Appl Biochem Biotechnol (2010) 162:795–804
DOI 10.1007/s12010-009-8845-z

Synthesis of Coenzyme Q_{10} and β-carotene by Yeasts Isolated from Antarctic Soil and Lichen in Response to Ultraviolet and Visible Radiations

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Received: 13 July 2009 / Accepted: 29 October 2009 /
Published online: 20 November 2009

Abstract The effect of different doses of visible (Vis), ultraviolet-A (UVA), and mixed light (UVA+Vis) upon coenzyme Q_{10} (CoQ_{10}) and β-carotene synthesis and biomass yield by the *Sporobolomyces salmonicolor* AL_1, *Cryptococcus albidus* AS_{55}, *Cryptococcus laurentii* AS_{56}, and *C. laurentii* AS_{58} strains isolated from Antarctic samples was investigated. The β-carotene concentration in the red strain biomass increased by 52% under irradiation with 11 J/cm^2 Vis, and the CoQ_{10} concentration rose by 37% in relation to the control quantity obtained through dark cultivation. Under irradiation with 6 J/cm^2 UVA, the *S. salmonicolor* AL_1 strain synthesized 15% more β-carotene; *C. albidus* AS_{55}, 22%; *C. laurentii* AS_{56}, 44%; and *C. laurentii* AS_{58}, 35% in relation to the control quantity. Irradiation with a low UVA+Vis dose significantly stimulated β-carotene biosynthesis by the strains of the *Cryptococcus* genus (87%, 138%, and 100%), whereas *S. salmonicolor* AL_1 increased the β-carotene content to a smaller degree (55%). Higher doses of all three irradiation types inhibited β-carotene accumulation. Vis suppressed CoQ_{10} biosynthesis in the *Cryptococcus* strains, whereas UVA and UVA+Vis inhibited it in all four strains. The *S. salmonicolor* AL_1 strain pre-treated with 0.02 J/cm^2 UVA synthesized twice as much CoQ_{10} and β-carotene when cultivated in the presence of Vis light in an 11-J/cm^2 dose.

Keywords *S. salmonicolor* AL_1 · *C. albidus* AS_{55} · *C. laurentii* AS_{56} · *C. laurentii* AS_{58} · β-carotene · Coenzyme Q_{10} · UV · Vis

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Introduction

Yeasts isolated from Antarctic [1] and freshwater ecosystems [2] have become the focus of attention for researchers who study the biosynthesis of photoprotective substances as a barrier against ultraviolet radiation (UV). UV has wavelength in the range of 200–400 nm and visible radiation (Vis) from 400 to 800 nm. In recent years, UV reaching the earth due to the reduction of the ozone layer has been noticed in terms of potential health hazard on humans, animals, and environment. High UV exposure causes damages to DNA, proteins, including cell membrane lipoproteins and organelles [3].

Terrestrial microorganisms in habitats exposed to high UV produce different pigments potentially protecting against UVB damages. UV-screening compounds provide a passive method for the reduction of UV-induced damage, and they are widely distributed across the microbial, plant, and animal kingdoms [4]. The synthesis of carotenoids that quench oxygen-free radicals generated by UV-induced photochemical reactions [5] is also an important response in preventing UV-induced damage to a wide diversity of biological macromolecules.

Carotenoids are important natural pigments that are found widely distributed in plants and microorganisms. Animals depend on vegetation for carotenoids because they cannot synthesize these pigments de novo. Carotenoids play an essential role as accessory light-harvesting pigments and, especially, in protection against damage by photosensitized oxidation [6]. Dietary studies have shown that β-carotene is useful in combating various types of cancers and other diseases owing to its antioxidant and provitamin-A potential [7, 8]. Several yeast genera—*Rhodotorula*, *Sporobolomyces*, *Rhodosporidium*, and *Cryptococcus*—produce β-carotene, torulene, torularhodin, which also belong to the carotenoid group, and coenzyme Q_{10} (CoQ_{10}) [9–11]. CoQ_{10} has a similar isoprenoid chain in its structure. It is also an interesting product for biotechnology. CoQ_{10} is present in all cells and membranes, and in addition to being a member of the mitochondrial respiratory chain, it also has several other functions of great importance for the cellular metabolism, such as participation in the extra-mitochondrial electron transport (plasma membranes and lysosomes), regulation of the mitochondrial permeability of transition pores, and regulation of the physicochemical properties of membranes [12]. CoQ_{10}, especially, is widely used as an essential component of ATP generation in the oxidative phosphorylation process and as an antioxidant preventing lipid peroxidation and scavenging superoxide. It has been proved that the CoQ_{10} yeast is much better absorbed by the skin than the synthetic CoQ_{10}. Peroxide reduction in the stratus corneum is considerably more pronounced after yeast CoQ_{10} application [13]. Therefore, research efforts on the production of CoQ_{10} by microorganisms focus on the development of potent strains by conventional mutagenesis and metabolic engineering, analysis and modification of the key metabolic pathways, and optimization of fermentation strategies [14]. Various microorganisms, including bacteria (e.g., *Agrobacterium*, *Rhodobacter*, and *Paracoccus*) and yeasts (e.g., *Candida*, *Rhodotorula*, and *Saitoella*), are reported as CoQ_{10} producers in patented laid-open applications [15] purposely applied in pharmaceutical and cosmetic industry.

The aim of the present study was to examine the effect of ultraviolet-A radiation, Vis light, and a combination of both on CoQ_{10} and β-carotene biosynthesis by the *Sporobolomyces* and *Cryptococcus* genera isolated from Antarctic samples.

Materials and Methods

Microorganisms

The four strains included in this study were isolated from soil and lichen from Livingston Island, Antarctica. *Sporobolomyces salmonicolor* AL_1 NBIMCC 8290 has been preserved in the National Bank for Industrial Microorganisms and Cell Cultures. *Cryptococcus albidus* AS_{55}, *Cryptococcus laurentii* AS_{56}, and *C. laurentii* AS_{58} were identified according to the yeast classification criteria proposed by Kurtzman and Fell in 1998 in a previous investigation [16].

Media and Growth Conditions

The fermentation medium contained (g/l) sucrose, 30; $(NH_4)_2SO_4$, 1.0; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.01; $CaCl_2 \cdot 2H_2O$, 0.1; and yeast extract, 1.0. The initial pH was adjusted to 5.3, and the medium was sterilized at 112 °C for 30 min. The inoculum was obtained on a rotary shaker (220 rev/min) in 500 ml Erlenmeyer flasks containing 50 ml of Sabouraud medium (Merck, Germany) on a shaker at 24 °C, 48 h. The fermentation medium was inoculated with 10% (*w*/*v*) of inoculum. The cultivation was carried out in 500 ml Erlenmeyer flasks containing 50 ml of the fermentation medium on a rotary shaker (220 rev/min) at 22 °C for 96 h. The biomass was separated by centrifugation (6,000 g, 30 min), washed twice with distilled water, and lyophilized.

Treatment with Light

The CoQ_{10} and β-carotene synthesis was carried out under deep cultivation in the dark, in Vis, under ultraviolet-A (UVA), and mixed (UVA+Vis) irradiation with different doses. The β-carotene, CoQ_{10}, and biomass quantities obtained during dark cultivation of the strains were accepted as control quantities.

An F15T8/D_{ALTO} Daylight Full Spectrum Fluorescent (Sylvania Danvers, MA, USA) lamp and two F15T8/BLB lamps with emission range from 345 to 400 nm, peak 365 nm (Sylvania Danvers, MA), placed at 20 cm above the culture medium were used as irradiation sources. Working periods of 3 or 12 h ensured the respective irradiation doses. Total irradiance was measured using an Almemo2690-8 data logger with the respective sensors: radiation measuring head Type FLA613VLM with spectral sensitivity from 360 to 760 nm and UV400 filter, and UVA radiation probe head Type FLA613UVA with spectral sensitivity from 310 to 400 nm in mW/cm^2, all from Ahlborn (Germany).

Four experiments were carried out using differing irradiation treatment. In the first experiment, the strains were cultivated under irradiation with 11, 24, 58, 69, and 97 J/cm^2 Vis. In the second experiment, the cultures developed under irradiation with 6, 12, 24, 45, and 52 J/cm^2 UVA doses. The fermentations in the third experiment were carried out under irradiation with 3+16, 5+25, and 6+32 J/cm^2 doses of UVA+Vis, respectively. In the fourth experiment, the *S. salmonicolor* AL_1 strain was pre-treated with 0.02, 0.08, 0.15, 0.30, 0.45, and 0.90 J/cm^2 UVA doses, then each of the treated variants was deep cultivated in two ways: in the dark and under irradiation with an 11-J/cm^2 dose of Vis.

Extraction and Analysis of CoQ_{10} and β-carotene

The extraction procedure for CoQ10 and β-carotene was adapted from Gimeno et al. [17]. Twenty milligrams of dry biomass was finely ground and transferred into a dark polypropylene tube (Herolab, Germany); 2 ml acetone was added, and it was vortexed for 2 min and centrifuged for 5 min at 3,000 g (Hettich EBA 20, Germany). The extraction procedure was repeated. The combined extracts were evaporated dry on a rotary vacuum evaporator (Büchi, Swetzerland) fitted with high vacuum pump E2M-1 (Edwards, England). The dry residue was dissolved in 1 ml mobile phase A, then filtered through a 0.20-μm syringe filter (Acrodisc, Germany), and 20 μl of the filtrate was injected for analysis. All sample preparation stages were carried out quickly and in dim light.

The high-performance liquid chromatography (HPLC) system was composed of a ProStar 230 solvent delivery module and photo diode array detector model 335, Microsorb-MV C18 column (150×4,6 mm, 5 μm particle size), all from Varian (Australia). A solvent system including methanol, n-hexane, 2-propanol in proportions 70:25:5 *v*/*v* (a), and acetonitrile (b) was used in gradient condition from 30A:70B to 90A:10B. The flow rate was 1 ml/min and detection at 450 nm for β-carotene and 270 nm for CoQ10, respectively. All HPLC-grade solvents were obtained from Labscan (Ireland).

The compounds of interest were identified according to their retention times determined using authentic CoQ_{10} and β-carotene standards (Sigma, USA). They were quantified using an absolute calibration curve. Star Chromatography Workstation Version 6.30 (build 5) software was used.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences for Windows software, Version 11.0. Statistically significant differences between groups were determined by analysis of variance. When the differences were significant, Duncan's multiple range test was performed. Means were considered significantly different at $p<0.05$.

Results and Discussion

The effect of different Vis doses upon β-carotene and CoQ_{10} synthesis and biomass yield by *S. salmonicolor* AL_1, *C. albidus* AS_{55}, *C. laurentii* AS_{56}, and *C. laurentii* AS_{58} strains was investigated. The results have been shown in Fig. 1.

Under dark cultivation conditions, β-carotene was synthesized in the following quantities: 63.8 μg/g by *S. salmonicolor* AL_1, 23.2 μg/g by *C. albidus* AS_{55}, 23.3 μg/g by *C. laurentii* AS_{56}, and 25.5 μg/g by *C. laurentii* AS_{58}. Maximum β-carotene concentration in the red strain biomass was reached at a dose of 11 J/cm^2 Vis making up 52% increase in relation to the control. The *Cryptococcus* strain exhibited insignificant increase at 24 J/cm^2 Vis. The β-carotene quantities decreased considerably in all strains with the increase in the irradiation doses up to 97 J/cm^2 Vis.

The CoQ_{10} control quantities varied within a narrow range: 201.6 μg/g for *S. salmonicolor* AL_1, 210.6 μg/g for *C. albidus* AS_{55}, 190.8 μg/g for *C. laurentii* AS_{56}, and 197.6 μg/g for *C. laurentii* AS_{58}. Culture reaction differed under the influence of different Vis doses. The CoQ_{10} concentration in the *S. salmonicolor* AL_1 biomass rose by 37% in relation to the control quantity under treatment with an 11-J/cm^2 dose and decreased with the increase in the irradiation dose. Vis irradiation suppressed CoQ_{10} synthesis by the *Cryptococcus* strains

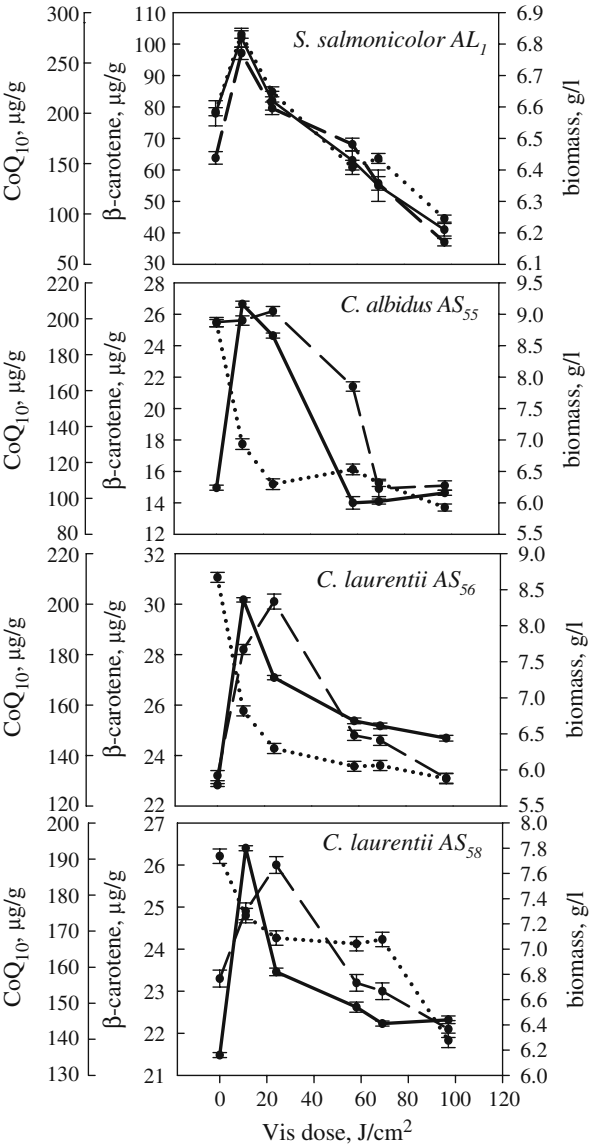

Fig. 1 Quantities of CoQ_{10} (*dotted line*), β-carotene (*dashed line*), and biomass (*solid line*) synthesized under irradiation with visible radiations

leading to a concentration lower than the control quantity. The strains studied, especially those of the *Cryptococcus* genus, exhibited a high biomass yield at an 11-J/cm^2 Vis irradiation dose. The yield decreased with the increase in doses.

The results shown in Fig. 2 demonstrate that UVA in a 6-J/cm^2 dose was favorable to β-carotene formation in Antarctic yeast, while with the increase in irradiation doses to 52 J/cm^2, its content was significantly reduced. At a lower irradiation dose, *S. salmonicolor* AL_1 synthesized 15% more β-carotene in comparison with the control quantity, whereas the strains of the *Cryptococcus* genus increased the carotenoid content by 22%, 44%, and 35% for *C. albidus* AS_{55}, *C. laurentii* AS_{56}, and *C. laurentii* AS_{58}, respectively. UVA inhibited CoQ_{10}

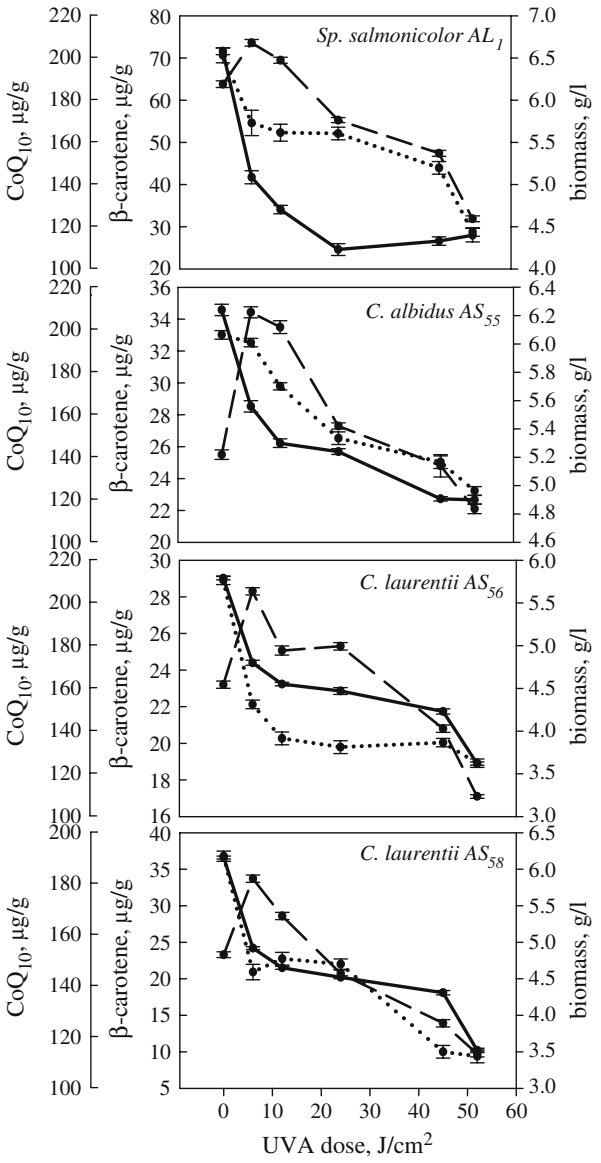

Fig. 2 Quantities of CoQ_{10} (*dotted line*), β-carotene (*dashed line*), and biomass (*solid line*) synthesized under irradiation with ultraviolet-A

biosynthesis and suppressed yeast development, higher doses leading to reduced quantities of synthesized biomass.

The results of the experiment involving irradiation of Antarctic yeast with mixed light (UVA+Vis) have been shown in Fig. 3. Mixed light stimulated β-carotene biosynthesis in psychrophilic yeast. The *C. albidus* AS_{55}, *C. laurentii* AS_{56}, and *C. laurentii* AS_{58} strains, where β-carotene levels are constitutionally low (23–25 μg/g), increased its concentration considerably by 87%, 138%, and 100% in relation to the control quantity when irradiated with a dose of 3+16 J/cm^2 (UVA+Vis). The *S. salmonicolor* AL_1 strain

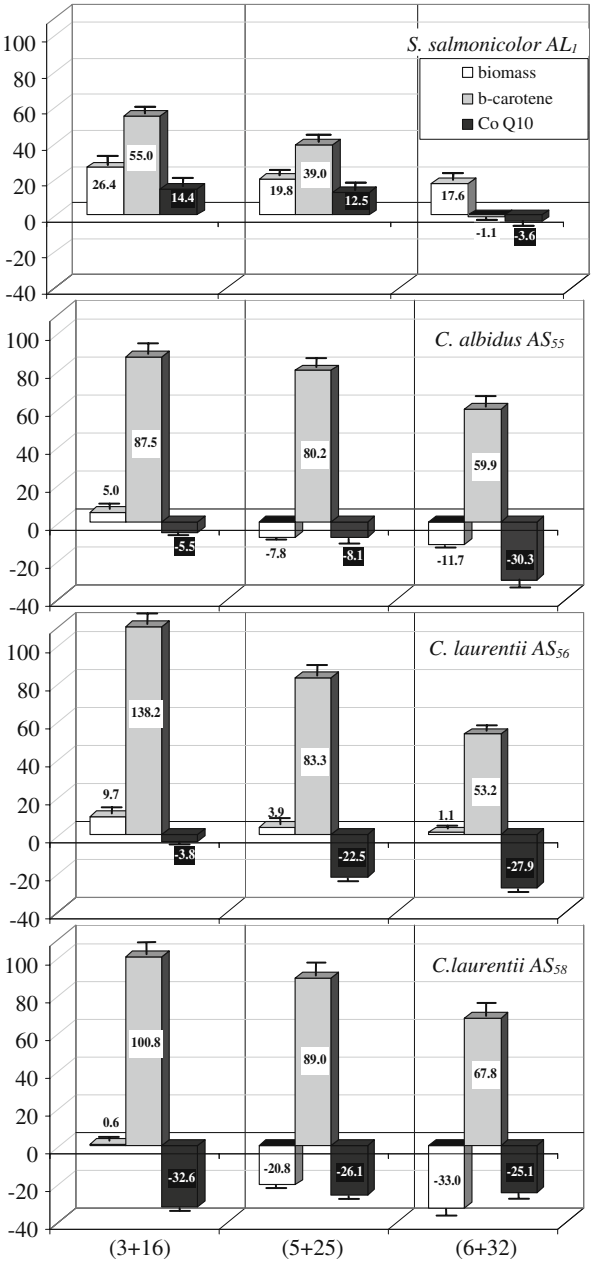

Fig. 3 Changes in the biomass, β-carotene, and CoQ_{10} quantities obtained under mixed irradiation (ultraviolet-A+visible radiations) J/cm^2, in percent according to the control quantities

with constitutionally 2.5 times more β-carotene in comparison to the strains of the *Cryptococcus* genus showed a smaller increase in its content (55%) under the effect of mixed irradiation. After irradiation with the two doses 3+16 J/cm^2 (UVA+Vis) and 5+25 J/cm^2 (UVA+Vis), the CoQ_{10} content increased slightly in the biomass of *S. salmonicolor* AL_1 only.

Irradiation with a 3+16-J/cm^2 (UVA+Vis) dose stimulated biomass accumulation in all strains. The highest yield was obtained by the *S. salmonicolor* AL_1 strain: 26.4% in relation to the control quantity, followed by *C. laurentii* AS_{56} with 9.7%. When the irradiation dose was increased to 5+25 J/cm^2 (UVA+Vis) and 6+32 J/cm^2 (UVA+Vis), *S. salmonicolor* AL_1 kept developing, whereas the same doses inhibited the strains of the *Cryptococcus* genus.

The next experiment was conducted with a *S. salmonicolor* AL_1 strain pre-treated with different UVA doses and deep cultivated in the dark and in the light, at a dose of 11 J/cm^2 Vis. The results have been shown in Fig. 4.

The preobtained dose of 0.02 J/cm^2 UVA was a positive factor in β-carotene and CoQ_{10} induction during the subsequent strain cultivation both in the dark and in the presence of light. The β-carotene and CoQ_{10} quantity was twice as large (63.8–132.9 and 201.6–430.9 μg/g, respectively) under light cultivation and over 1.5 times as large (63.8–103.5 and 201.6–325.4 μg/g, respectively) under dark cultivation. With the rise in the UVA irradiation dose, the values of the β-carotene and biomass indices decreased, whereas the CoQ_{10} level increased even at a dose of 0.45 J/cm^2.

Our study proved the ability of psychrophilic yeast of the *Sporobolomyces* and *Cryptococcus* genera isolated from Antarctic samples to synthesize β-carotene and CoQ_{10} when irradiated with Vis, UVA, and mixed light. The biomass quantities accumulated by *S. salmonicolor* AL_1, *C. laurentii* AL_{58}, *C. albidus* AL_{55}, and *C. laurentii* AL_{56} were larger under irradiation with low Vis doses than those accumulated under dark cultivation. White light stimulated β-carotene and CoQ_{10} biosynthesis in pigment-forming *S. salmonicolor* AL_1 strain, while the β-carotene quantity in the strains of the *Cryptococcus* genus was not affected by the cultivation conditions. Studying the effect of light on carotenogenesis in six strains of *Phaffia rhodozyma*, Vazquez proved they all produced greater amounts of total carotenoids in the light than in the dark, and the illumination influenced not only total

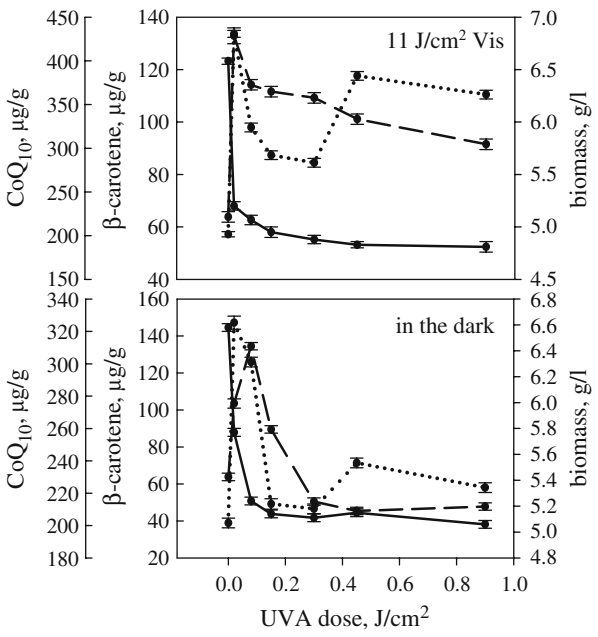

Fig. 4 Quantities of CoQ_{10} (*dotted line*), β-carotene (*dashed line*), and biomass (*solid line*) synthesized by *Sporobolomyces salmonicolor* AL_1 pre-treated with ultraviolet-A irradiation

carotenoid concentrations but also the carotenoid profile, biomass, and xylitol concentrations [18]. A research on the effect of weak white light on the growth and carotenogenesis of *Rhodotorula glutinis* showed that red strain growth was slightly inhibited, but the biosynthesis of carotenoids, torularodin in particular, was stimulated [19]. Carotenoid production and accumulation are reported to be positively affected by white-light irradiation in algae, fungi, and bacteria. However, the intensity and protocol of illumination varies with the microorganism [20].

Antarctic yeasts, in particular, the highly pigmented species, were essentially resistant to UVA radiation, at least up to 0.5 J/cm^2, whereas less than 5% can survive a similar dose of UVB. It has been suggested that resistance may be associated with pigmentation, possibly carotenoids and related pigments, that may afford important protection [1]. In our experiments, UVA radiation stimulated β-carotene biosynthesis in small doses only and suppressed CoQ_{10} accumulation. The rise in β-carotene in the *Cryptococcus* strains was more pronounced compared to *S. salmonicolor* AL_1, which demonstrated the defense reaction of Antarctic yeasts against UVA irradiation and was consistent with Libkind's conclusions [2].

The same tendency was observed with the biosynthesis of the investigated substances under the effect of mixed light. The β-carotene quantity in the biomass of *C. laurentii* AS_{56} and *C. laurentii* AS_{58} at a dose of 3+16 J/cm^2 (UVA+Vis) exceeded the controls by 100%. Carotenoid synthesis was clearly stimulated in six red yeast strains out of the 12 strains studied for their ability to produce photoprotective compounds—carotenoids and mycosporines—upon exposure to Vis or UV+Vis [21].

The highest CoQ_{10} and β-carotene quantities were obtained during the preliminary irradiation of the *S. salmonicolor* AL_1 strain with UVA, and the subsequent deep cultivation at a dose of 11 J/cm^2 Vis (430.9 μg/g) and in the dark (325.4 μg/g). These results can be interpreted in the light of the UVA effect on DNA expressed mainly as a change in pyrimidine bases [22] and activation of the photolyase enzyme under the effect of Vis. DNA photolyase is a flavoprotein that uses Vis light energy to split UV-induced cyclobutane pyrimidine dimers in damaged DNA [23].

The biomass quantity decreased with all yeast strains studied when they were subjected to UVA radiation. This effect was also observed by Tosi et al. during experiments with Antarctic soil fungi assemblages. They showed better growth when protected against UV radiation [24].

Conclusions

The investigations discussed and their results led to the conclusion that irradiation up to certain UVA and Vis doses stimulated CoQ_{10} and β-carotene biosynthesis by Antarctic yeast, whereas even low doses of UVA hindered the development of the four strains. This is the first report of CoQ_{10} presence in the biomass of the *S. salmonicolor* AL_1 and the first investigations of the effect of UVA and Vis irradiation upon its biosynthesis.

The presence of CoQ_{10} and β-carotene in the biomass of the *S. salmonicolor* AL_1 strain and the evidence of the stimulating UVA and Vis effect on their synthesis revealed some possibilities of their isolation and study with a view to their application to medicine, cosmetics, and agriculture.

Acknowledgements The study was supported by Grant B-1615 from the National Fund Scientific Investigation.

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