MEDIUM COMPOSITION EFFECT ON COENZYME Q PRODUCTION BY METABOLIC ENGINEERED ESCHERICHIA COLI STRAINS

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Abstract - Coenzyme Q (CoQ) applications have increased significantly in the past few years. Then, there is a strong interest in developing better production systems and processes. In the present work, the use of metabolically modified Escherichia coli was evaluated using different culture media for the accumulation of CoQ. The results showed that glycerol and succinate significantly increased CoQ accumulation in the control and modified E. coli strains in comparison to glucose, with glycerol showing the highest productivity in both strains.

Index Terms - coenzyme Q, Escherichia coli, metabolic engineering, ubiquinone

I. INTRODUCTION

Coenzyme Q (CoQ) is part of the respiratory chain of aerobic organisms and has important antioxidant properties. In particular, CoQ10 has been used in the treatment of different diseases including Parkinsons, Alzheimer and cardiovascular diseases. Additionally, it is used as a dietary supplement and in cosmetic applications, such anti-aging creams, due to its antioxidant property. Escherichia coli is easy to culture and relatively easy to modify genetically which makes it suitable for the development of an industrial-scale process. CoQ is composed of a benzoquinone ring and an isoprenoid chain. We constructed strains unable to produce demethylmenaquinone (DMK) and menaquinone (MK), compounds that compete for both chorismate, precursor of the benzoquinone ring, and the isoprenoid chain. In addition, the mutant strain was unable to produce enterobactin, high affinity siderophore, synthesized from chorismate. This strain was designed as platforms for the generation of novel CoQ-producing strains. In the present work, the production of CoQ was assessed in the mentioned using different carbon sources in a Lab-Scale Bioreactor.

Strains

The strains used in the present work were E. coli BW25113 and E. coli BW25113 □menF □entC. The latest was constructed using the Datsenko and Wanner (2000) method [1].

Culture medium and inoculum preparation

The effect of the culture medium on CoQ production was assessed using a defined M9 medium (Na_2HPO_4*7H_2O 12.8 g/L, KH_2PO_4 3 g /L, NaCl 0.5 g/L, NH_4Cl 1 g/L , $MgSO_4$ 0.24 g/L, $CaCl_2$ 0.011 g/L, tiamine 0.001 g/L) supplemented with 4 g/L of glucose, glycerol or succinate. The inoculum was prepared in 500 mL shake flasks with 100 mL of the corresponding culture medium according to the experiment. The cultures were incubated at 37°C and 250 rpm in an orbital shaker overnight.

Bioreactor experiments

Bioreactor experiments were performed in a 3 L Bioflo 115 (New Brunswick) bioreactor using 2 L of culture medium. The operating conditions were 37°C, pH 7.0, 600 rpm and an air flow rate of 2 L/min. Each bioreactor experiment was performed in duplicate.

Biomass determination

Culture growth was followed by spectrophotometer measurements at 600 nm. Dry cell weight (DCW) was calculated using a calibration curve.

Glucose, glycerol and succinate analysis

Glucose, glycerol and succinate were analyzed using a HPLC system (PU-4180, Jasco) equipped with an Aminex HPX-87H column(Biorad Labs), a differential refractive index (RI) detector (RI 4030, Jasco) and a ultraviolet detector (UV-4075, Jasco) 210 nm. The column was operated at 50°C. The mobile phase was 5 mM H_2SO_4 solution at a 0.6 mL/min flow rate. The column was operated at 55°C.

Coenzime Q_{10}analysis

Frozen pellets were resuspended in 6 mL of ice-cold methanol (Merck) followed by 6 mL of petroleum ether (boiling point 40-60°C, Merck). The mixture was vortexed for 1 min and then centrifuged at 10000 rpm, 5 min and 4°C. The upper petroleum ether phase was removed and transferred to a clean tube. Another 3 mL of petroleum ether were added to the lower phase. Then, the mixture was vortexed and centrifuged as described earlier and the petroleum ether phases were combined and let evaporate to dryness under a nitrogen flow. The dried extracts were stored at -20°C. Then, dried extracts were resuspended in 500 □l ethanol HPLC grade (Merck), filtrated (22 mm) and 10 □l were injected into the HPLC system (Perkin Elmer, serie 200), using a Symmetry C18 column 5□m 4.6x150 mm (Waters,Milford, MA, USA) at room temperature, UV detector 243 nm, ethanol:methanol (70:30) as mobile phase at a flow

rate of 1 mL/min. Calibration curves were prepared using CoQ_{10} standard (Sigma-Aldrich Co., St. Louis, MO, USA).

II. RESULTS AND DISCUSSION

The results from Bioreactor experiments are shown in Fig. 1 and Table 1.

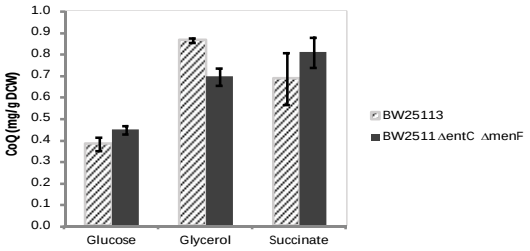


Fig. 1. Coenzyme Q content in Escherichia coli strains using different carbon sources.

Strain	Carbon Source	Productivity (mg CoQ L^{-1} h^{-1})
BW25113	Glucose	0.09 ± 0.03
	Glycerol	0.20 ± 0.06
	Succinate	0.12 ± 0.12
BW2511 □entC□menF	Glucose	0.07 ± 0.03
	Glycerol	0.11± 0.17
	Succinate	0.04 ± 0.07

Table 1. Coenzyme Q productivity in bioreactor experiments using metabolic engineered E. coli strain.

The metabolic engineered E. coli strain showed significantly higher CoQ content when glucose was used as carbon source as compared to the control strain (BW25113). On the other hand, the use of other carbon sources, succinate and glycerol, showed significant increase in CoQ accumulation in both strains, where the control strain showed the highest CoQ content when glycerol was used and the modified strain showed the highest content when succinate was used. However, the productivity was the highest when glycerol was used in both strains, mainly due to the higher biomass reached by both strains in comparison with the other tested carbon sources (data not shown).

CONCLUSION

The metabolic engineered E. colistrain (BW25113 Δ*menF*Δ*entC*)showed higher CoQ content as compared with the control strain when glucose or succinate was used as carbon source. The carbon source had a significant effect on coenzyme Q accumulation in E. coli strains, with glycerol been the best substrate under the condition tested.

REFERENCE

[1] K.A. Datsenko, B.L. Wanner BL, "One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products". Proc. Natl. Acad. Sci. U. S. A. vol. 97, pp. 6640-6645,April 2000.

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