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RESISTANCE OF THE FUNCTIONAL ACTIVITY OF *DUNALIELLA SALINA IPPAS D-294* CELLS MODIFIED WITH 2,6 DI-*TERT*-BUTYL PHENOL TO THE ACTION OF ACUTE DOSES OF UV-B RADIATION UNDER OPTIMAL AND HIGH SALINITY CONDITIONS

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Abstract. The article presents the results of the study of bioproductivity, functional activity in *Dunaliella salina IPPAS D-294* cells grown under optimal and high salinity conditions of the mineral environment. It is shown that under optimal conditions during the day the bioproductivity of the population increases by 3.5-4 times. In conditions of high salinity (3.0 M NaCl) of the mineral medium, the bioproductivity of cells decreases by 37,5% relative to the optimal cultivation regime. Modification of cells with 2,6 di*tert*-butyl phenol leads to stimulation of culture growth: in optimal 1,8-3,3%; in conditions of high salinity 3,4-4,7%. It was revealed that increasing the concentration (25-50 mkM) of the antioxidant reduce the photosynthetic activity of control cells to 2-8.2%, and under conditions of high salinity of the mineral medium by 1.2-3%. It was found that the functional stability of cells shifts to higher values of acute doses of UV-B radiation.

Keywords: *Dunaliella, bioproductivity, synthetic antioxidant 2,6 di-tert-butyl phenol, salinity, functional stability.*

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1. Introduction

Plants have developed complex mechanisms for perceiving environmental signals and developing appropriate and coordinated responses to abiotic and biotic stresses. Salt stress is one of the most serious environmental stresses (Kreslavsky *et al*., 2007; Rozentsvet *et al*., 2017; Selivanova, 2012). In general, the susceptibility or tolerance of plants to stress caused by high salt content is a coordinated action of several genes responding to stress (Kolupaev & Karpets, 2017; Kolupaev *et al*., 2018; Kreslavsky *et al*., 2012). Radiation in the UV region of the spectrum effectively induces direct and mediocre ROS (reactive oxygen species) of photo-oxidative damage.

The study of the ecological-physiological, biochemical and cytological features of *Dunaliella* species allows us to shed light on the mechanism of halophilia. It is known that these organisms develop under conditions of extremely high salinity of the medium (1-4 M NaCl), which makes it interesting to clarify the features of its structural and functional organization, study the mechanisms of osmoregulation, adaptation of algae to

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the osmotic action of the medium and under certain environmental conditions.

The photosynthetic apparatus (FA), first of all, photosystem II (FS II), is one of the most stress-sensitive physiological systems, in particular, to UV radiation. Therefore, the study of the mechanisms of action of stress factors and the protection of FA from them is an urgent problem.

Significant direct UV-induced damage is attributed to the action of radiation in the region of 280-320 nm (UV-B). It is possible to significantly reduce oxidative stress and its consequences by adding synthetic antioxidants to the mineral medium, such as 2,6 di-*tert*-butyl phenol belonging to the class of spatially hindered phenols (Jalilova, 2022).

In this regard, the aim of our work was to study the effect of 25 mkM and 50 mkM concentrations of the synthetic antioxidant 2,6 di-*tert*-butyl phenol on the growth, photosynthetic activity of *Dunaliella salina IPPAS D-294* algae and their role in functional resistance to acute doses of UV radiation in optimal and high salinity conditions.

2. Materials and methods

The object of the study was a halophilic green microalgae *Dunaliella salina IPPAS D-294* isolated from the salt lake Masazyr, located in the north-west territory of Baku.

Algae were grown at a temperature of 27^0C in glass photoreactors (250 ml), on a plant for growing cultures of unicellular algae. The mineral medium contained (g/l): NaCI – 87,5 (1,5 M) and 175 (3,0 M); KNO_3 – 5,0; KH_2PO_4 – 1,25; $MgSO_4$ – 50; $FeSO_4$ – 0.009 trace element 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. The suspension of cells in photoreactors was illuminated with white light (16 W/m^2) per day and continuously purged with a mixture (air + 1,5% CO_2) with a temperature of 27^0C. The source of UV-B radiation was a mercury lamp SVD-120. The cells were grown in intensive accumulative cultivation mode and illuminated around the clock.

The growth of the culture was determined by periodic counting of the number of cells in the Goryaev chamber under a microscope or by nephelometric measurement of the optical density of the suspension on a photoelectrocolorimeter. The work used 25 mkM and 50 mkM concentrations of the antioxidant -2,6 di-*tert*-butyl phenol. Photosynthetic activity of cells was measured on a polarographic device using a platinum Clark electrode (Gavrilenko *et al*., 1975). The experiments were repeated at least 6 times. The values were considered reliable when the significance levels were less than 0,05 ($p < 0.05$).

3. Results

Figure 1 shows quantitative indicators of daily biomass growth of cells modified with different (25 mkM and 50 mkM) concentrations of 2,6 di-*tert*-butyl phenol in optimal (A, B, C) and high salinity (D, E, F) cultivation. As can be seen from the figure, under optimal conditions (1,5 M NaCl) for 24 hours, the optical density of the control cell suspension increases by 3,5-4 times: from 0.3± 0,05 •10^6 cell /ml to 1,2± 0,05• 10^6 cell /ml (column A), with an increase in the salinity of the mineral medium, the bioproductivity of the population increases only 3 times: from 0,3 ± 0,05 • 10^6 cell/ml to

0,75± 0,05• 10^6 cell/ml (column D). This population growth trend continues in subsequent repeated variants of growing control suspensions.

The presence of 2,6-di-*tert*-butyl phenol in different concentrations (25 mkM and 50 mkM) in a growing medium with 1,5 M NaCl (A; B; C) and 3,0 M NaCl (D; E; F) markedly changes the growth dynamics of *Dunaliella*. At concentrations of 25 mkM (column B) and 50 mkM (column C) of 2,6 di-*tert*-butyl phenol in the optimal (1,5 M NaCl) cultivation mode, the antioxidant stimulates cell growth up to 1,22±0,05•10^6 cell/ml (1,7%) and up to 1,22 ±0,05•10^6 cell/ml (3 %), respectively, in relation to the control. Based on the conducted studies, it was found that increasing the concentration of NaCl to 3,0 M, the NaCl cells of *Dunaliella* have a lower growth rate of 0,45±0,05 •10^6 cell/ml (25%) and exhibit lower productivity.

In figure 1, columns D, E and F show the results of the growth dynamics of the culture modified by different (25 mkM and 50 mkM) concentrations of the antioxidant under conditions of high salinity (3,0 M NaCl) of the mineral medium. When 25 mkM (column E) and 50 mkM (column F) of antioxidant concentrations are added to the mineral medium, the dynamics of cell culture growth is stimulated to 0,776±0,05•10^6 cell/ml (103%) and to 0,787±0,05•10^6 cell/ml (107%), respectively, in relation to the control suspensions. This means that 2,6 di-*tert*-butyl phenol at low concentrations of 25 mkM and 50 mkM is comparable to the activity of conventional phytohormones. When the mineral environment is salted, the bio-synthesis of glycerol and the amount of carotenoids increases.

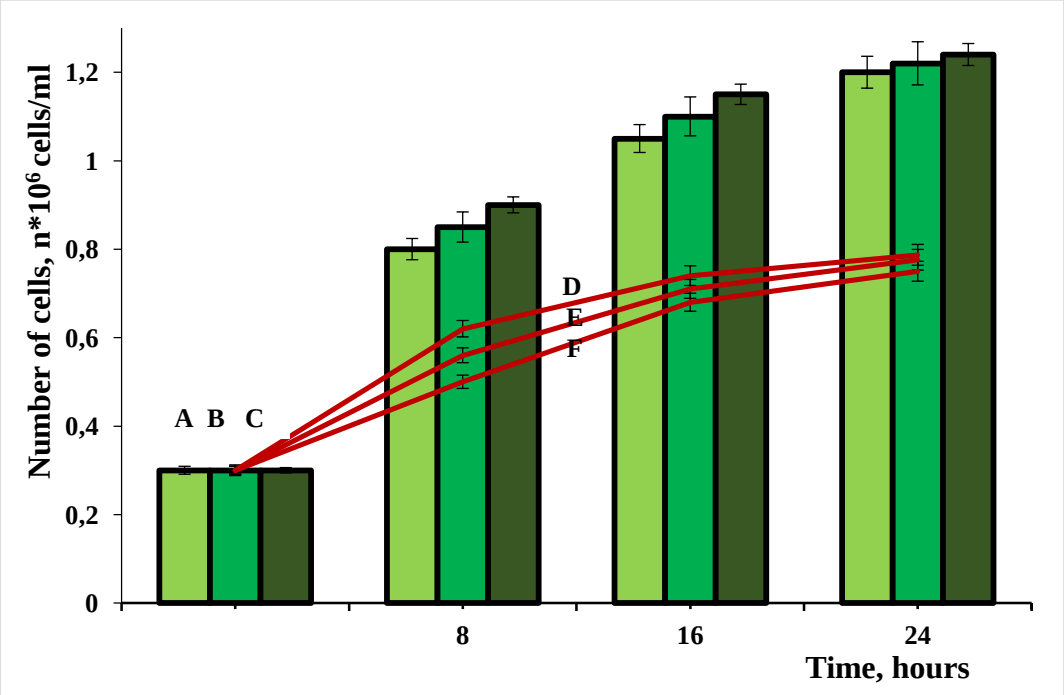

Fig. 1. Dependence of the growth dynamics of the *Dunaliella salina IPPAS D-294* cell population in a mineral medium with 1,5 M NaCl (A; B; C) and 3,0 M NaCl (D; E; F): A; D - control; B; E -25 mkM 2,6 di-*tert*-butyl phenol: C; F - 50 mkM of 2,6 di-*tert*-butyl phenol. Temperature 27^0C, light intensity 16 W/m^2

Table 1 shows quantitative indicators of photosynthetic oxygen release by control and modified with different concentrations (25 mkM and 50 mkM) of 2,6 di-*tert*-butyl

phenol cells grown under optimal (1,5 M NaCl) and high salinity (3,0 M NaCl) conditions of the mineral medium. As can be seen from the table, the photosynthetic oxygen release of control cells (1,5 M NaCl) and cells grown in high salinity (3,0 M NaCl) mineral medium differ significantly (about 17%).

In the presence of an antioxidant in a mineral medium of 25 mkM and 50 mkM concentrations in the optimal mode (1,5 M NaCl) of cultivation, the photosynthetic activity of cells is suppressed by 2% and 8.3%, respectively, in relation to the control suspensions. The increasing of salinity (3,0 M NaCl) in the presence of 2,6 di-*tert*-butyl phenol in the mineral medium at concentrations of 25 mkM and 50 mkM, the quantitative indicators of functional activity decreases by 7% and 8%, respectively, in relation to the control suspensions.

Table 1. Photosynthetic oxygen release by Dunaliella salina IPPAS D-294 cells grown at different concentrations of 2,6 di-tert-butyl phenol in the presence of 1,5 M NaCl and 3,0 M NaCl in a mineral medium

The degree of salinity of the mineral medium	**Oxygen release, rel. units**		
	Concentration, mkM		
	Control cells	**+ 25 мкМ**	**+ 50 мкМ**
1,5 M NaCl	1,20±0,05	1,18±0,05	1,10±0,05
3,0 M NaCl	1,00±0,05	0,97±0,05	0,88±0,05

Note: Temperature 40^0C, light intensity 100 W/m^2

Continuing the experiments, the modified cells were subjected to acute doses of UV-B radiation and the photosynthetic oxygen release of the culture (1,5 M NaCl and 3,0 M NaCl) was traced.

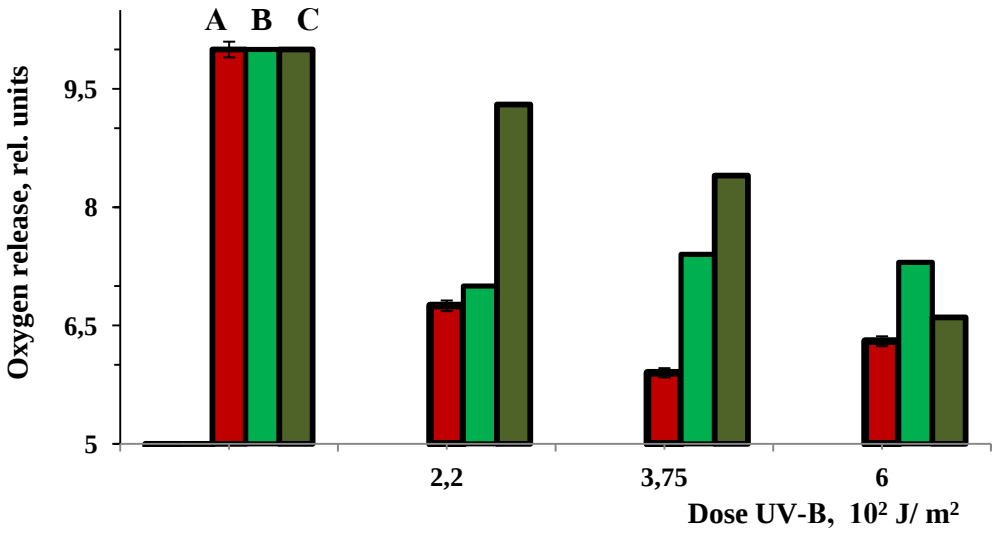

Fig. 2. Photosynthetic oxygen release by control cells and cells grown in the optimal mode (1,5 M NaCl) of cultivation and various concentrations of 2,6 di-*tert*-butyl phenol, when irradiated with acute doses of UV-B light: A- control; B- 25 mkM 2,6 di-*tert*-butyl phenol; C- 50 mkM 2,6 di-*tert*-butyl phenol. Temperature 40^0C, light intensity 100 W/m^2

Figure 2 shows the results of photosynthetic oxygen release by control cells irradiated with various acute doses of UV-B light, and cells modified with 25 mkM and 50 mkM with 2,6 di-*tert*-butyl phenol. The figure shows that in the pre-irradiated cells in the range of 2,2*102 J/m^2 – 6,0*102 J/m^2 with acute doses of control cells, the relative amount of photosynthetic oxygen release is sharply suppressed (column A) and a weak protective reaction of the functional activity of cells modified with 25 mkM concentration is manifested. The effect of an acute dose of 2,2*102 J/m^2 of UV-B light contributes to the manifestation of weak (at the control level), and high doses of 3,75* 10^2 and 6*102 J/m^2 more confident protection of the photosynthetic apparatus, which differs from the control cells (column B). With an increase in the protective function of the antioxidant in the range of up to 50 mkMs concentrations, a smooth decline in functional activity is observed with an increase in the acute dose of UV-B radiation (column C).

Figure 3. shows the results of photosynthetic oxygen release by control cells irradiated with various acute doses of UV-B light, and cells modified with 25 mkM and 50 mkM with 2,6 di-*tert*-butyl phenol, under optimal conditions and high salinity. As can be seen from the figure, in pre-irradiated in the range of 15*10^2 J/m2 – 21*10^2 J/m^2 with acute doses of control cells, functional activity is sharply suppressed (column A). Here, too, the antioxidant at 25 mkM concentration shows a weak protective reaction of functional activity.

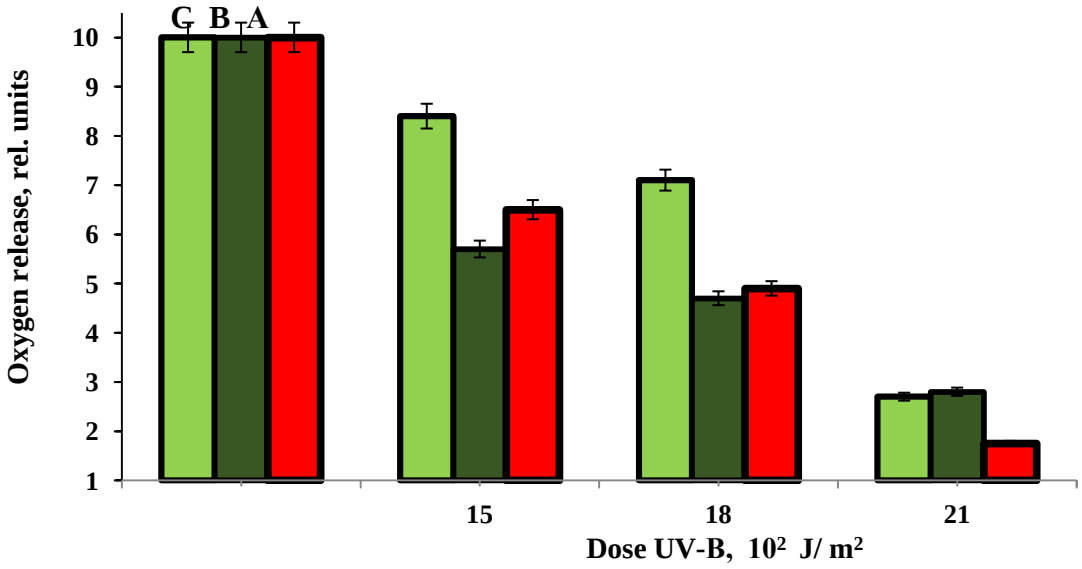

Fig. 3. Photosynthetic oxygen release by control cells and cells grown in an environment with high salinity (3,0 M NaCl) and different concentrations of 2,6 di-*tert*-butyl phenol, when irradiated with acute doses of UV-B light: A- control; B- 25 mkM of 2,6 di-*tert*-butyl phenol; C- 50 mkM of 2,6 di-*tert*-butyl phenol. Temperature 40^{0}C, light intensity 100 W/m^2

UV-B radiation at an acute dose of 15*10^2 J/m^2 contributes to the manifestation of weak (at the control level), and higher doses of 18*10^2 and 21*10^2 J/m^2 more confident protection of functional activity, compared with control cells (column B). The antioxidant in the concentration range of 50 mkM exhibits increased protective activity with a smooth decline in functional activity with an increase in the acute dose of UV-B radiation (column C). Thus, at an acute dose of 15*10^2 J/m^2 of UV-B radiation, the

protection of the functional activity of cells is 65% versus 57% (in control cells), and at an acute dose of $18*10^2$ J/m^2, functional stability (49%) differs slightly from control cells - 47% (column B). The effect of an acute dose of $21*10^2$ J/m^2 of UV-B light contributes to the suppression of the functional activity of modified cells below the control (column B). With an increase in the protective function of the synthetic antioxidant in the range of up to 50 mkM concentrations, a smooth decline in functional activity is observed with an increase in the acute dose of UV-B radiation (column C).

4. Conclusion

Thus, it can be assumed that when the high salinity of the mineral environment affects the cell population, disturbances occur due to the accumulation of reactive oxygen species in the structures of the photosynthetic apparatus, which affects the photosynthetic activity and growth of microalgae. A decrease in the bioproductivity of the cell population is accompanied by an increase in their resistance to the subsequent action of acute doses of UV-B radiation. The analysis of kinetic curves of photosynthesis of *Dunaliella salina IPPAS D-294* cells makes it possible to obtain information about the nature of damage to the plant organism under the action of high salinity and 25 mkM and 50 mkM concentrations of 2,6 di-*tert*-butyl phenol.

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