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Improvement of cultivation medium for enhanced production of coenzyme Q10 by photosynthetic *Rhodospirillum rubrum*

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ABSTRACT

Coenzyme Q10 (CoQ10), a potent antioxidative dietary supplement, was produced by submerged fermentation using an anaerobic photosynthetic bacteria *Rhodospirillum rubrum* ATCC 25852 instead of chemical synthesis or solvent extraction. Five nutritional factors, including malic acid, yeast extract, $(NH_4)_2SO_4$, $MgSO_4{\cdot}7H_2O$, and ferric citrate, were optimized for CoQ10 production using response surface methodology (RSM) in static test tube cultures. The optimal medium for CoQ10 production were (g/l): malic acid, 2.5; yeast extract, 1.29; $(NH_4)_2SO_4$, 1.34; $MgSO_4{\cdot}7H_2O$, 0.20; 0.90 g/l K_2HPO_4, 0.90; KH_2PO_4, 0.60; ferric citrate, 0.08; and EDTA, 0.02. The highest yield of CoQ10 was 9.76 mg/l, in agreement with the RSM predicted yield (9.63 mg/l). The yield of CoQ10 in a 3-l fermentor was higher than that achieved in the static culture and reached 10.81 mg/l, which could be attributed to the constant agitation (400 rpm) that enhanced cell–substrate contact throughout the fermentation. The optimal medium compositions acquired in the present study provide a solid foundation for further improvement and optimization of fermentation processes that can be suitable for industrial-scale production of CoQ10.

1. Introduction

Coenzyme Q10, aka ubiquinone or CoQ10, is an oil-soluble, vitamin-like substance containing 10 units of the isoprenoid side-chain [1]. It is an essential component of the electron transfer system in the plasma membrane of prokaryotes and the inner mitochondrial membrane of eukaryotes, where it plays a key role as an electron donor/acceptor between complex I/II and complex III [2]. Known functions of CoQ10 include boosting energy, enhancing the immune system, and acting as a free radical scavenger [3,4], whereas positive clinical evidences continue to accumulate when CoQ10 is supplemented for treatment of hypertension and heart disease [5,6], breast cancer [7–9], and Alzheimer's and Parkinson's disease [10,11]. Superior bioavailability of CoQ10 via oral ingestion [12] has made it a popular dietary supplement, consequently leading to extensive attempts to increase the production of CoQ10 to meet the growing demands.

CoQ10 can be produced by chemical synthesis [13], semi-chemical synthesis [14], extraction from biological tissues [15], and microorganism fermentation [16]. In the wake of environmental awareness the first three options became least desirable due to the inherent uses of solvents and chemicals in the process. Microbial fermentation, on the contrary, offers an environmentally benign option based on the enzymatic catalysis at the cellular level for CoQ_{10} assembly. Moreover, this approach is attractive to the industry because the process is easy to control at a relatively low production cost [17,18]. A variety of microorganisms have been employed to produce CoQ10, including bacteria (e.g. *Pseudomonas*, *Agrobacterium*, *Paracoccus*) [16,19,20], molds (e.g. *Neurospora*, *Aspergillus*) [21], and yeasts (e.g. *Candida*, *Rhodotorula*, *Saitoella*) [22]. Via screening of wild-type strains it has been demonstrated that photosynthetic bacteria (PSB) such as *Rhodospirillum* [23,24], *Rhodobacter* [16,25–27] and *Pseudomonas* [19] are superior CoQ10 producers, with the highest CoQ10 content reached in *Rhodospirillum rubrum* [28]. *R. rubrum* is a purple non-sulfur photosynthetic bacterium, which was widely used to produce hydrogen [29,30]. Currently, economical production of CoQ10 by microbes has become more important because of the growing demands of the pharmaceutical industry [26]. To date, there have been some studies of the CoQ10 fermentation using PBS such as *Rhodobacter*

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sphaeroides [25–27], but few studies of the CoQ10 fermentation using *R. rubrum* with the highest CoQ10 content.

It is well recognized that, when developing industrial fermentation, optimization of all elements involved for the growth of the microorganism and production of the bioproduct in question is the primary task because they could strongly impact the product yield [31]. The composition of carbon and nitrogen sources, as well as inorganic salts and growth factors should all be considered [32]. Ha et al. [20] investigated the effect of carbon and nitrogen sources for CoQ10 production by *Agrobacterium tumefaciens* using the one-factor-at-a-time approach. However, the statistical interactions between carbon and nitrogen sources were not attained. Moreover, other important nutritional factors, including inorganic salts and growth factors, were not investigated in their study. Therefore, the objective of this research was to systematically characterize the effects of carbon and nitrogen sources as well as growth factors and inorganic salts on the growth of *R. rubrum* and the production of CoQ10. The medium composition was optimized using the response surface methodology (RSM) to handle the multiple factors that affect CoQ10 production.

2. Materials and methods

2.1. Microorganism and media

Freeze-dried *R. rubrum* ATCC 25852 (ATCC, Manassas, VA), an anaerobic photosynthetic bacterium, was hydrated with 9 ml sterilized water and inoculated into ATCC medium. The base medium contained (g/l): malic acid 2.5, yeast extract 1, $(NH_4)_2SO_4$ 1.25, $MgSO_4{\cdot}7H_2O$ 0.2, $CaCl_2{\cdot}2H_2O$ 0.07, K_2HPO_4 0.9, KH_2PO_4 0.6, ferric citrate 0.01, and EDTA 0.02. One milliliter trace element solution and 7.5 ml vitamin solution were also added to each liter of ATCC medium. The trace element solution contained (g/l): ferric citrate 0.3, $MnSO_4{\cdot}H_2O$ 0.002, H_3BO_3 0.001, $CuSO_4{\cdot}5H_2O$ 0.001, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.002, $ZnSO_4$ 0.001, EDTA 0.05, and $CaCl_2{\cdot}2H_2O$ 0.02. The composition of vitamin solution was (g/l): nicotinic acid 0.2, nicotinamide 0.2, thiamine·HCl 0.4, and biotin 0.008. After incubation in AnaeroGen system (AN0025, Columbia, HBA, Oxoid) under a tungsten lamp (100 W, luminous flux = 1130 lumens) at 35 °C for 48 h, the stock of *R. rubrum* was prepared by mixing the broth with sterilized glycerol (10%, v/v) and stored at −70 °C.

2.2. Batch fermentation

Initial optimizations were conducted using test tubes containing 10 ml medium sterilized at 121 °C for 15 min. The trace element solution and the vitamin solution were supplemented by filtration employing a 0.22 μm membrane. The composition of the medium and the quantities of constituents used in the medium varied according to the design of the matrix (see below). The initial pH was maintained at 6.9. The culture was statically incubated in an anaerobic atmosphere generation system (AN0025, Columbia, HBA, Oxoid) under a tungsten lamp at 35 °C for 96 h.

The optimal medium compositions identified above were confirmed using a 3 l fermentor (Applikon, Schiedam, Netherlands). After sterilization (121 °C for 15 min), the medium was cooled to scheduled temperature (35 °C) and the trace element solution and the vitamin solution were supplemented by filtration employing a 0.22 μm membrane. The medium was inoculated with 5% (v/v) inoculum. The fermentor was operated under a tungsten lamp at 35 °C for 96 h. The agitation speed was 400 rpm. Carbon dioxide was introduced at a rate of 1 l/min to keep the anaerobic condition inside the fermentor.

2.3. Optimization of fermentation medium

2.3.1. Carbon and nitrogen sources

The effects of carbon and nitrogen sources on CoQ10 synthesis were evaluated using single factor design. Different carbon sources at concentration of 2 g/l, namely glucose, sodium acetate, malic acid, sucrose, citric acid, and fructose, were employed. Various nitrogen sources at concentration of 2 g/l commonly used in microbial fermentation, including yeast extract, casitone, ammonium sulfate, tryptone, proteose peptone, yeast extract + ammonium sulfate, casitone + ammonium sulfate, and tryptone + ammonium sulfate were studied.

2.3.2. Mineral sources

Screening of the most significant mineral sources affecting CoQ10 production was performed using the Plackett–Burman design [33]. Based on Plackett–Burman design, each independent variable was tested at high (+1) and low (−1) levels. Table 1 illustrates the experimental with eight variables, whereas, Table 2 shows the design matrix. In the present study, 12 experimental runs were conducted.

2.3.3. Concentration optimization of screened components

A Box–Behnken design [34] of RSM was conducted in the optimum vicinity to locate the true optimum concentrations of malic acid, yeast extraction, ammonium sulfate, $MgSO_4{\cdot}7H_2O$ and ferric citrate for CoQ10 production. The range and center point values of these five factors are shown in Table 3. The Box–Behnken design consisted of 46 experiments including six replicates of the central point (Table 4). For statistical calculations, the relation between the coded values and real values are described as follows:

$$x_i = \frac{X_i - X_0}{\Delta X}$$

where x_i is dimensionless coded value of the variable X_i, X_0 is the real value of the X_i at the center point; and ΔX is the step change of variable. The behavior of the system was explained by the following

Table 1
Levels of the variables and statistical analysis of Plackett–Burman design.

Factors (mg/l)	Code	Low level (−1)	High level (+1)	Effect	Coefficient	*t*-Value	*P*-Value
$MgSO_4$	x_1	100	200	2.3500	1.1750	7.06	0.006
$ZnSO_4$	x_2	70	140	−0.1267	−0.0630	−0.38	0.729
Ferric citrate	x_3	50	100	−1.2000	−0.6000	−3.61	0.037
EDTA	x_4	20	60	0.0233	0.0117	0.07	0.949
$CuSO_4$	x_5	0.1	0.4	−0.1900	−0.0950	−0.57	0.608
$CaCl_2$	x_6	70	100	0.5400	0.2700	1.62	0.203
$MnSO_4$	x_7	0.2	0.8	−0.2267	−0.1133	−0.68	0.545
KH_2PO_4	x_8	600	900	0.4967	0.2483	1.49	0.232

R^2(adj) = 84.65%; R^2 = 95.81%.