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REVIEW

Enhanced production techniques, properties and uses of coenzyme Q10

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Abstract Coenzyme Q10 (CoQ10) is an essential component of the respiratory chain which produces ATP. It is formed from the conjugation of a benzoquinone ring with a hydrophobic isoprenoid chain. Efforts on the production of CoQ10 by microorganisms focus on the development of potent strains by conventional mutagenesis and metabolic engineering especially in *Escherichia coli*, analysis and modification of the key metabolic pathways and optimization of fermentation strategies. CoQ10 has excellent antioxidant properties and is beneficial in the treatment of several human diseases. The present review covers the current strategies used to improve and/or engineer CoQ10 production in microbes, the yields obtained in light of the current knowledge on the biosynthesis of this molecule. It also highlights the medical effects of CoQ10.

Keywords Coenzyme Q10 · Metabolic engineering · Mevalonate and non-mevalonate pathways · Mutagenesis · Ubiquinone

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Introduction

Coenzyme Q (CoQ), also called ubiquinone, is an essential component of the respiratory chain, which produces ATP (Kawamukai 2002; Kazunori et al. 2004; Takahashi et al. 2006). It is composed of a quinonoid nucleus and a side chain of isoprenoids (Cluis et al. 2011; Lu et al. 2013a). CoQ10 can be produced via chemical synthesis, semi-chemical synthesis and microbial biosynthesis. Currently, microbial fermentation is the most viable method for CoQ10 production (Lu et al. 2013a; Qiu et al. 2012; Tian et al. 2010; Yuting et al. 2010) because of the ability to produce biologically potent CoQ10 without optical isomers and at reduced costs (Tian et al. 2010).

Normally, all wild type strains are excellent CoQ10 producers (Bule and Singhal 2009; Ha et al. 2007a), but among them *Rhodospirillum* presents the highest specific CoQ10 content (Tian et al. 2010). The biological reactor appeared to be one of the most important methods of achieving low cost and high yields of CoQ10 production via fed-batch fermentation (Qiu et al. 2012).

With the expanding applications of CoQ10 in the pharmaceutical and cosmetic industries, CoQ10 is growing in demand (Parmar et al. 2013). Therefore, it is highly desirable to improve the yield of CoQ10 production in these biotic producers to meet the increasing demand (Cluis et al. 2011; Lu et al. 2013a).

Genetically-engineered *Escherichia coli*, moulds like *Neurospora*, *Aspergillus* and yeasts such as

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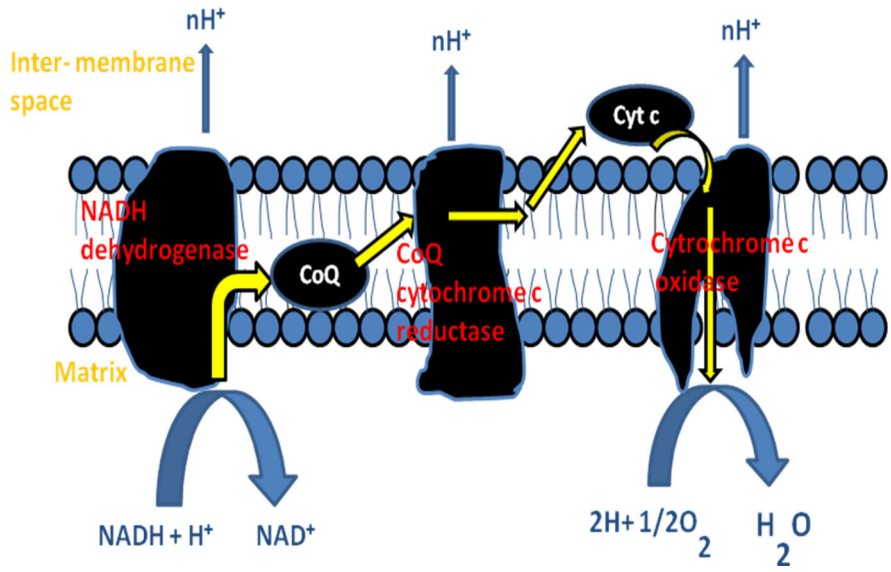

Fig. 1 Electron-transfer system in yeasts (Kawamukai 2009)

Candida, Rhodotorula, Saitoella have been reported as CoQ10 producers (Tian et al. 2010). However, until the present, the productivity of the engineered *E. coli* is still not competitive with the levels of produced by the natural producers by constitutive over-expression of 3-demethyl ubiquinone-9 3-methyltransferase under a Tac promoter in *Rhodobacter sphaeroides* (Lu et al. 2013; Ha et al. 2007a). CoQ10 has been intensively reviewed on its properties, functions, biosynthesis and different approaches used for production of CoQ10.

CoQ10 description, properties and functions

It is a lipid-soluble molecule located in the hydrophobic domain of the phospholipid bilayer of virtually all cellular membranes (Ha et al. 2007b, c; Yen and Shih 2009). It plays a central role in normal cell respiration and function, and thus a deficiency in its availability or endogenous production disrupts normal cellular functions. Such cellular disruption may lead to abnormal patterns of cell division and may produce an oncogenic response (Kawamukai 2002; Meganathan 2001; Potgieter et al. 2013).

CoQ also plays role in energy generation; it has several other functions including the removal of reactive oxygen species, the regulation of gene expression, and the control of the redox status of cells by adjusting the NAD^+/NADH ratio. In humans, CoQ10 can be used to prevent and treat several diseases, as well as aging symptoms (Priemé et al. 1997; Oytun et al. 2000; Cocheme et al. 2007; Zahiri et al. 2006a; Rosenfeldt et al. 2007; Yang et al. 2010; Gao et al. 2012a).

CoQ transfers electrons from NADH dehydrogenase and succinate dehydrogenase to CoQ: cytochrome *c* reductase in the respiratory chain (Fig. 1) (Ernster and Dallner 1995; Thai et al. 1996; Choi et al. 2009; Kawamukai 2009). The other roles of CoQ are disulfide-bond formation, sulfide oxidation and pyrimidine metabolism (Lee et al. 2004; Kawamukai 2009).

Coenzyme Q10 biosynthesis

Typically, the pathway for CoQ10 biosynthesis is composed of three parts, including synthesis of a quinonoid ring, synthesis of decaprenyl diphosphate and quinonoid ring modification (Jeya et al. 2010). In bacteria, the quinoid ring is formed from the *p*-hydroxybenzoate (PHB) its precursor derived from shikimate pathway which is the key pathway for synthesis of aromatic amino acids through chorismate. Chorismate is converted to PHB which is used for prenylation and ring modification by a reaction catalyzed by chorismate pyruvate lyase encoded by ubiC in *E. coli*. In mammals, tyrosine as an essential amino acid has to be supplied owing to their lack of the shikimate pathway. In lower eukaryotes, such as

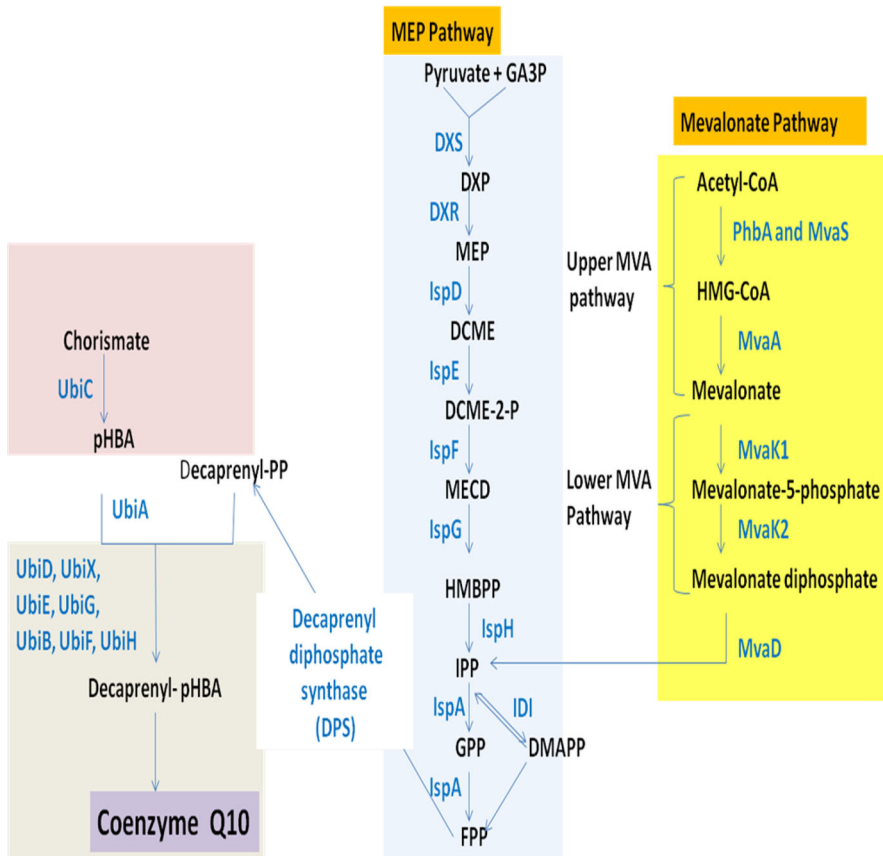

Fig. 2 Metabolic pathway involved in the synthesis of CoQ10 (Jeya et al. 2010). Decaprenyl diphosphate synthesized by DPS combines with pHBA and undergoes a series of modification reactions to produce CoQ10. 4-Diphosphocytidyl-2-C-methyl-D-erythritol (DCME) 2-phosphate, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MECD), 4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP). *Green* MEP pathway, *yellow* MVA pathway, *pink* chorismate pathway, *gray* ubiquinone pathway

Saccharomyces cerevisiase, PHB can be synthesized from both tyrosine and shikimate (Clarke 2000; Choi et al. 2005; Kawamukai 2009; Jeya et al. 2010).

The formation of the isoprenoid side chain is catalyzed by a group of enzymes, called polyprenyl diphosphate synthases, which deploy an allylic diphosphate as a priming molecule for consecutive additions of isopentenyl diphosphate (IPP) up to a certain length. The final length of the product is determined by the class of polyprenyl diphosphate synthases. These enzymes fall into four categories that include enzymes of class I whose products are short (C10, C15, and C20), those of class II whose products are medium (C30 and C35), and those of class III whose products are long (C40, C45, and C50) chain polyprenyl diphosphates. All the enzymes catalyze the formation of double bonds in trans stereoconfiguration (all-E). The products of class IV polyprenyl diphosphate synthases are generally long and, in contrast to other classes, have double bonds in *cis*-stereoconfiguration (Takahashi et al. 2006; Zahiri et al. 2006a; Jiazhou et al. 2013).

IPP is derived from two different ways (see Fig. 2): one is the mevalonate (MVA) pathway and the other is the non-MVA pathway (Choi et al. 2005; Jeya et al. 2010; Lee et al. 2007).

The enzymes of the non-MVA pathway are 1-deoxy-D-xylulose 5-phosphate synthase (DXP synthase) encoded by *dxs* catalyzing the formation of DXP by the condensation of pyruvate and D-glyceraldehyde 3-phosphate, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXP reductoisomerase) the first committed enzyme of the non-MVA pathway and encoded by the dxr gene catalyzing the synthesis of 2-C-methyl-D-erythritol 4-phosphate from DXP by intramolecular rearrangement and reduction in this single enzyme and the IPP isomerase encoded by the idi gene catalyzes the interconversion between IPP and dimethylallyl diphosphate (DMAPP), which is another five-carbon building block. The genes coding

for the enzymes involved in the other five reactions of the non-MVA pathway are *isp*D, *isp*E, *isp*F, *isp*G and *isp*H found in *E. coli* (Choi et al. 2005; Jeya et al. 2010; Lu et al. 2013a). The MVA pathway which is responsible for the production of isoprenoids, constitutes a large group of essential molecules involved in various cellular processes including CoQ10. The initial part of the MVA pathway constitutes a sequence of reactions that forms farnesyl pyrophosphate (FPP) from acetyl-coenzyme A, the last common substrate for the biosynthesis of CoQ10 and several other end products like cholesterol, dolichol and isoprenylated proteins.

Acetyl-CoA condenses with acetoacetyl-CoA resulting in the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The HMG-CoA is reduced in a two step reaction requiring 2 mol NADPH to MVA. HMG-CoA reductase catalyses the conversion of HMG-CoA to MVA (Forsman et al. 2010; Bentinger et al. 2010; Potgieter et al. 2013). MVA is then converted to MVA diphosphate by two phosphorylations mediated by MVA kinase and phospho-MVA kinase. MVA diphosphate undergoes dehydration–decarboxylation in an ATP-requiring reaction, resulting in IPP. An IPP isomerase catalyzes the isomerization of IPP to DMAPP. In the next step of the pathway, farnesyl diphosphate (FPP) synthase catalyzes the sequential 1–4 coupling of IPP with DMAPP and geranyl diphosphate (GPP) resulting in the formation of GPP and FPP, respectively. The product is elongated by decaprenyl diphosphate synthase (DPS) (Meganathan 2001; Jeya et al. 2010). The non-MVA pathway is frequently used by bacteria while eukaryotic microorganisms use the MVA pathway. However, there are exceptions; certain eukaryotic microbes, such as the green algae and the malaria parasite, *Plasmodium falciparum*, appear to possess the non-MVA pathway, while a few bacteria contain the MVA pathway. Eukaryotic microorganisms, like fungi and yeasts, lack the non-MVA pathway and rely on the MVA pathway with the few exceptions mentioned above (Meganathan 2001; Makoto 2002; Opitz et al. 2014).

The ring modification comprises the following reactions: the prenylation which is mediated by a membrane-bound enzyme, 4-hydroxybenzoate polyprenyltransferase, that transfers various types of polyprenyl diphosphate with little substrate specificity. Three hydroxylation and three methylation reactions, take place in different orders in prokaryotes and eukaryotes. All methyl groups are derived from *S*-adenosylmethionine and all oxygens come from O_2 (Meganathan 2001; Forsman et al. 2010; Choi et al. 2005; Lu et al. 2013a).

HMG-CoA reductase is the central regulatory enzyme of the MVA pathway. This enzyme primarily regulates cholesterol synthesis (Buhaescu and Izzedine 2007; Bentinger et al. 2010). HMG-CoA reductase is regulated through feedback mechanisms. Full suppression of the reductase occurs in the presence of cholesterol which is normally derived exogenously from plasma low density lipoprotein (LDL) and if an excess of MVA is concomitantly supplied (Thurnher et al. 2013). This can explain the action mechanism of one of our most common classes of drugs, the statins. Statins competitively inhibit HMG-CoA, the first committed enzyme of the MVA pathway, blocking the conversion of HMG-CoA to MVA. The molecular mechanism of inhibition of HMG-CoA reductase by statins is a catalytic mechanism. The statins molecules occupy the catalytic portion of HMG-CoA reductase, specifically the binding site of HMG-CoA, thus blocking access of this substrate to the active site. Since HMG-CoA reductase is the rate-controlling step of cholesterol biosynthesis, its inhibition by statins represents an effective strategy to lower cholesterol levels in patients with cardiovascular disease. (Rundek et al. 2004; Stocker et al. 2006; Buhaescu and Izzedine 2007; Gao et al. 2012b; Bentinger et al. 2010; Bhardwaj et al. 2013; Thurnher et al. 2013; Jiménez-Santos et al. 2014).

Mevalonate kinase (MK) is the second essential enzyme of the isoprenoid/cholesterol biosynthesis pathway, after HMG-CoA reductase, catalyzing the phosphorylation of mevalonic acid into phosphomevalonate. Although MK has not the rate-limiting properties of HMG-CoA reductase, MK activity is regulated via feedback inhibition by intermediates in the isoprenoid/cholesterol pathway geranylpyrophosphate, farnesylpyrophosphate and geranylgeranylpyrophosphate (Buhaescu and Izzedine 2007).

Techniques for coenzyme Q10 production

Coenzyme Q10 production in wild and mutant strains

The preferable approach for CoQ10 production is the use of natural high producers of CoQ-10 which

include strains of *Agrobacterium tumefaciens* or *Agrobacterium radiobacter*, *Paracoccus denitrificans* and *R. sphaeroides* or *Rhodopseudomonas sphaeroides* (Yen and Shih 2009; Choi et al. 2005; Cluis et al. 2007; Jeya et al. 2010). Other microorganisms which can be used in fermentation process for CoQ10 production are *Cryptococcus laurentii*, *Trichosporon* sp., *Sporobolomyces salmonicolor*, and *R. sphaeroides* and other types of yeasts such as *Candida, Rhodotorula* and *Saitoella* (Choi et al. 2005; Jeya et al. 2010).

The productivity of these strains ranges from 30 to 130 mg CoQ-10/l. However, a commercially viable strain should produce yields higher than 500 mg/l, which means that titers from natural high producers are insufficient for industrial purposes (Cluis et al. 2007). Therefore different approaches for enhancing CoQ10 production have been investigated. Improvements in CoQ10 production could be achieved through optimization conditions, cellular-regulatory mechanisms and mutagenesis in wild types or natural producers.

The oxidation–reduction potential (ORP) of the fermentation medium is an important factor that regulates CoQ biosynthesis in these strains. Low ORP, which is established by limiting the O_2 supply to growing cultures, increases CoQ-10 production in *A. tumefaciens* and *R. sphaeroides*. This should be due to the fact that the decrease of ORP might shift the ratio between oxidized CoQ and reduced $CoQH_2$ towards $CoQH_2$. This might trigger cells to synthesize more CoQ to compensate for this imbalance, to restore respiratory functions and/or to scavenge toxic electrons in the membrane (Cluis et al. 2007).

Improvements in CoQ-10 production were achieved also by chemical mutagenesis, enabling CoQ-10 yields up to 770 mg/l in *R. sphaeroides*. This high level production was reached by optimization of conditions where the optimal conditions for low viscosity and high CoQ10 production were 8 % (w/v) sugar and 0.16–0.26 % ammonium nitrogen. High cell-growth rates were obtained over −150 mV and high specific production rates at −200 mV. By maintaining the ORP around −200 mV in the last phase of fermentation, industrial production in a 80,000 l fermenter resulted in a final concentration of 770 mg/1 with 14 mg/g dry cell weight specific content after 150 h fermentation (Sakato et al. 1992; Choi et al. 2005; Jeya et al. 2010). High CoQ-10-producing mutants were selected based on several indirect phenotypes, such as growth on structural-analogue inhibitors of the pathway or alteration in pigment production. The rationale was to find mutants with an upregulated CoQ-10 pathway by selecting for growth on pathway or respiration inhibitors. For example, *A. tumefaciens* mutants with higher CoQ-10 content than the parent strain were identified based on their ability to grow in the presence of the structural analogues of CoQ, daunomycin and menadinone. Especially, selection studies of wild-type strains have shown *A. tumefaciens* ATCC 4452, *R. sphaeroides* FERM-P4675 and *P. denitrificans* ATCC 19367 to be excellent producers of CoQ10 and further strain development by chemical mutagenesis was performed for the above strains (Cluis et al. 2007).

According to the report from Pradipta et al. (2013), mutagenesis helped to solve the problem of CoQ10 production by *A. tumefaciens* ATCC 4452. During the production of CoQ10, the culture broth became highly viscous due to excessive synthesis of exopolysaccharides. This hindered the CoQ10 yield and complicated the downstream process. This problem was tackled by media modification and mutation. The mutation showed a significant reduction (6.3-fold) in viscosity development in the broth. The newly-developed mutant strain produced 48.9 mg CoQ10/l with a specific CoQ10 content of 1.87 mg/g DCW at 25 °C, 500 rpm agitation and 0.2 vvm aeration using continuous fed-batch fermentation and a newly-formulated cane molasses medium (Tokdar et al. 2013).

Another alternative explanation for the increase in CoQ-10 synthesis observed in carotenoid-mutant strains is that the cells are compensating for the loss of carotenoids as antioxidants by increasing the levels of a different antioxidant, CoQ-10, to protect themselves from oxidative damage (Choi et al. 2005; Gu et al. 2006; Cluis et al. 2007; Jeya et al. 2010).

After the strain development, optimizing fermentation conditions and environmental parameters which can be involved in improving CoQ10 production are temperature, aeration, carbon/nitrogen ratio, oxygen supply and viscosity (Choi et al. 2005; Jeya et al. 2010). For the commercial production of CoQ10 in batch-type fermentations and in a 150 l fermenter using a mutant strain of *R. sphaeroides,* the optimum temperature and initial aeration rate were 30 °C and 2 vvm, respectively (Kien et al. 2010). Table 1 summarizes CoQ10 production by wild types, chemical mutants and a recombinant strain.

Table 1 CoQ10 production in wild types, chemical mutants and a recombinant strain

Source	CoQ10 level (mg/l)	Specific CoQ10 content (mg/g DCW)	Type of strain	Reference
Pseudomonas N84	2.02	1.2	Wild type	Jeya et al. (2010)
Protaminobacter ruber	2.84	1.52	Wild type	Jeya et al. (2010)
Rhodospirillum rubrum ATCC 25852	20.16	–	Wild type	Tian et al. (2010)
Escherichia coli BL21/ pACDdsA	25.5	0.29	Recombinant strain	Choi et al. (2005)
Paracoccus denitrificans ATCC 19367	27.6	0.86	Wild type	Choi et al. (2005)
Pseudomonas diminuta NCIM 2865	29.22	–	Wild type	Bule and Singhal (2009)
Spingomonas sp. ZUTEO3	441.65		Wild type	Qiu et al. (2012)
A. tumefaciens KY-8593	75.0	1.2	Wild type	Cluis et al. (2007)
Agrobacterium tumefaciens ATTC4452	87.6	1.9	Wild type	Jeya et al. (2010)
Rhodobacter sphaeroides FERM-P4675	97.2	2.7	Wild type	Choi et al. (2005)
A. tumefaciens AU-55	110.0	9.6	Chemical mutants	Choi et al. (2005)
Agrobacterium sp.	180	1.96	Chemical mutants	Jeya et al. (2010)
Rhodobacter sphaeroides	350	8.7	Chemical mutants	Jeya et al. (2010)
R. spheroides Co-22 - 11 car-	346.8	2.6		Cluis et al. (2007)
R. sphaeroides Co-22-11	347.0	2.5	Chemical mutants	Choi et al. (2005)
A. tumefaciens KCCM 10413	458.0	8.54	Chemical mutants	Cluis et al. (2007)
Agrobacterium tumefaciens KCCM 10413	638	9.71	Chemical mutants	Jeya et al. (2010)
Rhodopseudomonas spheroides KY8598	770.0	8.7	Chemical mutants	Choi et al. (2005)

From the data presented in Table 1, the mutant strains exhibit higher productivities than wild-type strains. Moreover, some conditions are necessary for the increase of the CoQ-10 production. Aeration affects CoQ-10 production in *Agrobacterium* and *Rhodobacter* strains. But the reason why the mutant produced a larger amount was not clarified. This was confirmed when an ethionine-resistant mutant (M-37), derived from *A. tumefaciens* KY-3085, promoted increased production of CoQ-10 at 20 % higher than the parent. Also a green mutant (carotenoid-deficient mutant, Co-22-11) derived from *R. sphaeroides* KY-4113 produced 350 mg CoQ-10/l under culture conditions with a limited supply of air. The CoQ-10 content was 8.7 mg/g dry cell. In this case, the amount and content corresponded to 2.8 and 3.6 times larger than those given by the wild-type strain, respectively. Through the observation of multiple-layer structure of cell membrane under electronic microscope (Yoshida et al. 1998; Choi et al. 2005; Cluis et al. 2007; Hong and Tsu 2009).

The use of effective precursors can also improve CoQ10 production. In normal conditions *Sphingomonas* sp. ZUTE03 can produce about 43 mg/l but, by the addition of solid solanesol as a precursor to the organic phase for every 8 h batch, CoQ10 could accumulate and reach a yield of 171.5 mg/l. The yield can reach 442 mg/l by the addition of solid solanesol and *para*-hydroxybenzoic acid as precursors after 20 repetitions (Qiu et al. 2012).

The over-expression of enzymes, DXS, DXR, IDI and IspD, together with the application a self-regulation system combining a set of RBSs for adjusting the expression of the LacIq protein, could be another important way of improving CoQ10 production by increasing the decaprenyl diphosphate supplement in *R. sphaeroides*. The constitutive over-expression of the enzymes, DXS, DXR, IDI and IspD, under the control of the Tac promoter in *R. sphaeroides* and by applying a self-regulation system combining a set of ribosome binding sites for adjusting the expression of the LacIq protein to tune the expression of the four genes, resulted in improved CoQ10 production. Finally, another copy of the tac promoter with the UbiG gene (involved in the ubiquinone pathway of CoQ10 biosynthesis) was introduced into the engineered

pathway. By optimizing the expression level of both the upstream and downstream pathway, CoQ10 production in the mutants was improved up to 93.3 mg/l (7.16 mg/g DCW), about twice that of the wild-type (48.3 mg/l, 3.24 mg/g DCW) (Lu et al. 2013b).

The pH and the dissolved O_2 (DO) levels could be the key factors affecting CoQ10 production. When the pH and DO levels were controlled at 7 and 0–10 %, respectively, a dry cell weight (DCW) of 48.4 g/l and a CoQ10 production of 320 mg/l were obtained after 96 h of batch culture, corresponding to a specific CoQ10 content of 6.61 mg/g DCW. In a fed-batch culture using sucrose, the DCW, specific CoQ10 content, and CoQ10 production increased to 53.6 g/l, 8.54 mg/g DCW, and 458 mg/l, respectively. CoQ10 production was scaled up from a laboratory scale (5 l fermentor) to a pilot scale (300 l) and a plant scale (5,000 l) using the impeller tip velocity (V_{tip}) as a scale-up parameter (Ha et al. 2007a).

Metabolic engineering for CoQ10 production

Most of the efforts to engineer a CoQ10 pathway are focused on *E. coli* because *E. coli* is well suited for genetic modifications and large-scale fermentation (Jeya et al. 2010). The types CoQ are not the same in all species of organisms. Their CoQ differs by the length of the side chain. For example, most cereal crops produce CoQ9, in human CoQn exists mainly in the form of CoQ10 with 10 isoprene repeats in the side chain and *S. cerevisiae* produces CoQ6 and *Escherichia coli*, CoQ8 (Lee et al. 2004; Bhagavan and Chopra 2006; Dawei et al. 2007). Therefore metabolic engineering is mainly about the expression of the decaprenyl diphosphate synthase (DdsA) which catalyses the production CoQ10 in various organisms (Ha et al. 2007a; Parmar et al. 2013).

Escherichia coli naturally synthesizes CoQ8 rather than CoQ10. The biosynthesis of CoQ10 by *E. coli* is made possible by the expression of an E-decaprenyl diphosphate synthase (DdsA) cloned from a CoQ-10-producing microbe, along with the optional deletion of ispB, coding for E-octaprenyl diphosphate (OPP) synthase, in order to eliminate the synthesis of CoQ8. The improvement of CoQ10 in engineered *E. coli* has been carried out by the development of rational genetic engineering strategies such as the overexpressing 1-deoxy-D-xylulose 5-phosphate synthase (DXS) of the methylerythritol phosphate pathway (MEP) also known as the non-MVA pathway with the goal of increasing the availability of IPP and DMAPP (Cluis et al. 2007). The expression of a foreign MVA pathway in *E. coli* could also result in a higher CoQ10 content. The over-expression of UbiC, which converts chorismate into PHB, also results in an increase in CoQ production. However, the over-expression of selected enzymes of this pathway suggests that UbiA is rate limiting, while the over-expression of UbiB, UbiG and UbiH has little impact on CoQ production. Despite its great potential as a production host, the CoQ10 content currently obtained in engineered *E. coli* (2.5 mg/g DCW) still falls below those obtained in mutant strains of *A. tumefaciens* (11.8 mg/g DCW) and *R. sphaeroides* (8.7 mg/g DCW) (Cluis et al. 2007, 2011).

Blocking the biosynthesis of competing molecules, in particular of menaquinone (MK) and demethylmenaquinone (DMK), might also increase CoQ yields in *E. coli*. MK and DMK share the quinone pool with CoQ and are formed of OPP and a naphthoquinone derived from chorismate. Under aerobic conditions, the biosynthesis of CoQ is favored over that of DMK and MK because it forms 65 % of the quinone pool. However, the ratio between the different quinones is expected to shift rapidly with changes in oxygen availability, even under standard batch conditions. Therefore, blocking or down-regulating the biosynthesis of MK and DMK might be used as a metabolic engineering strategy to favor the production of CoQ in *E. coli*. Other pathways competing for chorismate and isoprenoid precursors could be considered in a similar manner (Cluis et al. 2007).

Escherichia coli DH5a was metabolically engineered for CoQ10 production by the introduction of decaprenyl diphosphate synthase gene (ddsA) from *A. tumefaciens* (Zahiri et al. 2006b). When grown in 2YTG medium (1.6 % tryptone, 1 % yeast extract, 0.5 % NaCl, and 0.5 % glycerol) with an initial pH of 7, the recombinant produced CoQ10 up to 470 μg/g DCW. This value could be further elevated to 900 mg/g DCW simply by increasing the initial culture pH from 7 to 9. However, engineering of a lower MVA semi-pathway so as to increase the IPP supply of the recombinant strain using exogenous MVA efficiently increased the CoQ10 production.

Metabolic engineering of *E. coli* can induce a bottleneck in MVA pathways. In this process the native regulation of the carbon flux through the

pathway may be lost thereby leading to imbalances in the pathways. Metabolic engineering of *E. coli* produces large quantities of isoprenoids by creating a MVA-based, isopentenyl pyrophosphate biosynthetic pathway where the strain produces high levels of isoprenoids but, upon further investigation, it was discovered that the accumulation of pathway intermediates limited flux and that high level expression of the MVA pathway enzymes inhibited cell growth. This is from the link of the growth inhibition phenotype with the accumulation of the pathway intermediate 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA). Such an accumulation implies that the activity of HMG-CoA reductase was insufficient to balance flux in the engineered pathway. By modulating HMG-CoA reductase production, the pathway bottleneck has been eliminated and increased MVA production (Pitera et al. 2007).

Outlook

Wild type strains are excellent CoQ10 producers, but *Rhodospirillum* presents the highest specific CoQ10 content with 20.2 mg/l and *A. tumifaciens* exhibits the highest CoQ10 yield in a fed-batch fermentation process. The microbial fermentation is the most viable method for CoQ10 production. Improvements in CoQ10 production were achieved by chemical mutagenesis which gives CoQ10 yields up to 770 mg/l in *R. sphaeroides*. Metabolic engineering of *E. coli* did not significantly increase this yield but further knowledge of the biosynthetic enzymes and of regulatory mechanisms modulating CoQ production will certainly increase the yield in the future.

The better precursors which could be combined for more CoQ10 production needs future studies. New methods for development of CoQ10 production in a better microorganism, which could produce high CoQ10 yield, could also be evaluated in the future. Finally, a type of biofilm reactor that provides high cell concentrations, high productivity, and easy separation of the products could be determined from further research. Protein engineering on *cis*-prenyl transferases should also be investigated in further research as the inhibition of *cis*-prenyl transferases could reduce the isoprenylated proteins production hence increase CoQ10 in mevalonate pathways.

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