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<sub>10</sub> 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, S. cryophilus, and S. octosporus. S. pombe has been extensively studied in genetic, molecular biological, biochemical, and cytological analyses, but studies of the other three species are limited. The S. pombe whole genome was completely sequenced by 2002, and the whole genomes of the other three species were determined in 2011.

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. S. japonicus was isolated in 1928 in Japan, and is currently undergoing re-evaluation because of its unique properties. Nuclear organization and division have been investigated in S. japonicus, 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), 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 and the S. pombe fission yeast, and these knowledge extended to higher eukaryotes, 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$_{10}$. 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.
The type of CoQ such as CoQ₆ in *S. cerevisiae* and CoQ₁₀ in *S. pombe* is determined by the supplied prenyl diphosphate synthesized by the species specific polyprenyl diphosphate synthase. After synthesis, the isoprenoid is transferred to *p*-hydroxy benzoate (PHB) by Coq2 (Ppt1) (PHB-polyprenyl diphosphate transferase).

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). Almost all CoQ synthetic genes in humans and *Arabidopsis thaliana* can function to complement each of the corresponding *S. pombe* gene deletion mutants. Biotechnology approaches have successfully enhanced CoQ₁₀ biosynthesis in *S. pombe* fission yeast. Therefore, an analysis of CoQ biosynthesis in other fission yeast species may provide insights for the utilization of fission yeast for commercial production of CoQ₁₀.

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. Yeast cells were transformed using either lithium acetate or electroporation. General genetic methods used for *S. pombe* have been described previously. 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.\textsuperscript{27} Oligonucleotides used in this study are listed in Table S1. \textit{Escherichia coli} strain DH5\textalpha{} 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 \textit{SalI} and \textit{BamHI} sites of pREP41. The pREP1-Sjdp1, pREP41-Sjdp1, and pREP2-Sjdp1 plasmids were constructed by amplifying a fragment using the Sjdp1-Sall-F and Sjdp1-BamHI-R primers, and inserting the amplified product into the \textit{SalI} and \textit{BamHI} sites of pREP1, pREP41, and pREP2, respectively. The pREP1-Sjdlp1 and pREP2-Sjdlp1 plasmids were constructed by amplifying a fragment using the Sjdlp1-Sall-F and Sjdlp1-BamHI-R primers, and inserting the amplified product into the \textit{SalI} and \textit{BamHI} sites of pREP1 and pREP2, respectively. The pREP41-dps1 and pREP2-dps1 plasmids were constructed inserting the \textit{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 \textit{Sjnmt1} promoter, and Spppt1-Sjnmt1-24-F-New and Spppt1-BamHI-R primers for \textit{Spppt1} coding gene. Amplified fragments were fused by PCR reaction, and the product was cloned into the \textit{KpnI} and \textit{BamHI} sites of pSJU11.

**Spot assay**

Cells were grown on YES plates for 3 days at 30\degree{}C, and then resuspended in water to a density of $2 \times 10^6$ cells/ml. Cell suspensions were serially diluted (1:10), spotted onto YES or EMMU plates, and incubated for 3–5 days at 30\degree{}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\degree{}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 \(\mu\)l of methanol:2-propanol (4:1) solution and filtered with YMC Duo-Filter XQ DUO 04 (pore size, 0.2 \(\mu\)m), and 8 \(\mu\)l 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 \(\times\) 50 mm, 1.7 \(\mu\)m 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, \(S. \text{pombe}\) and \(S. \text{japonicus}\) 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 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 $1 \times 10^9$ 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 (Δppt1) 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$_2$O$_2$. To determine the *S. japonicus* oxidative stress sensitivity, we determined the sensitivity to H$_2$O$_2$ and paraquat (PQ). *S. japonicus* was sensitive to both H$_2$O$_2$ 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. 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.\textsuperscript{19} SjPpt1 was identified, but the annotation stated that the first methionine was absent. When we carefully searched the \textit{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 \textit{S. japonicus} NIG5091 genome. Then, we tested Sjppt1 expression in the \textit{S. pombe} \textit{ppt1} strain. The delayed growth of \textit{S. pombe} \textit{ppt1} in minimal medium was complemented by the \textit{S. japonicus} SjPpt1 gene (Fig. 7A). SjPpt1 functioned well and restored CoQ\textsubscript{10} production in \textit{S. pombe} \textit{ppt1} (Fig. 7B). We also expressed \textit{S. pombe} \textit{ppt1} in \textit{S. japonicus}, but did not observe any significant increase in CoQ\textsubscript{10} (Fig. S4). Furthermore, we observed that addition of PHB increases the CoQ\textsubscript{10} levels in \textit{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 \textit{Ppt1} function.

\textit{S. japonicus} Sjdps1 and Sjdlp1 are homologous to \textit{dps1} and \textit{dlp1}, respectively, which are highly likely to encode prenyl diphosphate synthases.\textsuperscript{14,28,33} We tested the functionality of \textit{S. japonicus} \textit{dps1} and \textit{dlp1} in the corresponding \textit{S. pombe} deletion mutants. When Sjdps1 was expressed in the \textit{S. pombe} \textit{dps1} deletion mutant, it restored growth in minimal medium (Fig. 8A) but produced little CoQ\textsubscript{10} (Fig. 8B). When Sjdlp1 was expressed in the \textit{S. pombe} \textit{dlp1} deletion mutant, it restored growth in minimal medium (Fig. 9A) and produced normal levels of CoQ\textsubscript{10} (Fig. 9B). When Sjdps1 and Sjdlp1 were expressed in the \textit{S. pombe} \textit{dps1} \textit{dlp1} double mutant, they restored growth in minimal medium (Fig. 10A) and produced equivalent CoQ\textsubscript{10} levels to those produced by the homomer \textit{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\textsubscript{10} production. These combined results indicate that Sjppt1, Sjdps1, and Sjdlp1 are functional in \textit{S. pombe}, suggesting that \textit{S. japonicus} possesses functional genes.

**Ethanol production by \textit{S. japonicus}**

\textit{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*. 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. We found that *S. japonicus* produces very low levels of CoQ₁₀, is sensitive to oxidative stress, does not produce hydrogen sulfide as in *S. pombe* CoQ-deficient mutants, 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 CoQ₁₀ 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 CoQ₁₀ 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*. 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\textsubscript{10} by analyzing the CoQ biosynthetic genes in the whole-genome sequence of *S. japonicus*.\textsuperscript{6)} All genes [\textit{dps1}, \textit{dlp1}, \textit{ppt1} (\textit{coq2}-\textit{coq9})] involved in CoQ synthesis were identified in the whole-genome data (Table 3). We performed complementation analyses of *Sj*\textit{dps1}, *Sj*\textit{dlp1}, and *Sj*\textit{ppt1} in the corresponding *S. pombe* mutants \textit{dps1}, \textit{dlp1}, and \textit{ppt1}. The results show that the *S. japonicus* genes are functional and complement the *S. pombe* strains to produce CoQ\textsubscript{10}, which is consistent with the finding that *S. japonicus* naturally produces CoQ\textsubscript{10} despite its level is very low. *SjDps1* and *SjDlp1* function as decaprenyl diphosphate synthases, similar as in *S. pombe* and *H. sapiens*.\textsuperscript{14,15)} All \textit{coq} genes, \textit{dps1}, and \textit{dlp1} were confirmed by RNA seq analysis,\textsuperscript{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\textsubscript{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,\textsuperscript{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\textsubscript{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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highly efficient transformation procedure for the fission yeast
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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 CoQ9 and CoQ10 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 CoQ10.

**Fig. 3. Respiration deficiency of S. japonicus.** (A) S. pombe wild type (PR110), S. pombe Δppt1, 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 Δppt1 (square), and S. japonicus wild type NIG5091 (triangle).

**Fig. 4. Growth under anaerobic conditions.** S. japonicus (NIG2028, NIG5091), S. pombe (WT (PR110) and Δppt1), and S. cerevisiae (WT (W303-1A) and Δcoq2) 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 Δppt1, and S. japonicus NIG5091 were grown on YES medium containing 1 mM H2O2 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 Δppt1 (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 Δppt1 strain.** S. pombe wild type (PR110) harboring pREP41 and S. pombe Δppt1 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 Δdps1 strain.** S. japonicus dps1 was expressed in S. pombe Δdps1 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 Δdlp1 strain.** S. japonicus dlp1 was expressed in S. pombe Δdlp1 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 Δdlp1Δdps1 double mutant.** S. japonicus dlp1 and dps1 were expressed in the S. pombe Δdps1Δdlp1 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).
Fig. 1

7 × IPP + FPP → Dps1 + Dlp1

Ppt1 (Coq2)

COOH

PHB

COOH

[CH₃][₁₀H]

Coq6 Coq3 ? ? Coq5 Coq7 Coq3

Coenzyme Q₁₀
Fig. 2

(A) Absorbance at 275 nm over retention time for different CoQ derivatives from S. pombe, S. japonicus, S. octosporus, and S. cryophilus.

(B) MSMS spectra for CoQ10 and S. japonicus, showing m/z values and peak intensities.
(A) Carbon sources

<table>
<thead>
<tr>
<th></th>
<th>+3% Glucose</th>
<th>+2% Glycerol+1% EtOH</th>
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<tbody>
<tr>
<td><em>S. pombe</em></td>
<td>W. T.</td>
<td></td>
</tr>
<tr>
<td><em>Δpplt1</em></td>
<td>W. T.</td>
<td></td>
</tr>
<tr>
<td><em>S. japonicus</em></td>
<td>W. T.</td>
<td></td>
</tr>
</tbody>
</table>

(B) Graph showing the amount of dissolved oxygen (%) over time (sec).
**S. japonicus**
NIG2028 (W. T.)
NIG5091 (W. T.)

**S. pombe**
W. T.
Δppt1

**S. cerevisiae**
W. T.
Δcoq2

---

**YES**

- $O_2$

---

**YPAD**

- $O_2$

---

Fig. 4
Fig. 5

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>+1mM H₂O₂</th>
<th>+1mM PQ</th>
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<td><strong>S. pombe</strong></td>
<td>W. T.</td>
<td>Appt1</td>
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<tr>
<td><strong>S. japonicus</strong></td>
<td>W. T.</td>
<td></td>
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</tbody>
</table>
Fig. 6

(A) Number of cells (cells/ml) vs. time (h)

(B) H₂S (nM) vs. time (h)
(A)  

<table>
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<tr>
<th>S. pombe W. T.</th>
<th>vector</th>
<th>EMMU</th>
<th>+Cys</th>
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<td>vector</td>
<td>Spppt1</td>
<td>Sjppt1</td>
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(B)  

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<th>S. pombe W. T.</th>
<th>CoQ₁₀</th>
<th>vector</th>
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<td>CoQ₁₀</td>
<td>Spppt1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sjppt1</td>
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</tbody>
</table>

Fig. 7
(A) EMMU + Cys

(B) Absorbance 275nm

Retention time (min)

vector

Spdps1

Sjdp1s-1

Sjdp1s-2

CoQ10

S. pombe Δdps1

Fig. 8
(A)  
EMMU  
+ Cys

(B)  
vector  
Spdlp1  
Sjdlp1-1  
Sjdlp1-2

S. pombe Δdlp1

Fig. 9
Fig. 10

(A) EMM + Cys

(B) S. pombe Δdps1Δdlp1

CoQ_{10}
Fig. 11
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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<tr>
<td><strong>S. pombe</strong></td>
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<tr>
<td>L972</td>
<td>$h^-$</td>
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<tr>
<td>PR110</td>
<td>$h^+ ura4-D18 leu1-32$</td>
<td>Lab stock</td>
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<tr>
<td>LJ1030</td>
<td>$h^+ leu1-32 ura4-D18 dps1::kanMX6$</td>
<td>33)</td>
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<td>RM19 (KH2)</td>
<td>$h^+ leu1-32 ura4-D18 ppt1(coq2)::kanMX6$</td>
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<tr>
<td>LA1</td>
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<td></td>
<td>$dps1::kanMX6$</td>
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<tr>
<td>S. japonicus NIG2028</td>
<td>$h^-$</td>
<td>1)</td>
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<td>S. japonicus NIG5091</td>
<td>$h^- \Delta ura4$</td>
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<tr>
<td>S. octosporus yFS286</td>
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<td>6)</td>
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<td>S. cryophilus OY26</td>
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<td>6)</td>
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<td><strong>S. cerevisiae</strong></td>
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<td>kyokai No. 9</td>
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<td>Brewing Society of Japan</td>
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<tr>
<td>W303-1A</td>
<td>$MAT a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1$</td>
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<td>W303Δcoq2</td>
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Table 2. Plasmids used in this study

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<th>Plasmid</th>
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<td>pREP41</td>
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<td>pREP2</td>
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<td>pREP1-TP45ddsA</td>
<td>Mitochondrial transit peptide</td>
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<td>(pRDDSA)</td>
<td><em>G. suboxydans ddsA</em> in pREP1</td>
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<td>pSLF272L-GFP-Ppt1</td>
<td><em>S. pombe ppt1</em> in pSLF272L-GFP&lt;sub&gt;S65A&lt;/sub&gt;</td>
<td>20&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>(pSLF272L-GFP&lt;sub&gt;S65A-Dlp1&lt;/sub&gt;)</td>
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<td>pREP41-dps1</td>
<td><em>S. pombe dps1</em> in pREP41</td>
<td>this study</td>
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<td>pREP2-dps1</td>
<td><em>S. pombe dps1</em> in pREP2</td>
<td>this study</td>
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<td>pREP1-dlp1</td>
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Table 3. CoQ biosynthetic genes in four fission yeasts

<table>
<thead>
<tr>
<th></th>
<th><em>S. pombe</em></th>
<th><em>S. japonicus</em></th>
<th><em>S. octosporus</em></th>
<th><em>S. cryophilus</em></th>
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<tbody>
<tr>
<td><em>dps1</em></td>
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<td><em>coq3</em></td>
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