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The Condition of Antioxidant Sistems in Dunaliella Cellls under the Low Temperature Stress

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Abstract: *In the work have been presented the results of the investigations of bioproductivity, biosynthesis of carotenoids, catalase activity of MDA formation amounts in control and in cells, grown by blowing air mixture into the photoreactors under the low positive temperature (5^0C) (low temperature stress). It was shown that, in the low temperature stress condition and treating cells with dyes (methylene blue 2mkM) the bioproductivity of suspension in intensive-accumulative regime of cultivation considerably suppressed (25-30%), compared with control cells, which untreated with dyes .*

It has been determined that, low temperature stress and the treatment of Dunaliella cells with methylene blue, increase the activity of antioxidant system which is connected with the biosynthesis of carotenoids and catalase activity, and also increase peroxidation of lipids.

Keywords: *green microalgae, bioproductivity, low temperature stress, biosynthesis of carotenoids, catalase activity, peroxidation of lipids.*

1. INTRODUCTION

Nowadays, exist a large number of references on the problem of lively organism stability against low temperature. The value of the problem is that, the considerable part of the territorial land (65%) plants suffers from destructive action of low temperature [1]. Plant response against low temperature stress concludes in various alterations of metabolic and physiological processes, which must lead to the adaptation of plant organisms to changeable conditions [2]. By that, the energy outlay of cells increases, occurs the efficiency amplification of photosynthesis and breathing and that lead to the structural-functional changes of photosynthetic and mitochondrial apparatus [1], occurring as a response to low temperature influence. It is necessary to note that, a certain part of damage under the low temperature stress is connected with the influence forming in the cells during the stress of active oxygen form resulting in the activation processes of lipid peroxidation, causing membrane damage [3]. Plant cells have a strong defense system against oxidative stress, which extends in cells of thermophilic plants under the low positive temperature influence [1, 4]. Low temperature stress leads to the amounts of changes and activity of enzyme protection, also non-enzymatic elements, as carotenoids, flavonoids, α-tocopherol, ascorbate and others [3]. The accumulation of antioxidants can lead to the emergence of general nonspecific defense reactions of cells against low temperature stress [4, 5].

The aim of the present work is to study the bioproductivity, biosynthesis of carotenoids, catalase activity and peroxidation of lipids in control and in cells, grown in conditions of low positive temperature influence (5^0C) (low temperature stress) and in the cells treated with dyes (methylene blue 2mkM).

2. MATERIALS AND METHODS

The object of the investigation was the green unicellular algae *Dunaliella salina* IPPAS D-294, separated from the saline lake in Apsheron and introduced into the culture. The algae were grown under the 27^0C in glass photo reactors, with the volume 250 ml, in the installation for growing culture of unicellular algae. The mineral medium contained (g/l): NaCI - 87,5; KNO_3-5,0;

KN_2PO_4-1,25; $MgSO_4$-50; $FeSO_4$-0,009 and the solution of microelements, 1 ml/l. The suspension of cells in photoreactors day and night was illuminated by white light (16 Vt/m^2) and incessantly blown by the mixture (air+1,5 CO_2) under the temperature 27^0C for the control and 5^0C for the experimental suspension. The rate of the culture growth has been determined periodically by counting the number of cells in the camera Goryayev under the microscope or nephelometrically, measuring optic density of suspension.

Cellular suspension which was prepared for the measuring the content of carotenoids, catalase activity, was adjusted to 10^6cells/ml (optic density, OD=0, 8).
The content of carotenoids in cellular extract (100% acetone) was measured in the spectrophotometer and calculated on base of Vetshtain coefficient [6].

To measure the catalase activity of cells the suspension was cooled by centrifugation (3000 rev/min). The sediment was transferred into the solution with 0,5 g $CACO_3$, added 5 ml distilled water and triturated to homogenous mass. Then obtained mass quantitatively transferred into the glass, capacity 50 ml to the mark and infused periodically stirring within 3-4 hours. During that period, the extraction of enzyme becomes from plant material. After infusion the suspension was filtered in a dry glass. The catalase activity was measured by gazometric method, which based on a certain capacity after adding to aqueous extract of algae, containing catalase, hydrogen peroxide [7].
The evaluation degree of lipid peroxidation (POL) was conducted by the method of determination of MDA content in cells *Dunaliella salina*, based on the reaction of thiobarbituric acid.

The cell suspension (35ml) was centrifuged at 3000 v/min within 10 min. The obtained sediment homogenized at 20 ml 0,1% TCA. Homogenate was centrifuged at 3000 rev/min within 10 min. To 1 ml supernatant was added 4 ml 20 % TCA, containing 0, 5 % TBA. The mixture was heated in aqueous bath in 95^0C within 30 min. and immediately was cooled in running water. After centrifugation the mixture at 3000 rev/min within 10 min., was identified the optic density of supernatant 532 nm [8].

3. RESULTS AND DISCUSSION

The growth of control suspension of cells at strict stabilization in optimal conditions (temperature 27^0C, light intensity 16 Vt/m^2, partial pressure of carbon dioxide, mineral medium) in 250 ml glass photoreactors and supplying with air mixture and temperature 25^0C in periodically-accumulating regime the cultivation within 24 hours showed that ,the optic density of cellular suspension increases 3,5-4 times. By adding the dyes into the nutritive medium –generator the active form of oxygen AFO (methylene blue 2mkM) [9], the growth rate and bioproductivity in control suspension decreased to 8-10%. In low temperature stress conditions and treating the cells with dyes (methylene blue 2mkM), the bioproductivity of the suspension significantly suppressed (20-25%), compared with control cells, which untreated with dyes.

The indications of biosynthesis of enzymes, catalase acvtivity and the amounts of MDA formation in *Dunaliella* cells of control and in cells treated with dyes methylene blue within 24 hourly cultivation of control and experimental suspension of cells have been presented in table 1.

Table 1. *The indications of growth, enzyme formation, catalase activity and the quantity of MDA formation in Dunaliella cells in control and in cells treated with methylene blue*

Growth, OD			Catalase activity, time, min.				Quantity of enzymes, mg/l			Activity, mol/g
			5	10	15	20	Ca	Cb	Ccar.	
K	0,3	1,2±0,03	1,4	2,0	2,5	2,9	3,7±0,05	1,5±0,05	1,5±0,1	$0,75*10^{-3}$ ±0,05
O	0.3	1,1±0,03	1,6	2,8	3,0	3,7	4,1±0,05	2,2±0,05	1,6±0,1	$0,75*10^{-3}$ ±0,05

Note: *the optic density is OD=0, 8*

As seen from the table 1 in control suspension due to the growth and population development, the indications of biosynthesis of enzymes particularly carotenoids in cells differ from experimental ones. By treating the cells with dyes methylene blue (2mkM) are formed active oxygen species in experienced suspensions of cells during the growth process, which lead to the growth retardation and biosynthesis of increased amounts of low molecular antioxidants, particularly carotenoids. It is known that, in plant cells besides low molecular antioxidants also participate highly active highly molecular units (catalase, peroxidase and superoxide dismutase), which are able to inhibit the active oxygen species and free radical processes.

In table 1 were presented the indications of catalase activity in *Dunaliella* cells, grown in control and in the cells treated with the dyes methylene blue. The treatment of cells with dyes methylene blue, distinctly increase the catalase activity of experienced cells, and the difference with the control is 20-22%. So, the treatment of cells with the dyes methylene blue, in intensive cultivation of *Dunaliella* cells suspension decreases the bioproductivity of algae, increases the activity of antioxidant systems, that affects on biosynthesis of carotenoids and catalase activity. The results of studies of the influence of generator AFO methylene blue on the amounts of MDA formation of experienced suspension showed that during the influence of dyes in cells the process of peroxidation of lipids (POL) wasn't observed.

As follows from the literature data, during the influence of unfavorable temperature on the plant cells in them occur numerous structural-functional changes, part of which involved to the process of increased stability formation. One of the more important modern tradition in the explanation of exquisite mechanisms of plant stability to changeable environment, is concluded particularly in establishing interaction character between the processes of indications of carotenoid biosynthesis and catalase activity, can be discussed the development degrees of damage processes of active oxygen species (AOF) in *Dunaliella* cells. The response cell reaction against low temperature stress – that is some increase of carotenoid synthesis during the growth, and also of catalase activity. It would be interesting to study the indications of carotenoid synthesis, catalase activity and quantitative definitions of MDA in low temperature stress conditions and of cells treated with dyes-methylene blue (generator AOF). In our investigations was exactly shown the increase of carotenoid concentration, catalase activity and amounts of MDA formation.

Table 2. *The indications of growth, enzyme formation, catalase activity and amounts of MDA formation in Dunaliella cells in control and during the influence of low temperature stress and in the cells treated with dyes methylene blue*

Growth, OD			*Catalase activity, time,min.*				*Quantity of enzymes, mg/l*			*Activity, mol/g*
			5	*10*	*15*	*20*	*Ca*	*Cb*	*Ccar.*	
K	*0,3*	*1,2±0,03*	*2,9*	*4,6*	*5,5*	*6,0*	*3,1±0,1*	*1,7±0,1*	*0,9±0,1*	*0,75*10^{-3}±0,05*
O	*0.3*	*0,9±0,02*	*3,4*	*5,5*	*6,9*	*7,9*	*3,5±0,1*	*2,0±0,1*	*1,1±0,1*	*1,0*10^{-3}±0,05*

Note: *optic density OD=0, 8*

In table 2 have been presented the indications of enzyme formation, catalase activity, amounts of MDA formation in *Dunaliella* cells in control, under the influence of low temperature stress and in the cells treated with dyes methylene blue. As seen from the schedule, the influence of low temperature stress and methylene blue on *Dunaliella* cells lead to the increase of MDA formation amounts, catalase activity (25%) and MDA formation amounts, which weren't observed within the influence with dyes, individually.

Thus, during the intensive cultivation of algae in optimal conditions, low temperature stress and the treatment of cells with methylene blue (2mkM) decrease the cell bioproductivity. In those conditions, the intercellular activity of antioxidant systems increases, which affects on carotenoid biosynthesis and catalase activity, and also on the peroxidation of lipids (POL) in cells.

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