Advance Journal of Food Science and Technology 17(3): 48-53, 2019
DOI:10.19026/ajfst.17.6012
ISSN:2042-4868; e-ISSN: 2042-4876

Submitted: March 6, 2019 Accepted: April 01, 2019 Published: May 25, 2019

Research Article

Production of Functional Coenzyme Q_{10} from Genetic Engineered *Rhodobactersphaeroides*

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Abstract: The aim of this study was to enhance the Coenzyme Q_{10} (CoQ_{10}) production from *Rhodobactersphaeroides*. CoQ_{10} acts as an electron carrier and plays an important role in the aerobic respiration for the production of ATP. CoQ_{10} has been proven to be effective for treatment of human diseases and widely used in food and pharmaceutical industry. In the present study, the *ubiE* gene located in CoQ_{10} biosynthesis pathway was overexpressed in *Rb. sphaeroides* under strong *puf*operon promoter and micro-aerobic growth conditions to enhance CoQ_{10} production. The growth curve implied that effect of overexpression of *ubiE* on the growth of host cells was slight. The crude CoQ_{10} production was enhanced by 80.61%, which was increased much higher than reported literature. The *ubiE* mRNA level was significantly increased compared to the wild type harboring empty vector as measured by quantitative RT-PCR. Furthermore, the crude CoQ_{10} possessed strong hydroxyl radical scavenging activity as measured in *vivo* by zone of inhibition assay. The in *vitro* assay indicated that the crude CoQ_{10} exhibited much stronger hydroxyl radical scavenging activity than normally used antioxidant vitamin C.

Keywords: Anti-oxidation, CoQ_{10}, genetic engineering, hydroxyl radical *Rb. sphaeroides*, scavenging activity, *ubiE*

INTRODUCTION

CoQ_{10} (2, 3-dimethoxyl, 5-methyl, 6-decaisoprene parabenzoquinone), also referred as to ubiquinone 10, is a lipid-soluble material wide-spread in the plasma membrane of prokaryotes and inner mitochondrial membrane of eukaryotes. It has been proposed that CoQ_{10} has multiple functions, including transferring electron in the electron transport chain of aerobic respiration (Garrido-Maraver *et al*., 2014; Lenaz *et al*., 2007). Furthermore, CoQ_{10} is a well-known anti-oxidant since it can scavenge free radicals, which are generally harmful to phosphate lips, proteins and DNA (Cluis *et al*., 2007; Rizvi *et al*., 2015). CoQ_{10}has been used to treat and prevent many diseases, including Parkinson's disease, Alzheimer's disease and mitochondrial diseases (Cluis *et al*., 2007; Hargreaves, 2014; Negida *et al*., 2016). CoQ_{10} becomes a good additive for functional foods because of its excellent anti-oxidant activity (Lee *et al*., 2013). However, external source of CoQ_{10} is required because dietary uptake for CoQ_{10} will be limited. Furthermore, endogenous synthesis in the body will be decreased with the age and under various diseases. With the applications of CoQ_{10} in many fields, demands for the CoQ_{10} are growing very rapidly and solutions for enhancing CoQ_{10} production have being tried by researchers.

Currently, CoQ_{10} is mainly produced by three approaches, chemical synthesis, semi-chemical synthesis and microbial fermentation. Among which, the microbial fermentation approach is becoming more and more popular due to the advantages that the bio-produced CoQ_{10} compared to chemical synthesis. It is thus that the microbial fermentation is attracting more and more attentions from researchers. At the beginning, much attention has been paid to the construction of high-yield microbial producer by metabolic engineering *E. coli* strains. However, genetic engineered *E. coli* strains could not enhance the production of CoQ_{10}efficiently because of low fermentation titer and emergence of byproduct like CoQ_8 and CoQ_9 (Cluis *et al*., 2012; Park *et al*., 2005).

Among all the microbial producers employed for natural CoQ_{10} production, *Rb. sphaeroides* is one of the most promising microorganisms (Zahiri *et al*., 2006; Zhu *et al*., 2017). The whole genome of *Rb. sphaeroides* has been completely sequenced, which greatly benefits the researches on the enzyme encoding genes involved in the CoQ_{10} biosynthesis pathway. Biosynthesis of CoQ_{10} in *Rb. sphaeroides* generally includes three pathways, the 2-C-Methyl-D-Erythritol 4-Phosphate (MEP) pathway, the shikimate pathway and the Quinine Modification Pathway (QMP). Researches have been intensively performed and progress on

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improving CoQ_{10} production in *Rb. sphaeroides* has been made in the past several years. However, the performed researches were mainly focused on optimization of fermentation procedures. UbiE is a methyltransferase, participating in catalyzing 2-Decaprenyl-6-methoxyphenol into 2-Decaprenyl-3-methyl-5-hydroxy-6-methoxy-1, 4-benzoquinone (Lu *et al*., 2015).

On the other hand, *Rb. sphaeroides* has been an excellent model for studying both photosynthesis and membrane development (Kiley and Kaplan, 1987). The *puf*operon and *puc*operon encodes light-harvesting complexes 1 (LH1), Reaction Center (RC) and LH2, respectively (Hu *et al*., 2002). Both *puf* and *puc* promoters are strong, which are regulated by oxygen tension (Hu *et al*., 2010). A strong promoter and optimal growth conditions are very important for the production of CoQ_{10} in genetic engineered *Rb. sphaeroides*.

Currently, enhancement of CoQ_{10} in *Rb. sphaeroides* by overexpression of *ubiE* under strong *puf*operon promoter and micro-aerobic growth condition has not been reported. In the present study, we overexpressed the *ubiE* in *Rb. sphaeroides* under strong *puf*operon promoter at micro-aerobic growth condition. The production of CoQ_{10} from the genetic engineered strain was enhanced by 80.61%, which was increased much higher than reported literature (Lu *et al*., 2015). Our study will promote the application of *Rb. sphaeroides* for large scale production of functional CoQ_{10}.

MATERIALS AND METHODS

Bacterial strains and growth conditions: *Rb. sphaeroides* strains were grown at 30°C in malate minimal medium (Remes *et al*., 2014). Growth under micro-aerobic conditions was performed as described in our previous study (Hu *et al*., 2010). *E. coli* strains were grown aerobically at 37°C in Luria-Bertani medium. Antibiotics were added to the growth media at the following concentrations when necessary: 200 μg/mL ampicillin, 20 μg/mL tetracycline for *E. coli* and 1.5 μg/mL tetracycline for *Rb. sphaeroides*.

Construction of expression vector: The *ubiE* was first amplified from *Rb. sphaeroides* genomic DNA by PrimeSTAR HS DNA polymerase (TAKARA) with the primers of *ubiE*-F (5'-CGGGATCCATGAGCGACGA AACTTCC-3') and *ubiE*-R (5'-GGGGTACCTCAGAT CTTCCAGCCGG-3') and ligated into cloning vector pMD18-T (TAKARA). After sequence, the pMD18-*ubiE* plasmid was digested by *Kpn*I and *Bam*HI and the *ubiE* fragment was subsequently purified by gel extraction and ligated into pRK*puf* (Hendrischk *et al*., 2009) digested by *Kpn*I-*Bam*HI, resulting in the pRK *ubiE* overexpression vector.

Construction of the genetic engineered strain: The constructed plasmid DNA was mobilized into *Rb. sphaeroides*2.4.1 by using *E. coli* S17-1 as the donor as described in our previous study (Hu *et al*., 2010).

Determination of the high yield CoQ_{10} genetic engineered strain: After conjugation, three colonies of the conjugant were selected and cultured respectively in three 50 mL-flasks containing 40 mL of malate minimal media with 1.5 μg/mL tetracycline and grown under micro-aerobic conditions in the dark at 30°C until OD_{660} reached approximately 0.6. Pre-cultures were respectively inoculated into three 100-mL flasks containing 80 mL of malate minimal media with 1.5 μg/mL tetracycline at the ratio of 1% and grown under micro-aerobic conditions in the dark at 30°C for 48 h. Crude CoQ_{10} was extracted from the cell cultures and quantified as described by Chen *et al*. (2006), respectively.

Quantitative RT-PCR: Total RNA for qRT-PCR was isolated from cell cultures using the Tiangen Bacteria RNA Isolation Kit (#DPN430) as described by the manufacturer. mRNA from genetic engineered strain and wild type strain harboring empty vector was considered sample mRNA and control mRNA, respectively. To further confirm the absence of DNA, PCR was performed targeting *gloB* (RSP_0799). qRT-PCR was performed as described previously (Remes *et al*., 2014) in a Bio-Rad CFX96 Real Time system by using the primers, *ubiE*-real-F (5'-GTTCCGCTTCCTCAAGCG-3') and *ubiE*-real-R (5'-GACGTCGAACGTGTTCGA G-3'). Relative mRNA expression levels were normalized to the reference gene *rpoZ* (Zeller *et al*., 2007) according to the formula given by Pfaffl (2001).

Hydroxyl radical scavenging activity: Hydroxyl radical was generated by the Fenton system (Sachindra *et al*., 2007). The A_0 reaction mixture contained 1, 10-Phenanthroline monohydrate (1 mL, 0.75 mM), PBS solution (3 mL, pH7.4, 0.2 M), H_2O (1 mL), $FeSO_4$ (1 mL, 0.75 mM), H_2O_2 (1 mL, 0.01%). The A_1 reaction mixture was the same as A_0 reaction mixture without the H_2O_2, which was replaced by H_2O. However, the A_2reaction mixture was the same as A_0 reaction mixture without 1 mL of H_2O, which was replaced by various concentrations of crude CoQ_{10} or vitamin C. All the reaction mixtures were incubated at 37°C for 60 min. The OD was measured at 510 nm and the results were used to calculated the scavenging percent as follows:

$$\text{Scavenging (\%)} = [(A_2-A_0)/(A_1-A_0)]\times 100\%$$

Zone of inhibition: Zone of inhibition assay was performed as described in our previous study (Zhao *et al*., 2018). Filters soaked with 5 μL of 700 mM H_2O_2 were placed on the top of the plates.

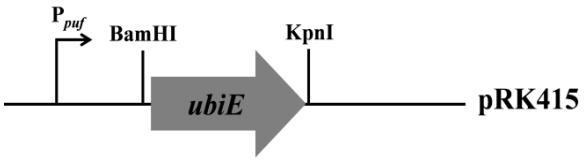

Fig. 1: Construct of *ubiE* overexpression vector. The *ubiE*gene was activated by the strong *puf*operon promoter from *Rb. sphaeroides*

Data analysis: All experiments were repeated three times. Turkey test and GraphPad Prism software were used to analyze the data trend.

RESULTS AND DISCUSSION

Construction of the expression vector: The expression vector used in this study was constructed as shown in Fig. 1. The UbiE is an important catalyzed enzyme in the ubiquinone pathway for the biosynthesis of CoQ_{10} in *Rb. sphaeroides* (Lu *et al*., 2015), which is a dimethyl menaquinone methyltransferase, involved in catalyzing 2-Decaprenl-6-methoxyphenol into 2-Decaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquione. *puf*operon encodes the LH1 and reaction center in *Rb. sphaeroides* (Eisenhardt *et al*., 2018; Gong and Kaplan, 1996). The *puf*operon promoter is a strong promoter responsible for two transcripts, a 2.7-kb transcript for *pufBALMX* and a 0.5-kb transcript for *pufBA* (Gong *et al*., 1994). Under the micro-aerobic growth conditions, the *puf*operon promoter exhibits stronger activity. On the other hand, the pRK415 vector (Billenkamp *et al*., 2015) is a broad host range expression vector. Consequently, the *ubiE* will be efficiently expressed in *Rb. sphaeroides* under the strong *puf*operon promoter grown at micro-aerobic growth conditions.

Construction of the genetic engineered strain for overexpression of UbiE: The constructed expression vector was mobilized into *Rb. sphaeroides* by conjugation via S17-1 as the donor. To test whether overexpression of UbiE affects the growth of the host cells, the growth curves for wild type 2.4.1, 2.4.1/pRK*puf* and 2.4.1/pRK*ubiE* were constructed as shown in Fig. 2. It was obvious that significant differences between the three different strains were not observed. Especially for the first 24 h, the growth curves were nearly overlapped, suggesting that the three different strains were grown at the same rate. In the following 12 h, the growth of all the strains nearly stayed the same tendency. It has been suggested that the CoQ_{10} plays indispensable roles not only in energy generation but also in many other processes that are important for cell's survival (Zahiri *et al*., 2006). However, in the present case, production of CoQ_{10} probably did not affect the growth of the host cells.

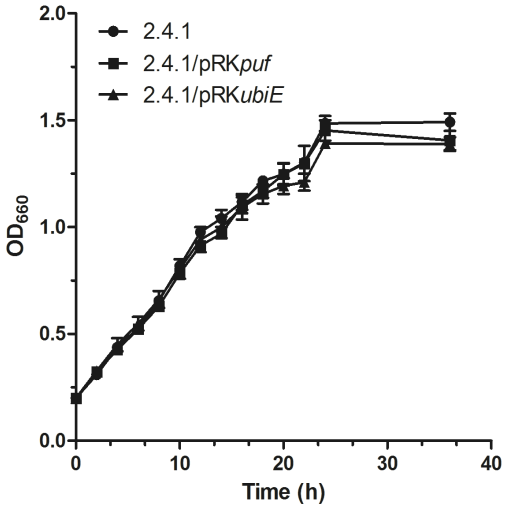

Fig. 2: Growth curve of wild type 2.4.1, 2.4.1/pRK*puf* and 2.4.1/pRK*ubiE*. Effect of overexpression of *ubiE* on the growth of host cells was minor

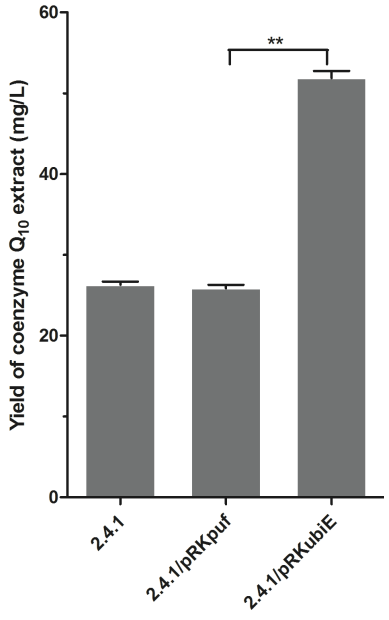

Fig. 3: Quantitative measurement of crude CoQ_{10}. The content of crude CoQ_{10} from wild type 2.4.1, 2.4.1/pRK*puf* and 2.4.1/pRK*ubiE* was 26.305, 25.869 and 51.887 mg/L, respectively; **indicates a highly significant change ($p \leq 0.01$)

It is reasonable to demonstrate that overexpression of *ubiE* gene do enhance the CoQ_{10} production in *Rb. sphaeroides*. Consequently, crude CoQ_{10} was extracted from the genetic engineered strain, as shown in Fig. 3. It was evident that production of crude CoQ_{10} from the genetic engineered strains 2.4.1/pRK*ubiE* was increased a lot compared to the wild type strain 2.4.1 and 2.4.1/pRK*puf*. The production of crude CoQ_{10} from the 2.4.1 and 2.4.1/pRK*puf* was nearly the same,

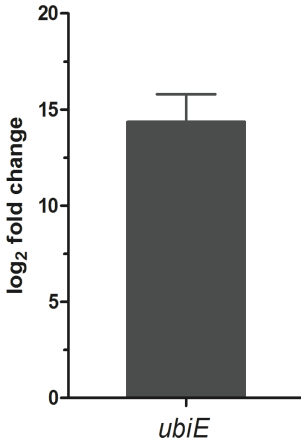

Fig. 4: Quantitative analysis of *ubiE* mRNA expression levels. The *ubiE* expression level in 2.4.1/pRK*ubiE* was significantly increased with log_2 fold change of approximately 13 compared to that in the 2.4.1/pRK*puf*. mRNA from 2.4.1/pRK*ubiE* and 2.4.1/pRK*puf* was considered sample and control mRNA, respectively

indicating that expression of the empty vector did not affect the production of crude CoQ_{10}. Compared to the wild type, the CoQ_{10} production in 2.4.1/pRK*ubiE*, was increased by 80.61%, which was much higher than the reported study (Lu *et al*., 2015). It can be suggested that overexpression of the *ubiE* lead to the obvious enhancement of the CoQ_{10} production under the strong *puf*operon promoter and micro-aerobic growth conditions, suggesting the strong activation by the *puf*operon promoter (Lu *et al*., 2015). On the other hand, the expression vector used in this study is a broad-host range vector to a wide range of Gram-negative bacteria.

Quantitative RT-PCR analysis for *ubiE* gene: To further test the reasons caused the obvious enhancement of CoQ_{10} from the genetic engineered strain 2.4.1/pRK*ubiE*, qRT-PCR was employed, as shown in Fig. 4. As expected, the *ubiE* mRNA level was significantly increased, with log_2 fold change of approximately 13. UbiE is a methyltransferase, participating in catalyzing 2-Decaprenyl-6-methoxyphenol into 2-Decaprenyl-3-methyl-5-hydroxy -6-methoxy-1, 4-benzoquinone (Lu *et al*., 2015). The UbiE expression level should be increased because of the increased *ubiE* mRNA expression levels and CoQ_{10} production.

Hydroxyl radical scavenging activity: From the in *vivo* experiment of zone of inhibition, it could be concluded that the strain 2.4.1/pRK*ubiE* possessed much higher hydroxyl radical scavenging activity than that of the control strain 2.4.1/pRK*puf*, as revealed in Fig. 5A. The H_2O_2 is a normally used oxidant produced

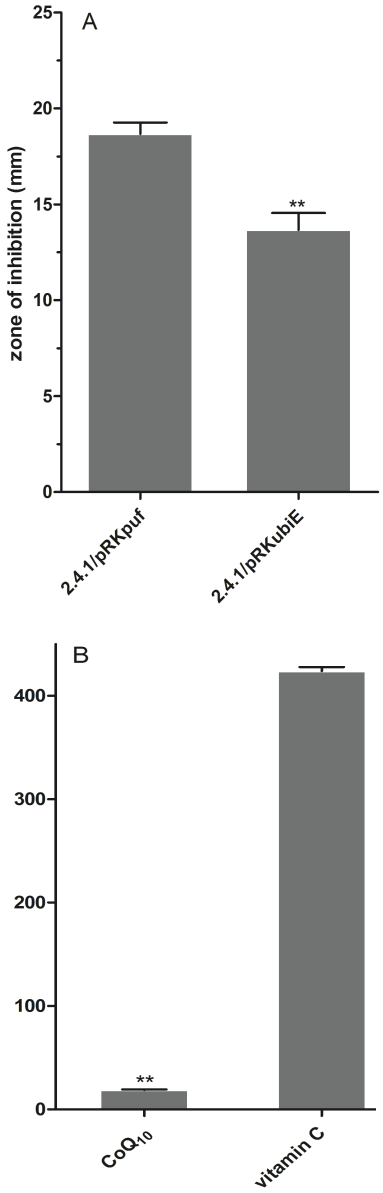

Fig. 5: In *vivo* (A) and in *vitro* (B) hydroxyl radical scavenging activity assay of 2.4.1/pRK*puf* and 2.4.1/pRK*ubiE*. The zone diameter for 2.4.1/pRK*ubiE* and 2.4.1/pRK*puf* was 13.667 and 18.667 mm, respectively. The IC_{50} of H_2O_2 hydroxyl radical scavenging for crude CoQ_{10} and vitamin C was 17.78 and 423.89 µg/mL, respectively; **indicates a highly significant change ($p \leq 0.01$)

·OH radical by Fenton reaction (Fischbacher *et al*., 2017). Obviously, the control strain 2.4.1/pRK*puf* was more sensitive to H_2O_2 than that of the sample strain 2.4.1/pRK*ubiE*, because the size of zone for 2.4.1/pRK*puf* was bigger than that of 2.4.1/pRK*ubiE*. On the other hand, CoQ_{10} exhibited much higher hydroxyl radical scavenging activity produced by H_2O_2 in *vitro* than that of the commonly used antioxidant vitamin C, since the IC_{50} for crude CoQ_{10} was much

lower than that of vitamin C, as shown in Fig. 5B. It was surprised that the crude CoQ_{10} had so much higher hydroxyl radical scavenging activity, indicating the crude CoQ_{10} was functional and thus possessed the potential for commercial utility in food, pharmaceutical and cosmetic industries after further purification by HPLC or other techniques.

CONCLUSION

In the present study, we constructed a genetic engineered *Rb. sphaeroides* for overexpression of *ubiE* to enhance functional CoQ_{10} production. The *ubiE* gene was expressed under the strong *puf* operon promoter in *Rb. sphaeroides*at micro-aerobic growth conditions. Production of the crude CoQ_{10} from 2.4.1/pRK*ubiE* was enhanced by 80.61%, which was increased much higher than reported literature. The crude CoQ_{10} exhibited strong hydroxyl radical scavenging activity.

ACKNOWLEDGMENT

This study was supported by Research Project of Sichuan University of Science & Engineering (2015RC27), Open Fund of Meat Processing Key Laboratory of Sichuan Province (16R-27), Sichuan Province Undergraduate Training Programs for Innovation and Entrepreneurship (201610622008). We thank Prof. Dr. Gabriele Klug (Justus-Liebig-University Giessen, Germany) for supplying the expression vector pRK*puf*.

CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

REFERENCES

Billenkamp, F., T. Peng, B.A. Berghoff and G. Klug, 2015. A cluster of four homologous small RNAs modulates C1 metabolism and the pyruvate dehydrogenase complex in *Rhodobactersphaeroides* under various stress conditions. J. Bacteriol., 197(10): 1839-1852.

Chen, D., Y. Han and Z. Gu, 2006. Application of statistical methodology to the optimization of fermentative medium for carotenoids production by *Rhodobactersphaeroides*. Process Biochem., 41(8): 1773-1778.

Cluis, C.P., A.M. Burja and V.J. Martin, 2007. Current prospects for the production of coenzyme Q_{10} in microbes. Trends Biotechnol., 25(11): 514-521.

Cluis, C.P., D. Pinel and V.J. Martin, 2012. The production of coenzyme Q_{10} in microorganisms. Subcell. Biochem., 64: 303-326.

Eisenhardt, K.M.H., C.M. Reuscher and G. Klug, 2018. PcrX, an sRNA derived from the 3'-UTR of the *Rhodobactersphaeroides puf* operon modulates expression of puf genes encoding proteins of the bacterial photosynthetic apparatus. Mol. Microbiol., 110(3): 325-334.

Fischbacher, A., C. von Sonntag and T.C. Schmidt, 2017. Hydroxyl radical yields in the Fenton process under various pH, ligand concentrations and hydrogen peroxide/Fe(II) ratios. Chemosphere, 182: 738-744.

Garrido-Maraver, J., M.D. Cordero, M. Oropesa-Avila, A.F. Vega, M. de la Mata, A.D. Pavon *et al*., 2014. Clinical applications of coenzyme Q_{10}. Front Biosci (Landmark Ed), 19: 619-633.

Gong, L. and S. Kaplan, 1996. Translational control of *puf* operon expression in *Rhodobactersphaeroides* 2.4.1. Microbiology, 142(Pt8): 2057-2069.

Gong, L., J.K. Lee and S. Kaplan, 1994. The Qgene of *Rhodobactersphaeroides*: Its role in *puf*operon expression and spectral complex assembly. J. Bacteriol., 176(10): 2946-2961.

Hargreaves, I.P., 2014. Coenzyme Q_{10} as a therapy for mitochondrial disease. Int. J. Biochem. Cell Biol., 49: 105-111.

Hendrischk, A.K., S.W. Fruhwirth, J. Moldt, R. Pokorny, S. Metz, G. Kaiser, A. Jager, A. Batschauer and G. Klug, 2009. A cryptochrome-like protein is involved in the regulation of photosynthesis genes in *Rhodobactersphaeroides*. Mol. Microbiol., 74(4): 990-1003.

Hu, X., T. Ritz, A. Damjanovic, F. Autenrieth and K. Schulten, 2002. Photosynthetic apparatus of purple bacteria. Q Rev. Biophys., 35(1): 1-62.

Hu, Z., Z. Zhao, Y. Pan, Y. Tu and G. Chen, 2010. A powerful hybrid *puc*operon promoter tightly regulated by both IPTG and low oxygen level. Biochemistry (Mosc), 75(4): 519-522.

Kiley, P.J. and S. Kaplan, 1987. Cloning, DNA sequence and expression of the Rhodobactersphaeroides light-harvesting B800-850-alpha and B800-850-beta genes. J. Bacteriol., 169(7): 3268-3275.

Lee, B.J., Y.F. Tseng, C.H. Yen and P.T. Lin, 2013. Effects of coenzyme Q_{10} supplementation (300 mg/day) on antioxidation and anti-inflammation in coronary artery disease patients during statins therapy: A randomized, placebo-controlled trial. Nutr. J., 12(1): 142.

Lenaz, G., R. Fato, G. Formiggini and M.L. Genova, 2007. The role of Coenzyme Q in mitochondrial electron transport. Mitochondrion, 7 Suppl: S8-33.

Lu, W., L. Ye, X. Lv, W. Xie, J. Gu, Z. Chen, Y. Zhu, A. Li and H. Yu, 2015. Identification and elimination of metabolic bottlenecks in the quinone modification pathway for enhanced coenzyme Q_{10} production in Rhodobactersphaeroides. Metab. Eng., 29: 208-216.

Negida, A., A. Menshawy, G. El Ashal, Y. Elfouly, Y. Hani, Y. Hegazy, S. El Ghonimy, S. Fouda and Y. Rashad, 2016. Coenzyme Q_{10} for patients with Parkinson's disease: A systematic review and meta-analysis. CNS Neurol. Disord. Drug Targets, 15(1): 45-53.

Park, Y.C., S.J. Kim, J.H. Choi, W.H. Lee, K.M. Park, M. Kawamukai, Y.W. Ryu and J.H. Seo, 2005. Batch and fed-batch production of coenzyme Q_{10} in recombinant Escherichia coli containing the decaprenyl diphosphate synthase gene from *Gluconobacter suboxydans*. Appl. Microbiol. Biotechnol., 67(2): 192-196.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res., 29(9): e45.

Remes, B., B.A. Berghoff, K.U. Forstner and G. Klug, 2014. Role of oxygen and the OxyR protein in the response to iron limitation in Rhodobactersphaeroides. BMC Genomics, 15: 794.

Rizvi, A., S. Chibber and I. Naseem, 2015. Cu(II)-vitamin D interaction leads to free radical-mediated cellular DNA damage: A novel putative mechanism for its selective cytotoxic action against malignant cells. Tumour. Biol., 36(3): 1695-1700.

Sachindra, N.M., E. Sato, H. Maeda, M. Hosokawa, Y. Niwano, M. Kohno and K. Miyashita, 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. J. Agr. Food Chem., 55(21): 8516-8522.

Zahiri, H.S., K.A. Noghabi and Y.C. Shin, 2006. Biochemical characterization of the decaprenyl diphosphate synthase of *Rhodobactersphaeroides* for coenzyme Q_{10} production. Appl. Microbiol. Biotechnol., 73(4): 796-806.

Zeller, T., M.A. Mraheil, O.V. Moskvin, K. Li, M. Gomelsky and G. Klug, 2007. Regulation of hydrogen peroxide-dependent gene expression in *Rhodobactersphaeroides*: Regulatory functions of OxyR. J. Bacteriol., 189(10): 3784-3792.

Zhao, Z., T. Peng, J. Oh, J. Glaeser, L. Weber, Q. Li and G. Klug, 2018. A response regulator of the OmpR family is part of the regulatory network controlling the oxidative stress response of *Rhodobactersphaeroides*. Environ. Microbiol. Rep., 11(2): 118-128.

Zhu, Y., L. Ye, Z. Chen, W. Hu, Y. Shi, J. Chen, C. Wang, Y. Li, W. Li and H. Yu, 2017. Synergic regulation of redox potential and oxygen uptake to enhance production of coenzyme Q_{10} in *Rhodobactersphaeroides*. Enzyme. Microb. Technol., 101: 36-43.