Research Article

Identification and characterization of a novel coenzyme Q_{10}-producing strain, *Proteus penneri* CA8, newly isolated from Ebolowa, Cameroon

Li Zhong[1], Zhuoyi Kong[1], Tengfei Bian[2], Changhe Cheng[2], Min Shu[2], Yuping Shi[3], Jiale Pan[1], Bidja Abena Marie Therese[4] and Weihong Zhong[1*]

[1]*College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou, China*
[2]*China Tobacco Zhejiang Industrial Co. Ld., Hangzhou, PR China*
[3]*Second Affiliated Hospital Zhejiang University College of Medicine, Hangzhou, PR China*
[4]*International College, Zhejiang University of Technology, Hangzhou, China*

_____

ABSTRACT

Coenzyme Q_{10} (CoQ_{10}) is used as an oral prophylaxis and therapy for cardiovascular and mitochondrial respiratory-chain diseases, among others. This study aims to isolate and optimize the culture conditions of novel bacteria with higher coenzyme Q_{10}-production activity. A novel CoQ_{10}-producing bacterial strain, CA8, was isolated from a soil sample from Ebolowa, Cameroon using a selective medium that contained isoprene as the sole carbon source. Morphological, biochemical/physiological, and phylogenetic analyses of the 16s rRNA sequence showed that CA8 belonged to genus Proteus, family Enterobacteriaceae. CA8 had the closest relationship with Proteus penneri among all reported strains. Thus, it was identified as P. penneri CA8. Under optimized culture conditions, the CoQ_{10} yield of P. penneri CA8 reached 108.2 mg l^{-1} after 36 h of cultivation in a flask. P. penneri CA8 exhibited potential application in the industrial-scale biotechnological production of CoQ_{10}. This report is the first one on CoQ_{10} production by Proteus. The potential industrial applications of CoQ_{10} production by CA8 warrant further exploration.

Keywords: Coenzyme Q_{10}, Isolation, 16S rRNA, *Proteus penneri* CA8, Culture conditions
_____

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}; also known as ubiquinone-10) is an electron carrier with antioxidant activity and is involved in the respiratory chain. It has been successfully used as an oral prophylaxis and therapy for cardiovascular disease and mitochondrial respiratory-chain diseases, among others[1]. CoQ_{10} is also usually recommended as a supplement to 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), which are drugs commonly given to patients with severe heart failure [1,2,3,4]. The use of CoQ_{10} as a complementary therapy in heart failure increases in proportion to the growth of the aging population and the increase in statin consumption.

Production CoQ_{10} follows one of the three routs: chemical synthesis[5], semichemical synthesis[6], or biological synthesis[7]. Biological synthesis is the most viable method for industrial CoQ_{10} production. So far, a number of microorganisms have been reported as capable of producing CoQ_{10}, such as *Pseudomonas* sp., *Gluconobacter* sp., *Agrobacterium* sp., *Rhodobacter* sp., *Paracoccus* sp. and *Sphingomonas* sp..[8,9,10,11].The optimization of strategies and environmental parameters has resulted in improved CoQ_{10} yield in mutant strains[12,13, 14,15] is been chosen for further strain development. Recombinant cells of various microorganisms that contain the key genes involved in CoQ_{10} biosynthesis[16,17,18,19,20,21] and in the metabolic modification of microorganisms also improve the CoQ_{10} yield[22,23,24,25,26].

However, a low-cost, high-yield CoQ_{10} production method that uses biological processes remains a major research aim in biochemical engineering[27]. Process simplification and integration are favorable pathways toward the economic production of valuable metabolites by microbes. For example, we previously designed a coupled fermentation-extraction process and succeeded to improve the CoQ_{10} yield of *Sphingomonas* sp. ZUTE03 [11] However, other metabolites or residual components in media can be extracted by hexane from the mixed fermentation broth phase and result in increased CoQ_{10} purification cost. Biotransformation has several advantages over chemical synthesis including reaction specificity, regio-specifi-city, stereo-specificity, and mild reaction conditions [28].

Therefore, we further designed a conversion-extraction coupled process for CoQ_{10} production by *Sphingomonas* sp. ZUTE03 directly from precursors in a two-phase conversion system. In this method, fewer metabolites are extracted into the organic phase, which leads reduced downstream purification cost [29,30]. The conversion-extraction coupled process can enhance CoQ_{10} production in a three-phase fluidised bed reactor (TPFBR) [31]. However, the CoQ_{10} yield by *Sphingomonas* sp. ZUTE03 is moderate compared with that in the fed-batch process in previous reports (Table 1). Further optimization, particularly in the use of microorganisms with high CoQ_{10} content, is necessary to improve the CoQ_{10} yield. The selection of a highly productive strain leads to higher CoQ_{10} production at lower costs, which lead to improved industrial production by microbes.

In this study, a novel CoQ_{10}-producing strain, *Proteus penneri* CA8, with high CoQ_{10}-production activity was isolated on a selective medium from a soil sample collected from Ebolowa, Cameroon. To our knowledge, this report is the first one on CoQ_{10} production by microbes that belong to the genus *Proteus*.

EXPERIMENTAL SECTION

Chemicals
CoQ_{10} (>99.9 % purity) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Genomic DNA extraction and PCR kits were purchased from Invitrogen Corp. (Shanghai, China). The 16S rRNA primers were also designed and synthesized by Invitrogen Corp. All other chemicals were locally purchased.

Media and culture conditions
CoQ is composed of a benzoquinone ring that is prenylated with an isoprenoid chain of various lengths. CoQ_{10}-producing strains might utilize isoprene as a sole carbon source. In our previous study, a CoQ_{10}-producing strain Sphingomonase sp. ZUTE03 was isolated via this method[32]. Thus, a selective agar medium was designed and consisted of isoprene 0.5 g l^{-1}, 1.0 g l^{-1} $(NH_4)_2SO_4$, 4.5 g l^{-1} KH_2PO_4, 21.6 g l^{-1} $Na_2HPO_4 \cdot 12H_2O$, and 15–20 g l^{-1} agar. The initial pH of this medium was adjusted to 7.0 using 2 M NaOH. The prepared medium was then autoclaved at 115 °C for 30 min.

The seed medium contained 5 g l^{-1} yeast extract, 10 g l^{-1} peptone, and 10 g l^{-1} NaCl.

The initial production medium consisted of 20 g l^{-1} glucose, 10 g l^{-1} peptone, 10 g l^{-1} yeast extract, 0.5 g l^{-1} KH_2PO_4, 1.5 g l^{-1} Na_2HPO_4, and 0.5 g l^{-1} $MgSO_4 \cdot H_2O$.

To evaluate the effect of carbon source on CoQ_{10} production by CA8, the glucose of the initial production medium was replaced by different carbon sources (glucose, sucrose, maltose, and lactose) at a concentration of 20 g l^{-1}. For evaluating the effect of nitrogen source on CoQ_{10} production, the peptone and yeast extract of the initial production medium was replaced different nitrogen sources [$(NH_4)_2SO_4$, peptone, yeast extract, and com steep liquor] at a concentration of 10 g l^{-1}. For furthere evaluating the effect of the initial concentrations of lactose and yeast extract, the concentrations of lactose (10, 20, 30, 40, and 50 g l^{-1}) and yeast extract (10, 20, 30, 40, and 50 g l^{-1}) in the initial production medium were adjusted as required. One loop of strain CA8 from a slant was inoculated into a 50 ml seed medium in a 250 ml flask, and then incubated at 30 °C for 24 h at 180 rpm. For the flask culture, 5 ml of the seed medium was inoculated in a 500 ml flask that contained 100 ml of the production medium. The culture was then grown at 30 °C and 180 rpm for 2 d.

Isolation and identification of novel CoQ_{10}-producing strains
Soil samples (10 g) from a corn farm in Ebolowa, Cameroon were added to a flask that contained 100 ml of physiological saline and glass beads. After shaking for 30 min at 30 °C and 180 rpm, 0.2 ml of the mixture was spread onto the solid selective plates that contained 0.5 g l^{-1} isoprene. The plates were then incubated at 30 °C for 48 h. The colonies were collected and repeatedly streaked onto a new plate until a pure isolate was obtained. The cell morphology was examined under a transmission electron microscope. The physiological characteristics were determined using standard methods[33].

_____

The genomic DNA of strain CA8 was extracted using a rapid extraction kit (Guangzhou Dongsheng Biotechnology Co. Ltd., China). The 16S rRNA sequence was amplified by polymerase chain reaction (PCR) using Taq polymerase (Takara, Japan) and universal primer sequences (forward primer: 5'-agagtttgatcctggctcag-3'; reverse primer: 5'-cggctaccttgttacgacttc-3'). The PCR conditions were as follows: 5 min at 94 °C, 34 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, a final incubation at 72 °C for 10 min, and storage at -20 °C. The amplified DNA sequence was identified by Shanghai Invitrogen Biological Technique Co. Related sequences were obtained from the GenBank database using BLAST. The determined 16S rRNA gene sequences and the reference sequences obtained from GenBank databases were aligned using the CLUSTALX software. A phylogenetic tree was constructed using the MEGA4 software.

Analytical methods
To determine the CoQ_{10} content, the cells were harvested by centrifugation at $12000 \times g$ at 4 °C for 15 min with a centrifuge (Sanyo, Japan). After washing the cell pellet twice with distilled water, CoQ_{10} was extracted with alcohol by ultrasonication at 500 W for 8 min.

CoQ_{10} measurements were performed according to previously reported methods[29]. The CoQ_{10} concentrations in all liquid samples were analysed by a high-performance liquid chromatography (HPLC) system (SPD-10AVP, SHIMADZU, Japan) equipped with an Agilent SB-C18 column (4.6×150 mm^2). A mixture of methanol and hexane (83:17 by volume) was used as the mobile phase at a flow rate of 0.6 ml·min^{-1}. The samples were detected using a UV detector at a wavelength of 275 nm. The sample volume was 20 μl.

CA8 cell growth was determined as the dry cell weight (DCW), which could be calculated according to a standard curve of the relationship between the cell optical density in the broth and the CA8 DCW. For the standard curve and each measurement, the cells were harvested by centrifugation ($12000 \times g$, 10 min, 4 °C) from 2 ml of liquid culture, washed three times, and finally suspended in 5 ml of 50 mM potassium phosphate buffer (pH 7.0). The cell optical density of the suspension was determined at 600 nm using a spectrophotometer (752, Shanghai, China). The DCW for the standard curve was determined after the cells were dried overnight at 100 °C to a constant weight.

Effects of medium components and culture conditions on CoQ_{10} production by CA8
On a shaking flask level, the optimum carbon sources, nitrogen sources, medium volume, temperature, and pH were determined by single-factor experiments on the fermentation culture conditions. The effect of the carbon source on CoQ_{10} synthesis was investigated using different carbon sources (glucose, sucrose, maltose, and lactose) at a concentration of 20 g l^{-1}. The effect of the nitrogen source on CoQ_{10} synthesis was determined using different nitrogen sources [$(NH_4)_2SO_4$, peptone, yeast extract, and com steep liquor] at a concentration of 10 g l^{-1}. The initial pH of the aforementioned medium was adjusted to 7.0 using 2 M NaOH. The effects of various cultures on the CoQ_{10} production in 500 ml flasks were determined at 30 °C using medium volumes of 50, 80, 100, 130, and 150 ml. The effect of temperature was investigated at a cultivation pH of 7.0, and the effect of pH was investigated at a cultivation temperature of 30 °C.

Time course of CoQ_{10} production by CA8
Under the determined optimal culture conditions, the time course of CA8 cell growth and CoQ_{10} production was determined in a 500 ml flask. The sampling time intervals that were used to detect the biomass and CoQ_{10} content were 6 h (before 48 h culturing was reached) and 12 h (after 48 h culturing), respectively.

Statistical analysis
Each sample in the experiment was analyzed in triplicate. The Origin 8.0 software was used to draw the figures, including error bars.

Nucleotide sequence accession number
The resultant CA8 16S rRNA gene sequences were submitted to Genbank under the following accession number JX141365.

RESULTS
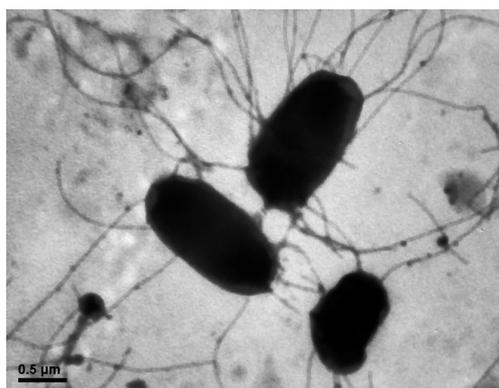
Fig. 1 Transmission electron microscopy image of CA8 bacterial strain (30000×)

Isolation and identification of novel CoQ$_{10}$-producing strains

Among all strains isolated from the soil samples from Ebolowa, Cameroon, the Gram-negative and spore-negative isolate CA8 was the only one with the ability to grow on a selective medium that contained isoprene as the sole carbon source. The colonies of this strain on the LB agar plate were translucent, sticky, and convex. Electron microscope observations showed that strain CA8 had rod-shaped cells with peritrichous flagella (Fig. 1). After standard biochemical/physiological identification and carbon source utilization analyses using a Biolog GEN III Microstation MicroStation, the characteristics of strain CA8 (Table 2) were found similar to those of *P. penneri* or *P. vulgaris*. Similarity analysis of the partial 16S rRNA gene sequence of strain CA8 (Genbank accession number:JX141365) revealed its 99 % similarity to *P. penneri*. Phylogenetic tree analysis (Fig. 2) demonstrated that strain CA8 had the closest relationship with *P. penneri* among all other reported strains. Therefore, strain CA8 was identified as *P. penneri* CA8 and then preserved at the China Center for Type Culture Collection (Access No. CCTCC NO. M2012208).
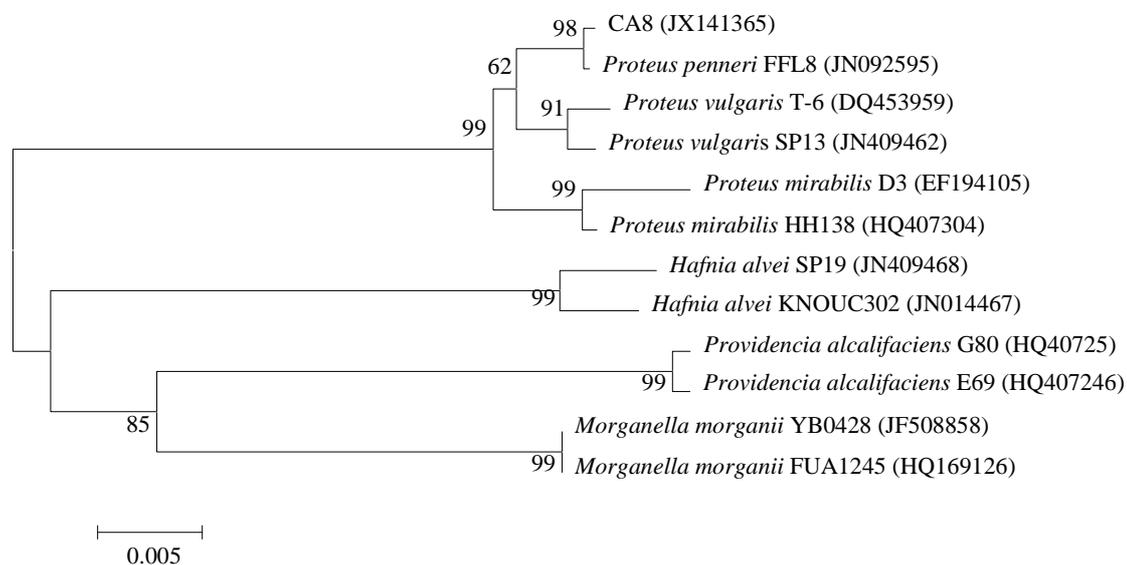
Fig. 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain CA8 and representatives of related species. The numbers in parentheses represent the sequence accession numbers in GenBank. Bootstrap proportions, >50%. Bar, 0.005 substitutions per nucleotide position

It was reported that Enterobacteriaceae such as *Proteus vulgari* generally produces CoQ$_8$[34]. As one species of *Proteus*, it is essential to ensure CoQ type produced by *Proeus penneri* CA8. Thus, an internal standard experiment of CoQ$_{10}$ was designed to prove the peak represented exactly CoQ$_{10}$. The results (Figure 3) showed that *Proeus penneri* CA8 could produce CoQ$_{10}$, the peak of which was same to that of the standard CoQ$_{10}$ on HPLC.

_____

Fig. 3 HPLC analysis of the sample without (a) and with (b) internal standard of CoQ$_{10}$

Effects of carbon and nitrogen sources on CoQ$_{10}$ production by *P. penneri* CA8

Among the tested carbon sources (20 g l^{-1} glucose, sucrose, maltose, and lactose), lactose exerted the greatest improvement effect on the CoQ$_{10}$ yield (35.8 mg l^{-1}) by CA8 (Fig. 4a). Although a higher CoQ$_{10}$ yield was observed when the initial lactose concentration was 40 g l^{-1}, the change in the initial lactose concentration exhibited no significant effect on CoQ$_{10}$ production by CA8 (Fig. 4b).

Fig. 4 Effects of different (a) carbon sources and (b) lactose concentrations on CoQ$_{10}$ production by CA8 at 30 °C and 180 rpm for 48 h: (a) DCW (white), CoQ$_{10}$ concentration (grey), and specific CoQ$_{10}$ content (black); (b) DCW (■), CoQ$_{10}$ concentration (●), and specific CoQ$_{10}$ content (▲). The bars represent the standard deviations of triplicate experiments

Fig. 5 Effects of different (a) nitrogen sources and (b) yeast exact concentrations on CoQ$_{10}$ production by CA8 at 30 °C and 180 rpm for 48 h. (a) DCW (white), CoQ$_{10}$ concentration (grey), and specific CoQ$_{10}$ content (black); (b) DCW (■), CoQ$_{10}$ concentration (●), and specific CoQ$_{10}$ content (▲). The bars represent the standard deviations of triplicate experiments

Peptone, yeast extract, $(NH_4)_2SO_4$, and corn steep liquor (10 g l^{-1}) were used to determine the effect of the nitrogen source type on CoQ$_{10}$ production by CA8. The results are shown in Fig. 5a. Peptone and yeast extract were more suitable than $(NH_4)_2SO_4$ and com steep liquor as the nitrogen sources for CA8 cell growth and CoQ$_{10}$ production. However, yeast extract was the most suitable nitrogen source for CoQ$_{10}$ production because of its ower cost. The effect of the initial yeast extract concentration (10, 20, 30, 40, and 50 g l^{-1}) on cell growth and CoQ$_{10}$ production was also evaluated (Fig. 5b). The maximal CoQ$_{10}$ yield (84.0 mg l^{-1}) was achieved when the initial yeast extract concentration was increased to 40 g l^{-1}.

Effects of cultivation conditions on CoQ$_{10}$ production by CA8
The cultivation conditions, including the medium volume in the 500 ml flask, temperature, and initial pH, are the basic factors that affect cell growth and metabolism. Thus, the effects of these cultivation conditions were also evaluated in the flasks.

First, the effects of temperature (25, 30, and 37°C) on cell growth and CoQ$_{10}$ production were determined. Cell growth and CoQ$_{10}$ production at 30 and 37 °C were higher than that under 25°C. However, the CoQ$_{10}$ concentration and specific content were maximal (73.2 mg l^{-1} and 11.1 mg g^{-1} DCW, respectively) at 30 °C (Fig. 6a).
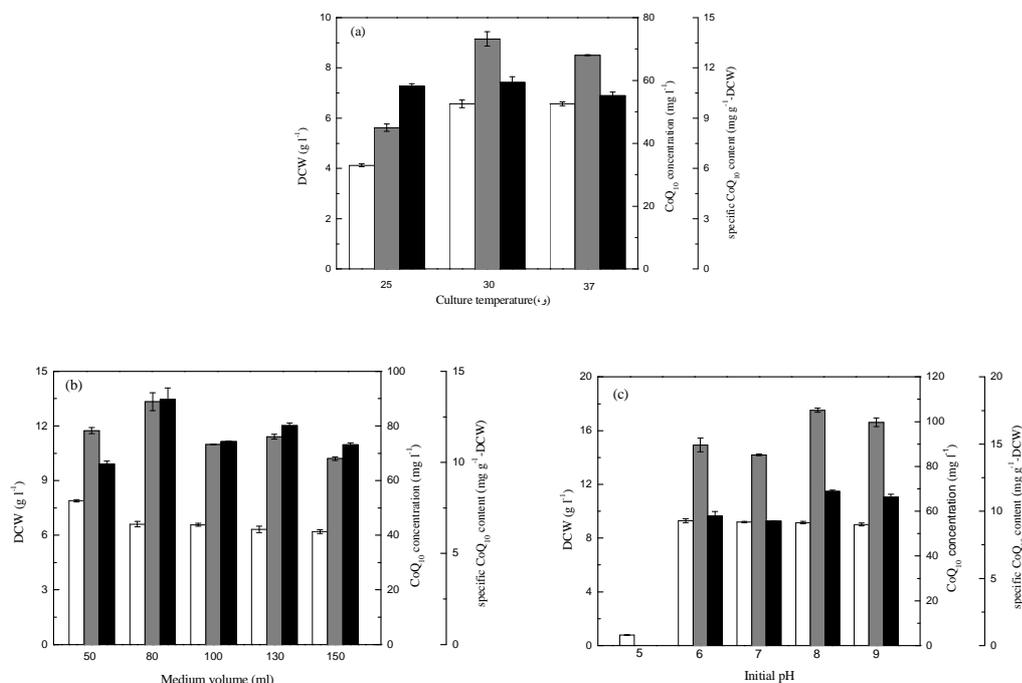
Fig. 6 Effects of (a) culture temperature, (b) medium volume, and (c) initial pH on CoQ$_{10}$ production by CA8 at 180 rpm for 48 h. DCW (white), CoQ$_{10}$ concentration (grey), and specific CoQ$_{10}$ content (black). The bars represent the standard deviations of triplicate experiments

The effects of the medium volume (50, 80, 100, 130, and 150 ml) on CoQ_{10} production were determined at 30 °C. The results (Fig. 6b) suggested that an extremely low or extremely high medium volume in the flask was not suitable for cell growth and CoQ_{10} production. The maximum CoQ_{10} concentration (88.8 g l^{-1}) was achieved at 80 ml medium volume.

The effect of the initial medium pH (ranging from 5.0-9.0) on cell growth and CoQ_{10} production was also determined. The results (Fig.6c) suggested that the pH range of 6–9 exerted no significant effect on cell growth. However, the maximum CoQ_{10} concentration (105.1 mg l^{-1}) was achieved only at pH 8.0.

In summary, the optimal cultivation conditions for CoQ_{10} production by CA8 is 80 ml medium volume in a 500 ml flask at an initial pH of 8.0 and a cultivation temperature of 30 °C.

Time course of CoQ_{10} production by CA8 during flask-shaking fermentation
The time course of cell growth and CoQ_{10} production of CA8 under optimal culture conditions was determined. The results are shown in Fig. 7. The cell growth in the given medium showed the typical growth trend of a single-cell microorganism. However, cell growth directly entered the exponential growth phase after 24 h without a lag phase. After the cells entered the stationary phase, the CoQ_{10} yield reached the maximal value of 108.2 mg l^{-1} at 36 h. However, the specific yield of CoQ_{10} reached the highest value after 12 h, suggesting that CoQ_{10} was rapidly produced during rapid cell growth and when the total biomass remained low during the mid-exponential growth phase.
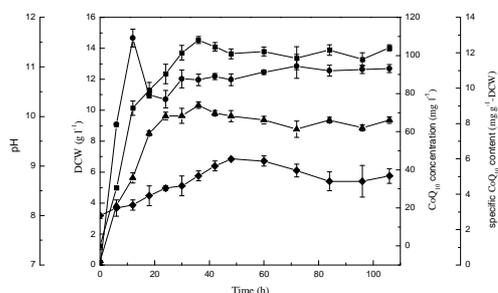
Fig. 7 Time course of CoQ_{10} production by CA8 during flask-shaking fermentation at 30 °C and 180 rpm. DCW (▲), CoQ_{10} concentration (■), specific CoQ_{10} content (●), and pH (◆). The bars represent the standard deviations of triplicate experiments

DISCUSSION

Analyses of the phenotypic, biochemical/physiological, and phylogenetic characteristics of CA8 based on the 16 rRNA sequence show that the strain is a novel CoQ_{10}-producing bacteria that should be assigned to the genus *Proteus*[35,36], family Enterobacteriaceae. The major characteristics of strain CA8 (Table 2) were similar to those of *P. penneri/vulgaris*. Similarity analysis of the partial 16S rRNA gene sequence of strain CA8 and phylogenetic tree analysis (Fig. 2) show that strain CA8 has the closest relationship with *P. penneri* among all reported strains. Therefore, strain CA8 is identified as *P. penneri* CA8.

Under the optimal culture conditions, the CoQ_{10} yield of *P. penneri* CA8 reaches 108.2 mg l^{-1} after 36 h of fermentation in a flask. The CA8 CoQ_{10} yield is higher than those obtained from other wild microorganisms (*Sphingomonas* sp. ZUTE03 and *Paracoccus dinitrificans* NRRL B-3785) via batch processing in a flask (Table 1). However, the yield is lower than those of other mutant microorganisms via batch processing [*Rhodobacter sphaeroides* Shenzhou6 (mutant)] and of some other wild microorganisms via the fed-batch process [*Rhodopseudomonas spheroids* (mutant)]. These results suggest that strain CA8 can improve the CoQ_{10} yield. However, further optimization of several factors is required to improve the CoQ_{10} yield of CA8.

First, the medium component and culture conditions of CA8 require further optimization. Second, the CoQ_{10} production activity of the wild strain can be improved by various mutation methods, including spaceflight mutation. Third, a fed-batch process can be used to obtain a high-cell density culture of CA8, which results in a high CoQ_{10} yield. Conversion in a two-phase system (CITPS; also called coupled conversion-extraction), which has been successfully used to improve the CoQ_{10} yield in our previous study [11,29,31], should also be evaluated in terms of improving the CoQ_{10} production by CA8. The addition of precursors may also improve CoQ_{10} production by CA8[30]. Finally, the feasibility of a scale-up process in a bioreactor coupled with a conversion-extraction process in the industrial application and economic production of CoQ_{10} by CA8 should be investigated.

Table 1 Coenzyme Q₁₀ (CoQ₁₀) production by various microorganisms via different processes

Process	Microorganism	CoQ$_{10}$ yield (mg l^{-1})	Specific CoQ$_{10}$ yield (mg g^{-1}-DCW^a)	Working volume (l), time (h)	Volumetric Productivity (mg l^{-1} h^{-1})	Reference
Batch	*Agrobacterium tumefaciens* KCCM 10413	320	6.61	2, 96	3.33	(Ha et al. 2007)
Batch	*Paracoccus dinitrificans* NRRL B-3785	14.12	0.806	0.05, 109	0.147	(Bule and Singhal 2011)
Batch	*Rhodobacter sphaeroides* Shenzhou6 (mutant)	800	NG	NG, 168	4.76	(Lei et al. 2012)
Fed-batch	*Rhodopseudomonas spheroids* (mutant)	770	14.5	30, 150	5.13	(Sakato et al. 1992)
Fed-batch	*Rhodobacter sphaeroides*	45.65	4.4	4, 24	1.9	(Yen and Shih 2009)
Batch	*Sphingomonas* sp. ZUTE03	1.14	0.48	0.15, 30	0.04	(Zhong et al. 2009)
CFEP^b	*Sphingomonas* sp. ZUTE03	43.2	32,5	0.15, 30	1.44	(Zhong et al. 2009)
CITPS (free cell)	*Sphingomonas* sp. ZUTE03	60.8	40.6	0.15, 8	7.6	(Zhong et al. 2011)
CITPS (entrap cell)	*Sphingomonas* sp. ZUTE03	51.6	38.5	0.15, 8	6.45	(Zhong et al. 2011)
CITPS (entrap cell, repeat batch)	*Sphingomonas* sp. ZUTE03	>40	>23	0.15, 8	>5.00	(Zhong et al. 2011)
CITPS (entrap cell in TPFBR^c fed-batch)	*Sphingomonas* sp. ZUTE03	441.65 380	81.67 70.37	0.20, 144 80	3.07 4.75	(Qiu et al. 2012)
Batch	*Proteus penneri* CA8	108.2	11.5	0.08, 36	3.01	This study

a. DCW: dry cell weight; b. CITPS: conversion in a two-phase system; c. TPFBR: three-phase fluidized bed reactor

Table 2 Physiological and biochemical characteristics of the CA8 bacterial strain

Characteristic	CA8	*Proteus vulgaris*	*Proteus mirabilis*	*Proteus penneri*
Oxidase test	-	-	-	-
Voges–Proskauer test	-	-		-
Gelatin liquefaction (22 ℃)	-	+	+	v
Indole test	-	+	-	_
Phenylalanine deaminase	+	+	+	+
Citrate utilisation (Simmons)	-	v	+	v
Motility	+	+	+	v
Maltose	+	+	-	+
Acid production from:				
D-Salicin	-	+	-	-
Cellobiose	-	-	-	-
Glycerol	+	v	+	+
Lactose	-	-	-	-
D-Mannitol	-	-	-	-
D-Mannose	-	-	-	-
Raffinose	-	-	-	-
L-Rhamnose	-	-	-	-
D-Sorbitol	-	-	-	-
Sucrose	+	+	v	+

+, Positive; -, negative; v, variable between strains

REFERENCES

[1] Choi, J.H., Ryu, Y.W., Seo. J.H, *Appl Microbiol Biotechnol*,**2005**, 68(1):9-15.

[2] Folkers, K., Langsjoen, P., Willis, R., Richardson, P., Xia, L.J., Ye, C.Q., Tamagawa, H. Lovastatin, Proc *Natl Acad Sci* U S A, **1990**, 87(22):8931-8934.

[3] Mortensen, S.A., Leth, A., Agner, E., Rohde, M.., *Mol Aspects Med*, **1997**, 18:S137-S144.

[4] Overvad, K., Diamant, B., Holm, L., Holmer, G., Mortensen, S.A., Stender, S., *Eur J Clin Nutr*, **1999**,53(10):764-770.

[5] Negishi, E., Liou, S.Y., Xu, C.D., Huo, S.Q., *Org Lett,* **2002**, 4(2):261-264.

[6] Lipshutz, B.H., Mollard, P., Steven, S., Pfeiffer, Chrisman W. , Am Chem Soc, **2002**, 124(48):14282-14283.

[7] Yajima, K., Kato, T., Kanda, A., Kitamura, S., Y, Ueda., Processes for producing coenzyme Q10. patent US 20050069996A1. **2002**.

[8] Yoshida, H., Kotani, Y., Ochiai, K., Araki, K., J Gen Appl Microbiol, **1998**, 44(1):19-26.

[9] Huembelin, M., Lopez-Ulibarri, R., Perkins, J.B., Schyns, G, DSM IP Assets, B.V.,assignee (**2004**) Oct. 14, **2008**. Microblal production of CoQ10. patent US 7,435,571 B2.

[10] Yen, H.W., Chiu, C.H., *Enzyme Microb Technol*, **2007**, 41(5):600-604.

[11] Zhong, W.H., Fang, J.J., Liu, H.G., Wang, X. , *J Ind Microbiol Biotechnol*, **2009**, 36(5):687-693.

[12] Sakato, K., Tanaka, H., Shibata, S., Kuratsu, Y. , *Biotechnol Appl Biochem*, **1992**, 16(1):19-28.

[13] Wu, Z.F., Du, G.C., Chen, J., *World J Microbiol Biotechnol*, **2003**, 19(9):925-928.

[14] Gu, S.B., Yao, J.M., Yuan, Q.P., Xue, P.J., Zheng, Z.M., Wang, L., Yu, Z.L, *Appl Microbiol Biotechnol*, **2006**, 72(3):456-461.

[15] Yen, H.W., Shih, T.Y., *Bioprocess Biosyst Eng*, **2009**, 32(6):711-716.

[16] Lee, J.K., Her, G., Kim, S.Y., Seo, J.H, *Biotechnol Progr*, **2004**, 20(1):51-56.

[17] Park, Y.C., Kim, S.J., Choi, J.H., Lee, W.H., Park, K.M., Kawamukai, M., Ryu, Y.W., Seo, J.H., *Appl Microbiol Biotechnol,* **2005**, 67(2):192-196.

[18] Zhang,D.W., Li, Z., Wang, F.H., Shrestha, B., Tian, P.F., Tan, T.W., *Enzyme Microb Technol*, **2007,** 41(6-7):772-779.

[19] Zhang, D.W., Shrestha, B., Li, Z.P., Tan, T.W., *Mol Biotechnol*, **2007,** 35(1):1-14.

[20] Zhang, D.W., Shrestha, B., Niu, W.N., Tian, P.F., Tan, T.W., *J Biotechnol*, **2007,** 128(1):120-131.

[21] Cheng, B., Yuan, Q.P., Sun, X.X., Li, W.J, *Appl Biochem Biotechnol* , **2010** ,160(2):523–531.

[22] Matthews, P.D., Wurtzel, E.T. , *Appl Microbiol Biotechnol*, **2000**, 53(4):396-400.

[23] Wu, Z.F., Du, G.C., Chen, J., *Microbiologica Sinica*, **2005**, 45(2):231-235.

[24] Zahiri, H.S., Yoon, S.H., Keasling, J.D., Lee, S.H., Kim, S.W., Yoon, S.C., Shin, Y.C., *Metab Eng,* **2006**, 8(5):406-416.

[25] Lee, P.C., Salomon, C., Mijts, B., Schmidt-Dannert, C, *Appl Environ Microbiol*, **2008**, 74(22):6908-6917.

[26] Choi, J.H., Ryu, Y.W., Park, Y.C., Seo, J.H, *J Biotechnol*, **2009**, 144(1):64-69.

[27] Jeya, M., Moon, H.J., Lee, I.W., Lee, J.K, *Appl Microbiol Biotechnol*, **2010,** 85:1653-1663.

[28] Wang, Z.F., Huang, Y.L., Rathman, J.F., Yang, S.T. , *J Chem Technol Biotechnol*, **2002**,77:1349- 1357.

[29] Zhong, W.H., Wang, W.J., Kong, Z.Y., Wu, B., Zhong, L., Li, X.Z., Yu, J., Zhang, F.M., *Appl Microbiol Biotechnol,* **2011**, 89(2):293-302.

[30] Qiu, L.Q., Wang, W.J., Zhong, W.H., Zhong, L., Fang, J.J., Li, X.Z., Wu, S.J., Chen, J.M., *J Microbiol Biotechnol*, **2011**, 21(5):494-502.

[31] Qiu, L.Q., Ding, H.B., Wang, W.J., Kong, Z.Y., Li, X.Z., Shi, Y.P., Zhong, W.H. , *Enzyme Microb Technol,* **2012**, 50(2):137-142.

[32] Liu, H.G., F, J.J., Jin, L., Zhong, W.H., Ye, Z.J., *Acta Microbiologica Sinica*, **2008**, 48(2):157-163.

[33] Shen, P., Fan, X., Li, G, Microbiology Experiment, Beijing: High Education Press, **2004**.

[34] Collins, M.D., Jones, D, *Microbiol Rev*, **1981**, 45(2): 316–354.

[35] Costas, M., Holmes, B., Frith, K.A., Riddle, C., Hawkey, P.M, *J Appl Microbiol*, **1993**, 75(5):489-498.

[36] Hickman, F.W., Steigerwalt, A.G., Farmer III, J.J., Brenner, D.J., *J Clin Microbiol* , **1982**, 15(6):1097- 1102.