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Review Article

Medical Significance of CoQ10 and Its Enhanced Production Using Potential Producer Microorganisms

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Abstract: Coenzyme Q10 is a polyphilic molecule resembling to vitamin structure. In the cell, CoQ10 is produced from hydrophobic isoprenoid chain conjugation with benzoquinone ring. Naturally, various ubiquinone (UQ) can be produced by yeast and microorganisms and present in human as CoQ10. It plays a critical role in human health given its use as medicine, dietary supplement and in cosmetics. Currently, CoQ10 biosynthesis is highly studied due to its critical contribution in the electron transport chain and cellular respiration which produces energy in the form of ATP. It has antioxidant activity and highly used as supplement to human with various diseases depicting high need on the market. Its high scale production is based on engineering of natural producing strains through putative techniques of metabolic engineering including mutagenesis and/or metabolic pathways modification followed by fermentation strategies optimization. *Escherichia coli* and *Saccharomyces cerevisiae* are the most–used and genetically optimized. However, to date, more other strains such as *Sphingomonas sp., Agrobacterium sp.,* and *Rhodobacter sphaeroides* are entirely explored with significant improvement and utility since their CoQ are similar to that produced in humans. This review will first focus on strategies used to improve CoQ10 yield with the use of preferred genetic engineering of potent strain producers and culminate with pointing out the latest medical significance of CoQ10 in light of its deficiency towards advocating its use in mitigating mitochondrial associated dysfunction, neurodegenerative and cardiovascular diseases.

Keywords: CoQ10, Metabolic Engineering, Mevalonate and Non-Mevalonate Pathway, Potent, CoQ10 Producers, Medical Functions

1. Introduction

Coenzyme Q10 (CoQ10) is a ubiquinone, redox active compound, soluble in lipids constituted with ten units isoprenoid repeats in the side chain. It is present in yeast, microorganism and all tissues of human encoded by COQ4 gene. It is found in 3 redox states depending on whether is fully or partially oxidized namely ubiquinone and semi-ubiquinone respectively, or fully reduced as ubiquinol. Ubiquinone (UQs) compounds are found in human mainly as UQ10, *E. coli* as UQ8, in yeast as UQ6, and in rodents as UQ9 [1].

In eukaryotes, CoQs works in the electron transport chain as 2 electrons carrier between ubiquinone and ubiquinol and 1 electron carrier between semi-quinone and other compounds depicting its role as an antioxidant through Reactive Oxygen Species (ROS) inhibition mainly for DNA, proteins and membrane phospholipids protection [2]. Additionally, as a supplement, CoQ10 oral or intravenous administration is more effective to prevent severe progressive encephalomyopathy, ataxia, neurodegenerative diseases, cardiomyopathy and diabetes. Furthermore, it boosts vitamin E, which has antioxidant activity too; hence enhancing the heart muscles through efficient energy storage, protection against free

radicals and increase nitric-oxide blood vessels relaxation (block arteries) [3, 4]. CoQ10 possesses tremendous benefits, thus its large scale production is of great importance. Numerous researches of synthetic, biosynthetic and improved biotechnological production are undergone. However, up to date no cost effective chemical production of CoQ10, except putative ways of natural producers microorganisms and few vegetable oils which gives few content (only 0.025 mg kg^{-1})[5, 6] since it was highly produced through fermentation engineering processes using various potent microorganisms and yeast [7]. Currently, pharmaceuticals and nutraceuticals are produced using natural producers' microorganisms and chemical synthesis [8]. The latter is commonly used because metabolic engineering may have imbalances in the production pathways due to the host's pathways manipulation through homologous or heterologous expression which may disturb the native carbon flux regulation pathway. The engineered strains for enhanced expression level of the mevalonate pathway enzymes would produce high amount of isoprenoids, a major source of CoQ10; but accumulating pathway intermediates able to limit molecules flux leading to the cell growth inhibition [9]. In this process, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) is the pathway intermediate which accumulates and in return causes the flux discontinuity. The modulation of HMG-CoA reductase assist the mevalonate pathway and consequently could reverse the imbalance flux in the engineered pathway [10, 11].

The *In vivo* biosynthesis of CoQ10 is mediated by an enzymatic cascade. Here, Decaprenyl Diphosphate Synthase (DPS) catalyze the synthesis of the decaprenyl tail with isopentenyl diphosphate group. The DPS condenses isopentenyl diphosphate and allylic diphosphate substrates [12]. A DPS encoding gene exists in *Schizosaccharomyces pombe, Aspergillus clavatus, Gluconobacter suboxydans, Leucosporidium scotti, Agrobacterium tumefaciens and Rhodobacter sphaeroides*. Among these microorganisms, *R. sphaeroides*, can produce CoQ with a side chain composed of ten isoprenoid units which is highly similar to human produced CoQ10 [1]. This review aims to focus on the production of CoQ10 by natural producing microorganisms, emphasizing on potent strains obtained through conventional mutagenesis and metabolic engineering followed by analysis and modification of the key metabolic pathways and optimization of fermentation strategies and culminating by giving the medical significance of CoQ10 usage in people with its deficiency.

2. Enhanced Production of CoQ10 Through Selection of Potent Strains

The selection of CoQ10-producing microorganisms, DPS gene cloning and identification and improvement of fermentation strategies are major prerequisites. Additionally, chemical mutagenesis of natural CoQ10 producers' bacteria improve the yield during fermentation process [13]. In strains selection process, the wild type bacteria of *A. tumefaciens* ATCC 4452, *R. sphaeroides* FERM-P4675 and *P. denitrificans* ATCC 19367 showed excellent CoQ10 production (Table 1). At this selection process, mutagenic compound-N-Methyl-N′-nitro-N-nitrosoguanidine combined with various chemicals cause selection pressure to L-ethionine (an analogue of L-methionine), which is a precursor for the methoxy moiety of coenzyme Q, daunomycin and menadinone (vitamin K3). *A. tumefaciens* AU-55 mutant strain, obtained after mutagenesis process by employing chemical treatment and resistance selection yielded 180 mg L^{-1} of CoQ10 in 58 h and 4.5 mg g^{-1} was specific CoQ10 content from dry cell weight (DCW). Similarly, this mutagenesis protocol offered a *R. sphaeroides* Co-22-11 mutant with 346.8 mg L^{-1} concentration yield of CoQ10 whereby a 8.7 mg g^{-1} DCW was obtained and both were produced in the Erlenmeyer flasks under simplified condition of less aeration and final product recovery using internal membranes without baffle plates [11, 14]. Moreover, investigation on the mutant *Pseudomonas diminuta* NCIM 2865, showed also that aeration and agitation affect CoQ10 production, volumetric mass transfer coefficient, glycerol utilization and biomass formation and this strain has capacity to produce CoQ10 up to 42.85 mg L^{-1} [15]. The current efforts associated to improved genetic, metabolic engineering and fermentation mechanistic steps ultimately proves at some extent beneficial effect in the gap refilling of highly needed CoQ10. For example, the process coupled fermentation-extraction has enhanced potentially the CoQ10 yield in *Sphingomonas sp.* It is of worth to notice that the chemical mutagenesis and other strain improvement strategies achieved good production. *A. tumefaciens* and *R. sphaeroides* are preferred candidates and the most characterized natural producers [16], thus its current continuous utilization in the CoQ10 production.

3. CoQ10 Biosynthetic Pathway

CoQ10, like all isoprenoids, is synthesized from common building units produced from two putative pathways namely mevalonate (MVA) and non-mevalonate (Non-MVA)/ methylerythritol phosphate (MEP). The isoprene subunits are built by two essential precursors namely isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) [17] via the MVA pathway also known as isoprenoid/HMG-CoA reductase pathway in both prokaryotic and eukaryotic cells are well characterized [18]. Through this pathway several biomolecules are made such as sterol isoprenoids like cholesterols, steroids-derived hormones; and non-sterol isoprenoids, such as heme-A dolichol, isopentenyl tRNA, vitamin K as well as ubiquinone are made from acetyl-coA. However, as an alternative to MVA pathway exists in plants, most bacteria, such as *E. coli* and *Mycobacteria tuberculosis* and some parasites like *plasmodium sp.* are capable to produce isoprenoids chains using Non-MVA or MEP pathway to produce high IPP and DMAPP, however the involved enzymes are totally different

[19, 20]. Thus, MEP pathway is considered since microorganisms such as *A. tumafaciens* and *R. sphaeroides* were reported as the potent CoQ10 natural producing strains. Both universal pathways produce IPP and DMAPP which serves as precursors of various cellular components including CoQ10 (Fig. 1). Taking into account the combination of those two pathways, tremendous content of CoQ10 is achieved by their manipulation in possessing or non-possessing organisms. However, both pathways coexist naturally only in plants; elsewhere the MVA can be found in archaea, animals depicting the critical role of using microorganisms because they may have either MVA or its alternative MEP pathway [21]. This is mediated by condensation reactions from initially produced pyruvate and glyceraldehydes-3-phosphate. In summary, the enzymatic cascade reactions in the MEP pathway consist of IPP and DMAPP synthesis through GAP substrates (Fig. 1 A). Next, several additional reaction steps such as MEP isomerization and 2-C-methyl-d-erythritol-2, 4-cyclodiphosphate synthesis occurs [22, 23]. Dxs enzyme initiate the MEP pathway [24] by which converts MEP to cMEDP through an enzymatic cascade of reaction such as 4-diphosphocytidyl-2-C-methyl-d-erythritol (CDP-ME) synthase, CDP-ME kinase, and 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate (cMEDP) synthase. During the first reduction reaction, the ring in the cyclic diphosphate opens for 1-hydroxy-2-methyl-2-buten-4-yl diphosphate (HDMAPP) generation, followed by the second reduction consisting of the removal of the hydroxyl group to obtain IPP and DMAPP mixture. The enzymatic cascade reactions mediate those molecule conversions. Here, the IspD enzyme transfers the CMP unit in CTP to the terminal phosphate moiety of MEP to generate CDP-ME. Kinase enzyme like IspE catalyzes phosphorylation of the C-2 hydroxyl group in CDP-ME to CDP-MEP, followed by the IspF-catalyzed cyclization to give MEcPP. This is followed by ispD conversion of MEcPP to HMB-PP which finally generates CoQ10 precursor isomers IPP or DMAPP via ispH enzyme [23, 25]. The IPP and/or DMAPP are both important precursors of CoQ10 and other related compounds like hemes and lipids (Fig. 2).

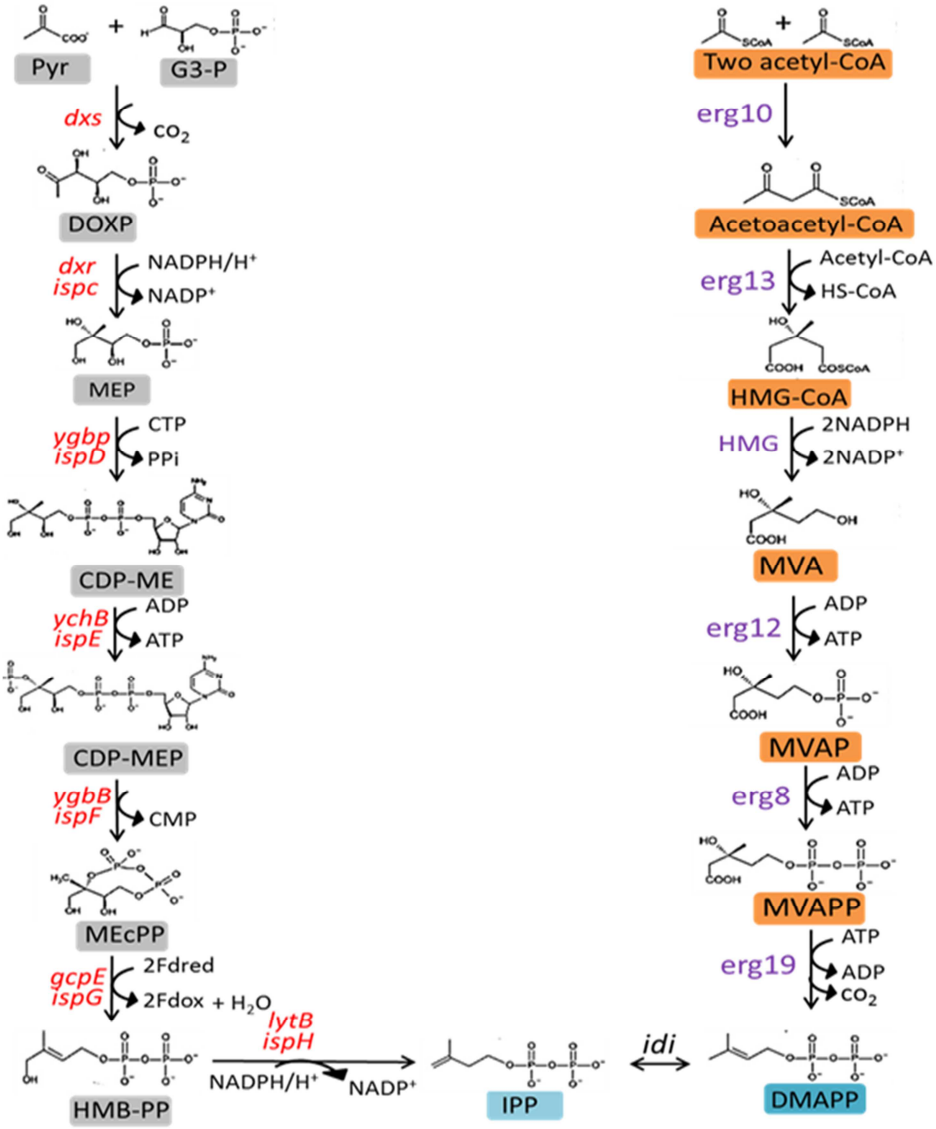

Figure 1. *Isoprenoid biosynthesis through MVA (left) and Non-MVA/MEP (right) pathways.*

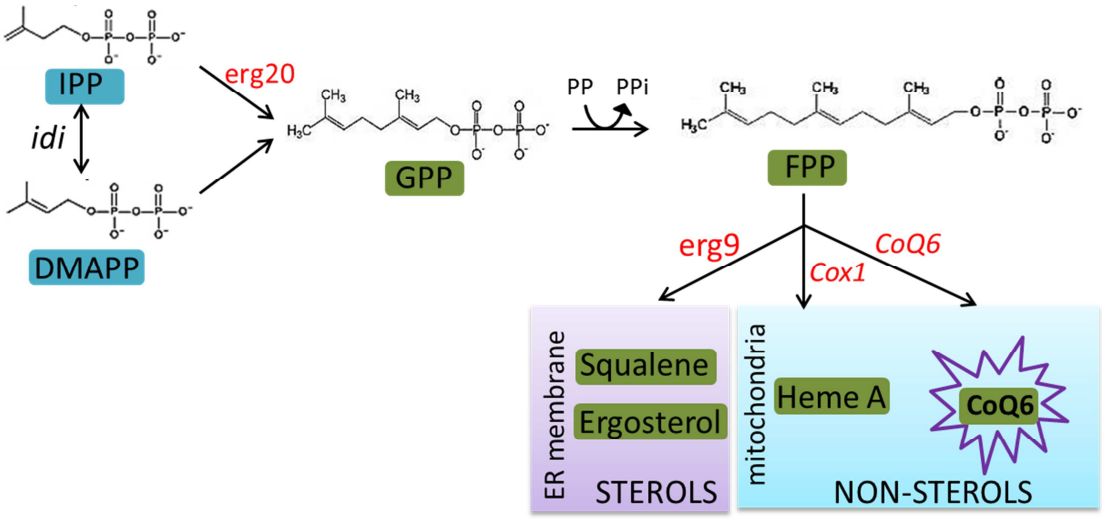

Figure 2. *The final stage of CoQ10 production in the Isoprenoid biosynthesis pathways.*

4. Influence of Over-Expression of Enzymatic Genes on CoQ10 Yield

CoQ10 has proved beneficial to people with heart failure, ageing problems and so on, thus its economic production of CoQ10 is highly required due to the pharmaceutical demands. Microorganisms and yeast are a major source of CoQ10. Conventional mutagenesis and metabolic engineering are mainly used to engineer potent producing strains which basically analyze and modify the key metabolic pathways [26]. Particularly, random mutants with drug resistance obtained by metabolic engineering techniques improve CoQ10 production [14]. Here generally, the yield is compared between the wild-type strains, chemically and/or genetically engineered recombinant strains. Owing to the sophistication and the elusiveness of coenzyme Q10 biosynthetic pathway, the efficient metabolic pathways design is highly needed. In another way, CoQ10 is produced by biotechnological techniques combining systematic approaches and expression of various genes enhancing metabolic pathways followed by the optimization of the fermentation key steps.

Most used CoQ10 microorganisms producers are *Agrobacterium tumefaciens, Rhodobacter sphaeroides, Rhodotorula glutinis* and *Cryptococcus laurentii*; though *A. tumefaciens* showed the uppermost UbiQ10 yield [27].

The in vivo catalytic activity of the enzyme (Rsdds) was further compared with that of *A. tumefaciens* (Atdds) in *E. coli*. The CoQ10 production and the relative amount of CoQ9 formation were respectively used as criteria for the evaluation of in vivo catalytic activity and product specificity of the enzymes. Accordingly, it was shown that the catalytic activity of Rsdds was less than that of Atdds; however, its product specificity was higher. The induction of pTrsdds in *E. coli* PTR resulted in a two times increase in CoQ10 production, whereas the induction of pTatdds in *E. coli* PTA decreased the CoQ10 production [28]. When the exogenous *dxs* gene was overexpressed, *A. tumefaciens* pGX11 exhibited a significant increase in UbiQ10 production and in the specific activity of Dxs.

Despite the recent accomplishments in metabolic engineering of *E. coli* cells for UbiQ production, production levels are not yet competitive with the levels presently produced by fermentation or isolation and much efforts are done through projects [29]. Although one of the major limitations for UbiQ production in *E. coli*, supply of isoprenoid precursors, can be addressed by metabolic engineering, solutions for the second major limitation, inadequate storage capability for lipophilic compounds, have yet to be found. Metabolic engineering of other microorganisms such as *A. tumefaciens*, which already synthesize significant amounts of UbiQ10 in their intracellular membranes, could provide an alternative route for the high production of UbiQ10. Although metabolic engineering of *A. tumefaciens* has not yet been explored widely, the strategy using engineered *A. tumefaciens* as a host for UbiQ10 production is promising as demonstrated in this report. However, Protein engineering on cis-prenyl transferases should also be investigated as the inhibition of cis-prenyl transferases could reduce the isoprenylated proteins production hence increase CoQ10 in mevalonate pathways and also biofilm reactor that can provide high cell concentrations, high productivity, and easy separation of the products could be determined from further research. The production of CoQ10 is improved through overexpression of the dxs gene encoding 1-deoxy-d-xylulose 5-phosphate (DOXP) synthase and reductoisomerase. This gene is important in the second step of the MEP pathway because it catalyzes the condensation reaction of carbon 2 and 3 of pyruvate and glyceraldehyde 3-phosphate respectively to yield DOXP (table 1). Moreover, the CoQ10 building block depends on one or two major components mainly quinone ring and/or prenyl tail; produced in there above pathways [13].

The recombinant *A. tumefaciens* could produce a high amount of CoQ10 more than the recombinant *E. coli* cells and the non-recombinant *A. tumefaciens*. This has been demonstrated during cloning of *dxs11* gene from the potential producer of CoQ10 (*A. tumefaciens* KCCM 10413) in *E. coli*

[30]. The overexpression of DPS and *ddsA* genes coding for decaprenyl diphosphate synthase in *E. coli* have unexpectedly shown a very low level of CoQ10 production compared to the level of industrial scale production. This is due to the fact that the recombinant *E. coli* produces CoQ10 in addition to CoQ8. Conversely, a recombinant *A. tumefaciens* pGX11 has shown a CoQ10 content with significant yield up to 8.3 mg g^{-1} DCW, final concentration (502.4 mg L^{-1}), and productivity (4.5 mgL^{-1} h^{-1}), relative whereas for *A. tumefaciens* KCCM 10413 the content was 6.3 mg/g DCW with concentration (412.3 mg L^{-1}) and productivity (3.7 mg L^{-1} h) when grown in the same conditions [22, 31]. Recently, the overexpression of the glyceraldehyde 3-phosphate dehydrogenase and subsequent addition of hydroxybutyrate to the *A. tumefaciens* culture media enhanced NAD/NAD+ ratio from 0.8 to 1.2 which resulted in yield increase up to 5.27 mg g^{-1} DCW [32]. Nevertheless these efforts in significant increase of CoQ10 production, still these modifications and strategy are insufficient to meet its demands thus other optimizations of conditions like in the fermentation process.

Rhodospirillum sp. showed the highest ability in CoQ10 production and *A. tumefaciens* exhibits the highest CoQ10 yield in a fed-batch fermentation process (table 2). 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^{-1} 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.

Table 1. *Level of CoQ10 production by natural producers through over expression of key genes encoding enzymes involved in non mevalonate pathways.*

Microorganism	Over expresseion gene	Coenzyme Q10 concentration (mgL^{-1}) before gene overexpression	Coenzyme Q10 content (mgL^{-1}) before gene overexpression
A. tumefaciens pGX11	*Dxs*	412.3	6.3
A. tumefaciens	GAPDH	-	-
R. sphaeroides	-	65.8 mgL^{-1}	47.6 mgL^{-1}
E. coli BL21 (DE3)ΔispB/pAP1	*Dxs*	0.55–0.89mgg^{-1}	1.40mgg^{-1}

Table 1. *Continue.*

Microorganism	Coenzyme Q10 concentration (mgL^{-1}) after gene overexpression	Coenzyme Q10 content (mgL^{-1}) after gene overexpression	References
A. tumefaciens pGX11	502.4	8.3	[22]
A. tumefaciens	211	5.1	[32, 33]
R. sphaeroides	-	-	[34] [14, 35]
E. coli BL21 (DE3)ΔispB/pAP1	-	-	[36]

5. Optimization Conditions in Fermentation Process for CoQ10 Production

After producing a potent strain, fermentation process and fermentor parameters optimization namely oxidation–reduction potential (ORP), temperature, carbon/nitrogen and oxygen ratio demonstrated a significant increase of CoQ10 yield since these factors are important in allowing mutant like *A. tumefaciens* and *R. sphaeroides* welfare [23].

The optimization of temperature at 32–34°C, carbon/nitrogen ratio like 8% sugar and 0.16–0.26% ammonium nitrogen respectively in the *Agrobacterium sp.* mutant strain can display high yield and reduced viscosity of the culture broth owing to extracellular polysaccharides production. This mutant exhibit an optimal oxygen absorption rates (OAR) of 0.84 mmol O_2 L^{-1} min^{-1} to generate 66 mg L^{-1} of CoQ10 whereas the use of 0.58 mmol O_2 L^{-1} min^{-1} yielded 3.2 mg g^{-1} DCW of CoQ10. Though, the low OAR levels showed slow cell growth, it suggests the optimization of OAR. Through comparison of specific respiratory rate and ORP, a mutant strain *Rhodopseudomonas sphaeroides* KY 8598 was investigated for. The optimum conditions obtained for high cell-growth rates were over −150 mV and −200 mV for high specific production rates. The use of −200 mV (ORP) in the last phase of fermentation, produced 770 mg L^{-1} of final concentration with 14 mg g^{-1} DCW specific content within 150 h of fermentation in an 80,000 L industrial fermenter [14, 23].

As the microbial fermentation is the most practical method for CoQ10 production owing to its cost effective and high yield. Various species of Sphingomonas, Agrobacterium, Paracoccus, Pseudomonas and Rhodobacter have been employed, however, *A. tumefaciens* possess tremendous yield of CoQ10 in fed-batch fermentation reactor [22, 37]. Both batch fermentation and fed-batch fermentation yield depend on cell growth. In batch fermentation, the expression of the low copy number plasmid harboring ddsA gene generates 0.97 mg L^{-1} of CoQ10 content while the fed-batch fermentation with high load of *E. coli* BL21/pACDdsA ~103 g L^{-1} enhanced the CoQ10 yield up to 25.5 mg L^{-1} and 0.67 mg L^{-1} h^{-1} of productivity. This improved the production up to 26.0 and 6.9 times respectively when compared to fed-batch fermentation [13]. Furthermore, the yield has been enhanced by the usage of conventional reactors like the three-phase fluidized bed reactors (TPFBRs) because of its low cost and high efficiency. *Sphingomonas sp.* cells produce CoQ10 via fermentation-extraction coupled process. Moreover, the CoQ10 yield was boosted via a conversion–extraction coupled process with gel-entrapped cells of *Sphingomonas sp.* [37, 38].

The mixture of the two phases are mixed in the bioreactor could reach the CoQ10 content of 441.65 mgL^{-1} after 152 h in the organic phase when fed-batch is used. Interestingly, this method simplifies downstream process i.e. recovery and purification of end products, which reduces the cost. Additionally, the combination of conversion–extract with TPFBR becomes crucial in the improvement of CoQ10 yield. However, until now its production remains moderate (Table 2). Though the TPFBR similarity to CITP in CoQ10 production, the TPFBR can be used both alone and/or also with CITP inclusion. Only TPFBR use could accumulate CoQ10 which is separated from the organic phase. Thus the fermentation process is enhanced by this kind of reactor not only through yield improvement but also alleviates the drawbacks of flask culturing. However, other optimizations of more frequently reported drawbacks of TPFBR like the gel beads malformation of calcium alginate during the conversion process. Intriguingly this could be lessened by the use of cell immobilization technology; though, it is also observed that immobilized cells may lose activity after long period usage. Additionally, the TPFBR is troubled by the decrease of the condensation efficiency which could result on organic phase evaporation. Qiu *et. el.* suggests that the use of Butylated Hydroxytoluene (BHT) precursor and/or in combination with solanesol which works as isoprene units provider and also with improved potent strain producer can boost the CoQ10 production in the TPFBR [37]. Therefore, the combination of all those factors and the use of decent biofilm reactor could be of high importance.

Other extrinsic factor like temperature on CoQ10 production has been reported in a 1-l reactor culture of *R. sphaeroides* mutant cells. The latter becomes green in the fermentation media as protective mechanism against menandinone byproducts which are likely to inhibit wild type strains. For that reason, most methods rely on mutant strains in conjunction with optimized conditions. The temperature and dissolved oxygen (DO) level control in fermentation are frequently characterized by a significant cell growth up to 24 h followed by a gradual growth decrease. However, the DO level can reduce due to foaming in the reactor; which causes its dropping up to 0.9 mg L^{-1} in 3 to 4 h. Nevertheless, it could be retained below 1.0 mg L^{-1} up to 24 h of fermentation, and being aeration-free with the slow increase. More specifically, the stirrer speed adjustment could be better than aeration control and the optimized DO of 10-20% could be enough. In this experiment the high yield of CoQ10 was also achieved dependently on the maximum dry cell weight and optimized temperature. Here 30°C was obviously reliable condition due to its displayed yield up to 4.66 mg/g DCW. Other findings revealed that *Rhodopseudomonas gelatinosa* is better grown at 35°C could in CoQ10 production; however, this temperature increase over 30°C affected the yield [39]. This depicts variability of photosynthetic bacteria, thus different working conditions in the fermenter. The maximal Q10 production reached 4.66 mgg^{-1} (DCW) with CoQ10 productivity of 0.569 $mgL^{-1}h^{-1}$ which is found to have the same yield as *R. sphaeroides* [40]. However, it was revealed that consecutive experiments on same mutant could not always give high [41]. Principally, this is caused by mutant reversion when compare to the wild type. Though some experiments showed insignificant reduction in yield for better tackling this major bottleneck of productivity [16].

Next, one should bear in mind that the initial rate of aeration is crucial in CoQ10 production mainly by controlling the DO level during fermentation. When the *R. sphaeroides* is cultured in high yield fermenter, the desired production should be proportional to the invested aeration rates (Table 2). The yield of CoQ10 together with the aeration and DO rate and cell growth rate follow the same trend. Taking into account the balance of these conditions, 6.34 mg g^{-1} of CoQ10 content was produced with 0.798 mg/L^{-1} per hour at a 2vvm aeration rate. This depicts the crucial importance of air flow rate increase targeting high yield by even surpassing the 1L fermenting reactor. This was due to the limited air-flow rate found in small and medium reactors compared to larger ones; though an additional increase of the initial aeration rate of 1 vvm didn't improve the productivity in a 150 L, contrary reduced followed by low DCW. This depicts the effect of high aeration on the cell growth since the DCW reduced, this may be attribute to high aeration stress on cells, which leads to a low CoQ10 productivity [16]. This is consistent with previous reports showing that the increase of aeration rate induces cell growth, but less productivity of CoQ10 [16, 42].

During batch fermentation process, the best conditions in a 150L reactor were optimized. First, the fermentation was initially done at 30°C, 200 rpm, 2 vvm and 50 lux followed by rapid decrease of the DO level in the reactor to 1.5 mg L^{-1} within 3 h, and then further decreased to 0.6 mg L^{-1} around 7 h which resulted foam. At an early phase of cell growth, the DO level decreased enormously in 150L reactor than in a 1L reactor, indicating the need of a high oxygen level in larger reactors at early log phase. During the log phase (6-8hours), the DO level was maintained at a low rate (less than 1.0 mg L^{-1}) and it seemed increasing slightly after one day in absence of aeration suggesting that *R. sphaeroides* requires oxygen as a facultative microbe [16].

In addition, the PH condition is a crucial point. The previously described study of high yield production of CoQ10 (6.34 mgg^{-1} DCW) showed that the increase of pH up to 9.35 during 24 h of fermentation, which decreased to some extent in the following hours. In the first 24 hours, the pH increase is directly proportional to the cell increase in lower alkaline environment; though it is followed by little decrease. This could affect the production depicting that the slight decrease of pH could affect the yield. This may be associated with primary metabolite produced in late growth phase and is consistent with other finding on *Rhodobacter sp.* as well [43] showing that CoQ10 is largely produced during the log phase, hence, the harvest of *R. sphaeroides* mutant cells should be done at the late-exponential growth phase to prevent undesired metabolites.

The scale-up in a bioreactor is of great importance and

depends most mainly on the bioreactor conditions and type of producing strain. In view of that, various main factors like oxygen should be adjusted for high yield improvement. Through active fermentation, the CoQ10 high yield were achieved when poorer conditions of aeration-agitation are applied, showing significant detrimental effect of high DO on cell and CoQ10 yield in *A. tumefaciens* [29], *Rhizobium radiobacter* [44], and *R. sphaeroides* [45]. Therefore, it is recommended to culture *R. sphaeroides* in obscurity at 0% DO for CoQ10 scale-up, however the aeration should be maintained sufficiently at early phase and being shifted late exponential phase of cell growth at of the growth [16].

During fed-batch fermentor, Kien *et. al.* adjusted the pH, DO, DCW, and monitored the CoQ10 yield every 12 hours up to 70 h. After feeding 10 L of fresh culture medium to the 150 L fermenter, they maintained Cell activity. This study achieved specific CoQ10 content of 8.12 mg g^{-1} DCW in 70 h with increased DCW which is greater than corresponded to 1.28 times more than that achieved from batch operation. However, the increase of CoQ10 production in the fed-batch fermentation was not high, compared with the result of fed-batch culture by Ha et al. (1.3 times higher) or Gu et al. (3.5 times higher), which was carried out in laboratory-scale fermenters. This was caused probably by difficulty of scale-up and/or feeding strategy in fed-batch operation [16].

In most studies, *R. sphaeroides* was found to be able to grow photosynthetically under strictly anaerobic conditions and aerobically in either light or dark conditions, which may lead to make scale-up of the CoQ10 fermentation difficult. This is because the main bottleneck in scale-up of phototrophic fermentation has been found to be the low efficiency of light energy conversion to the desired product, which is caused by an excessive dissipation of light energy to heat. Even though photosynthetic bacteria have been diversely applied, especially to the hydrogen production to meet the increasing demand for energy in recent years, a few of their pilot-scale studies have been reported to date. Although an interest in CoQ10 has been recently renewed due to the growing demands of the pharmaceutical industry, a few scale-up studies of the CoQ10 fermentation have been conducted using microorganisms other than photosynthetic bacteria: optimization of culture conditions and scale-up to pilot and plant scales using *A. tumefaciens* and statistical optimization of culture conditions and operation parameters in a 150 L fermenter using *Paracoccus denitrificans*. As a result, a pilot scale fermentation of *R. sphaeroides* is necessary to verify its potential for commercial CoQ10 production. The identified high-coenzyme-Q10-producing *R. sphaeroides* mutant of an isolated strain were deposited into the Korean Agricultural Culture Collection (KACC) as *R. sphaeroides* KACC 91339P. Therefore, in this study, a scale-up fermentation was attempted from a 1L fermenter to a 150L fermenter, upon which the optimum fermentation conditions and operation parameters were investigated for high CoQ10 production using a mutant strain of *R. sphaeroides* [16].

Table 2. *Production of CoQ10 by potent strains through various improved techniques.*

Process	Microorganism	CoQ10 yield, mgL^{-1}	Specific CoQ10 yield, mgg^{-1} DCW*	Working volume, time	Volumetric productivity, $mgL^{-1}h^{-1}$	References
Fed-batch	*P. denitrificans*	-	1.63	1L, 120 h	-	[46]
Fed-batch	*A. tumefaciens*	71.5	2.1	2 L, 96 h	0.74	[47]
Fed-batch	*A. tumefaciens*	626.5	9.25	160 L, 120 h	5.22	[48]
Fed-batch	*A. tumefaciens*	562.3	9.1	2.8 L, 96 h	5.86	[49]
Fed-batch	Recombinant *E. coli*	25.5	0.247	1 L, 38 h	0.67	[13]
Fed-batch	Recombinant *E. coli*	99.4	1.41	33 h	3.01	[36]
Fed-batch	Recombinant *A. tumefaciens*	30.8	1.38	3 L, 180 h	0.17	[37, 50]
Fed-batch	Recombinant Fission yeast	23	0.403	3 L, 90 h	0.26	[37, 51]
Fed-batch	*R. sphaeroides*	770	14.5	30 L, 150 h	5.13	[52]
Fed-batch	*Sphingomonas sp.*	1.14	0.48	0.15 L, 30 h	0.04	[53]
CFEP**	*Sphingomonas sp.*	43.2	32.5	0.15 L, 30 h	1.44	[37]
CITPS*** (free cell)	*Sphingomonas sp.*	60.8	40.6	0.15 L, 8 h	7.6	[54]
CITPS (entrap cell)	*Sphingomonas sp.*	51.6	38.5	0.15 L, 8 h	6.45	[54]
*^{v}CITPS (entrap cell in TPFBR)	*Sphingomonas sp*	380-441.65	70.37-81.67	0.200 L, 80-144h	3.07-4.75	[37]

*DCW: dry cell weight, **CFEP: coupled fermentation–extraction process, ***CITPS: conversion in two-phase system and *^{v}TPFB: three phase fluidized bed reactor.

6. Clinical Significance of CoQ10 and Its Associated Deficiency Diseases

CoQ10 is phenolic compound and has antioxidant activity. It endows cells protection against reactive oxygen species and prevents superoxide anions accumulation in the aerobic respiration [55] and the lipid oxidation via its transportation in the blood with LDL cholesterol [56, 57].

In several organisms, the CoQ10 deficiency is triggered by mutation occurring in the CoQ biosynthetic pathway which consequently leads to low CoQ10 levels. The latter is directly associated with different ailments such as cerebellar ataxia Leigh syndrome, and encephalomyopathy [7] and other

mitochondrion-linked dysfunctions [58].

For example, CoQ10 reduces superoxide anion levels and aging in *Caenorhabditis elegans* through lowering of oxidative stress [59]. Similarly, in *S. cerevisiae,* COQ3, but not CoQ5 cause

deficiency in CoQ10 synthesis which render the yeast hypersensitive to lipid peroxide; however, the expression of a COQ3 gene within a single-copy plasmid rescue the strain [60]. Moreover, when added in cosmetics products, CoQ10 blocks oxidants present on the skin or in another [14].

CoQ10, as a mitochondrion coenzyme plays a critical role in the synthesis of ATP. The CoQ10 deficiency mediates different organs ailments mainly cells requiring high energy. The disruption of CoQ5-depending proteins complex reduces the total content of CoQ10 resulting in the mitochondrion-energy deficiency and membrane integrity weakening; interestingly, the reconstitution supplementation reverse the case to normal [61]. In cardiology, the intravenous injunction of CoQ10 induces intensification in NO-linked blood vessels relaxation [3].

More importantly, several trials in the treatment of cancer [62, 63], Alzheimer and Parkinson's diseases [64], mitochondrion dysfunction [58, 65], and refractory corneal ulcers [66, 67] have been undertaken. Owing to its broad application as shown by [68], various techniques of CoQ10 delivery in the body are developed to enhance its stability and targeting the specific area of treatment. Due to the CoQ10 unique characteristics like high redox activity and lipophilicity; the CoQ10-micelle loaded system is developed which is stable at temperature below 25°C and 24 months lifespan [69].

7. Conclusion and Future Perspectives

Owing to the beneficial role of CoQ10 much effort in CoQ10 production have been intensified. To date, the main techniques for its production are based on biosynthesis pathway manipulation by mutagenesis and inhibitor selection of strains which doesn't satisfy CoQ10 high demand. One solution is the wide exploration of CoQ10 production mediating enzymes. For example, the cloning of different enhancing genes from different organisms like DPS1 gene cloning, but not dlp1, from various fungi showed the coenzyme production improvement [70]. However, to date, the much better yield by two fold increase is obtained when there is a combination of expression of CoQ specific producing genes (which give rise to ~10%) and overexpression of genetically modified MVA-Non-MVA pathways intermediate enzymes such as HMG-Coenzyme reductase, 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase and chorismate lyase [71]. This could be adopted alongside with the metabolic engineering of natural producers like *A. tumefaciens* and *R. sphaeroides* followed by the fermentation parameters optimization obtained high yield; however *A. tumefaciens* exploration is still elusive. Moreover, all strains are not malleable and very conducive like natural producers providing an open question for more exploration of CoQs producing genes in various organisms. The last but not least, so far only a couple of methods are reported in the delivery of CoQ10 to the organ of interest; depicting that it is still needed more attempts of metabolic engineering, condition optimization for specific high content of CoQ10 in recombinant hosts and rational design of more and feasible delivery systems.

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List of Abbreviations

Dxs: 1-deoxy-d-xylulose 5-phosphate synthase
HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA
IPP: isopentyl pyrophosphate
FPP: fanesyl pyrophosphate
Idi: isopentyl diphosphate isomerase
MVAP: mevalonate-5 phosphate
MVAP: mevalonate-5 pyrophosphate
DMAPP: dimethylallyl pyrophosphate
IPP: isopentyl pirophosphate
HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA
CoQ10: coenzyme Q10
UQ: ubiquinone
ROS: reactive oxygen species
HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A
DPS: decaprenyl diphosphate synthase
DCW: dry cell weight
MEP: methylerythritol phosphate
DMAPP: dimethylallyl pyrophosphate
cMEDP: 2-c-methyl-D-erythritol 2, 4-cyclodiphosphate synthase
HDMAPP: 1-hydroxy-2-methyl-2-buten-4-yl diphosphate
DOXP: 1-deoxy-d-xylulose 5-phosphate synthase
ORP: oxidation–reduction potential
OAR: oxygen absorption rates
TPFBRs: three-phase fluidized bed reactors
BHT: butylated hydroxytoluene
DO: dissolved oxygen
KACC: Korean agricultural culture collection
CFEP: coupled fermentation–extraction process
CITPS: conversion in two-phase system
TPFB: three phase fluidized bed reactor

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