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Research Article

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Optimization of production procedures for coenzyme Q_{10} from *Rhodobacter sphaeroides*

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ABSTRACT

In the present study, single factors including inoculate amounts, fermentation temperature, fermentation duration, ratio of fermentation media volume to total flask volume were optimized for enhancing the production of coenzyme Q_{10} from Rhodobacter sphaeroides. The experimental results suggested that optimal single factor was: inoculate amounts 2%, fermentation temperature 30 ºC, fermentation duration 72 h, fermentation media volume to total flask volume 60%. The production of CoQ_{10} was 28.22 mg/L, enhanced by 141.6% compared to the conditions before optimization. The present study will promote the large scale production of CoQ_{10} from microorganisms.

Key words: CoQ_{10}, *Rhodobacter sphaeroides*, optimization, fermentation, extraction

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INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble molecule found in prokaryotes plasma membrane and eukaryotes inner mitochondrial membrane[1]. It is composed of a quinone head and a tail of ten repeated isoprene units[2]. CoQ_{10} plays very important roles in aerobic respiration and electron transfer system[3]. Furthermore, CoQ_{10} can boosts energy, increase the ability of immune system and act as an active antioxidant against diseases[4]. Because of its excellent physiological activities, it is now widely used in pharmacy and food industry[5]. Consequently, there is an rapidly increasing demands for CoQ_{10}. Chemical synthesis, semi-chemical synthesis and microbial production are the considered as major ways to produce CoQ_{10}[6]. Currently, microbial pathway is the most viable method to produce CoQ_{10} and thus attracts increasing attention from researcher's all over the world[7]. *Rhodobacter sphaeroides* has been identified as a good candidate for CoQ_{10} production. However, the current yield of CoQ_{10} from *Rb. sphaeroides* is relatively low. In the present study, we optimize the production process of CoQ_{10} production from *Rb. sphaeroides*.

EXPERIMENTAL SECTION

Effects of inoculate amounts on the yield of CoQ_{10}

A single colony was inoculated into a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in the dark at 30 °C until OD_{660} reaches 0.6. Precultures were inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 1%, 2%, 3%, 4% and 5% and grown under micro-aerobic conditions in the dark at 30 °C for 48 h. CoQ_{10} was extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of fermentation temperature on the yield of CoQ_{10}

A single colony was inoculated into a 50 ml-flask containing 40 ml of malate minimal media and grown under

micro-aerobic conditions in the dark at 30 °C until OD_{660} reaches 0.6. Precultures were inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 1:50 and grown in dark at 27, 30 and 33 °C for 48 h. CoQ_{10} was extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of fermentation duration on the yield of CoQ_{10}

A single colony was inoculated into a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C until OD_{660} reaches 0.6. Precultures were respectively inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 2% and grown in dark at 30 °C for 36, 48, 60, 72 and 84 h. CoQ_{10} was extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of oxygen tension (fermentation media volume to total flask volume) on the yield of CoQ_{10}

A single colony was inoculated into a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C until OD_{660} reaches 0.6. Precultures were respectively inoculated into five 100-ml flasks containing 50, 60, 70, 80 and 90 ml of malate minimal media at 2% and grown in dark at 30 °C for 48 h. CoQ_{10} was extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of pH value of acidic water on the extraction of CoQ_{10}

Five samples of 40 ml of cultures were used for the extraction of CoQ_{10}. In the process of CoQ_{10} extraction, pH values of acidic water were set as 2.0, 2.5, 3.0, 3.5 and 4.0. After extraction, CoQ_{10} was quantified, respectively. The experiment was repeated three times.

Effects of extraction temperature on the extraction of CoQ_{10}

Five samples of 40 ml of cultures were used for the extraction of CoQ_{10}. Optimal pH value of acidic water was employed. For the extraction temperature, 70, 80, 90 and 100 °C were used. After extraction, CoQ_{10} was quantified, respectively. The experiment was repeated three times.

RESULTS AND DISCUSSION

Effects of inoculate amounts on the yield of CoQ_{10}

Production of CoQ_{10} from the *Rb. sphaeroides* was influenced significantly by the inoculate amounts, as shown in Figure 1. Highest yield of CoQ_{10} was produced when the inoculate amounts was 2%, followed by 4% and 1%. Consequently, inoculate amounts of 2% was considered as the optimal inoculate amounts.

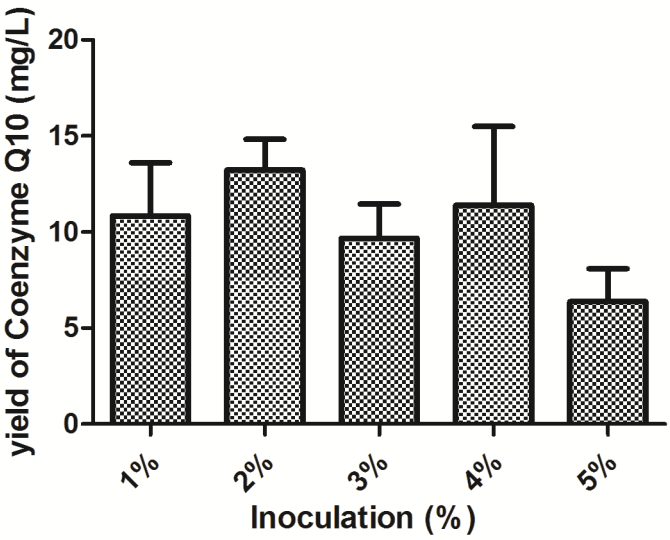

Figure 1. Effects of inoculate amounts on the production of CoQ_{10}

Effects of fermentation temperature on the yield of CoQ_{10}

Fermentation temperature affects the production of CoQ_{10} remarkably, as can been seen in Figure 2. Higher productions of CoQ_{10} was obtained when the fermentation temperature was 30 °C. The yield of CoQ_{10} from cultures grown under 27, 30 and 33 °C was 14.21, 16.36 and 11.16 mg/L. The yield of CoQ_{10} from cultures grown under 33 °C was reduced 31.78% compared to that of CoQ_{10} yield from cultures grown under 30 °C.

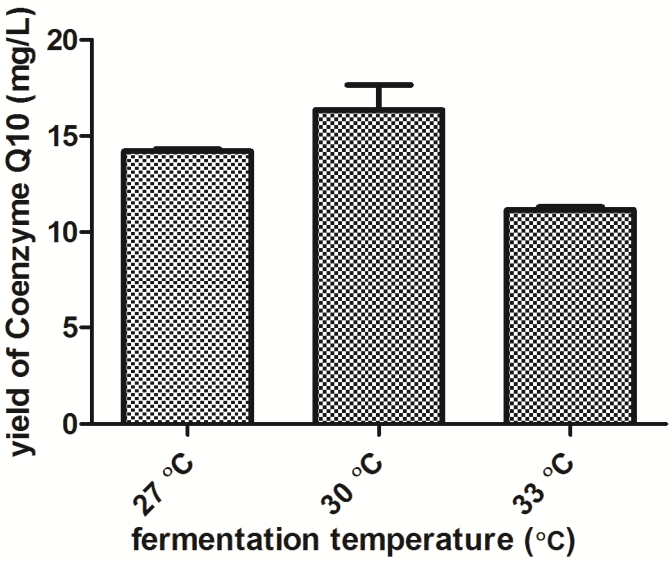

Figure 2. Effects of fermentation temperature on the production of CoQ_{10}

Effects of fermentation duration on the yield of CoQ_{10}

Effects of fermentation duration on the yield of CoQ_{10} was markedly, as revealed in Figure 3. Normally, the production will be increased with the extension of fermentation duration. However, in the present study, the yield of CoQ_{10} with the fermentation duration of 60 h was slightly lower than that of CoQ_{10} extracted from cultures fermented for 48 h. The highest production of CoQ_{10} was obtained with the fermentation duration of 84 h. However, the production with the fermentation duration of 84 h was slightly higher than that of 72 h. Consequently, fermentation for 72 h was considered as the optimal fermentation duration.

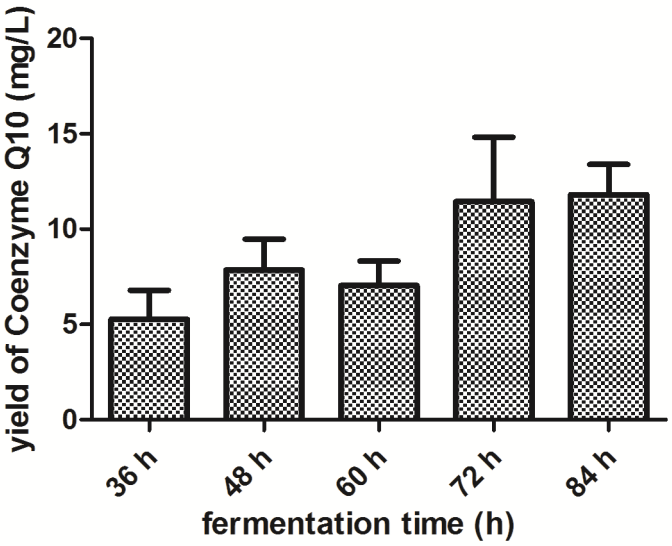

Figure 3. Effects of fermentation duration on the production of CoQ_{10}

Effects of fermentation media volume to total flask volume on the yield of CoQ_{10}

Oxygen tension plays very important roles in the biosynthesis of CoQ_{10}, as seen in Figure 4. Furthermore, the CoQ_{10} biosynthesis is sensitive to oxygen tension. The CoQ_{10} yield was 16.44 mg/L when the volume of media to flask total volume was 60%. While, the CoQ_{10} yield was 6.95 mg/L when the volume of media to flask total volume was 90%.

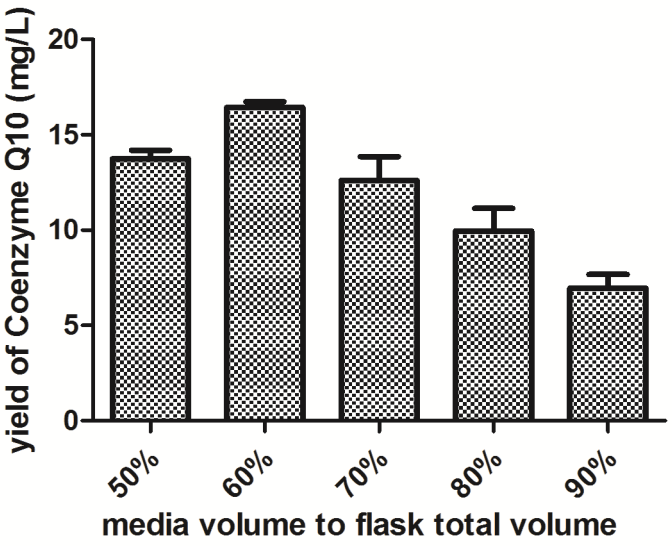

Figure 4. Effects of fermentation media volume to total flask volume on the production of CoQ_{10}

Effects of pH value of acidic water on the extraction of CoQ_{10}

The pH value of acidic water affected the extraction of CoQ10. However, it is not significant as the oxygen tension, as observed in Figure 5. The highest yield of CoQ_{10} was obtained when the pH value of acidic water was 3.0 and the yield was 15.1 mg/L. When the pH value of acidic water was 4.0, the yield of CoQ10 was 11.98 mg/L.

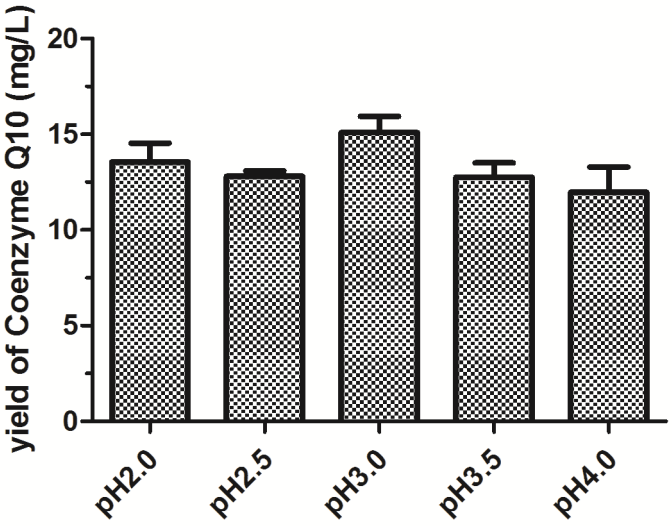

Figure 5. Effects of pH value of acidic water on the production of CoQ_{10}

Effects of extraction temperature on the extraction of CoQ_{10}

Extraction temperature slightly affected the yield of CoQ_{10}, as seen in Figure 6. The highest yield of CoQ_{10} was produced when the extraction temperature was 90 ºC with the yield of 14.03 mg/L. It was nearly no differences of the yield of CoQ_{10} between the extraction temperature of 70 and 80 ºC.

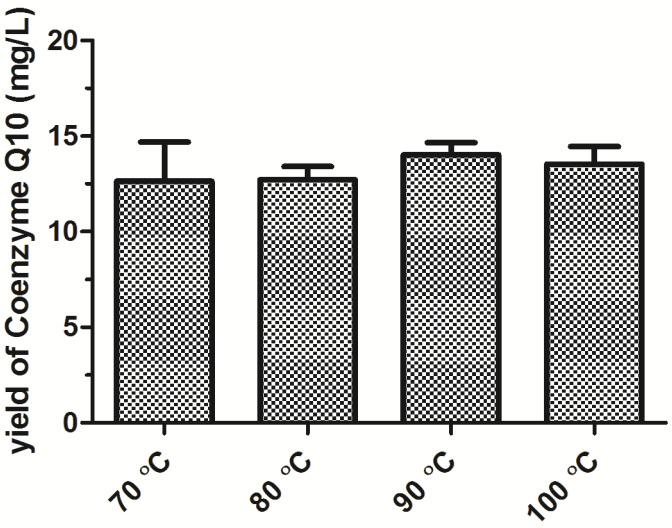

Figure 6. Effects of extraction temperature on the production of CoQ_{10}

Production of CoQ_{10} under the optimal conditions

The highest yield of CoQ_{10} was obtained under the optimal fermentation processes and extraction processes and the yield of CoQ_{10} was 28.22 mg/L. The yield was significantly enhanced compared to that of the original conditions by 141.6%, as observed in Figure 7.

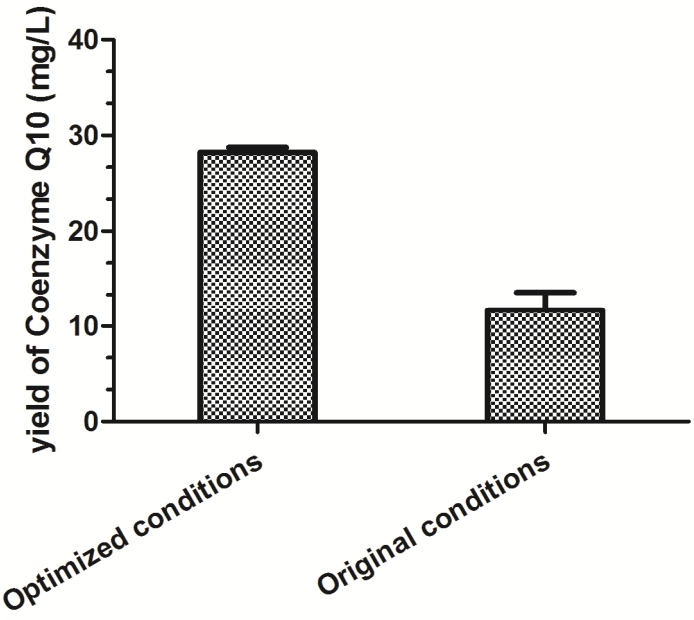

Figure 7. Yields of CoQ_{10} under optimal conditions and original conditions

CONCLUSION

The optimal processes for producing CoQ_{10} from *Rb. sphaeroides* were: inoculate amounts 2%, fermentation temperature 30 °C, fermentation time 72 h, volume of media to flask total volume 60%, extraction temperature 90 °C, pH value of acidic water pH3. Under the optimal conditions, the yield of CoQ_{10} was enhanced by 141.% compared to the conditions before optimization.

Acknowledgements

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REFERENCES

[1] W Lu; L Ye; H Xu; W Xie; J Gu; H Yu. *Biotechnol Bioeng*, **2014**, 111(4), 761-769.
[2]W Q Lu; Y J Shi; S W He; Y Z Fei; K Yu; H W Yu. *Biochem. Eng. J.*, **2013**, 72, 42-47.
DOI: 10.1016/j.bej.2012.12.019.
[3]S Takahashi; Y Ogiyama; H Kusano; H Shimada; M Kawamukai; K Kadowaki. *FEBS Lett*, **2006**, 580(3), 955-959.
[4] L Ernster; G Dallner. *Biochim Biophys Acta*, **1995**, 1271(1), 195-204.
[5] D Zhang; B Shrestha; W Niu; P Tian; T Tan. *J Biotechnol*, **2007**, 128(1), 120-131.
[6] N B Kien; I S Kong; M G Lee; J K Kim. *J. Ind. Microbiol. Biotechnol.*, **2010**, 37(5), 521-529.
[7] L Qiu; H Ding; W Wang; Z Kong; X Li; Y Shi; W Zhong. *Enzyme Microb Technol*, **2012**, 50(2), 137-142.