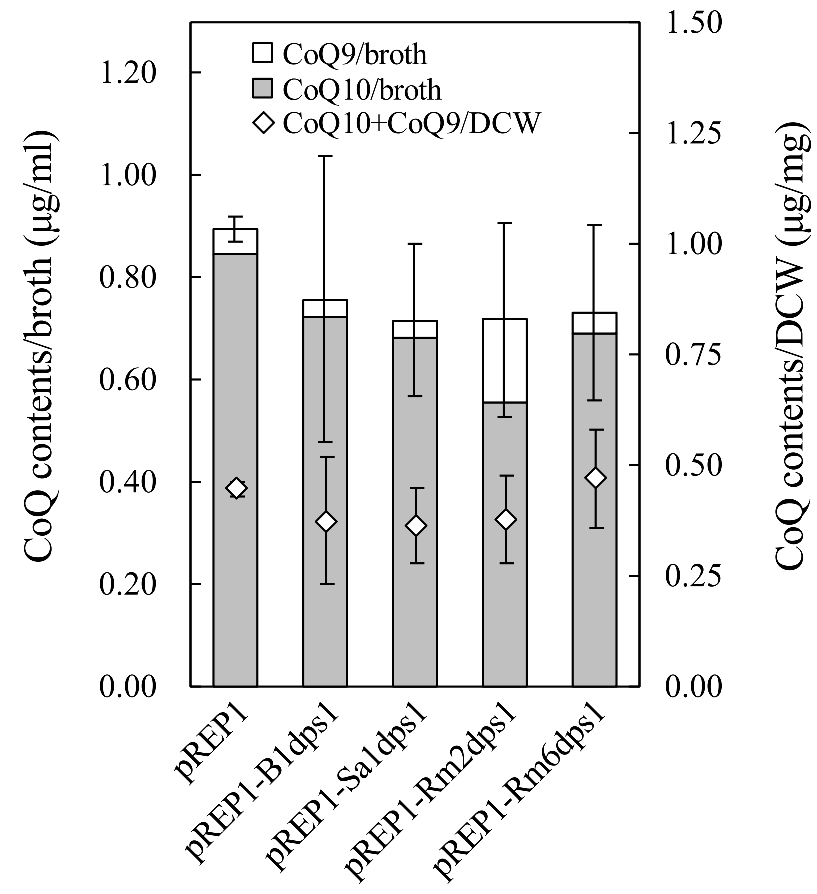


Fig. 9 Moriyama et al.