

STIMULATION OF BIOSYNTHESIS OF COENZYME Q10 BY *SACHAROMYCES CEREVISIAE* UNDER THE INFLUENCE OF VITAMIN B1

Vicaș Simona Ioana*, Vasile Laslo*, Diana Uivarosan**

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea; Romania, e-mail: sim_vicas@yahoo.com

** University of Oradea, Faculty of Medicine and Pharmacy, Oradea

Abstract

*Q10 coenzyme, also known as ubiquinone is a liposoluble which is biosynthesized by all living organisms, being an essential component for the health of all cells. One of the commercial methods of production of coenzyme Q10 is yeast fermentation. The coenzyme Q10 obtained by yeast fermentation is bio-identical with that which is produced by the human body. The aim of this study was to observe if the supplementation of the GYEP culture environment with different concentrations of vitamin B1 (2.37, 1.18 and 0.59 mM respectively) stimulates endogenous production of coenzyme Q10 by the *Saccharomyces cerevisiae* yeast strain as a result. Results show a positive correlation between the quantity of coenzyme Q10 which was biosynthesized by the yeasts and the quantity of vitamin B1 in the culture medium. A larger quantity of vitamin B1 in the culture medium resulted in an increased quantity of coenzyme Q10. Thus, the progression of endogenous biosynthesis of coenzyme Q10 in the 4 experimental variants was $W < V1 < V2 < V3$. In the experimental variant (V3) with the highest concentration of vitamin B1 (2.37 mM) we registered an increase of 43,3% when compared to the maritor.*

Key words: yeast, coenzyme Q10, vitamin B

INTRODUCTION

Coenzyme Q 10 (ubiquinone) is an essential electron carrier of the mitochondrial respiratory chain. Its main function is to accept electrons from the NADH- and succinate-coenzyme Q reductases and to donate them to the bc1 complex (Fig.1).

Biosynthesis of coenzyme Q in eukaryotes occurs in mitochondria. CoQ10 is characterized by a quinone ring (Fig. 2) attached to a repeating series of side-chain isoprene units (10 repeating isoprene units). The number of isoprene units is denoted by the coenzyme-X designation. In the case of CoQ10, there are Coenzyme Q was first discovered by researchers at the University of Wisconsin in 1957. Later, Wolf et al. (1958) reported the chemical structure of the compound.

Mitochondrial Electron Transport Chain

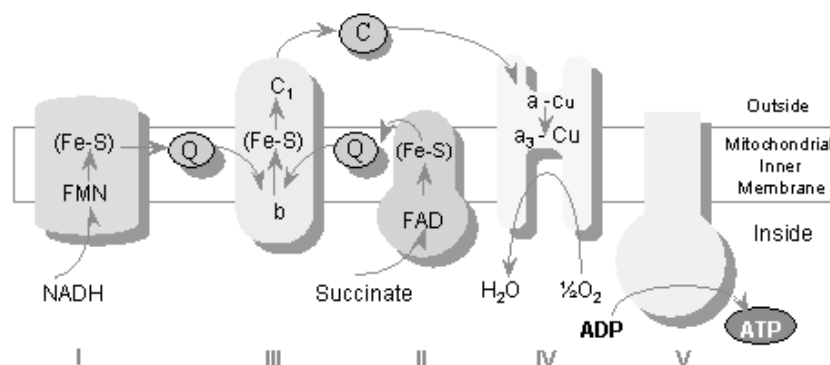


Fig. 1: Coenzyme Q10 is an important element in the electron transporting chain (<http://aix-150.ion.ucl.ac.uk/neurochemistry/nmu/Mitochondrial-Disorders.htm>)

The reduced biologically active forms of CoQ10, QH, and QH₂ are the result of protonation at the carbonyl moieties of the quinone ring (Fig. 2). CoQ10 is lipophilic and highly water-insoluble (Aberg et al., 1992).

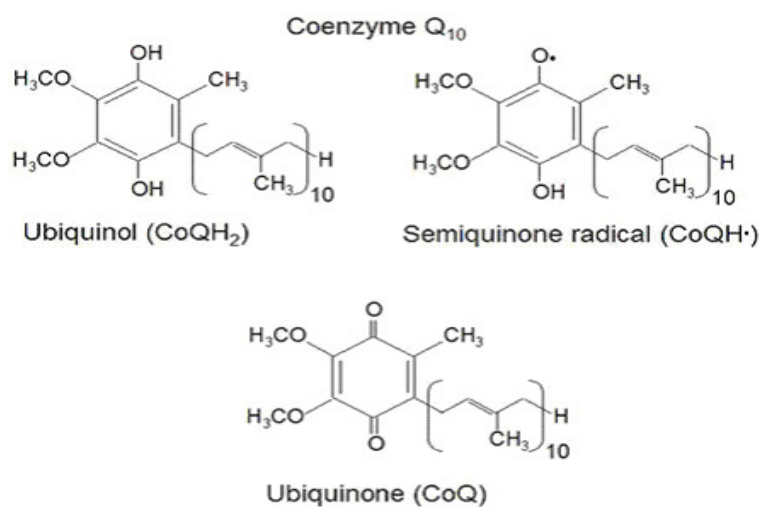


Fig.2 Chemical structure of coenzyme Q 10

CoQ10 is found in numerous cellular structures within the body, including the endoplasmic reticulum, lysosomes, other vesicles, and mitochondria, where it is an important part of the electron transport chain. Other purported beneficial effects of CoQ10 (Overvad și colab., 1999; Fuke și colab., 2000; Beal, 2002; Bliznakov și colab., 2004) include: the

prevention of lipid peroxidation initiation in plasma membranes, prevention of low-density lipoprotein oxidation, antihypertensive functions, migraine headache treatment, neurodegenerative disease treatment (Parkinson's Disease) (Shultz și colab., 2002; Kieburz, 2001), cardiovascular disease.

The purpose of this study is to observe if supplementing the culture medium with different concentrations of vitamin B1 stimulates production of coenzyme Q10 by the *Saccharomyces cerevisiae* yeast strain, as a result.

MATERIALS AND METHOD

This study was conducted in the Biotechnology Laboratory of the Faculty of Environmental Protection from the University of Oradea in 2009.

Preparation of the culture environment

The culture environment that we used is GYEP (10% glucose, 1% yeast extract, 1% peptone). For each experimental variant we prepared 250 ml of culture environment. Bioreactors along with culture environments were autoclaved, sterilized at 121°C for 20 minutes.

Yeast and culture conditions

The yeast we used for this study was a commercially available *Sacharomyces Cerevisiae*, Lovlin D47 strain.

Inoculation took place in two stages. In the first stage a single colony obtained in a Petri dish was inoculated in a small quantity of GYEP environment (10 ml) and then incubated for 24 hours in a thermostat set at 30°. At the end of the exponential growth phase, the culture was transferred (stage 2) in a 250 ml GYEP medium, resulting in 8.7×10^6 cellular density in all experimental variants (Table 1).

Table 1

Experimental variants

Experimental variants	Volume of GYEP culture environment (ml)	Vitamin B1 (mM)	Initial yeast suspension (cells/ml)
M	250	-	$8,7 \times 10^6$
V1	250	0.59	$8,7 \times 10^6$
V2	250	1.18	$8,7 \times 10^6$
V3	250	2.37	$8,7 \times 10^6$

To ensure air circulation of 3.5 l/h we used a type AC-9906 air pump. The air which was introduced to the bioreactors was filtered with textile filters.

Analytic methods

Yeast cell density within the culture environment was determined using a DEN-1 densitometer equipped with an internal gauging curve. When there were readings of cell density which were not included on the gauging curve, we proceeded to dilute appropriately, taking the dilutions into account in further cell density measurements.

Extraction of coenzyme Q10 from the yeast

For the extraction and then identification of coenzyme Q10, both in terms of quality and quantity, by use of the spectrophotometric method, after 42 hours of cellular growth, all four experimental variants (M, V1, V2 and V3) were subjected to freeze/thaw cycles during 4 days, every 12 hours. Finally the coenzyme Q10 was extracted by using n-propanol.

Identification and quantification of coenzyme Q10 using the spectrophotometric method

As a standard for CoQ10 we used a pharmaceutical product (*Coenzyme Q10 in box thorn oil ((Lycium barbarum)(food supplement)*, HOFIGAL, Bucharest (www.hofigal.eu)) as base for a 10 mg/ml stock solution, using n-propanol as solvent. We produced dilutions from the stock solution of coenzyme Q10, obtaining the following concentrations: 8mg/ml, 4 mg/ml and 2 mg/ml. For the stock solution of coenzyme Q10 we measured a spectrum with wavelengths between 190 nm and 1100 nm (UV-VIS spectrophotometer, UVmini-1240, Shimadzu), to identify the wavelength at which the absorbion of coenzyme Q10 peaks and the form it can be found in (oxidized or reduced). The wavelength at which we registered maximum coenzyme Q10 absorbion was 300 nm, in reduced form. Using the solutions of different concentrations of CoQ10 we determined the absorbion rate at 300 nm and we traced the standard curve, shown in **Figure 3**.

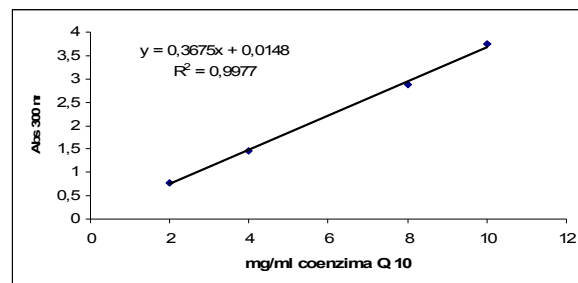


Fig 3: Standard curve for Coenzyme Q10.

RESULTS AND DISCUSSION

Cell density dynamics

The development of yeast cells over 42 hours, through the four stages (lag phase, logarithmic multiplication phase, stationary phase and decline phase) is shown in **Figure 4**. The lag phase (Fig. 1) lasts for approximately 6 hours in all experimental variants (M, V1, V2, V3), while the time it takes for the logarithmic multiplication phase to occur differs. This phase takes 15 hours in the martor experimental variant and 11 hours in the other three experimental variants. The Stationary phase lasts for 22 hours in the martor

variant and 25-27 hours in all the other experimental variants. The highest cell density was recorded 17 hours after inoculation in the culture environment in V3 (75×10^6 cells/ml), a value which remained almost constant throughout the rest of the experiment. This value is distinctly significant when compared to the martor (65×10^6 cells/ml) and significant when compared to V1 (66×10^6 cells/ml) and V2 (67×10^6 cells/ml).

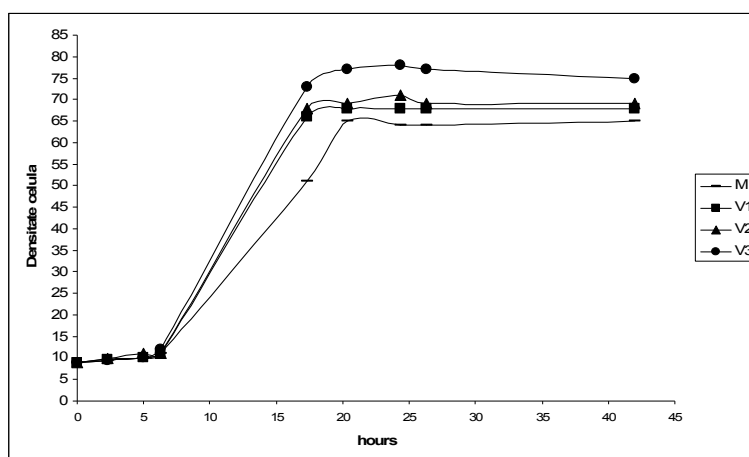


Fig. 4: Cell density of *Saccharomyces cerevisiae*, at different time intervals, in the four experimental variants

Identification and quantification of Coenzyme Q10 using the spectrophotometric method

Qualitative highlighting of the Coenzyme Q10 in the *Coenzyme Q10 in box thorn oil ((Lycium barbarum)(food supplement)*, HOFIGAL, Bucharest pharmaceutical product, as well as the 4 experimental variants was realized by measuring the absorbtion spectrum of extracts made in propanol (**Fig. 5**). In all cases the presence of Coenzyme Q10 was highlighted.

After calibrating the gauging curve of the coenzyme Q10 (**Fig. 3**) the regression equation $y = 0,3675 x + 0,0148$ was obtained, where y represents the absorbtion read at 300 nm and x represents the concentration in mg/ml. After extracting CoQ10 from the 4 experimental variants the absorbance at 300 nm was read and the obtained values were used in the regression equation to determine the concentration of CoQ10. The results obtained are shown in **Fig. 6**.

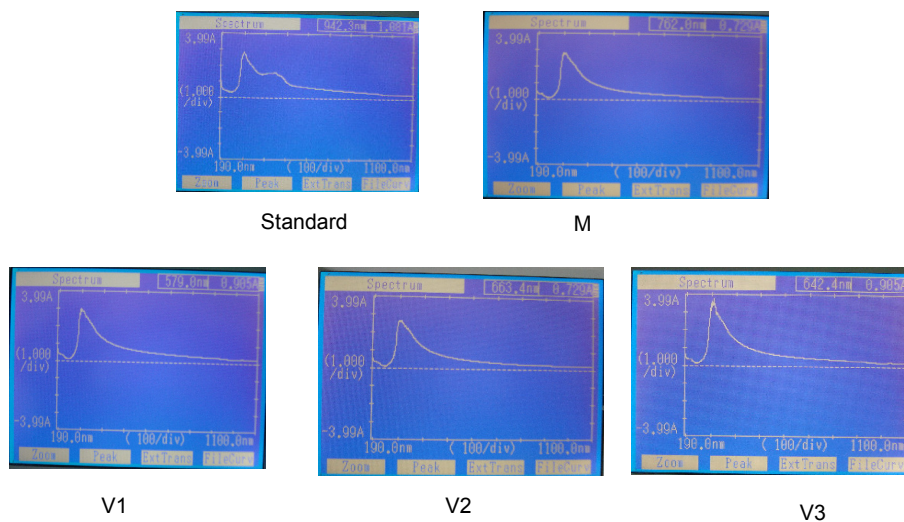


Fig. 5 Absorption spectrums of yeast extracts in propanol, grown in culture mediums with different concentrations of vitamin B1.

Results obtained after quantification of CoQ10 show that in the case of experimental variant V3 (in which the vitamin B1 content was highest), there was the largest quantity of CoQ10 (10,27 mg/ml). In the case of the martor, the CoQ10 quantity reached 7,17 mg/ml. In the case of experimental variants V1 and V2, the quantity of CoQ10 reached 7,67 ml/ml and 8,17 mg/ml respectively.

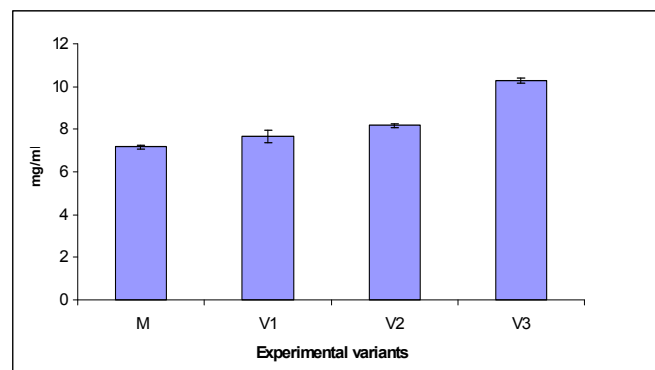


Fig. 6 Quantity of CoQ10 biosynthesized by *S. cerevisiae* in the four experimental variants (M, V1, V2, V3).

Endogenous biosynthesis of CoQ10 is a very complex process that requires numerous vitamins from the B vitamin group, folic acid, niacynamide, pantotenic acid and vitamin C, as well as the presence, in small quantities, of certain elements. Supplementing the culture environment with vitamin

B1 resulted in a stimulation of endogenous biosynthesis of CoQ10 by the *Saccharomyces cerevisiae* yeast strain.

CONCLUSIONS

The conclusions which can be drawn from this study are:

1. The logarithmic multiplication phase of yeast cells takes different amounts of time – 15 hours in the martor and 11 hours in the 3 experimental variants which contain different quantities of vitamin B (2.37; 1.18 and 0.59 mM respectively).
2. The highest cell density was recorded 17 hours after inoculation in the culture environment in V3 (75×10^6 cells/ml), a value which remained almost constant throughout the rest of the experiment. This value is distinctly significant when compared to the martor (65×10^6 cells/ml) and significant when compared to V1 (66×10^6 cells/ml) and V2 (67×10^6 cells/ml).
3. Results show a positive correlation between the quantity of coenzyme Q10 which was biosynthesized by the yeasts and the quantity of vitamin B1 in the culture medium. A larger quantity of vitamin B1 in the culture medium resulted in an increased quantity of coenzyme Q10. Thus, the progression of endogenous biosynthesis of coenzyme Q10 in the 4 experimental variants was $M < V1 < V2 < V3$.
4. In the experimental variant (V3) with the highest concentration of vitamin B1 (2.37 mM) we registered an increase of 43,3% when compared to the martor.
5. Endogenous biosynthesis of CoQ10 by *Saccharomyces cerevisiae* requires the presence of vitamin B in the culture environment.

REFERENCES

1. *** http://aix-150.ion.ucl.ac.uk/neurochemistry/nmu/Mitochondrial-Disorders.htm-Coenzyme_Q10 (11. aprilie 2009)
2. Aberg F, Appelkvist EL, Dallner G, Ernster L., 1992, Distribution and redox state of ubiquinones in rat and human tissues. Arch Biochem Biophys., 295, 230-234.
3. Beal M.F., 2002, Coenzyme Q10 as a possible treatment for neurodegenerative diseases. Free Rad Res, 36, 455-460.
4. Bliznakov E.G., Chopra R.K., Bhagavan H.N., 2004, Coenzyme Q10 and neoplasia: Overview of experimental and clinical evidence. In: Phytopharmaceuticals in Cancer Chemoprevention, Eds. Bagchi D, Preuss HG, CRC Press, Boca Raton, 599-622.
5. Fuke C, Krikorian S.A., Couris R.R., 2000, Coenzyme Q10: A review of essential functions and clinical trials. US Pharmacist, 25,1-12.
6. Kiebertz K. [The Huntington Study group], 2001, A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. Neurology, 57, 397-404.
7. Overvad K., Diamant B., Holm L., Holmer G., Mortensen S.A., Stender S., 1999, Coenzyme Q10 in health and disease. Eur J Clin Nutr., 53(10), 764-770.

8. Shultz C.W., Oakes D., Kieburtz K., Beal F.L., Haas R., Plumb S., Juncos J.L., Nutt J., Shoulson I., Carter J., Kompoliti K., Perlmutter J.S., Reich S., Stern M., Watts R.L., Kurlan R., Molho E., Harrison M., Lew M., and the Parkinson Study Group, 2002, Effects of coenzyme Q10 in early Parkinson Disease. *Arch Neurol.*, 59:1541-1550.
9. Wolf D.E., Hoffman C.H., Trenner N.R., Arison B.H., Shunk C.H., Linn B.D., McPherson J.F., Folkers K., 1958, *J. Am. Chem. Soc.*, 80, 4752–4758.