CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Online International Journal Available at http://www.cibtech.org/cjb.htm
2012 Vol. 1 (1) April-June 12, pp.36-39/Ali-Zadeh
Research Article

LOW TEMPERATURE STRESS INCREASES *DUNALIELLA* CELLS POPULATION RESISTANCE TO THE EFFECT OF CHRONIC DOZES OF UV-B RADIATION

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ABSTRACT
The objective of this investigation was to determine the resistence of Dunaliella cells population to various chronic dozes of UV-B radiation in intensive culture. The studies were carried out at $5C^0$, 15^0C, 25^0C temperatures of air mixture given to photoreacror. The results of investigation have showed that low temperature stress increases cells population stability to chronic dozes of UV-B radiation while reducing bioproductivity and increasing the quantity of synthesized carotenoids in the control population of cells.

Key Words: *Green Algae, Low Temperature, Bioproductivity, Resistance, Uv –B Radiation*

INTRODUCTION
In natural culture, UV-B radiation is one of the ecological factors causing different effect on the population of unicellular algae and plants. It is necessary to differentiate specific peculiarities and mechanism of UV-B radiation effect upon population level among other environmental factors Alizadeh and Nadjafov (2002); and Sideif-Zadeh *et al., (2008)*. The reaction of plants to low temperature stress leads to various metabolic and physiological processes that should finally result in adaptation of plant organisms to changeable conditions that increase both cell's energy consumption and intensification of breath effectiveness Semikhatova (1995). In its turn, intensification of breath effectiveness is associated with the structural-functional alterations in mitochondrial apparatus Voynikov (1987); Kislyuk *et al.,* (1995); and Khokhlova *et al.,* (1995) occurring against low temperature effect. A part of damages observed at low temperature stress is conditioned by the forming of an active oxygen state during cell's stress due to the activation of lipid peroxidation process causing membrane damage. Some available data have suggested that mitochondrene is one of the main sources of active oxygen at low temperature stress Purvis *et al.,* (1995). It is worth noting that in the plants acclimatized to 10^0 C temperatures, the intensity of lipid peroxide oxidation is lower than in the plants grown at 20^0 C temperatures. Oxidative stress is usually developed in the cells of heat consuming plants under low positive temperature effect. In this case, the damage can be associated with the inhibition of catalaze resulting in H_2O_2 accumulation in cells Zikova *et al.,* (2002). Stressed cells are capable of increasing antioxidants' content. Thus, under UV-radiation α-tocopherol accumulates in the cells of wheat plants Veselovsky (1982). As a result of the effect of spring and autumn frosts, the content of tocopherol in the wheat grain increases to 25% and 32%, respectively. According to some authors, hyperoxidaze activity increases in the cells of maize roots under hyperterm conditions Zikova *et al.,* (2002). Antioxidant accumulation can be considered as general unspecific protect reaction of cells to low temperature stress Zikova *et al.,* (2002).
The purpose of this work was, therefore, a study of 24-hours chronic effect of UV-B radiation on the population of *Dunaliella salina* cells in intensive culture giving air mixture (air + CO_2) with $5C^0$, 15^0C, 25^0C temperatures to photoreactor.

MATERIALS AND METHODS
Green halophile unicellular algae ***Dunaliella salina*** IPPAS D-294 was used. Algae were grown at 27^oC in glass (control suspension) and quarts (test suspension) photoreactors (250 ml) in special devices for growing unicellular algae culture Alizadeh *et al.,* (1999). Mineral culture consisted of (q/l): NaCI –87, 5 (1, 5 M); KNO_3 –5, 0; KH2PO4-1, 25; $MgSO_4$ –50; FeSO4-0,009 solution of microelements, 1 ml/l.

Suspension of cells in photoreactors was illuminated by white light of luminescent lamp (16 Wt/m^2) in a whole day, and was continuously blown by air mixture (air + 1, 5% CO_2), at various temperatures from 5^0 to 25^0 C.

Mercury lamp SVD-120 with UFS-2 lightfiltre was used as a source of UV-B radiation. Chronic UV-B radiation of cells was performed within a day using hour mechanism. Cells were grown within 24 hours, under intensive-accumulation culture regime.

The rate of culture growth was determined by periodic calculation of cells amount in Qoryaev camera under a microscope or by nefelometrical measurement of suspension optic density in spectrophotometer.

The content of pigments in cellular extracts (100% acetone) was measured by spectrophotometer and calculated on Vetshtain coefficients Qavrilenko *et al.,* (1975).

The grown algae were precipitated through centrifugation and transferred into newly prepared mineral medium to measure photosynthetic activity of cells. Cells' density was brought to 10^6 cell/ml (optic density D-0, 8).

The speed of the oxygen emitted from cells was measured polarographically by platinum Clark electrode illuminating suspension in thermostatized holes (40^oC) by the white light of saturating intensity (100 Wt/m^2).

RESULTS AND DISCUSSIONS

The curves of *Dunaliella* cell population survival under chronic UV-B radiation doze in intensive culture and at 5^0C, 15^0C and 25^0C temperatures of the air mixture giving to photoreactors are presented in Figure 1. As it can be seen from Figure 1, population survival decreases to 98% under 210 sec/hour of chronic UV-B radiation doze at 5^0C temperature (Figure1 A). The increase of the chronic doze of UV-B radiation up to 450 sek/hour rises the test suspension productivity, which is 103-104% compared to the control suspension. Stimulation of cell population division under chronic doze of UV-B radiation was achieved by us earlier Alizadeh GI and Nadjafov MG (2002).

It was shown that depending on medium salinity, the growth of this phenomenon was observed at low chronic doze. Under the chronic doze of 600 sec/hour a decrease in the speed of cell population growth was observed. It makes 97-98% of the control suspension. An increase of the temperature to 15^oC in photoreactors by air mixture reduces stability of cell population to UV-B radiation. Hence, less than 210 sek/hour chronic doze of UV-B radiation stimulation of cellular division is observed and the population productivity was 104-105% of that in the control suspension (Figure1B). An additional increase of chronic doze to 450 sec/hour reduces the population productivity to 94-95%. 1080 sec/hour of chronic doze of UV-B radiation reduces the algae productivity in photoreactors to 90-91%. Giving air mixture (air + CO_2) with 25^oC temperature to cellular suspension in photoreactor in intensive culture has shown that 5 sek/hour of chronic doze reduces population growth to 84-85%. Typical suppression is observed also during the increase of chronic dozes of UV-B radiation from 5 to 30 sec/hour. The examination of doze-effect curve provides information about negative effect of UV-B radiation on the *Dunaliella* cells population. Based on the above mentioned, we can say that low positive temperatures play an important role in the stability of population to chronic dozes of UV-B radiation. The decrease of air mixture temperature in photoreactors results in the increase of population stability to chronic dozes of UV-B radiation. This fact has also confirmed by the data derived from measurements of average daily biomass growth under chronic radiation of UV-B light of various dozes (Figure1).

In Figure 2, the data of dependence of *Dunaliella* cell population productivity and resistance to chronic dozes of UV-B radiation in intensive culture on the temperature of air mixture in photoreactors from 5 to 25^0C is presented.As it can be seen from the Figure 2, increase of temperature in photoreactor increases cell population bioproductivity. This value is increasing proportionally according to the increase of temperature from 5^0C to 25^0C (Figure 2,2). The increase of temperature of the air mixture passing through photoreactors from 5^0C to 25^0C decreases the stability of cell population to chronic dozes of UV-B radiation (Figure 2.1). The measurements, carried out to determine oxygen emission speed have shown

that photosynthetic activity of control cells was suppressed under low temperature stress. Photosynthesis of control population at studied interval of temperatures - 5^0C, 15^0C, and 250C was 60-62%, 72-75% and 100%, respectively.

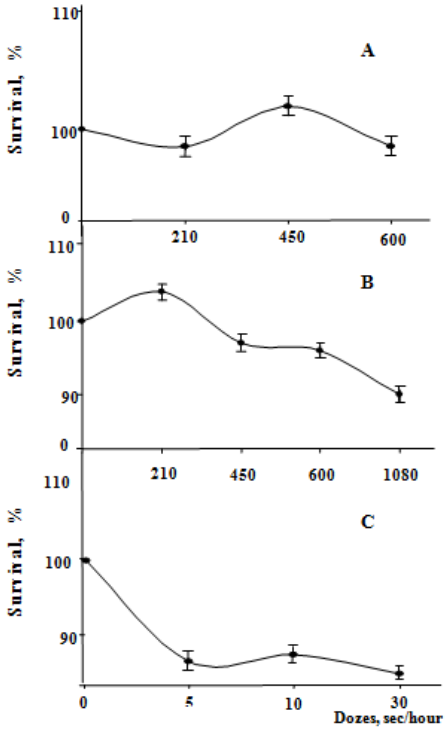

Figure 1: The survival of population in Dunaliella cells within the chronic doze of UV-B radiation in intensive culture and bliw through the photoreactors by air mixture in various temperatures; A- ^{0}C; B - 15^{0}C; C - 25^{0}C

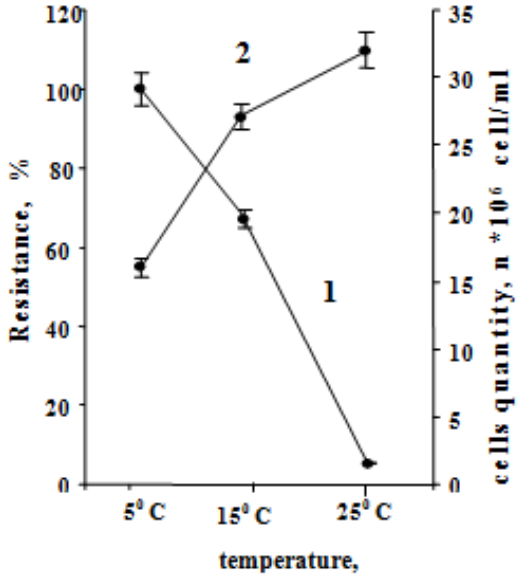

Figure2: The dependence of productivity (2) and resistance (1) to chronic dozes of UV-B radiation of population of *Dunaliella* cells in intensive culture on temperatures entering by air mixture through the photoreactors

The decrease of oxygen emission speed in control cells at low positive temperatures is the evidence of various metabolic and physiological processes, which affects both photosynthetic activity and bioproductivity of algae population.

Separately, a low temperature stress under permanent temperatures of photoreactors increases the quantity of synthesized carotenoids during control population (Table 1).

Table 1: The content of pigments in control cells of *Dunaliella*, in intensive culture by giving air mixture to photoreactors at 5^0 to 25^0 C

Temperature ^{0}C	Chlorophyll a, mq/l	Chlorophyll b, mq/l	Sum of Carotenoids, mq/l	Ratio Chlorophyll/Carotenoids
5	5,50 ± 0,1	2,45 ± 0,05	1,51 ± 0,05	5,2 ± 0,01
15	5,33 ± 0,1	2,35 ± 0,05	1,32 ± 0,05	5,8 ± 0,01
25	4,65 ± 0,1	2,35 ± 0,05	1,15 ± 0,05	6,1 ± 0,01

Note: Medium contains 1.5 M NaCI at 27^{o}C temperature and intensity of light 16 Wt/m^2.

It is known that the ratio of chlorophylls/carotenoids is one of the factors indicating the photosynthetic activity of cells, more this quantity and more the productivity in green plants. Low positive temperatures lead to the decrease in chlorophylls/carotenoids ratio due to the increase of carotenoid synthesis in the control population of cells.

Thus, UV-B radiation inhibits the growth of *Dunaliella* cells' population. In addition, it was established that UV-B radiation induces serious breach in population with the increase of temperatures of air mixture up to 25^0C in photoreactors. In these conditions, in the curves of cells' doze-effect population sharp suppression of growth (to80-85%) was observed. Accordingly, the photosynthetic emission of oxygen from cells suppresses.

Simultaneous application of two stresses (low temperature stress and chronic UV-B radiation) increases the biosynthesis of carotenoids in cells in intensive culture. This influences the resistance of algae population. It was determined that the stability of cells during simultaneous influence of two stressors is higher than each of them taken separately. The results presented here minimize uncertainties and suggest that the damage appearing by chronic UV-B radiation and low temperature stress is exactly associated with oxidizing processes.

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