Title

Schizosaccharomyces Japonicus Has Low Levels of CoQ 10 Synthesis, Respiration Deficiency, and Efficient Ethanol Production

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Schizosaccharomyces japonicus has low levels of CoQ_{10} synthesis, respiration deficiency, and efficient ethanol production

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Running title: Physiological properties of *S. japonicus*

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Abstract

Coenzyme Q (CoQ) is essential for mitochondrial respiration and as a cofactor for sulfide quinone reductase. *Schizosaccharomyces pombe* produces a human-type CoQ_{10}. Here, we analyzed CoQ in other fission yeast species. *S. cryophilus* and *S. octosporus* produce CoQ_9. *S. japonicus* produces low levels of CoQ_{10}, although all necessary genes for CoQ synthesis have been identified in its genome. We expressed three genes (*dps1*, *dlp1*, and *ppt1*) for CoQ synthesis from *S. japonicus* in the corresponding *S. pombe* mutants, and confirmed that they were functional. *S. japonicus* had very low levels of oxygen consumption and was essentially respiration defective, probably due to mitochondrial dysfunction. *S. japonicus* grows well on minimal medium during anaerobic culture, indicating that it acquires sufficient energy by fermentation. *S. japonicus* produces comparable levels of ethanol under both normal and elevated temperature (42°C) conditions, at which *S. pombe* is not able to grow.

Keywords: Fission yeast, *Schizosaccharomyces japonicus*, *S. pombe*, respiration, CoQ

Introduction

Schizosaccharomyces fission yeast species are believed to have diverged from *Saccharomyces* budding yeast species about a billion years ago. Fission yeasts are named based on their binary fission cell division pattern, in contrast to the cellular budding division pattern in *Saccharomyces*. Four fission yeast species are currently known, and all belong to the genus *Schizosaccharomyces*, including *S. pombe*, *S. japonicas*,[1] *S. cryophilus*,[2] and *S. octosporus*.[3] *S. pombe* has been extensively studied in genetic, molecular biological, biochemical, and cytological analyses,[4] but studies of the other three species are limited. The *S. pombe* whole genome was completely sequenced by 2002,[5] and the whole genomes of the other three species were determined in 2011.[6] Genomic differences among the four *Schizosaccharomyces* species were determined; 3,924 genes are common among the four species, whereas 133–401 genes are different.

S. japonicus is a dimorphic yeast, which can transit from unicellular yeast to long filamentous hyphae, and form ascospores with eight spores when starved.[1] *S. japonicus* was isolated in 1928 in Japan, and is currently undergoing re-evaluation because of its unique properties.[7] Nuclear organization and division have been investigated in *S. japonicus*,[1] but physiological studies of this yeast are limited. A prominent characteristic of *S. japonicus* is that it does not respire, and instead grows via fermentation. It was reported that *S. japonicus* does not produce Coenzyme Q (CoQ) ,[8] despite its essential role in respiration and oxidative ATP synthesis in mitochondria. CoQ synthesis in eukaryotes has been studied primarily in the *Saccharomyces cerevisiae* budding yeast[9-11] and the *S. pombe* fission yeast, and these knowledge extended to higher eukaryotes [12-14] , but has not been studied in *S. japonicus*. As CoQ is synthesized in mitochondria, it is interesting to know how *S. japonicus* adapted to deficiency of its synthesis, which causes respiration deficiency.

CoQ contains a quinone frame and isoprenoid side chain, with variable isoprene units in each organism. *S. cerevisiae* produces CoQ_6, whereas *S. pombe* and *Homo sapiens* produce CoQ_{10}.[10, 12, 15] CoQ isoprenoid side chains are synthesized by the homomeric form of Coq1 (hexaprenyl diphosphate synthase) in *S. cerevisiae*, and by the heterotetrameric form of Dps1 and Dlp1 (decaprenyl diphosphate synthase) in *S.*

pombe.[14] The type of CoQ such as CoQ_6 in *S. cerevisiae* and CoQ_{10} in *S. pombe* is determined by the supplied prenyl diphosphate synthesized by the species specific polyprenyl diphosphate synthase. [16, 17] After synthesis, the isoprenoid is transferred to *p*-hydroxy benzoate (PHB) by Coq2 (Ppt1) (PHB-polyprenyl diphosphate transferase).[18, 19] Prenylated PHB undergoes the following modifications: hydroxylation by Coq6 and Coq7, *O*-methylation by Coq3, *C*-methylation by Coq5, and decarboxylation by an unknown protein (Fig. 1).[13] Almost all CoQ synthetic genes in humans and *Arabidopsis thaliana* can function to complement each of the corresponding *S. pombe* gene deletion mutants.[20] Biotechnology approaches have successfully enhanced CoQ_{10} biosynthesis in *S. pombe* fission yeast.[21, 22] Therefore, an analysis of CoQ biosynthesis in other fission yeast species may provide insights for the utilization of fission yeast for commercial production of CoQ_{10}.

In this study, we investigated CoQ synthesis in *S. japonicus*, genes involved in CoQ synthesis in *S. pombe*, and yeast phenotypes associated with respiration and ethanol production. We show that evolutionally unique properties of *S. japonicus* which lost major mitochondrial function and enforced fermentation for energy acquirement.

Materials and Methods

Yeast strains and media

The genotypes of all yeast strains used in this study are listed in Table 1. *S. pombe* and *S. japonicus* strains were grown in YES medium (0.5% yeast extract, 3% glucose, and 225 mg/L each of adenine, leucine, uracil, histidine, and lysine hydrochloride), YPD medium (1% yeast extract, 2% glucose, and 2% polypeptone) or EMM synthetic medium containing nutritional supplements when necessary.[23] Yeast cells were transformed using either lithium acetate[24] or electroporation.[25] General genetic methods used for *S. pombe* have been described previously.[26] The thiamine-repressible *nmt1* promoter was repressed by adding 5 μg/ml thiamine to EMM medium.

DNA manipulation

Cloning, restriction enzyme analysis, and plasmid DNA preparation were performed essentially as described previously.[27] Oligonucleotides used in this study are listed in Table S1. *Escherichia coli* strain DH5α was used for plasmid construction and propagation. DNA sequences were determined using the dideoxynucleotide chain-termination method and the ABI377 DNA sequencer.

Plasmid construction

The plasmids used in this study are listed in Table 2, and the primers used for plasmid construction are listed in Table S1. The pREP41-Sjppt1 plasmid was constructed by amplifying a fragment using the Sjppt1(ORF)-SalI-F and Sjppt1-BamHI-R primers, and inserting the amplified product into the *Sal*I and *Bam*HI sites of pREP41. The pREP1-Sjdps1, pREP41-Sjdps1, and pREP2-Sjdps1 plasmids were constructed by amplifying a fragment using the Sjdps1-SalI-F and Sjdps1-BamHI-R primers, and inserting the amplified product into the *Sal*I and *Bam*HI sites of pREP1, pREP41, and pREP2, respectively. The pREP1-Sjdlp1 and pREP2-Sjdlp1 plasmids were constructed by amplifying a fragment using the Sjdlp1-SalI-F and Sjdlp1-BamHI-R primers, and inserting the amplified product into the *Sal*I and *Bam*HI sites of pREP1 and pREP2, respectively. The pREP41-dps1 and pREP2-dps1 plasmids were constructed inserting the *dps1* gene which was cut from pREP1-cloning plasmid by restriction enzymes into the same sites of pREP41 and pREP2, respectively. The pSJU11-Spppt1-15 plasmid was constructed by amplifying fragments using the Sjnmt1-897-F and Sjnmt1-24-R primers for *Sjnmt1* promoter, and Spppt1-Sjnmt1-24-F-New and Spppt1-BamHI-R primers for *Spppt1* coding gene. Amplified fragments were fused by PCR reaction, and the product was cloned into the *Kpn*I and *Bam*HI sites of pSJU11.

Spot assay

Cells were grown on YES plates for 3 days at 30°C, and then resuspended in water to a density of 2×10^6 cells/ml. Cell suspensions were serially diluted (1:10), spotted onto YES or EMMU plates, and incubated for 3–5 days at 30°C. Plates were placed in a sealed chamber under anaerobic conditions with AnaeroPack Kenki (Mitsubishi Gas Chemical Co., Inc., Tokyo), and incubated for 2 days at 30°C.

CoQ extraction and measurement

CoQ was extracted as described previously.[28] The CoQ crude extract was analyzed by normal-phase thin-layer chromatography (TLC) with authentic CoQ_6 or CoQ_{10} standards. Normal-phase TLC was conducted on a Kieselgel 60 F_{254} plate and developed with benzene. The plate was viewed under UV illumination, the CoQ band was collected, and the sample was extracted with chloroform/methanol (1:1, v/v). Samples were dried and solubilized in ethanol. Purified CoQ was subjected to high-performance liquid chromatography (HPLC) with ethanol as the solvent.

Liquid chromatography–mass spectrometry (LC-MS) analysis

The CoQ sample was extracted for liquid chromatography–mass spectrometry (LC-MS) analysis as described above. Samples were resuspended in 80 µl of methanol:2-propanol (4:1) solution and filtered with YMC Duo-Filter XQ DUO 04 (pore size, 0.2 µm), and 8 µl of sample was used for analysis. LC-MS data were obtained using a MassLynx system (Waters) coupled to a Xevo-TQS mass spectrometer (Waters). LC separation was performed on an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm particle size; Waters). The mobile phase was methanol:2-propanol (4:1) solution (buffer A) and methanol:2-propanol (4:1) solution containing 5 mM ammonium formate (buffer B). Chromatographic conditions were 98% buffer A and 2% buffer B. The flow rate was 0.5 ml/min. Matrix-assisted laser desorption/ionization–time of flight–mass spectrometry (MALDI-TOF MS) (SYNAPT G2-S; Waters) was performed to determine the precise molecular masses of compounds.

Sulfide measurement

Sulfide content was determined quantitatively using the methylene blue method as described previously.[14] Briefly, *S. pombe* and *S. japonicus* cells were grown in YES medium (50 ml) until the late log phase. Then, cells were collected and disrupted by glass beads, and cell extracts were resuspended in 0.1 ml of 0.1% dimethylphenylenediamine (in 5.5 N HCl) and 0.1 ml of 23 mM $FeCl_3$ (in 1.2 N HCl). The samples were incubated at 37°C for 5 min, and the sample absorbance at 670 nm was determined using a blank containing only the reagents.

Oxygen consumption

Oxygen consumption was measured in the medium where the tested strains were grown using the YSI model 53 oxygen monitor (YSI, Inc.). Cells were cultured until log phase in YES medium at 30°C. Cells were collected by centrifugation, washed in MilliQ water, and resuspended in water to a density of 1×10^9 cells/ml. Then, 3 ml of air-saturated culture was used to calculate the rate of oxygen consumption.

Ethanol measurement

Ethanol production by the tested strains was measured using a refractive index detector (Shimazu HPLC LC6AD) equipped with an ULTRON PS80-H column. Ethanol was quantified by differential refractive index with glycerol as a standard.

Results

Respiration is deficient in *Schizosaccharomyces japonicus*

We measured CoQ species and their contents in four fission yeast species: *S. pombe*, *S. cryophilus*, *S. octosporus*, and *S. japonicus*. *S. pombe* produces CoQ_{10}.[13, 14] We confirmed an earlier report that *S. octosporus* produces CoQ_9. The type of CoQ produced in *S. cryophilus* was unknown; we identified CoQ_9, similar as in *S. octosporus*. A previous study reported that *S. japonicus* does not produce detectable CoQ,[8] but we detected a very small amount of CoQ_{10} using HPLC analysis (Fig. 2A). The CoQ_{10} content was approximately 0.167 μg/1×10^9 cells or 0.3 μg/100 ml of culture, which is approximately 220 times lower than the CoQ_{10} content in *S. pombe* grown in YES medium (37 μg/1×10^9 cells or 69.5 μg/100 ml of culture). We subjected the sample to MS analysis (Fig. 2B). A peak appearing at 885.6797 *m/z* $[M+Na]^+$ corresponded with CoQ_{10}, and a peak at 197.0831 *m/z* $[M]^+$ by MS/MS corresponded with tropylium ion $[M]^+$[9]. These results verified that this product is CoQ_{10}.

Because the amount of CoQ was very low in *S. japonicus*, we measured the respiration capacity. We tested the growth of *S. japonicus* on non-fermentable carbon sources. *S. japonicus* and the *S. pombe* CoQ-deficient mutant (Δ*ppt1*) could not grow on 2% glycerol + 1% ethanol as carbon sources (Fig. 3A). Next, we measured oxygen consumption of *S. japonicus* and compared it with that of *S. pombe* wild type and *ppt1*

mutants (Fig. 3B). *S. japonicus* did not consume oxygen, which was similar to the *S. pombe* respiration-deficient mutant. These combined results suggest that *S. japonicus* can grow well under anaerobic conditions. We measured the growth of *S. japonicus* under oxygen-depleted conditions, and compared it with that of *S. pombe* wild type and CoQ-deficient mutants (Fig. 4). Under anaerobic conditions, *S. japonicus* grew much faster than *S. pombe* wild type and CoQ-deficient mutants. There was no difference in *S. japonicus* growth under aerobic and anaerobic conditions, whereas *S. pombe* and *S. cerevisiae* grew much faster under aerobic conditions, and growth of the *S. pombe* CoQ-deficient mutants was slow.

Sensitivity to oxidative stress

S. pombe coq deletion mutants are sensitive to H_2O_2.[29] To determine the *S. japonicus* oxidative stress sensitivity, we determined the sensitivity to H_2O_2 and paraquat (PQ). *S. japonicus* was sensitive to both H_2O_2 and PQ (Fig. 5). Wild-type *S. pombe* does not display oxidative stress sensitivity. *S. japonicus* has much greater oxidative stress sensitivity than *S. pombe ppt1* (*coq2*) mutants.

Sulfide production

S. pombe coq mutants produce higher sulfide levels than the wild type due to defective sulfide quinone reductase activity in mitochondria.[30-32] As *S. japonicus* produces very little CoQ_{10}, we assessed the amount of sulfide produced in *S. japonicus*. *S. japonicus* did not produce sulfide even though it produces almost no CoQ (Fig. 6B). These combined results indicate that the metabolic regulation of sulfide and CoQ in mitochondria of *S. japonicus* differs from that in *S. pombe*.

Expression of CoQ biosynthetic genes in *S. pombe*

We investigated possible reasons for low CoQ_{10} levels in *S. japonicus* by performing complementation assays of CoQ biosynthetic genes in *S. pombe*. We tested three genes involved in early steps of CoQ biosynthesis: *ppt1*, *dps1*, and *dlp1*. These genes were isolated from *S. japonicus* by searching databases using *S. pombe* homolog sequences for Ppt1, Dps1, and Dlp1 [National Center for Biotechnology Information (NCBI) BLAST program]. Homologous proteins [SJAG_06603 (named SjPpt1), SJAG_04568 (named

SjDps1), and SJAG_05776 (named SjDlp1)] were identified, and amino acid sequence alignments of these proteins are shown in Figs. S1, S2, and S3. Ppt1 (Coq2) condenses polyprenyl diphosphate with PHB.[19] SjPpt1 was identified, but the annotation stated that the first methionine was absent. When we carefully searched the *S. japonicus* genome data, the ATG codon was found in the 5′ upstream region of SJAG_06603 and no other ATG codon was found around there. We were able to find the real open reading frame (ORF) of SJAG_06603 in the *S. japonicus* NIG5091 genome. Then, we tested *Sjppt1* expression in the *S. pombe* Δ*ppt1* strain. The delayed growth of *S. pombe* Δ*ppt1* in minimal medium was complemented by the *S. japonicus Sjppt1* gene (Fig. 7A). SjPpt1 functioned well and restored CoQ_{10} production in *S. pombe* Δ*ppt1* (Fig. 7B). We also expressed *S. pombe ppt1* in *S. japonicus*, but did not observe any significant increase in CoQ_{10} (Fig. S4). Furthermore, we observed that addition of PHB increases the CoQ_{10} levels in *S. japonicus* (Fig. S5) and mitochondria show weak staining with Mitotracker (data not shown). We believe that the reason for the lack of CoQ synthesis is not due to Ppt1 function.

S. japonicus Sjdps1 and *Sjdlp1* are homologous to *dps1* and *dlp1*, respectively, which are highly likely to encode prenyl diphosphate synthases.[14, 28) 33)] We tested the functionality of *S. japonicus dps1* and *dlp1* in the corresponding *S. pombe* deletion mutants. When *Sjdps1* was expressed in the *S. pombe dps1* deletion mutant, it restored growth in minimal medium (Fig. 8A) but produced little CoQ_{10} (Fig. 8B). When *Sjdlp1* was expressed in the *S. pombe dlp1* deletion mutant, it restored growth in minimal medium (Fig. 9A) and produced normal levels of CoQ_{10} (Fig. 9B). When *Sjdps1* and *Sjdlp1* were expressed in the *S. pombe dps1 dlp1* double mutant, they restored growth in minimal medium (Fig. 10A) and produced equivalent CoQ_{10} levels to those produced by the homomer *ddsA* gene fused to the mitochondrial targeting sequence (Fig. 10B). We swapped the cloning vector of *Sjdps1* and *Sjdlp1*, but this did not affect growth or CoQ_{10} production. These combined results indicate that *Sjppt1*, *Sjdps1*, and *Sjdlp1* are functional in *S. pombe*, suggesting that *S. japonicus* possesses functional genes.

Ethanol production by *S. japonicus*

S. japonicus lacks respiration and grows by fermentation. Therefore, we expect that it might produce higher ethanol levels during fermentation. A previous study reported

ethanol production by *S. pombe*.[34] We measured the ethanol produced by the other three fission yeasts, *S. pombe*, *S. octosporus*, and *S. cryophilus* (Fig. 11). *S. japonicus* produces comparable ethanol levels to *S. pombe*, whereas *S. octosporus* and *S. cryophilus* did not produce ethanol as efficiently as *S. japonicus* and *S. pombe* (Fig. 11A). *S. japonicus* grew at 42°C (Fig. S6); therefore, we measured ethanol production at 42°C. At higher temperature, ethanol production was not as efficient as at 30°C, but significant ethanol was produced at 42°C (Fig. 11B). These results indicate that *S. japonicus* is potentially useful for ethanol production, especially at higher temperatures.

Discussion

In this study, we analyzed the physiological properties of the hyphal-forming fission yeast *S. japonicus*. We observed that *S. japonicus* did not respire, and it grew well under anaerobic conditions.[35] We found that *S. japonicus* produces very low levels of CoQ_{10}, is sensitive to oxidative stress, does not produce hydrogen sulfide as in *S. pombe* CoQ-deficient mutants,[30] and produces ethanol under higher temperatures (42°C). *S. japonicus* is quite different from *S. pombe* in its mitochondrial dependency, even though these two species are within the same genus. *S. japonicus* was first isolated from a strawberry field in Kyushu, Japan. The reason why *S. japonicus* lacks respiration is unknown. We also isolated a natural *S. japonicus* species (*S. japonicus* Kinzaki in Matsue City). This strain also produced only a low level of CoQ_{10} and had defective respiration (data not shown). At least two other strains of *S. japonicus* have been isolated from natural environments in Nagano and Hirosaki, Japan. These strains also produced only a low level of CoQ_{10} and had deficient respiration (data not shown). At least four independently isolated strains display the same properties, so it is unlikely that the phenotypes we observed in this study are specific to certain strains. We measured very low levels of CoQ of *S. japonicus*, although a previous study reported that CoQ was not detected in *S. japonicus*.[8] The low CoQ levels may cause the respiration deficiency, but this is not conclusive. Low CoQ levels may be a consequence of mitochondrial dysfunction, but not a reason for respiration deficiency. Mitochondrial dysfunction in *S.*

japonicus probably affects the production of hydrogen sulfide, which is synthesized in mitochondria.

We tried to determine why *S. japonicus* produces very little CoQ_{10} by analyzing the CoQ biosynthetic genes in the whole-genome sequence of *S. japonicus*.[6] All genes [*dps1*, *dlp1*, *ppt1* (*coq2*)-*coq9*] involved in CoQ synthesis were identified in the whole-genome data (Table 3). We performed complementation analyses of *Sjdps1*, *Sjdlp1*, *and Sjppt1* in the corresponding *S. pombe* mutants *dps1*, *dlp1*, and *ppt1*. The results show that the *S. japonicus* genes are functional and complement the *S. pombe* strains to produce CoQ_{10}, which is consistent with the finding that *S. japonicus* naturally produces CoQ_{10} despite its level is very low. SjDps1 and SjDlp1 function as decaprenyl diphosphate synthases, similar as in *S. pombe* and *H. sapiens*.[14, 15] All *coq* genes, *dps1*, and *dlp1* were confirmed by RNA seq analysis,[6] the expression levels in *S. japonicus* were within normal ranges, and the alignment of all Coq proteins was well-conserved. Although we did not test every gene related to CoQ biosynthesis, we think it unlikely that very low CoQ levels in *S. japonicus* are due to lack of specific CoQ genes. We also observed that addition of PHB increases the CoQ_{10} levels in *S. japonicus* (Fig. S5), which suggests that the whole enzymatic reaction is not disrupted. CoQ synthetic enzymes are active, but have weak activity. The low level of CoQ synthesis results from mitochondrial incompleteness. Mitochondrial DNA is present,[36, 37] and mitochondria show weak staining with Mitotracker (data not shown). Further analysis of mitochondrial function will be necessary to determine the reason for low CoQ_{10} synthesis in *S. japonicus*.

S. japonicus is a unique yeast in that it evolved limited respiratory function. It grows much faster than *S. pombe* under anaerobic conditions (fermentation). We found that *S. japonicus* produces more ethanol at higher temperatures than the other three fission yeasts. *S. japonicus* and *S. pombe* produce comparable ethanol levels at 30 and 37°C, but *S. japonicus* has much more efficient ethanol production at 42°C. Therefore, this yeast has great potential for ethanol production at higher temperatures or during fermentation to make beer or sake. *S. japonicus* smells better than *S. pombe* because it lacks hydrogen sulfide synthesis, which is a benefit for the production of alcoholic beverages.

Author contributions

K.T. S.M. and Y.T. performed the experiments and analyzed the data; M.K. and T.

K. designed the experiments and wrote the manuscript.

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References

1) Niki H (2014) *Schizosaccharomyces japonicus*: the fission yeast is a fusion of yeast and hyphae, Yeast, 31, 83-90.

2) Helston RM, Box JA, Tang W, Baumann P (2010) *Schizosaccharomyces cryophilus* sp. nov., a new species of fission yeast, FEMS Yeast Res, 10, 779-786.

3) Conti SF, Naylor HB (1960) Study of life-cycle of *Schizosaccharomyces octosporus* by means of ultra-thin sectioning and electron microscopy, Nature, 185, 633-634.

4) Hoffman CS, Wood V, Fantes PA (2015) An ancient yeast for young geneticists: a primer on the *Schizosaccharomyces pombe* model system, Genetics, 201, 403-423.

5) Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, Sgouros J, Peat N, Hayles J, Baker S, Basham D, Bowman S, Brooks K, Brown D, Brown S, Chillingworth T, Churcher C, Collins M, Connor R, Cronin A, Davis P, Feltwell T, Fraser A, Gentles S, Goble A, Hamlin N, Harris D, Hidalgo J, Hodgson G, Holroyd S, Hornsby T, Howarth S, Huckle EJ, Hunt S, Jagels K, James K, Jones L, Jones M, Leather S, McDonald S, McLean J, Mooney P, Moule S, Mungall K, Murphy L, Niblett D, Odell C, Oliver K, O'Neil S, Pearson D, Quail MA, Rabbinowitsch E, Rutherford K, Rutter S, Saunders D, Seeger K, Sharp S, Skelton J, Simmonds M, Squares R, Squares S, Stevens K, Taylor K, Taylor RG, Tivey A, Walsh S, Warren T, Whitehead S, Woodward J, Volckaert G, Aert R, Robben J, Grymonprez B, Weltjens I, Vanstreels E, Rieger M, Schafer M, Muller-Auer S, Gabel C, Fuchs M, Dusterhoft A, Fritzc C, Holzer E, Moestl D, Hilbert H, Borzym K, Langer I, Beck A, Lehrach H, Reinhardt R, Pohl TM, Eger P, Zimmermann W, Wedler H, Wambutt R, Purnelle B, Goffeau A, Cadieu E, Dreano S, Gloux S, Lelaure V, Mottier S, Galibert F, Aves SJ, Xiang Z, Hunt C, Moore K, Hurst SM, Lucas M, Rochet M, Gaillardin C, Tallada VA, Garzon A, Thode G, Daga RR, Cruzado L, Jimenez J, Sanchez M, del Rey F, Benito J,

Dominguez A, Revuelta JL, Moreno S, Armstrong J, Forsburg SL, Cerutti L, Lowe T, McCombie WR, Paulsen I, Potashkin J, Shpakovski GV, Ussery D, Barrell BG, Nurse P (2002) The genome sequence of Schizosaccharomyces pombe, Nature, 415, 871-880.

6) Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin MF, Heiman DI, Young SK, Furuya K, Guo Y, Pidoux A, Chen HM, Robbertse B, Goldberg JM, Aoki K, Bayne EH, Berlin AM, Desjardins CA, Dobbs E, Dukaj L, Fan L, FitzGerald MG, French C, Gujja S, Hansen K, Keifenheim D, Levin JZ, Mosher RA, Muller CA, Pfiffner J, Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Ekwall K, Kellis M, Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW, Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nusbaum C (2011) Comparative functional genomics of the fission yeasts, Science, 332, 930-936.

7) Klar AJ (2013) *Schizosaccharomyces japonicus* yeast poised to become a favorite experimental organism for eukaryotic research, G3 (Bethesda), 3, 1869-1873.

8) Yamada Y, Arimoto M, Kondao K (1973) Coenzyme Q system in the classification of the ascosporogenous yeast genus *Schizosaccharomyces* and yeast-like genus *Endomyces*, J. Gen. Appl. Microbiol., 19, 353-358.

9) Marbois B, Xie LX, Choi S, Hirano K, Hyman K, Clarke CF (2010) *para*-Aminobenzoic acid is a precursor in coenzyme Q6 biosynthesis in *Saccharomyces cerevisiae*, J. Biol. Chem., 285, 27827-27838.

10) Tran UC, Clarke CF (2007) Endogenous synthesis of coenzyme Q in eukaryotes, Mitochondrion, 7 Suppl, S62-71.

11) Allan CM, Awad AM, Johnson JS, Shirasaki DI, Wang C, Blaby-Haas CE, Merchant SS, Loo JA, Clarke CF (2015) Identification of Coq11, a new

coenzyme Q biosynthetic protein in the CoQ-synthome in *Saccharomyces cerevisiae*, J. Biol. Chem., 290, 7517-7534.

12) Kawamukai M (2009) Biosynthesis and bioproduction of coenzyme Q10 by yeasts and other organisms, Biotechnol. Appl. Biochem., 53, 217-226.

13) Kawamukai M (2016) Biosynthesis of coenzyme Q in eukaryotes, Biosci Biotechnol Biochem, 80, 23-33.

14) Saiki R, Nagata A, Uchida N, Kainou T, Matsuda H, Kawamukai M (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.

15) Saiki R, Nagata A, Kainou T, Matsuda H, Kawamukai M (2005) Characterization of solanesyl and decaprenyl diphosphate synthases in mice and humans, FEBS J., 272, 5606-5622.

16) Okada K, Suzuki K, Kamiya Y, Zhu X, Fujisaki S, Nishimura Y, Nishino T, Nakagawa T, Kawamukai M, Matsuda H (1996) Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone, Biochim. Biophys. Acta, 1302, 217-223.

17) Okada K, Kainou T, Matsuda H, Kawamukai M (1998) Biological significance of the side chain length of ubiquinone in *Saccharomyces cerevisiae*, FEBS Lett., 431, 241-244.

18) Suzuki K, Ueda M, Yuasa M, Nakagawa T, Kawamukai M, Matsuda H (1994) Evidence that *Escherichia coli ubiA* product is a functional homolog of yeast COQ2, and the regulation of *ubiA* gene expression, Biosci. Biotech. Biochem., 58, 1814-1819.

19) Uchida N, Suzuki K, Saiki R, Kainou T, Tanaka K, Matsuda H, Kawamukai M (2000) Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for *p*-hydroxybenzoate polyprenyl diphosphate transferase, J. Bacteriol., 182, 6933-6939.

20) Hayashi K, Ogiyama Y, Yokomi K, Nakagawa T, Kaino T, Kawamukai M (2014) Functional conservation of coenzyme Q biosynthetic genes among yeasts, plants, and humans, PLoS One, 9, e99038.

21) Moriyama D, Hosono K, Fujii M, Washida M, Nanba H, Kaino T, Kawamukai M (2015) Production of CoQ_{10} in fission yeast by expression of genes responsible for CoQ_{10} biosynthesis, Biosci Biotechnol Biochem, 79, 1026-1033.

22) Moriyama D, Kaino T, Yajima K, Yanai R, Ikenaka Y, Hasegawa J, Washida M, Nanba H, Kawamukai M (2017) Cloning and characterization of decaprenyl diphosphate synthase from three different fungi, Appl Microbiol Biotechnol, 101, 1559-1571.

23) Alfa C, Fantes P, Hyams J, McLeod M, Warbrick E (1993) Experiments with fission yeast., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

24) Okazaki K, Okazaki N, Kume K, Jinno S, Tanaka K, Okayama H (1990) High-frequency transformation method and library transducing vectors for cloning mammalian cDNAs by trans-complementation of *Schizosaccharomyces pombe.*, Nucleic Acids Res., 18, 6485-6489.

25) Prentice HL (1992) High efficiency transformation of *Schizosaccharomyces pombe* by electroporation, Nucleic Acids Res, 20, 621.

26) Moreno S, Klar A., Nurse P (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe.*, Methods Enzymol., 194, 795-823.

27) Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a lablatory manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

28) Suzuki K, Okada K, Kamiya Y, Zhu X, Tanaka K, Nakagawa T, Kawamukai M, Matsuda H (1997) Analysis of the decaprenyl diphosphate synthase (*dps*) gene in fission yeast suggests a role of ubiquinone as an antioxidant, J. Biochem. (Tokyo), 121, 496-505.

29) Miki R, Saiki R, Ozoe Y, Kawamukai M (2008) Comparison of a *coq7* deletion mutant with other respiration-defective mutants in fission yeast, FEBS J., 275, 5309-5324.

30) Zhang M, Wakitani S, Hayashi K, Miki R, Kawamukai M (2008) High production of sulfide in coenzyme Q deficient fission yeast, Biofactors, 32, 91-98.

31) Saiki R, Ogiyama Y, Kainou T, Nishi T, Matsuda H, Kawamukai M (2003) Pleiotropic phenotypes of fission yeast defective in ubiquinone-10 production. A study from the *abc1Sp* (*coq8Sp*) mutant, Biofactors, 18, 229-235.

32) Kawamukai M (2002) Biosynthesis, bioproduction and novel roles of ubiquinone, J. Biosci. Bioeng., 94, 511-517.

33) Zhang M, Luo J, Ogiyama Y, Saiki R, Kawamukai M (2008) Heteromer formation of a long-chain prenyl diphosphate synthase from fission yeast Dps1 and budding yeast Coq1, FEBS J., 275, 3653-3668.

34) Sakurai M, Tohda H, Kumagai H, Giga-Hama Y (2004) A distinct type of alcohol dehydrogenase, *adh4*+, complements ethanol fermentation in an adh1-deficient strain of *Schizosaccharomyces pombe*, FEMS Yeast Res, 4, 649-654.

35) Bulder CJ (1971) Anaerobic growth, ergosterol content and sensitivity to a polyene antibiotic, of the yeast *Schizosaccharomyces japonicus*, Antonie Van Leeuwenhoek, 37, 353-358.

36) Naehring J, Kiefer S, Wolf K (1995) Nucleotide sequence of the *Schizosaccharomyces japonicus* var. versatilis ribosomal RNA gene cluster and its phylogenetic implications, Curr Genet, 28, 353-359.

37) Svoboda A, Slaninova I (1997) Colocalization of microtubules and mitochondria in the yeast *Schizosaccharomyces japonicus* var. versatilis, Can J Microbiol, 43, 945-953.

38) Okada K, Ohara K, Yazaki K, Nozaki K, Uchida N, Kawamukai M, Nojiri H, Yamane H (2004) The *AtPPT1* gene encoding 4-hydroxybenzoate polyprenyl

455 diphosphate transferase in ubiquinone biosynthesis is required for embryo
456 development in *Arabidopsis thaliana*, Plant Mol. Biol., 55, 567-577.
457 39) Aoki K, Nakajima R, Furuya K, Niki H. (2010) Novel episomal vectors and a
458 highly efficient transformation procedure for the fission yeast
459 *Schizosaccharomyces japonicus*. Yeast, 27, 1049-1060.
460
461
462

Figure legends

Fig. 1. Proposed coenzyme Q (CoQ) biosynthetic pathway in *S. pombe*. The biosynthetic pathway that converts PHB into CoQ consists of eight steps in *S. pombe*. Decaprenyl diphosphate which is synthesized by decaprenyl diphosphate synthase (Dps1 + Dlp1) is transferred to PHB by PHP-decaprenyl diphosphate transferase (Ppt1 (Coq2)), and then seven modifications of the aromatic ring are performed in CoQ biosynthesis.

Fig. 2. CoQ contents in four fission yeasts. (A) HPLC analyses of CoQs from *S. pombe* PR110, *S. japonicus* NIG5091, *S. octosporus* yFS286, and *S. cryophilus* OY26 with CoQ_9 and CoQ_{10} standards. (B) MS analysis of CoQ produced in *S. japonicus*. Open triangle marks peak at 197.0831 *m/z*; closed triangle marks peak at 885.6797 *m/z*. It is identical to standard CoQ_{10}.

Fig. 3. Respiration deficiency of *S. japonicus*. (A) *S. pombe* wild type (PR110), *S. pombe* Δ*ppt1*, and *S. japonicus* wild type NIG5091 were grown on YES medium containing 3% glucose for 5 days or 2% glycerol + 1% ethanol for 7 days at 30°C. (B) Oxygen consumption was monitored in *S. pombe* wild type PR110 (diamond), *S. pombe* Δ*ppt1* (square), and *S. japonicus* wild type NIG5091 (triangle).

Fig. 4. Growth under anaerobic conditions. *S. japonicus* (NIG2028, NIG5091), *S. pombe* (WT (PR110) and Δ*ppt1*), and *S. cerevisiae* (WT (W303-1A) and Δ*coq2*) [38] strains were grown, serially diluted, and spotted on YES and YPAD for 2 days under aerobic and anaerobic conditions.

Fig. 5. Stress sensitivity of *S. japonicus*. *S. pombe* wild type PR110, *S. pombe* Δ*ppt1*, and *S. japonicus* NIG5091 were grown on YES medium containing 1 mM H_2O_2 and 1 mM paraquat (PQ) for 5 days at 30°C.

Fig. 6. Sulfide production. (A) Growth of *S. pombe* wild type PR110 (diamond), *S. pombe* Δ*ppt1* (square), and *S. japonicus* NIG5091 (triangle) was monitored by counting

cell numbers in YES medium. (B) Sulfide is measured in the same strains by the methylene blue method.

Fig. 7. Expression of *S. japonicus ppt1* in *S. pombe* Δ*ppt1* strain. *S. pombe* wild type (PR110) harboring pREP41 and *S. pombe* Δ*ppt1* harboring pREP41, pSLF272LGFP-Ppt1, or pREP41-Sjppt1 were grown in minimal medium with or without cysteine for 4 days at 30°C (A). Production of CoQ_{10} was measured by HPLC (B). CoQ_6 was used as standard.

Fig. 8. Expression of *S. japonicus dps1* in *S. pombe* Δ*dps1* strain. *S. japonicus dps1* was expressed in *S. pombe* Δ*dps1* strain (LJ1030). Cells were grown on minimal medium with or without cysteine for 6 days at 30°C (A), and synthesis of CoQ_{10} was measured by HPLC (B). Vector: LJ1030/pREP41; *Spdps1*: LJ1030/pREP41-dps1; *Sjdps1*-1 and *Sjdps1*-2: LJ1030/pREP41-Sjdps1-1 or pREP41-Sjdps1-2 (these plasmids were constructed independently, but used the same structure).

Fig. 9. Expression of *S. japonicus dlp1* in *S. pombe* Δ*dlp1* strain. *S. japonicus dlp1* was expressed in *S. pombe* Δ*dlp1* strain (RM19). Cells were grown on minimal medium with or without cysteine for 5 days at 30°C (A), and synthesis of CoQ_{10} was measured by HPLC (B). Vector: RM19/pREP1; Spdlp1: RM19/pREP1-dlp1; *Sjdlp1*-1 or *Sjdlp1*-2: RM19/pREP1-Sjdlp1-1 or pREP1-Sjdlp1-2 (these plasmids were constructed independently, but used the same structure).

Fig. 10. Expression of *S. japonicus dlp1* and *dps1* in the *S. pombe* Δ*dlp1*Δ*dps1* double mutant. *S. japonicus dlp1* and *dps1* were expressed in the *S. pombe* Δ*dps1*Δ*dlp1* double deletion strain (LA1). Cells were grown on the minimum medium with or without cysteine for 5 days at 30°C (A), and synthesis of CoQ_{10} was measured (B).

Fig. 11. Ethanol production. (A) The amount of ethanol produced in *S. cerevisiae* kyokai No. 9 (Sc), *S. pombe* L972 (Sp), *S. japonicus* NIG2028 (Sj), *S. octosporus* yFS286 (So), and *S. cryophilus* OY26 (Scryo) was measured by HPLC at 0 (white bar),

24 (light gray bar), 48 (dark gray bar), and 72 (black bar) hours. Cells were grown at 25°C in YPD (10% glucose). (B) The amount of ethanol produced in *S. cerevisiae* kyokai No. 9 (Sc), *S. pombe* L972 (Sp), and *S. japonicus* NIG2028 (Sj) was measured by HPLC at 0 (white bar), 24 (light gray bar), 48 (dark gray bar), and 72 (black bar) hours. Cells were grown either at 30 or 42°C in YPD (10% glucose).

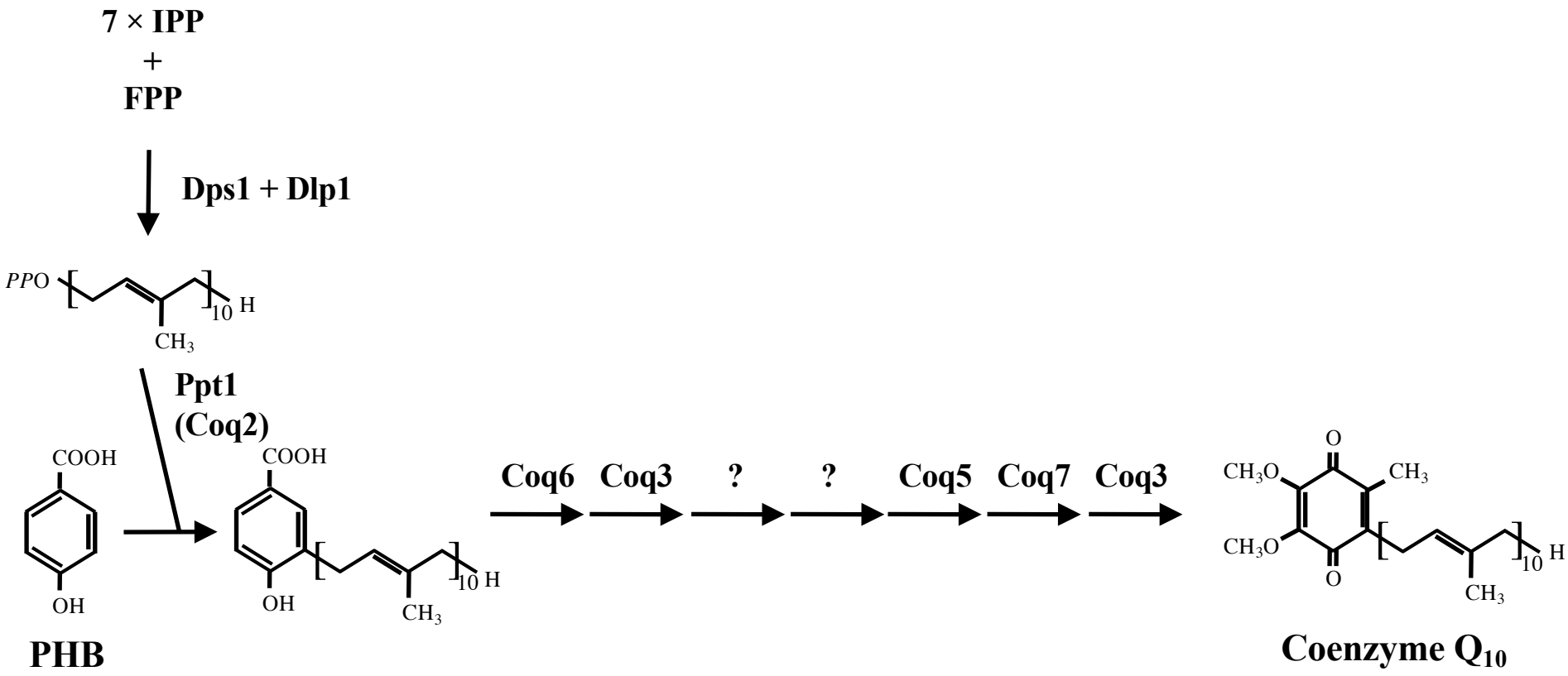
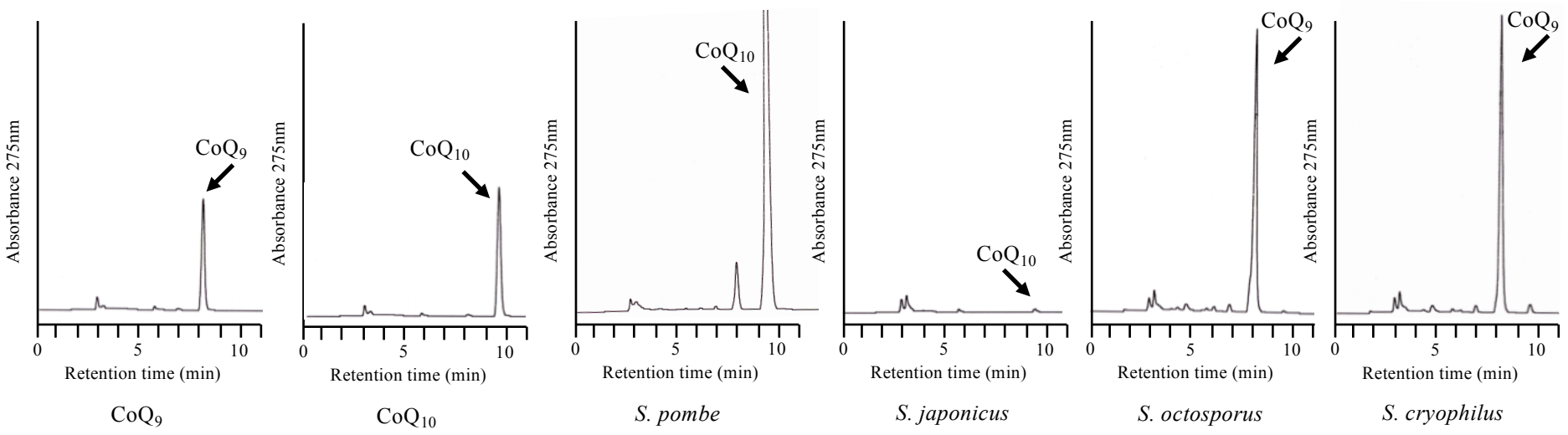

7 × IPP
+
FPP
Dps1 + Dlp1
PPO
10 H
CH3
Ppt1
(Coq2)
COOH
OH
PHB
COOH
OH
10 H
CH3
Coq6
Coq3
?
?
Coq5
Coq7
Coq3
O
CH3O
CH3
CH3O
O
10 H
CH3
Coenzyme Q10

Fig. 1

(A)


Absorbance 275nm
CoQ9
CoQ10
Retention time (min)
0
5
10
CoQ9
CoQ10
S. pombe
S. japonicus
S. octosporus
S. cryophilus

(B)

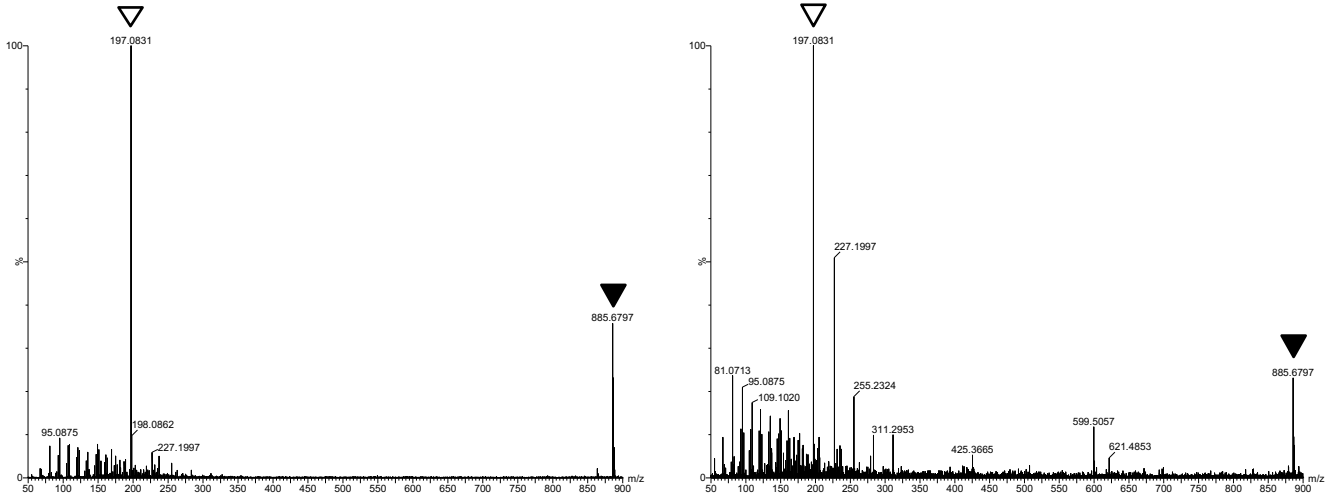
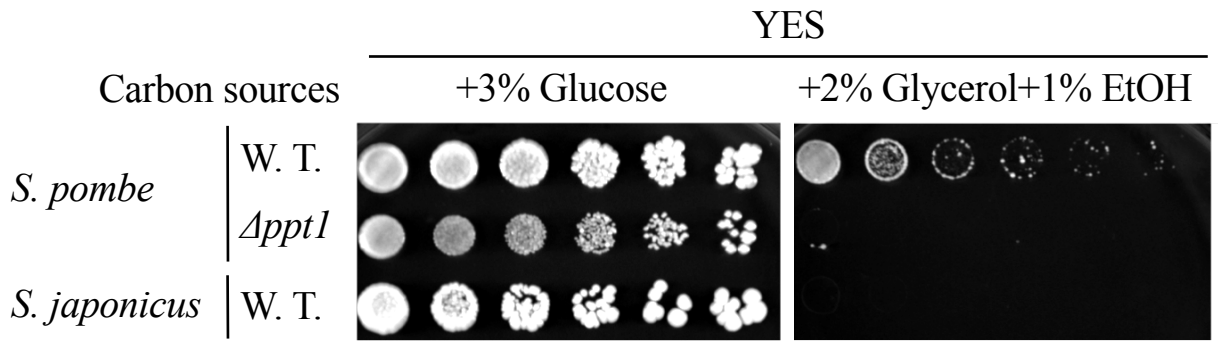

197.0831
885.6797
95.0875
198.0862
227.1997
81.0713
109.1020
255.2324
311.2953
425.3665
599.5057
621.4853
m/z
CoQ10
S. japonicus

Fig. 2

(A)


YES
Carbon sources
+3% Glucose
+2% Glycerol+1% EtOH
S. pombe
W. T.
Δppt1
S. japonicus
W. T.

(B)

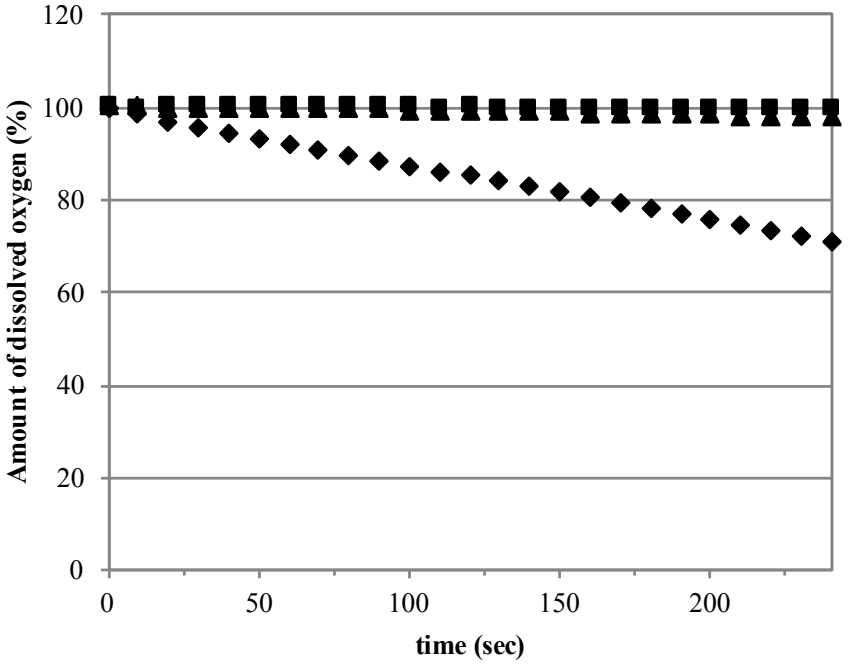

120
100
80
60
40
20
0
Amount of dissolved oxygen (%)
0
50
100
150
200
time (sec)

Fig. 3

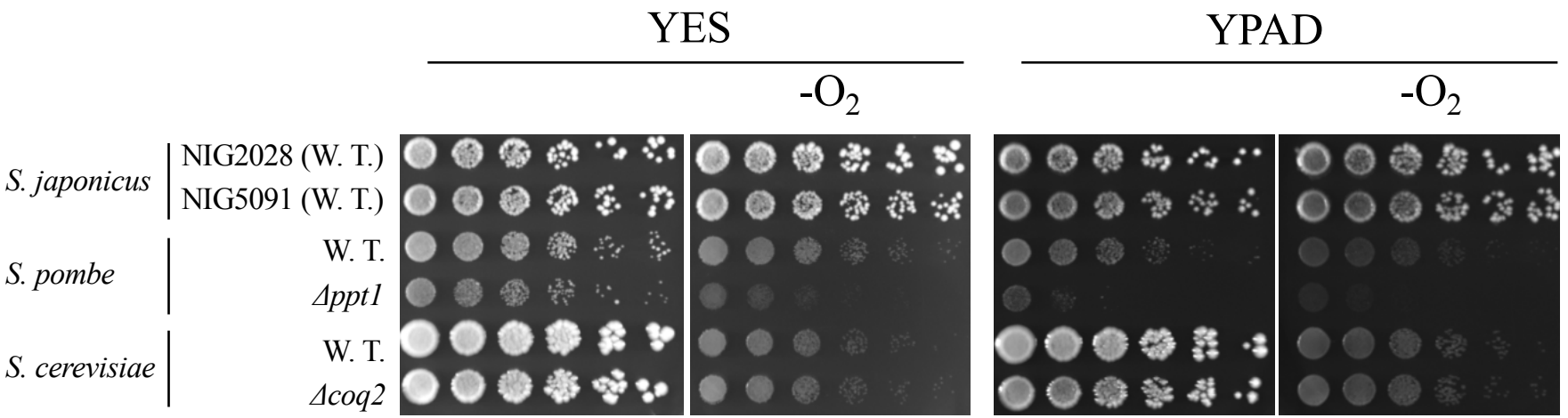

YES
YPAD
-O2
-O2
S. japonicus
NIG2028 (W. T.)
NIG5091 (W. T.)
S. pombe
W. T.
Δppt1
S. cerevisiae
W. T.
Δcoq2

Fig. 4

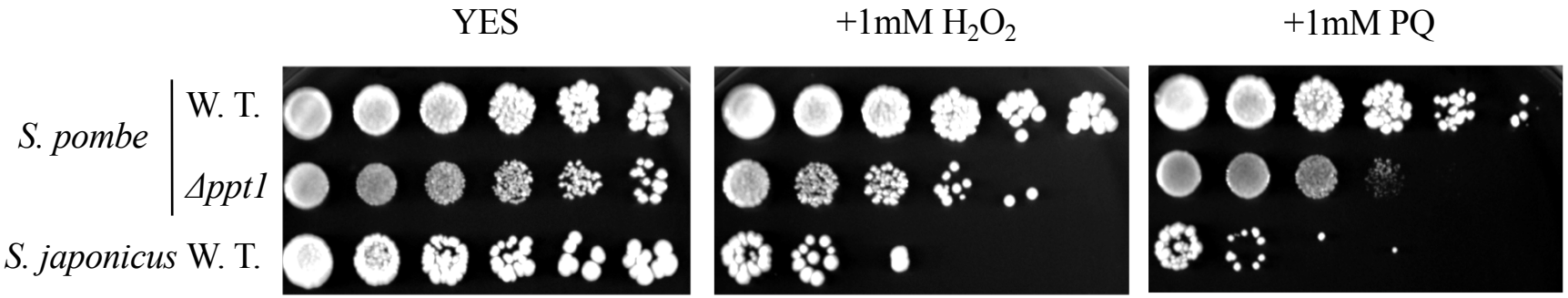
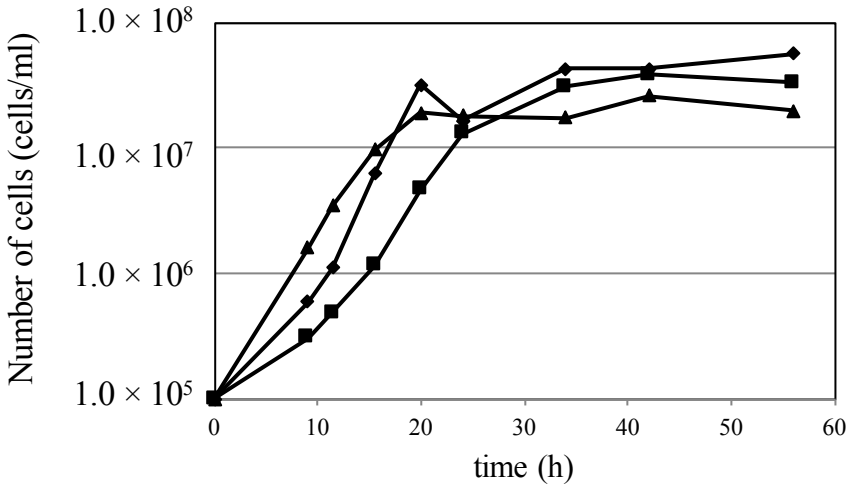


Fig. 5

(A)


Number of cells (cells/ml)
1.0 × 10^8
1.0 × 10^7
1.0 × 10^6
1.0 × 10^5
0
10
20
30
40
50
60
time (h)

(B)

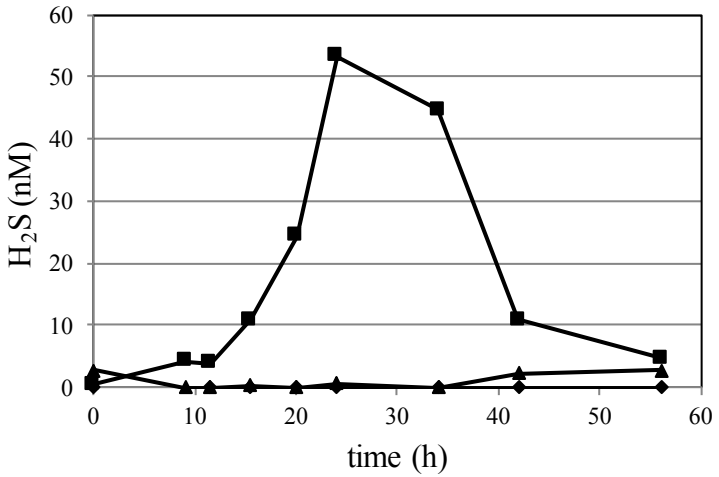
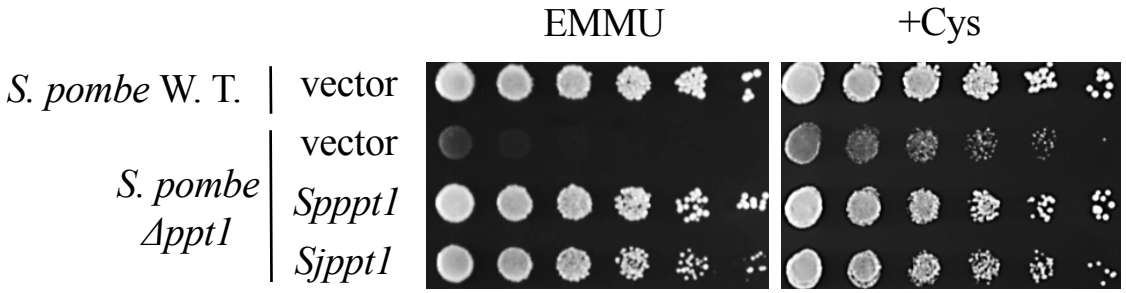

H2S (nM)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
time (h)

Fig. 6

(A)


EMMU
+Cys
S. pombe W. T.
vector
S. pombe Δppt1
vector
Spppt1
Sjppt1

(B)

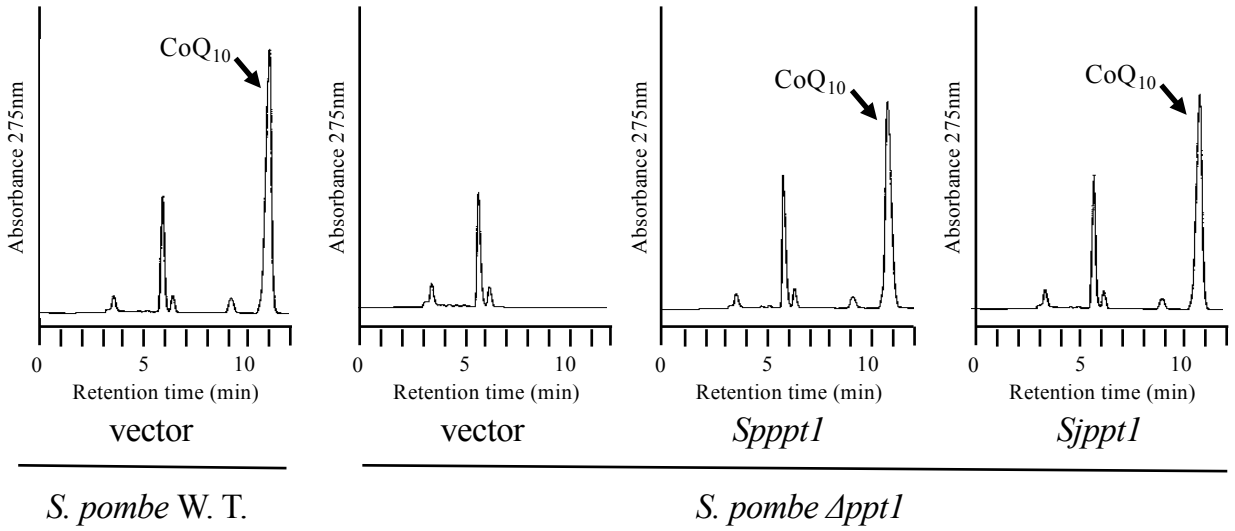
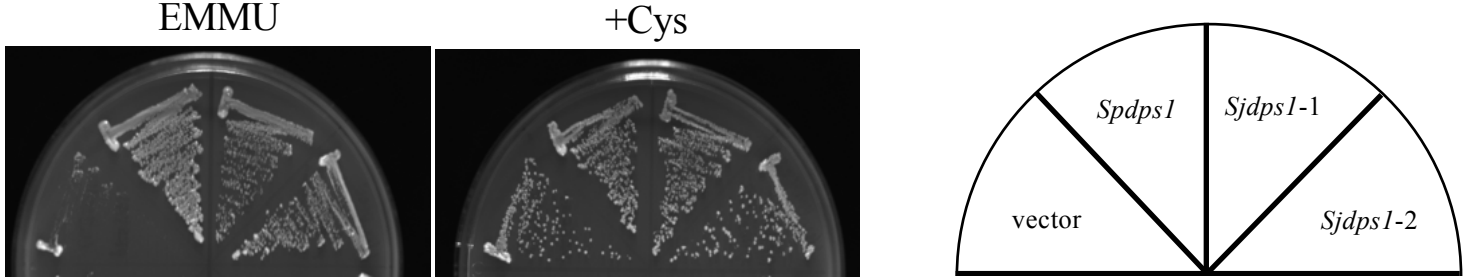
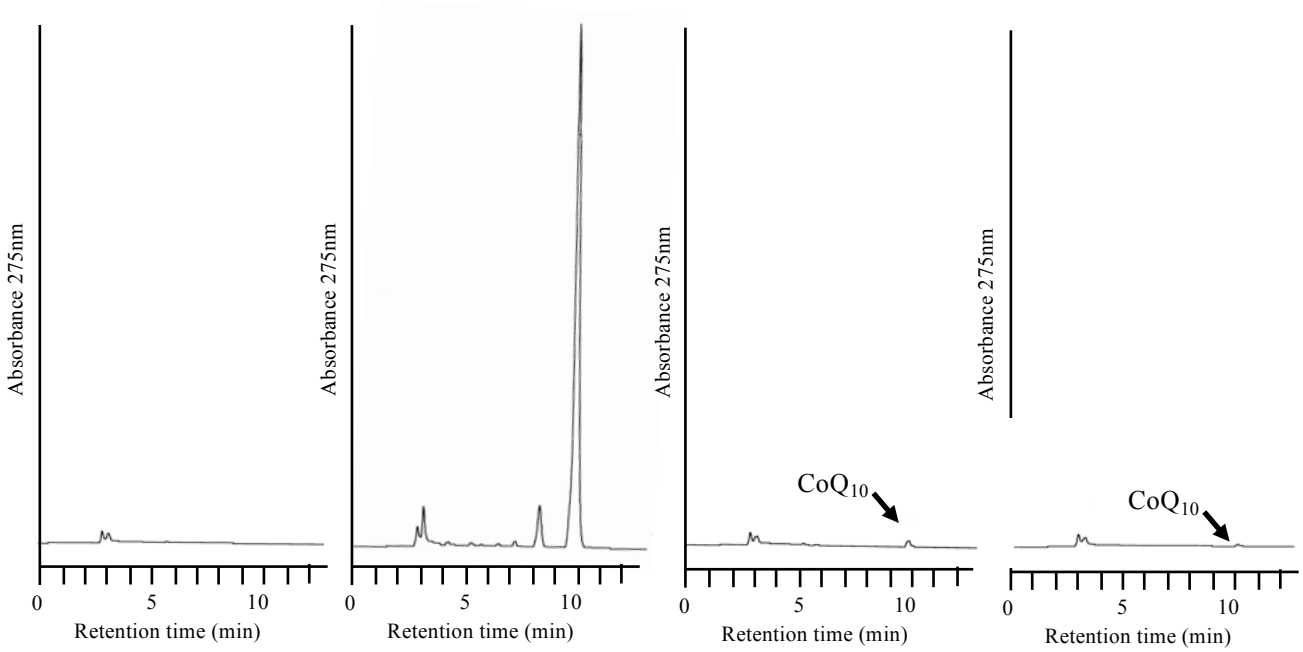

CoQ10
Absorbance 275nm
0
5
10
Retention time (min)
vector
vector
Spppt1
Sjppt1
S. pombe W. T.
S. pombe Δppt1

Fig. 7

(A)



(B)



S. pombe Δdps1

Fig. 8

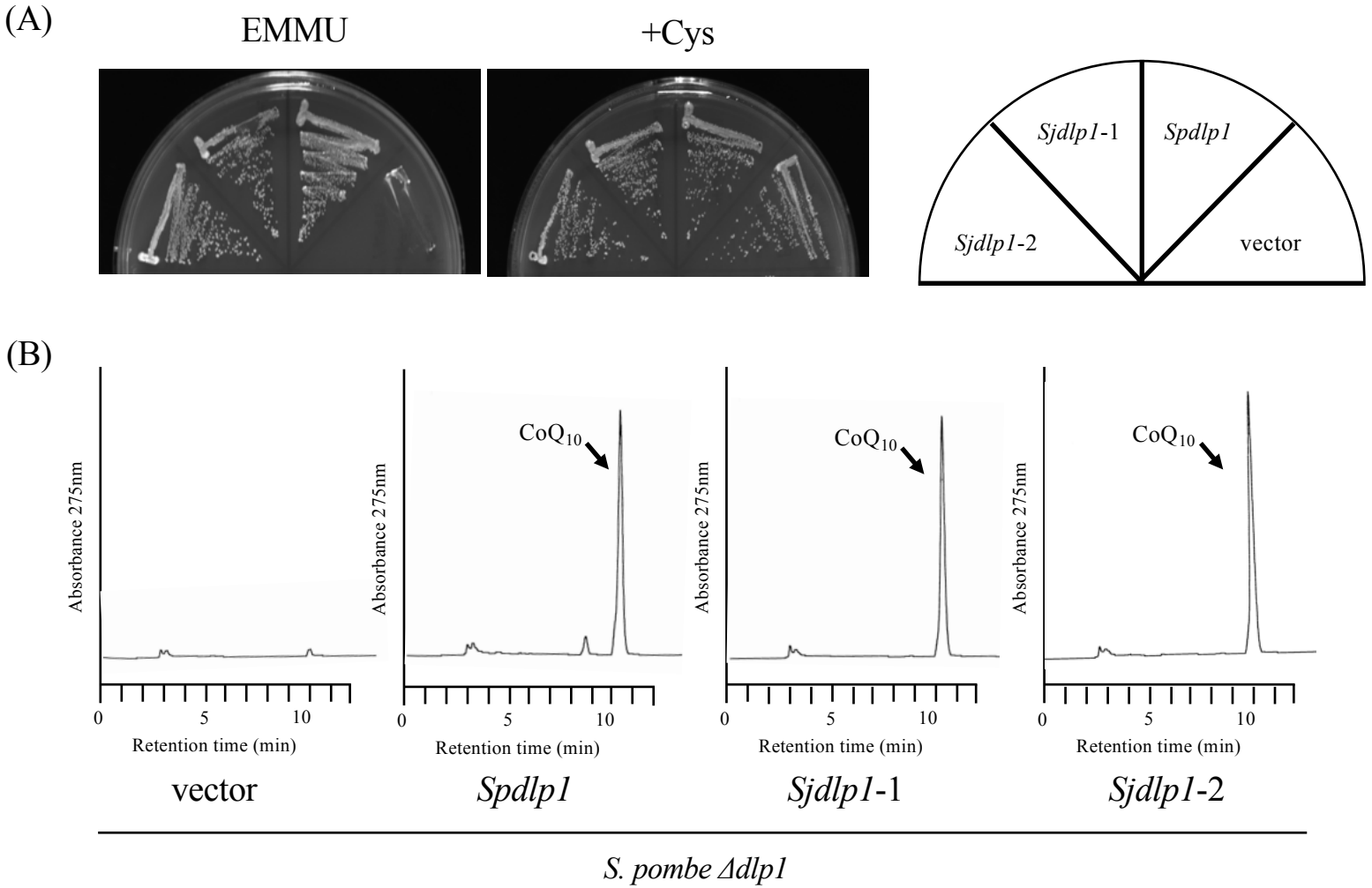
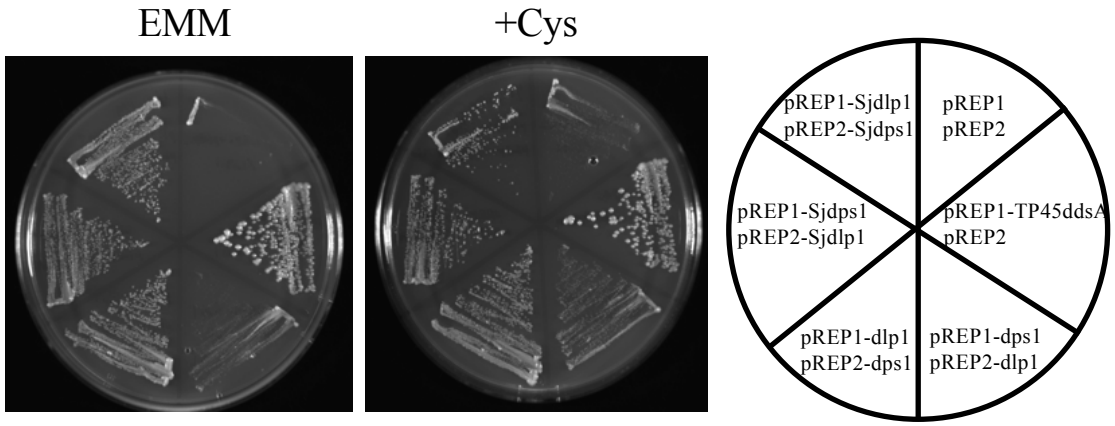
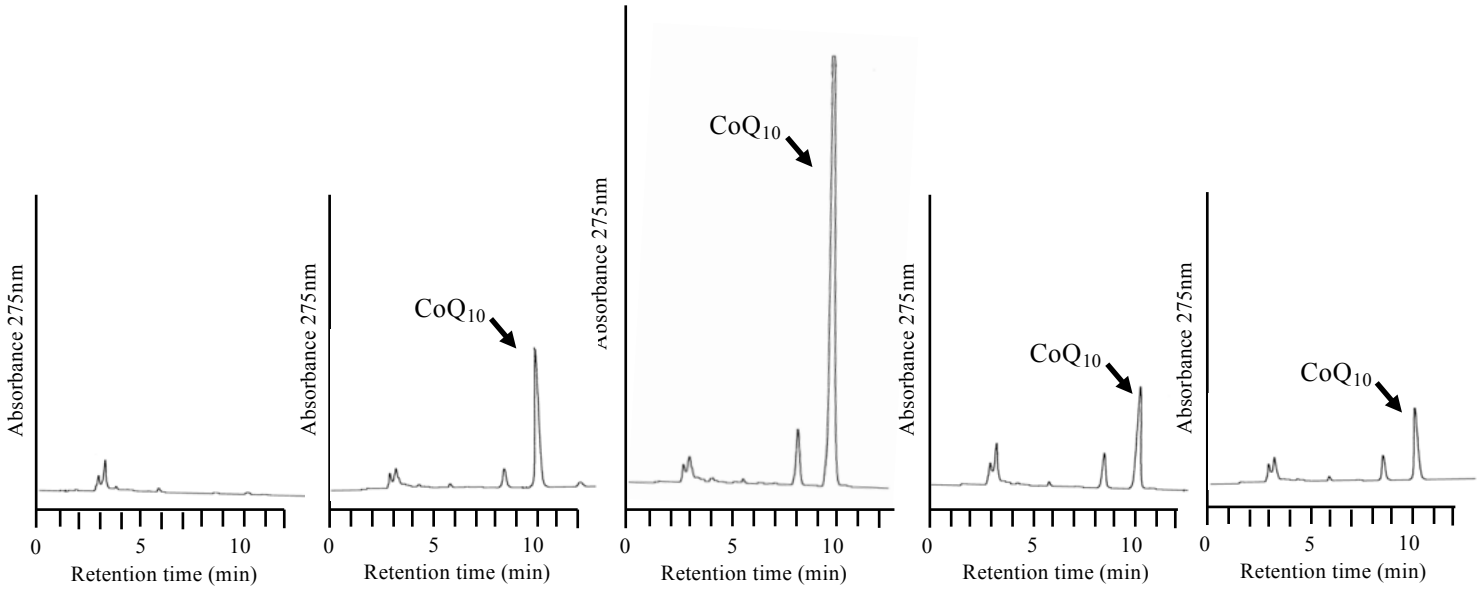

(A)
EMMU
+Cys
Sjdlp1-1
Spdlp1
Sjdlp1-2
vector
(B)
Absorbance 275nm
CoQ10
0
5
10
Retention time (min)
vector
Spdlp1
Sjdlp1-1
Sjdlp1-2
S. pombe Δdlp1

Fig. 9

(A)



(B)



S. pombe Δdps1Δdlp1

Fig. 10

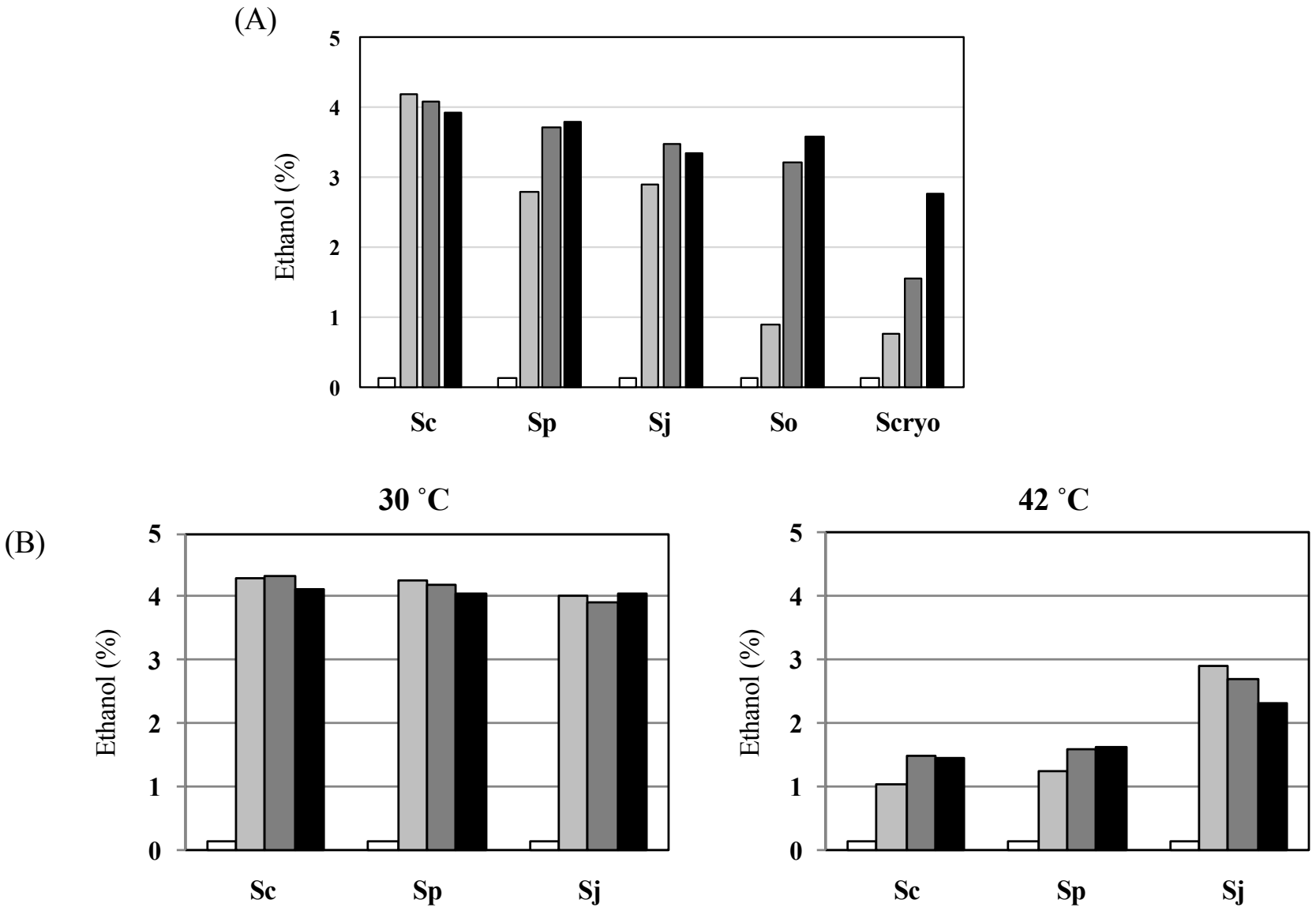

(A)
Ethanol (%)
0
1
2
3
4
5
Sc
Sp
Sj
So
Scryo
(B)
30 ˚C
42 ˚C
Ethanol (%)
0
1
2
3
4
5
Sc
Sp
Sj

Fig. 11

Table 1. Strains used in this study

Strain	Genotype	Reference
S. pombe		
L972	h^-	Lab stock
PR110	h^+ *ura4-D18 leu1-32*	Lab stock
LJ1030	h^+ *leu1-32 ura4-D18 dps1::kanMX6*	[33]
RM19 (KH2)	h^+ *leu1-32 ura4-D18 ppt1(coq2)::kanMX6*	[19]
LA1	h^+ *leu1-32 ade6-M210 ura4-D18 dlp1::ura4::ADE2 dps1::kanMx6*	[33]
S. japonicus NIG2028	h^-	[1]
S. japonicus NIG5091	h^- Δ*ura4*	[1]
S. octosporus yFS286	h^{90}	[6]
S. cryophilus OY26		[6]
S. cerevisiae		
kyokai No. 9		Brewing Society of Japan
W303-1A	*MAT a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	Lab Stock
W303Δ*coq2*	*MAT a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 coq2::HIS3*	[38]

Table 2. Plasmids used in this study

Plasmid	Relevant characteristics	Source or reference
pREP1	*ars1*, *LEU2*, *nmt1*-P, vector	lab stock
pREP41	*ars1*, *LEU2*, *nmt**-P, vector	lab stock
pREP2	*ars1*, *ura4*, *nmt1*-P, vector	lab stock
pREP1-TP45ddsA (pRDDSA)	Mitochondrial transit peptide *G. suboxydans ddsA* in pREP1	[14]
pSLF272LGFP-Ppt1 (pSLF272L-GFP_{S65A}-Dlp1)	*S. pombe ppt1* in pSLF272L-GFP_{S65A}	[20]
pREP41-dps1	*S. pombe dps1* in pREP41	this study
pREP2-dps1	*S. pombe dps1* in pREP2	this study
pREP1-dlp1	*S. pombe dlp1* in pREP1	[14]
pREP2-dlp1	*S. pombe dlp1* in pREP2	[33]
pREP41-Sjppt1	*S. japonicus ppt1* in pREP41	this study
pREP1-Sjdps1	*S. japonicus dps1* in pREP1	this study
pREP41-Sjdps1	*S. japonicus dps1* in pREP41	this study
pREP2-Sjdps1	*S. japonicus dps1* in pREP2	this study
pREP1-Sjdlp1	*S. japonicus dlp1* in pREP1	this study
pREP2-Sjdlp1	*S. japonicus dlp1* in pREP2	this study
pSJU11	*Spura4*	[39]
pSJU11-Sppt1-15	*S. japonicus nmt1* promoter -*S. pombe ppt1* in pSJU11	this study

Table 3. CoQ biosynthetic genes in four fission yeasts

	S. pombe	*S. japonicus*	*S. octosporus*	*S. cryophilus*
dps1	SPBPJ4664.01	SJAG_04568.4	SOCG_05355.5	SPOG_01333.3
dlp1	SPAC19G12.12	SJAG_05776.4	SOCG_05034.5	SPOG_02630.3
ppt1	SPAC56F8.04c	SJAG_06603.4	SOCG_02185.5	SPOG_00640.3
coq3	SPCC162.05	SJAG_06463.4	SOCG_02911.5	SPOG_02511.3
coq4	SPAC1687.12c	SJAG_00721.4	SOCG_02103.5	SPOG_00720.3
coq5	SPCC4G3.04c	SJAG_01043.4	SOCG_03809.5	SPOG_03428.3
coq6	SPBC146.12	SJAG_04000.4	SOCG_03515.5	SPOG_04223.3
coq7	SPBC337.15c	SJAG_00459.4	SOCG_03501.5	SPOG_04237.3
coq8	SPBC2D10.18	SJAG_00933.4	SOCG_00248.5	SPOG_02833.3
coq9	SPAC19G12.11	SJAG_01866.4	SOCG_05035.5	SPOG_02631.3