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Notes

Effects of pH and Light Irradiation on Coenzyme Q_{10} Production Using *Rhodobacter sphaeroides*

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To increase the level of CoQ_{10} production in mass culture, the effects of pH and light irradiation on CoQ_{10} production by *Rhodobacter sphaeroides* were investigated in a 1-L bioreactor. CoQ_{10} production was growth-associated, and the highest production of CoQ_{10} (1.69 mg/g dry cell) was obtained under uncontrolled pH; this production was 1.7 times higher than that obtained at controlled pH 7. Therefore, pH was a key factor affecting CoQ_{10} production. The effect of light irradiation on CoQ_{10} production was negligible. This result offers an advantage for mass production of CoQ_{10}.

Key words: pH, Light, *Rhodobacter sphaeroides*, Coenzyme Q_{10}

Introduction

Photosynthetic bacteria can utilize various types of organic matter as carbon and energy substrates, and they are thus common microorganisms in the natural environment (Sasaki et al., 1998). They have been widely applied in wastewater treatment and bioremediation of sediment mud (Takeno et al., 1999; Nagadomi et al., 2000). Recently, they have also been used for medical fields, since they can produce various types of physiologically active substances such as vitamin B_{12}, ubiquinone (Coenzyme Q_{10}), 5-aminolevulinic acid, porphyrins and RNA (Sasaki et al., 2005; Jeong et al., 2008). Notably, coenzyme Q_{10} (CoQ_{10}) and 5-aminolevulinic acid have been prepared and commercialized.

Ubiquinones, also referred to coenzyme Q, are membrane-bound lipid components. They are common materials in animals, plants and microorganisms as coenzymes involved in biological reactions. They play a vital role not only as electron carriers in the respiratory chain, but also as antioxidants and prooxidants (Ernster and Dallner, 1995; Grant et al., 1997; Wu et al., 2001; James et al., 2004). The number of isoprene units in the prenyl side chain of ubiquinones varies depending on the organism. CoQ_{10}, 2,3-dimethoxy-5-methyl-benzoquinone with a side chain of 10 monosaturated isoprenoid units, is the only ubiquinone homolog found in human organs (Gale et al., 1961). In humans, CoQ_{10} boosts energy, enhances the immune system, and acts as an antioxidant (Ernster and Dallner, 1995) Recently, CoQ_{10} has been widely used for pharmaceuticals, cosmetics, food supplements, etc. because of its various physiological activities (Takahashi et al., 2003; Sasaki et al., 2005; Zhang et al., 2007).

CoQ_{10} can be produced by chemical (Negishi et al., 2002), semi-chemical (Lipshutz et al., 2002) and biological syntheses. The biological synthesis of CoQ_{10} is more widely used than chemical and semi-chemical syntheses, since CoQ_{10} produced by chemical synthesis may not be desirable because the starting materials differ from those used in microorganisms and humans (Ha et al., 2007). Therefore, the commercial production of CoQ_{10} biologically synthesized from microorganisms has attracted increasing attention (Choi et al., 2005), and a genetically engineered microorganism has been constructed to synthesize CoQ_{10} (Lee et al., 2004; Park et al., 2005; Sakai et al., 2005). However, low yields from microbiological production of CoQ_{10} on an industrial scale have resulted in a high cost for CoQ_{10} (Ha et al., 2007). Despite the recent accomplishments in metabolic engineering of *Escherichia coli* for CoQ_{10} production, production levels are not yet competitive

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with the levels produced by isolation or fermentation (Park et al., 2005). To increase the level of CoQ_{10} production in mass culture, environmental conditions must be optimized. In this study, the effects of pH and light irradiation on CoQ_{10} production using *R. sphaeroides* were investigated.

Materials and Methods

Microorganism and medium

R. sphaeroides (GenBank Accession Number: AM69671) was isolated from the silt of the Nakdong River by our laboratory (Jeong et al., 2008). The microorganism was maintained on a solid agar plate that contained (per L): 1 g of malic acid; 2 g of casamino acid; 3 g of yeast extract; 1 mL of vitamin solution; 1 mL of mineral solution; and 15 g of agar. The vitamin solution contained (per L): 0.2 g of nicotinic acid; 0.4 g of thiamine-HCl; 0.2 g of nicotinamide; and 0.008 g of biotin. The mineral solution contained (per L): 3 g of $FeSO_4 \cdot 7H_2O$; 0.01 g of H_3BO_3; 0.01 g of $Na_2MoO_4 \cdot 2H_2O$; 0.02 g of $MnSO_4 \cdot H_2O$; 0.01 g of $CuSO_4 \cdot 5H_2O$; 0.01 g of $ZnSO_4$; and 0.5 g of ethylenediamine tetraacetic acid. The pH of the medium was adjusted to 7.2 before autoclaving, and the medium was sterilized at 121°C for 15 min. The microorganism was regularly checked under a microscope in order to eliminate any possible contaminants. The culture was stored on an agar plate at 4°C until used and was transferred to a fresh agar plate every two weeks.

Batch culture for CoQ_{10} production

A batch reaction for CoQ_{10} production was carried out in a 1-L bioreactor in a 600 mL working volume (Marubishi, Japan). The seed culture was cultivated in a 250-mL flask at 30°C, 180 rpm and 50 Lux; 60 mL of the broth culture, in which cells were grown to the end of the exponential growth phase, were used as inoculums. The bioreactor was operated under different pH (controlled at 7 and uncontrolled) and light irradiation (50 Lux and without light) conditions. The bioreactor was completely covered with aluminum foil after inoculation of the seed culture when the bioreactor was operated under no light. The agitation speed and aeration rate were set at 300 rpm and 5 vvm, respectively. 'Antifoam 204' (diluted 10-fold) was occasionally used when severe foaming occurred. Samples were taken periodically from the bioreactor to measure the concentrations of cells and CoQ_{10}. The pH and the concentration of dissolved oxygen (DO) were measured in real time using Labo Controller (Marubishi, Japan). With proper dilution, the numbers of viable cells in the samples were measured by counting colonies formed on agar plates. The dry-cell weight of the bacteria was determined by weighing the cell pellet after it was dried in an oven at 105°C for 12 hrs. The cell pellet was prepared by centrifuging a 20-mL sample of broth culture at 5,000 rpm for 10 min and then decanting the supernatant after two washes with distilled water.

Extraction and measurement of CoQ_{10}

CoQ_{10} extracted from the isolated photosynthetic bacterium was analyzed by the method of Matsumura et al. (1983) and Takahashi et al. (2003) with modifications. Ten grams of cells (wet weight), which were grown until the late-logarithmic phase, were suspended in 70 mL of methanol, and the slurry was heated at 55°C for 5 min. Chloroform (140 mL) was added, and the suspension was stirred at 30°C for 20 min and filtered through filter paper (Whatman No. 1). NaCl solution (0.58%, w/v) was added by at one-fifth of the filtrate volume. The filtrate and the NaCl solution were gently mixed and then allowed to separate into two phases. The lower phase was evaporated and resuspended in ethanol. CoQ_{10} was analyzed by HPLC (Agilent 1200, USA) on a Zorbax Eclipse Plus C18 column (100 mm×4.6 mm, 5 μm) with ethanol as the mobile phase at a flow rate of 1 mL/min. The CoQ_{10} was quantified by an external standard method, based on the peak area, and detected at 275 nm. The intracellular content of CoQ_{10} was estimated by the relationship between dry-cell weight and the amount of CoQ_{10} in the broth.

Results and Discussion

The microorganism, *R. sphaeroides,* had high CoQ_{10} content (1.55 mg/g dry cell) in our previous study (Jeong et al., 2008). Among the photosynthetic bacteria, *Rhodopseudomonas*, *Rhodobacter* and *Rhodospirillum* strains are known to produce CoQ_{10} (Urakami and Yoshida, 1993), and *Rhodobacter sphaeroides* has been used preferentially to produce CoQ_{10} (Gu et al., 2006). *R. sphaeroides* is a facultative microorganism that can be cultivated under many different growth conditions, including photoheterotrophy, photoautotrophy, chemoheterotrophy, and fermentation (Kokua et al., 2003). Thus, the effects of pH and light irradiation on CoQ_{10} production using *R. sphaeroides* were investigated for mass culture.

Under the conditions of 50 Lux, 30°C, 300 rpm, and 5 vvm aeration, *R. sphaeroides* was cultivated in a 1-L bioreactor either at pH 7 or at uncontrolled pH to observe the effect of pH on CoQ_{10} production. The

results are shown in Fig. 1 and Fig. 2, respectively. Under cultivation at pH 7 (Fig. 1), the DO level in the bioreactor was reduced to 0.3 mg/L within 6 hr, with the generation of foam. The DO level was subsequently recovered to 80%. This phenomenon may occur because a facultative microorganism, *R. sphaeroides*, needs oxygen in the early growth phase, but its metabolism would later switch to fermentation metabolism (Saunders and Johnes, 1974; Kokua et al., 2003). The biomass production increased as the cells were cultivated. The maximum dry-cell weight was obtained at 12 hr of cultivation, and the CoQ_{10} production was at its maximum (1.0 mg/g dry cell). After the stationary phase, the CoQ_{10} production was reduced as the dry-cell weight decreased. The CoQ_{10} production was growth-associated, possibly as a primary metabolite; the same result was observed in the study of *Rhodobacter* sp. by Yamada et al. (1991). Yen and Chiu (2007) also reported that CoQ_{10} biosynthesis by *R. sphaeroides* occurred predominantly during the exponential growth phase. Therefore, *R. sphaeroides* cells must be harvested at the late-exponential growth phase.

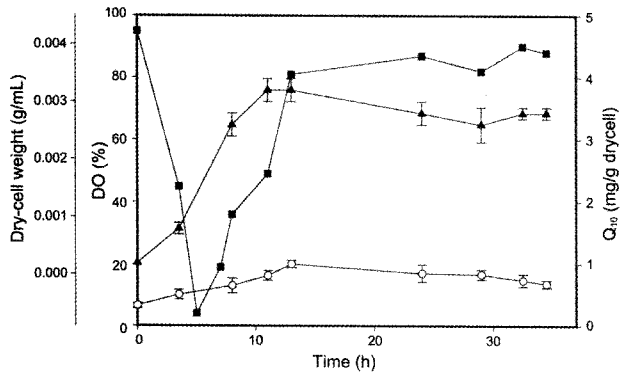

Fig. 1. Profiles of DO (-■-), dry-cell weight (-▲-) and CoQ_{10} (-○-) in a 1-L bioreactor under 50 Lux, 30°C, 300 rpm, 5 vvm aeration, and pH 7. Error bars: mean ± S.D. of three replicates.

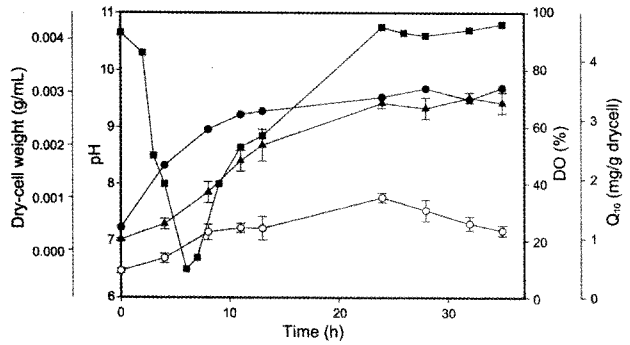

Fig. 2. Profiles of pH (-●-), DO (-■-), dry-cell weight (-▲-) and CoQ_{10} (-○-) in a 1-L bioreactor under 50 Lux, 30°C, 300 rpm, and 5 vvm aeration. Error bars: mean ± S.D. of three replicates.

At uncontrolled pH (Fig. 2), the change of DO level in the bioreactor was similar to that at pH 7, and foam was also generated to some degree. The pH steadily increased up to 9, and was constant after 10 hr. The maximum cell mass was obtained at 25 hr, along with the highest production of CoQ_{10} (1.69 mg/g dry cell), which was 1.7 times higher than that obtained at controlled pH 7. Even though the cellular growth was somewhat retarded, cultivation under uncontrolled pH increased CoQ_{10} production, which indicates cellular growth metabolism of a facultative microorganism, *R. sphaeroides*, under different environmental conditions (Kokua et al., 2003). This result agreed with that obtained from a study of CoQ_{10} production using *Agrobacterium tumefaciens* (Ha et al., 2007).

The effect of light irradiation on CoQ_{10} production using *R. sphaeroides* was also investigated in a 1-L bioreactor cultivated without light irradiation, and the result is shown in Fig. 3. As cultivation proceeded, cell growth with DO consumption and DO recovery was similar to that with light irradiation. Also, the maximum production of CoQ_{10}, 1.67 mg/g dry cell, was almost the same as that with light irradiation. In the study by Sasaki et al. (1998), CoQ_{10} production using *R. sphaeroides* was enhanced under microaerobic dark cultivation. In this study, CoQ_{10} production using *R. sphaeroides* isolated from silt of the Nakdong River was not enhanced under dark cultivation. This difference may have resulted from the culture conditions, since *R. sphaeroides* exhibits differrent cell growth under different culture conditions (Kokua et al., 2003). Our result offers an advantage for mass production of CoQ_{10}, since mass culture of cells with light irradiation is costly. Further study of

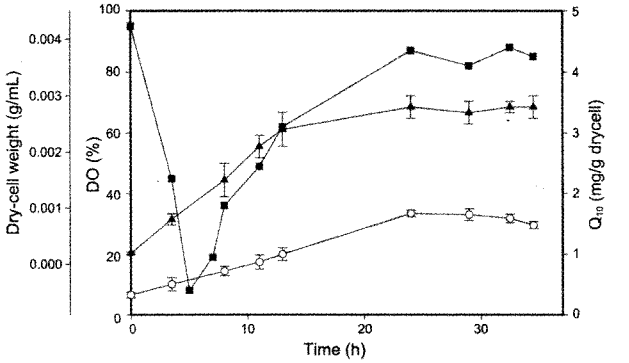

Fig. 3. Profiles of pH (-●-), DO (-■-), dry-cell weight (-▲-) and CoQ_{10} (-○-) in a 1-L bioreactor under 30°C, 300 rpm, 5 vvm aeration, and without light irradiation. Error bars: mean ± S.D. of three replicates.

economical mass production of CoQ_{10} is under way.

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References

Choi, J.H., Y.W. Seo and J.H. Seo. 2005. Biotechnological production and applications of coenzyme Q_{10}. Appl. Microbiol. Biotechnol., 68, 9-15.

Ernster, L. and G. Dallner. 1995. Biochemical, physiological and medical aspects of ubiquinone function. Biochim. Biophys. Acta, 1271, 195-204.

Gale, P.H., F.R. Koniuszy, A.G. Page Jr. and K. Folkers. 1961. Coenzyme Q. XXIV. On the significance of coenzyme Q_{10} in human tissues. Arch. Biochem. Biophys., 93, 211-213.

Grant, C.M., F.H. Maclver and I.W. Dawes. 1997. Mitochondrial function is required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*. FEBS Lett., 410, 219-222.

Gu, S.B., J.M. Yao, Q.P. Yuan, P.J. Xue, Z.M. Zheng and Z.L. Yu. 2006. Kinetics of *Agrobacterium tumefaciens* ubiquinone-10 batch production. Process Biochem., 41, 1908-1912.

Ha, S.J., S.Y. Kim, J.H. Seo, H.J. Moon, K.M. Lee and J.K. Lee. 2007. Controlling the sucrose concentration increases Coenzyme Q10 production in fed-batch culture of *Agrobacterium tumefaciens*. Appl. Microbiol. Biotechnol., 76, 109-116.

James, A.M., R.A.J. Smith and M.P. Murphy. 2004. Antioxidant and prooxidant properties of mitochondrial coenzyme Q. Arch. Biochem. Biophys., 423, 47-56.

Jeong, S.K., S.C. Ahn, I.S. Kong and J.K. Kim. 2008. Isolation and identification of a photosynthetic bacterium containing high content of coenzyme Q_{10}. J. Fish. Sci. Technol., 11, 172-176.

Kokua, H., I. Eroglu, U. Gunduz, M. Yucel and L. Turker. 2003. Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides*. Int. J. Hydrogen Energy, 27, 1315-1329.

Lee, J.K., G. Her, S.Y. Kim and J.H. Seo. 2004. Cloning and functional expression of the dps gene encoding decaprenyl diphosphate synthase from *Agrobacterium tumefaciens*. Biotechnol. Prog., 20, 51-56.

Lipshutz, B.H., P. Mollard, S.S. Pfeiffer and W. Chrisman. 2002. A short, highly efficient synthesis of coenzyme Q_{10}. J. Am. Chem. Soc., 124, 14282-14283.

Matsumura, M., T. Kobayashi and S. Aiba. 1983. Anaerobic production of ubiquinone-10 by *Paracoccus dentrificans*. Eur. J. Appl. Microbiol. Biotechnol., 17, 85-89.

Nagadomi, H., T. Kitamura, M. Watanabe and K. Sasaki. 2000. Simultaneous removal of chemical oxygen demand (COD), phosphate, nitrate and hydrogen sulphide in the synthetic sewage wastewater using porous ceramic immobilized photosynthetic bacteria. Biotechnol. Lett., 22, 1369-1374.

Negishi, E., S.Y. Liou, C. Xu and S. Huo. 2002. A novel, highly selective, and general methodology for the synthesis of 1,5-diene-containing oligoisoprenoids of all possible geometrical combinations exemplified by an iterative and convergent synthesis of coenzyme Q_{10}. Org. Lett., 4, 261-264.

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, 192-196.

Sasaki, K., T. Tanaka and S. Nagai. 1998. Use of photosynthetic bacteria for production of SCP and chemicals from organic wastes. In: Bioconversion of waste materials to industrial products (2nd Edition). Martin, A.M., Ed. Blackie Academic and Professionals, New York, 247-291.

Sasaki, K., M. Watanabe, Y. Suda, A. Ishizuka and N. Noparatnaraporn. 2005. Applications of photosynthetic bacteria for medical fields. J. Biosci. Bioeng., 100, 481-488.

Saunders, V.A. and O.T.G. Jones. 1974. Properties of the cytochrome *a*-like material developed in the photosynthetic bacterium *Rhodopseudomonas spheroides* when grown aerobically. BBA- Bioenergetics, 333, 439-445.

Takahashi, S., T. Nishino and T. Koyama. 2003. Isolation and expression of *Paracoccus dentrificans* decaprenyl diphosphate synthase gene for production of ubiquinone-10 in *Escherichia coli*. Biochem. Eng. J., 16, 183-190.

Takeno, K., K. Sasaki and N. Nishio. 1999. Removal of phosphorus from oyster farm mud sediment using a photosynthetic bacterium, *Rhodobacter sphaeroides* IL106. J. Biosci. Bioeng., 88, 410-415.

Urakami, T. and T. Yoshida. 1993. Production of ubiquinone and bacteriochlorophyll α by *Rhodobacter sphaeroides* and *Rhodobacter sulfidophilus*. J. Ferment. Bioeng., 76, 191-194.

Wu, Z.F., P.F. Weng, G.C. Du and J. Chen. 2001. Advances of coenzyme Q_{10} function studies. J. Ningbo Univ., 2, 85-88.

Yamada, Y., K. Haneda, S. Murayama and S. Shiomi. 1991.

Application of fuzzy control system fermentation. J. Chem. Eng., 24, 94-99.

Yen, H.W. and C.H. Chiu. 2007. The influences of aerobic-dark and anaerobic-light cultivation on CoQ_{10} production by *Rhodobacter sphaeroides* in the submerged fermenter. Enzyme Microb. Technol., 41, 600-604.

Zhang, D., B. Shrestha, W. Niu, P. Tian and T. Tan. 2007. Phenotypes and fed-batch fermentation of ubiquinone-overproducing fission yeast using *ppt1* gene. J. Biotechnol., 128, 120-131.

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