European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 5; Issue 5; September 2017; Page No. 41-46

Carotenogenesis in *Dunaliella* cells under stressed conditions

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Abstract
Dunaliella cells are known as carotenotransferring halotolerant green algae. In the work were presented the results of biosynthesis investigations of carotenoid amounts in *Dunaliella* cells, grown in mineral medium by adding 1,0M; 1,5 M and 3,0 M NaCl, their bioproductivity, culture resistance against chronic doses of UV-B rays, the influence of low temperature stress, and also their photosynthetic activity under high temperatures. It was shown that the cells, grown in intensive culture with 1,0M; 1,5M and 3,0 M NaCl in nutrition medium show various biosynthesis indications of carotenoid amounts, bioproductivity, and functional stability against high temperature. It was determined that the cells grown in medium with 3,0 M NaCl lead to the decrease of bioproductivity and increase of resistance against high temperature, related to the increase of synthesis of carotenoid amounts in cells. It has been also identified that the cells grown in medium with 1,5 M NaCI and in different chronic UV-B -rays in intensive culture increase the synthesized carotenoids and show high functional stability against high temperature. The decrease of NaCI (1,0M) concentration in nutrition medium leads to decrease of indications of biosynthesis of carotenoid amounts and growth rate to (10-15%. It was determined that low temperature stress decreases the cell bioproductivity and carotenoid amounts. One came into conclusion that, the population resistance against the chronic doses of UV-B-rays, functional stability against high temperature correlated by a number of synthesized carotenoids in those conditions.

Keywords: *dunaliella*, salinity, bioproductivity, carotenoids, uv-b-irradiance, low-temperature stress, high temperature, functional stability

Introduction

Lately a great interest was observed to the carotenoid microalgae synthesizing high amounts of carotenoids. Those are called unicellular green algae, usually accumulating under the influence of unfavorable conditions, high quantity (till 6% dry weight) of secondary carotenoids. The induction of high carotenoid quantity, called also carotenogenesis, is the characteristic respond of unicellular algae, especially the representatives of green algae types (*Chlorophyta*), against the stressor influence of various nature (intensive light, high salinity, UV irradiation, extreme temperature) [1-6]. It is considered that carotenogenesis, with the other answers against the stressor influence, is the adaptive reaction, supplying the surviving of microalgae in extreme conditions of habitat environment. So, to investigate the carotenogenesis as the object often used as called "snowy algae"-microalgae (*Chlorella nivalis, Chloromonas rubroleosa, Parietochloris* incise and other series), able to grow also in snowy slopes under bright sunny light in temperatures close to 0^0C [7, 8]. In those conditions the colors of algae change from green to various shades of yellow-orange and red color, as the result of carotenoid synthesis induction in great quantities. Among pigments, accumulated by various microalgae under stress conditions, often observed β-carotene (in representatives of *Dunaliella* types) [1], astaxanthin (in *Haematococcus* types) [4], also in other carotenoids and their derivatives.

B-carotenoid accumulation in green algae, as *Dunaliella salina* and *Dunaliella bardawil* [9], also *Haematococcus*, induced to strong light, high salinity in addition, deficient mineral nutrition and low temperature, due to conditions lead to lowering effectiveness of photosynthesis and increasing photo inhibition risk [10]. It is known that β-carotene in those algae can be induced artificially even in low irradiation, if treated their cells by dyes generators of AOS (methylene blue and Rose Bengal), on the contrary, the addition of extinguishers $^1O_2$ (histidine and eosin) inhibited carotenogenesis in *Haematococcus* under high irradiance and deficient mineral nutrition [11]. Thus, has been presented the probable participation formed during the photosynthesis $^1O_2$ in carotenogenesis induction in cells of unfavorable conditions [11, 12]. According to the over-producing of β-carotenoid was identified the coordinated regulation of various responds against the influence of stressors-synthesis of high quantity of carotenoids and protein expression of light-harvesting complex, binding zeaxanthin and also protein-stabilating lipid globul, in which postponed β- carotene [13].

The aim of investigation is to study the synthesis of carotenoid amounts of *Dunaliella* cells grown in mineral medium adding 1,0M; 1,5M and 3,0M NaCl, their bioproductivity, culture resistance to chronic doses of UV-B irradiation, the influence of low temperature stress, also their photosynthetic activity under high temperature.

Materials And Methods

The object of investigation is the halophile green microalgae *Dunaliella salina IPPAS D-294*, taken from saline lake Masazir locating in the North-East territory of city Baku.

In conditions of chronic doses of UV-B irradiation, the algae

were grown at 27^0C in photoreactors (250ml), of ordinary (control suspension) and quartz (experienced suspension) glass, in the installation to grow the culture of unicellular algae. The source of UV-B irradiation is mercury lamp supplied by light filter.

Chronic UV-B irradiation of cells was carried out 24 hourly (day and night) with the help of hourly mechanism.

Mineral medium contains (g/l): NaCI-58,5 (1,0M); 87,5 (1,5M) and 175,5 (3,0M); KNO_3-5,0; KH_2PO_4-1,25; $MgSO_4$-50; $FeSO_4$-0,009, the microelement solution (mg/l): – $Ca(NO_3)_2$ •H_2O- 735; H_3BO_3 -735; $ZnSO_4$ •$7H_2O$- 615; $(NH_4)MoO_4$- 100; $MnCl_2$ •$4H_2O$- 180. 1 ml solution of microelements is added into each liter of mineral medium. Cell suspension in photoreactors within 24 hours was irradiated by white light (16 Wt/m^2) and continuously was blown by the mixture (air+1, 5% CO_2) at the temperature 25ºC for the control and in the conditions of low temperature stress 10ºC; 5ºC for the experienced suspension.

The cells were grown within 24 hours, in intensive-accumulating regime of cultivation and was irradiated day and night (24 hourly). The growth of culture was determined periodically by counting the number of cells in the Goryayev chamber under microscope or by nephelometrically measurement of suspension optic density in the photoelectrocolorimeter.

The content of carotenoids in cellular extracts (100% acetone) was measured in the spectrophotometer and calculated on the base of Wetshtain coefficient [14].

In order to measure the photosynthetic activity of cells, the grown algae were cooled by centrifugation 3000 rev/min. within 10 minutes at room temperature and were transferred into newly prepared mineral medium. The density of cell suspension adjusted to10^6 cell/ml (optic density OD=0,8). The rate of cell oxygen evaluation was measured in the polarography installation, by using platinum electrode Klark, lighting the suspension in thermo-stated cellule, with white light saturating intensity (100 Wt/m^2).

Results and Discussions

The dependence of carotenoid accumulation in Dunaliella salina IPPAS D-294 cells on NaCI concentration in the medium. The investigation of eco-physiological, biochemical and cytological peculiarities of *Dunaliella* types, give an opportunity to shed light to the mechanism halotolerance. It is known that, those organisms develop in extremely high saline medium (1-4M NaCl), that make interesting the explanation of peculiarities of their structural-functional organization, the study of osmoregulation mechanisms, ability to directed synthesis of β-carotene. Preliminary practice of seed gives an opportunity to determine the limits of salt tolerance of *Dunaliella* cells and discover the growth concentration optimum. Under the influence of unfavorable factors of external environment (low temperature, overage salt) glycerin content increases in *Dunaliella* cells [15]. Thus, characteristic glycerin accumulation in cytoplasm, as osmoregulation substance, closely correlated with the salt concentration in medium of algae habitat. The investigation of growth kinetics of population in *Dunaliella salina IPPAS D-294* cells in various salinities of medium in intensive-accumulative cultivation regime showed that, the maximum productivity was observed with NaCl (1,5M) concentration in mineral medium (fig.1).

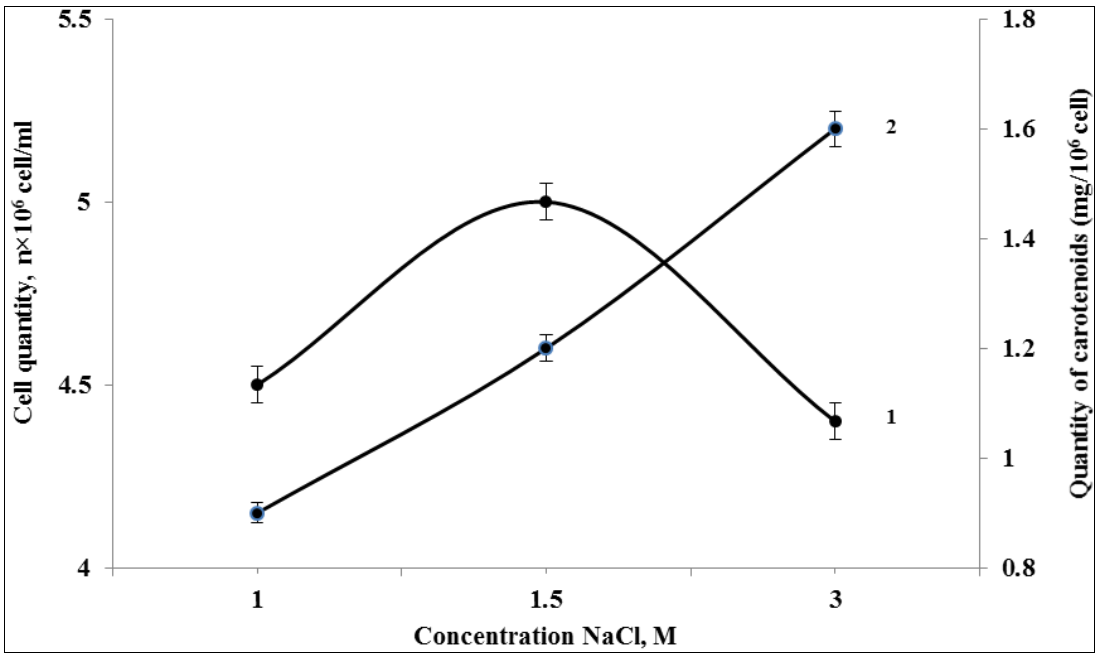

Fig 1: The dependence of growth indications (1) and biosynthesis of carotenoid amounts (2) in *Dunaliella* cells in concentration NaCl (M) in nutrition medium. Temperature 27^0C, light intensity 16 Wt/m^2.

It is interesting to note that, the decrease of NaCl (1,0M) concentration in mineral medium leads to the reduction of indications of carotenoid biosynthesis (12%) and growth temp of culture to (20-22%).

The high concentration of sodium chloride in nutrition medium 3,0M leads to considerable increase of synthesis of

carotenoid amounts (30%) and reduction of day and night growth rate of *Dunaliella* to 20-25%. It should be noted that, despite of the reduction of growth rate, the culture bioproductivity remains on the enough high level.

The investigation of a number of indications of synthesis of carotenoid amounts in *Dunaliella* cells in NaCl concentration showed that, in those conditions the cells keep their typical respond reaction, and by increasing NaCl concentration in medium the carotenoid amounts rise in them. Thus, the inverse correlation among accumulation of carotenoid amounts in cells and culture growth also attracts an attention.

The influence of various doses of chronic UV-B irradiation on the accumulation of carotenoid amounts in *Dunaliella salina IPPAS D-294.* Due to ozone depletion were observed the increase of UV-B irradiation dose on the Earth surface and inerruptional function of plant organism. UV-B irradiance is one of the ecological factors, affecting in different directions on unicellular algae population and plants. It is necessary to isolate the specific peculiarities and the mechanism of UV irradiance from totality of ecological factors on population level.

In fig. 2, was presented the dependence of growth indications and carotenoid amounts in *Dunaliella* cells under the chronic dose of UV-B irradiance in intensive cultivation. On the curve of dose-effect was observed the suppression of population growth in microalgae cells (fig.2, 1). The increase of UV-B irradiation in investigating range effects on the population survival, so under chronic dose of $15 \cdot 10^3$ Erg/mm^2 during an hour it constitutes 85 % of control.

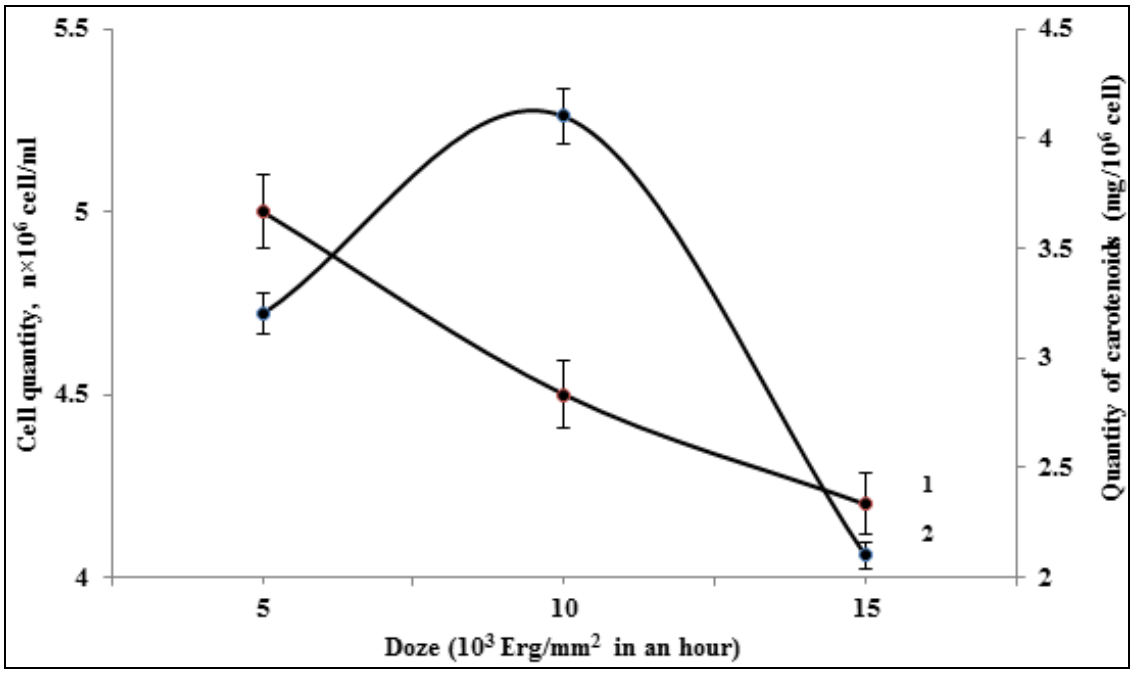

Fig 2: The dependence of growth indications (1) and biosynthesis of carotenoid amounts (2) in *Dunaliella* cells under chronic dose of UV-B irradiation in intensive culture. Temperature 27 ^{0} C, light intensity 16 Wt/m^2.

Under the influence of chronic dose of UV-B irradiance on population of *Dunaliella* cells have been gained the results of dependence of biosynthesis of carotenoid amounts on chronic dose of UV-B irradiation, shown in fig.2. (curve2). As seen from figure, that dependence is described by single-humped curve with maximum under chronic dose of UV-B radiation ($10 \cdot 10^3$ Erg/mm^2 in an hour). The biosynthesis of carotenoid amounts in experienced cells in intensive culture under chronic dose of UV-B irradiation $5 \cdot 10^3$ Erg/mm^2 within an hour is 3,2mg/10^6 cell. That quantity exceeds control cells (1, 5 mg/10^{6}cell) 2 times. Maximum quantity (4, 1 mg/10^{6}cell) of synthesized cells of carotenoid amounts was observed under chronic dose $10 \cdot 10^3$ Erg/mm^2 within an hour.

In intensive culture conditions, the increase of chronic dose of UV- rays to $15 \cdot 10^3$ Erg/mm^2 within an hour decreases the quantity of synthesis of carotenoid amounts in population of *Dunaliella* cells. On base of gained results we can conclude the following, the cultivation of algae in intensive culture under various chronic doses of UV-B irradiation, allows us to gain cells, enriched with carotenoid amounts.

The influence of low temperature stress on the synthesis of carotenoid amounts in cells of *Dunaliella salina IPPAS D-294* in intensive culture. Nowadays exists extensive literature on the problem of alive organism stability against low temperature. The significance of the problem conditioned by the fact, that considerable part of dry territory (65%) of plants suffers from the destructive influence of low temperature.

It is necessary to note that, a certain part of damage under low temperature stress conditioned by the influence formed in cells during the stress of active form of oxygen, resulting in activation of lipid peroxidation processes, causing structural membrane changes. The cultivation of control suspension of cells in optimal conditions (temperature 27^{0}C, light intensity 16 Wt/m^2, partial pressure of carbon dioxide, mineral medium) in 250 ml glass photoreactors and supplying with 25^{2}C air mixture in periodically- accumulative regime of cultivation within 24 hours showed that, optic density of

cellular suspension increases 3,5-4 times [16]. In figure 3 has been presented the dependence of growth indications and carotenoid biosynthesis in *Dunaliella* cells under air mixture temperature supplying in photoreactors in intensive-accumulating cultivation regime. As seen from the figure, the reduction of air mixture temperature, supplying in photoreactors to 10^0C and 5^0C, considerably suppresses the growth and cellular suspension bioproductivity to 10%-20% respectively (fig.3. curve1).

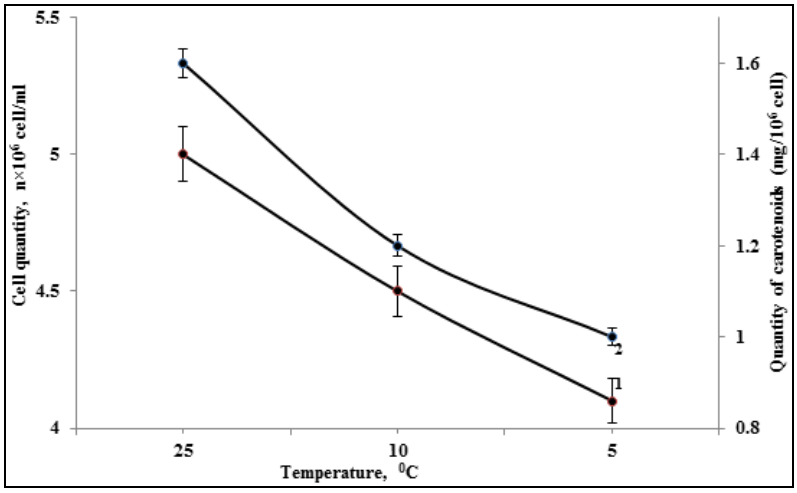

Fig 3: The dependence of growth indications (1) and biosynthesis of carotenoid amounts (2) in *Dunaliella* cells under air mixture temperature supplying in photoreactors in intensive-accumulative regime of cultivation. Temperature 27^0 C, light intensity 16 Wt/m^2.

As the result of low positive temperature influence (10^0C) on *Dunaliella* cells was observed the degradation of carotenoid amounts, relative to control cells (fig. 3.curve 2). It is necessary to note that, by lowering air mixture temperature, supplying in photoreactors to 5^0C, was observed considerable reduction of biosynthesis of carotenoid amounts in cells of *Dunaliella salina IPPAS D-294*. Thus, low positive temperature (10^0C) and (5^0C) of air mixture supplying in photoreactors, in intensive-accumulative cultivation regime decreases the population bioproductivity and the biosynthesis of carotenoid amounts in cells.

Carotenoids increase the functional stability in cells of *Dunaliella salina IPPAS D-294* at high temperatures. Plant cultivation seldom grows in optimal conditions. Usually they exposed to simultaneous effect of some stresses. Various plant respond reactions against those stresses can be additive, synergetic or antagonistic.

The aim of the investigation was the clarification of roles of synthesis of carotenoid amounts in *Dunaliella* cells, grown in various salinity of medium and chronic doses of UV-B irradiance, during investigation their functional stability to high temperature. The influence of unfavorable factors of external environment (low temperature, exceed salt, UV irradiation) strongly affected on functional activity of *Dunaliella* cells. In fig. 4 have been given the investigation results of indications of photosynthetic oxygen evolution dependence on *Dunaliella* cells, grown in intensive-accumulative cultivation regime of 1,5M NaCl in mineral medium (1); high concentrations of NaCl (3,0M) in mineral medium (2) and in conditions of chronic doses 10 Erg/mm^2 UV-B irradiation (3) under the temperature. As seen from the figure the photosynthetic activity of cells; grown in high concentrations of NaCl (3,0M); show relatively low speed indications of oxygen evolution, compared to cells, grown in 1,5M NaCl. That is connected with the high quantity of synthesized cells of carotenoid amounts, which affected the relations of chlorophylls/carotenoids, characterizing indications of photosynthetic activity.

The investigation of dependence of oxygen evolution in cells, grown in medium with 1,5M NaCl, under temperature showed that, 40^0C temperature of photosynthetic oxygen evolution for those algae is optimal. Under the ($42,5^0$C) temperature, photosynthetic oxygen evolution in cells decreases to (75%). Under the 45^0C temperature the value constitutes only 30% (fig.4. curve1). The investigation of temperature dependence of photosynthetic activity in cells, grown in high concentrations of NaCl (3,0M) showed that under 40^0C oxygen evolution in algae is 90% of maximum value, the temperature rise to $42,5^0$C increase the photosynthetic algae activity to maximum level. With further temperature increase to 45^0C oxygen evolution in cells suppressed and constitutes 95% of maximum indications (fig.4. curve3). The growth of cells in mineral medium with 3,0M NaCl, leads to the synthesis of higher amount of carotenoids. Those cells shift temperature maximum of photosynthetic oxygen evolution with 40^0C (under optimal concentration of 1,5M sodium chloride for the given strain) to $42,5^0$C. On base of that we can consider that, carotenoids synthesizing in cells perform the role of protectors, under extremely high temperatures.

The investigation of oxygen evolution in cells, grown in various chronic doses of UV-B light, under temperature showed that, at optimal temperature (40^0C), the control cells demonstrate high photosynthetic activity potential (fig.4.curve2). Preliminary investigations of photosynthetic activity in cells, grown under chronic doses of UV-B irradiation 5 Erg/mm^2 in an hour in intensive culture, by increasing temperature under optimal value 40^0C the algae population showed some functional stability.

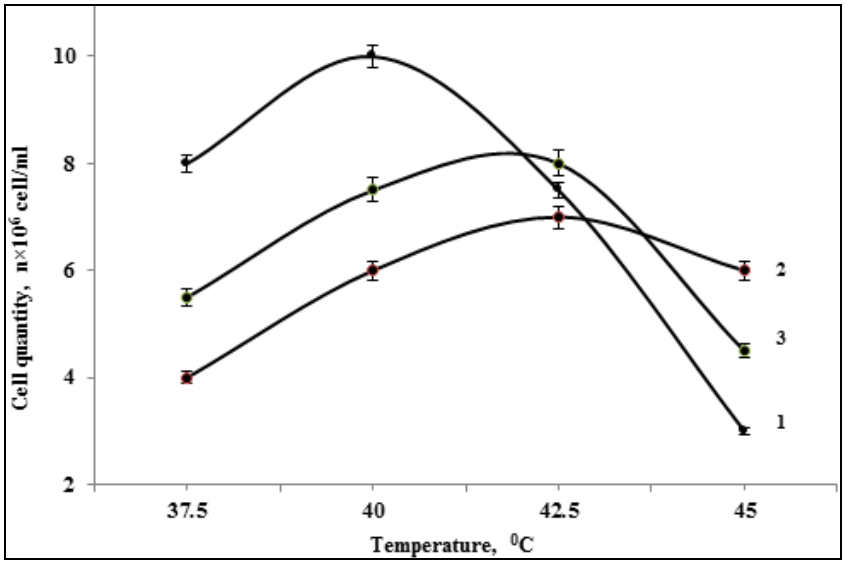

Fig 4: The dependence of indications of photosynthetic oxygen evolution in *Dunaliella*, grown in intensive-accumulative cultivation regime with 1,5M NaCl in mineral medium (1), in high concentrations of NaCl (3,0M) in mineral medium (2) and in conditions of chronic doses (10 Erg/mm^2 in an hour) of UV-B irradiation (3) under the temperature. Light intensity 100 Wt/m^2.

That functional stability can clearly be seen on kinetics of photosynthetic oxygen evolution in cells under 42,5^{0}C temperature, which exceeds control suspension to 6-9%. The increase of chronic doses to 10 Erg/mm^2 within an hour, somewhat reduces the growth rate in culture, but retains enhanced functional stability to the extreme temperature. Here has been observed the offset of maximum photosynthetic activity in cells to the side of high temperature 42,5^{0}C (fig.4.curve3). Further temperature increase to 45^{0}C suppresses oxygen evolution in cells to 65% level.

On base of gained data it can be concluded that, the cell stability to high temperature, probably is due to the UV-B light influence or enhanced quantity of synthesized algae of carotenoid amounts in the chronic UV-B irradiation conditions, that is synthesized carotenoids allow the algae to extend the range of temperature stability and protection of algae from stress, caused by the high temperature. Such suppositions have been made by authors [17], where said the carotenoids induce the reduction in fluidity at the periphery of thylakoid membranes, whereas lipid mobility in central hydrophobic part remains practically unchangeable. Such the thinning of membranes, being in the gel state, makes stiffer the membranes in the liquid-crystal phase, thus extend the range of temperature stability.

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