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学　位　論　文　要　旨

氏名:　　森山　大輔

題目:　Bioproduction of coenzyme Q_{10} in fission yeast *Schizosaccharomyces pombe*
（分裂酵母*Schizosaccharomyces pombe*でのコエンザイムQ_{10}の生産）

Coenzyme Q (CoQ), also called ubiquinone, is a well known component of the electron transport chain that participates in aerobic cellular respiration within mitochondria and is essential for ATP-dependent energy production. CoQ consists of a hydrophobic isoprenoid side chain and a quinone ring, CoQ delivers electrons through the conversion of quinol (reduced form) and quinone (oxidized form). CoQ acts as a fat-soluble antioxidant by this oxidation-reduction reaction, which contributes to the removal of lipid peroxidation, and CoQ plays the role of the electron donor during disulfide bond formation in *Escherichia coli*, and its reduction is coupled to sulfide oxidation in *Schizosaccharomyces pombe* and other organisms. Moreover, CoQ is required for de novo synthesis of UMP (uridine monophosphate) in many eukaryotes. It is known that the amount of CoQ_{10} synthesis of human decreases after peaking at 20 years old, so that the external supplementation of CoQ_{10} is considered to be important for keeping an active life. So currently, the intake of CoQ_{10} by supplements has become popular and therefore the demand for CoQ_{10} has been increasing.

Living organisms possess different species of CoQ depending on the length of the isoprenoid side chain. For example, humans and *S. pombe* produce CoQ harboring ten-unit isoprene (CoQ_{10}), *E. coli* produces CoQ_8, and *Saccharomyces cerevisiae* produces CoQ_6. Since *S. pombe* naturally produces CoQ_{10}, it is easier to manipulate gene expression to produce CoQ_{10} than other popular organisms such as *S. cerevisiae* and *E. coli*, which only produce CoQ_6 and CoQ_8, respectively. In this research, I aim to improve the productivity of CoQ_{10} in *S. pombe* which naturally produces CoQ_{10}, and look for decaprenyl diphosphate synthase genes useful for production of CoQ_{10} in heterologous hosts.

In chapter 1, the author described the general introduction of the thesis.

In chapter 2, I aimed to improve the productivity of CoQ_{10} in *S. pombe* by expression of a panel of biosynthetic genes. In order to improve the CoQ_{10} productivity, CoQ biosynthetic genes (*dps1-dlp1*, *ppt1*, *coq3-coq9*) were cloned and individually overexpressed in *S. pombe*, however, the CoQ productivity hardly changed. As neither one of the CoQ biosynthetic genes may not be rate-limiting, I therefore simultaneously enhanced the expression of multiple CoQ biosynthetic genes. However, the CoQ productivity was not improved in spite of 10 kinds of CoQ biosynthetic genes were simultaneously enhanced. I then attempted to improve CoQ_{10} productivity by increasing the supply

of the CoQ_{10} precursors PHB (*p*-hydroxybenzoate) and DPP (decaprenyl diphosphate). Nine different biosynthetic genes involved in the shikimate and mevalonate pathways were overexpressed in *S. pombe*. These include the genes which lost its regulation that is known to regulate the mevalonate and shikimate pathway, and the genes allow the efficient flow bypassing metabolic pathway. The overexpression of chorismate lyase from *E. coli* (*Eco_ubiC*) or truncated HMG-CoA reductase from *S. cerevisiae* (*Sce_thmgr1*) gene resulted in a CoQ_{10} productivity increase of approximately 30% and the overexpression of the feedback-inhibition-resistant DAHP (3-deoxy-D-arabinoheptulosonate 7-phosphate) synthase from *E. coli* (*Eco_aroF*^{FBR}) gene increased productivity by approximately 15%. Furthermore, the result of co-expression of these upstream genes, two-fold increase of a CoQ_{10} productivity was observed in the strains expressing *Sce_thmgr1* and *Eco_aroF*^{FBR}, *Sce_thmgr1* and *Eco_ubiC*. No further gain in productivity was observed when all three genes (*Sce_thmgr1*, *Eco_aroF*^{FBR}, and *Eco_ubiC*) were co-expressed. These results indicate that, in *S. pombe*, the overproduction of precursors in the CoQ biosynthetic pathway is an effective strategy for improving CoQ productivity.

In chapter 3, three decaprenyl diphosphate synthase genes from three CoQ_{10} producing fungi, *Bulleromyces albus*, *Saitoella complicata*, and *Rhodotorula minuta* were cloned. The predicted Dps1 proteins contained seven conserved regions (domains I-VII) typically found in long-chain trans-prenyl diphosphate synthases, and exhibited common DDXXD motifs for FPP (farnesyl diphosphate) and IPP (isopentenyl diphosphate) recognition. These fungal Dps1 were 528, 440, and 537 amino acids in length in *B. albus*, *S. complicata*, and *R. minuta*, respectively. The sequence similarities of *B. albus* Dps1, *S. complicata* Dps1, and *R. minuta* Dps1 with *S. pombe* Dps1 were 50, 51, and 46%. I and my collaborators characterized these three fungal Dps1 enzymes in *E. coli* and *S. pombe*. *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 a *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*. Furthermore, I tested whether the CoQ_{10} production in *S. pombe* was increased by expressing exogeneous *dps1* genes from *B. albus*, *S. complicata*, and *R. minuta*; however, no increase in CoQ_{10} yield was observed. It is possible that exogeneous Dps1 interferes with endogenous *S. pombe* Dps1 or Dlp1. These fungal Dps1 proteins possessed deca-PDS activity and functioned as homomeric enzymes, and the cloned *dps1* genes can be used to enhance efficient production of CoQ_{10} in a range of species.

From the results, I conclude that two things: (1) CoQ productivity was not improved by overexpression of CoQ biosynthetic genes, but it was mproved the supply of the CoQ_{10} precursors PHB and DPP by enhancement of upstream responsible genes. (2) By cloning novel decaprenly diphosphate synthases from three fungi, I proved that they are functional in *S. pombe*.