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Effect of Low Positive Temperature and Salinity on the Catalase Ferments Activity and Proliferation of *Dunaliella Salina* Ippas D-24 Cells

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Abstract

In this research was studied the effect of different level of salinity on the catalase ferments activity, proliferation, pigment composition and photosynthetic activity of *Dunaliella Salina* IPPAS D-24 cells. In the experiments were studied also the activity of catalase ferments of intensely culture green micro-algae in the regime different temperature (5^0C, 10^0C, 27^0C) composition. It is identified that the maximal proliferation temps and photosynthetic activity of *Dunaliella Salina* is observed in the 1,5M concentration of NaCl. The decrease (1,0 M NaCl and increase 3,0M NaCl) of salinity in the environment causes the fall of cell proliferation. Investigation of catalase activity of green micro-algae cultivated intensively in the nutritious medium of Abdullaev-Semenenko with various NaCl concentrations (1,0M, 1,5M, 3,0M) showed that the maximum activity of the enzyme is observed during cultivation in 1.5M NaCl concentrated medium. As the temperature of the air mixture inflicted on photoreactors decreases (temperature stress), cell populations' rates of reproduction decrease, at the same time stimulating catalase activity.

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Keywords

Green micro-algae, Salinity, Bio productivity, Low positive temperature stresses, Photo reactor, catalase activity

Introduction

The eco-physiological, biochemical and cytological studies of *Dunaliella Salina* IPPAS D-24 cells are important in the study of the mechanism of halophyte. According to the results of some research, the salt tolerant of the *Dunaliella Salina* cells has a wide amplitude of 30-300 g / l (Najafli 2011; Masyuk, 1973). In the review paper of Aharon Oren are given the characteristics of several types of *D. salina* and *D. viridis* that are common in different saline environments (Aharon, 2014).

It is important to determine in which concentration occurs optimal reproduction. As it is known, during the unfavorable effects of the external environment (low temperatures, excess salinity), the collection of glycerin in the *Dunaliella Salina* cells is also observed. The stimulation of the glycerin synthesis is the response to the increased amniotic salinity. Because in the cells of *Dunaliella Salina* to the glycerin are treated as osmotic regulators (Semenenko, 1980; Zykov, 2002). In the work of Alizadeh *et al.,* (2017) were studied the carotenoid amounts in *Dunaliella* cells in the medium which included the 1,0M; 1,5 M and 3,0 M NaCl. It was found that in this medium with 3,0 M NaCl the bioproductivity, synthesis of carotenoid increases on high temperature,

related to the increase of in cells. The carotenoid amounts and growth rate to (10-15%) was decreases on the low temperature stress. The functional stability population of *D. salina* against high temperature correlated by a number of synthesized carotenoids in those conditions (Alizadeh, 2017). In experiments of Amel *et al.,* (2011) studied the influence of salinity in the concentration interval of from 0.05 to 4 M NaCl on growth and antioxidant parameters *Dunaliella salina* and *Dunaliella tertiolecta*. It is shown that chlorophyll, carbohydrate contents and biomass yields were decreased at salinity extremes. But the protein contents under low salinities increased. The level of several enzymes such as catalase, peroxidase, superoxide dismutase, ascorbate peroidase was quantified (Amel, 2011). It is shown that the expression level of hsp70 Gene in *D. salina* depends on the salt stress. By influences of different salinity (0%, 5%, 12%, 20% and 30% NaCl) it was found that the maximum level of expression occurs at a 12% concentration of NaCl. The level of expression of hsp70 gene depends also on the time of salinity exposure (Bager *et al.,* 2016).

Low positive temperature stresses various changes in the metabolic and physiological processes of the cells, resulting in adaptation to changing conditions. At the same time, the energy costs of the cells increase and the respiration accelerates. The low positive temperature stresses activates of the defense enzymes, as well as effect non-fermentative protection systems: on the amount of carotenoids, flavonoids, a-tocopherol, etc. (Kuznetsov and Dimitrieva, 2011).

At present, it has been established that the formation of active forms of oxygen in the generation of general response responses to the effects of various stress factors (low temperature, salinity, UV-B, etc.) of the external environment is important. One of the main causes of injury of cells during various strokes is the activation of the peroxide oxidation processes of lipids, which are observed in the formation of active forms of oxygen in the cell and damage to bio membranes (Zykov, 2002). As it turns out, one of the main features of living things is not to protect itself from oxygen activation, but to regulate and keep under control the reactions which occur by oxygen activation. In the evolutionary process, different types of defense systems have emerged in the living organisms due to stress factors. The aim of the investigation is to study the effect of salinity changes in the nutrient environment and the effects of low positive temperatures on the growth rate of *Dunaliella Salina* IPPAS D-24 cells, pigment composition and catalase activity.

Materials and Methods

As the object of the research was use the DAP cells derived from salty lakes of the Absheron peninsula and was cultured. Micro-algae cells have been intensively profilated on the device by "UVKV" (microalgae cell cultivation plant). The diameter of the photoreactors used was 6.5 cm, volume 1L. For the intensive cultivation of micro-algae was used by artificial nutrient media of Abdullayev-Semenenko. The composition of the nutrient environment has been so: KNO_3 -5,0 g/l, KH_2PO_4 -1,25 g/l, $MgSO_4$ – 50 g/l, $FeSO_4$ – 0,009 g/l, EDTA, microelements solution -1 ml/l. In addition, in a nutritious medium was NaCl salt with concentrations 1,0M, 1,5M, and 3M. The cell culture is intensely profiled in the medium which included air with a light mixture of 1.5% CO_2, lighting (24vt/m^2) and 27^0 C temperature. The cell culture is intensely was breed in the medium which included air with a light mixture of 1.5% CO, lighting (24vt / m2) and 27C temperature. During the experiments, the temperature of the air mixture which discharged to the photo reactors was 5^{0}C, 10^0 C, and 27^0 C respectively.

The reproduction of the culture was determined by measuring the optical density of cell suspensions. The cells periodically were counted on the Qoryaev camera under a microscope and the optical density of cells suspensions was identified in the photoelectrocalorimeter (FEK) by nephlometric method. The content of pigments in the cell extracts extracted in 100% acetone was measured in the CF-46 spectrophotometer and calculated on the basis of the Wattstein coefficient. To determine the photosynthetic activity, cell suspensions with density d = 0.8 were prepared and stored in the thermostat at 27^0 C temperature, 20 minutes in the dark. The photosynthetic activity of cell suspension samples amount of 50 ml was measured and controlled by applying the platinum Clark electrode in the polyarografical device. To determine the activity of the catalase, 40 ml and density d = 0.8 of cell suspensions were taken and centrifuged at 4000 ppm for 5 minutes. After addition of 0.05 g of $CaCO_3$ on the sediment it was crushed, 10 ml of distilled water was added onto the collected mass and kept for 2-3 hours with filling into 50 ml flasks. During this time, the enzyme extraction from the cell material occurs. The suspension was then transferred to dry glass by filtration. The catalase activity

was determined by a gasometrical method based on the amount of oxygen released after the addition of hydrogen peroxide to the cell suspension.

Results and Discussions

Figure 1 shows the kinetics of proliferation of *Dunaliella Salina* IPPAS D-24 cells intensively cultivated in various saline nutrient environments. As can be seen from the picture, in the optimum temperature and light intensity of the of cells suspensions, depending on the different concentrations of the NaCl salt, has a very high rate of cell proliferation. The highest results was observed in cell cultures cultivated in a 1,5 M NaCl concentrate (curve-2). There is a significant decrease in cell culture growth rates during the reduction of concentration of nutrient medium (1.0M NaCl, curve-1) and an increase (3.0M NaCl, curve - 3). It should be noted that, while the cell growth rate in the 3.0M NaCl concentration was weakened (compared to cultured cells in 1.5M NaCl), the cultivation levels of the cultures were fairly high.

Table 1 shows the pigment content and photosynthetic activity of *Dunaliella Salina* IPPAS D-24 cells cultivated in various nutritive food environments and intensive-harvesting modes. As can be seen from the table 1, the biosynthesis of chlorophyll and carotenoids in intensive cultivated cells in a 1.5M NaCl concentrated medium has the maximum value in optimum temperature and light intensity. Although increased salt content (3.0 M NaCl) causes chlorophyll **a** and **b** to weaken biosynthesis, the amount of carotenoids remains high.

As the ratio of chlorophyll to carotenoids is one of the indications for photosynthetic activity for *Dunaliella Salina* cells, salinity in the environment is higher than the optimal concentration (1.5 M NaCl), which decreases the photosynthetic activity, which shows itself in the final bio production. The results show that *Dunaliella Salin* cells in the high salinity medium, have a low reproduction rate and photosynthetic activity. Reduced proliferation temp causes stimulation of carotenoid and glycerin synthesis, at the same time decreasing photosynthetic activity by 25% (Larsen 1988). Finally, it should be noted that *Dunaliella Salina* IPPAS D-24 cells are resistant to extreme environmental factors, but also to salinity changes. Maximal photosynthetic activity and bio-productivity are observed in intensive-harvesting mode when cultivated in a 1,5 M NaCl-rich medium for this culture.

Figure 2 shows the reproduction kinetics of reproduction micro- algae intensively cultivated in different temperature air mixtures (5^0 C, 10^0 C, 27^0C). İt is clear from Figure 2 that the micro-algae cells in the optimum medium have a higher rate of growth (temperature 27^0 C, light intensity of 24 wt/m^2 and air temperature 27^0 C). İncreased rate of intensive cultivated cells in 5^0C airflow mode decreased by 46% compared with control. In 10^0C airborne mode intensive cultivated cells also exhibit a marked decrease in reproduction rates and decreased by 24% compared with control.

It is known that during the effects of unfavorable temperatures on plant cells, numerous structural and functional changes occur, resulting in high tolerant. Response responses to low positive temperature stresses in *Dunaliella Salina* IPPAS D-24 cells show itself in the dynamics of increased carotenoid biosynthesis and catalase activity.

Table.1 Pigment composition of *Dunaliella Salina* IPPAS D-24 cells cultivated in various nutritive environments and intensive harvesting modes

Concentration of NaCl , (M)	Chlorofill**a** (mg/l)	Chlorofill**b** (mg/l)	Total amount of chlorophyll (mg/l)	Total amount of carotenoids (mg/l)	The ratio of chlorophyll to carotenoids	Photosynthetic activity (%)
1,0	2,46±0.05	1,27±0.02	3,73±0.01	1,3±0.02	2,83±0.01	90
1,5	3,22±0,01	1,77±0.01	5,0±0.05	1,7±0.01	2,93±0.01	100
3,0	2,41±0.01	1,09±0.01	3,5±0.02	1,7±0.02	2,07±0.01	75

Note: density of suspension – d=0, 8, (10^6 cells/ml); temperature -27^0C; intensity of light -24 wt/m^2

Table.2 The catalase activity of *Dunaliella Salina* IPPAS D-24 cells intensive cultivated in Abdullayev-Semenenko nutritive medium with different NaCl concentration

Concentration of NaCl in a nutritious medium	Catalase activity (by relative unit)			
	5 min	10 min	15 min	20 min
1,0 M	0,34	0,81	1,05	1,15
1,5 M	0,40	1,0	1,2	1,35
3,0 M	0,25	0,60	0,85	1,05

Table.3 The catalase activity of *Dunaliella Salina* IPPAS D-24 cells cultivated in various temperature air mixture

The temperature of air mixture	Catalase activity (by relative unit)			
	5 min	10 min	15 min	20 min
5^0C	0,45	1,05	1,45	1,80
10^0C	0,45	1,10	1,40	1,60
27^0C	0,40	1,0	1,20	1,35

Figure.1 The profilation kinetics of *Dunaliella Salina* IPPAS D-24 cells cultivated in various nutritive environments and intensive harvesting modes 1-1,0M NaCl; 2-1,5M NaCl; 3-3,0M NaCl
Temperature 27^0C, intensity of light 24 vt/m^2

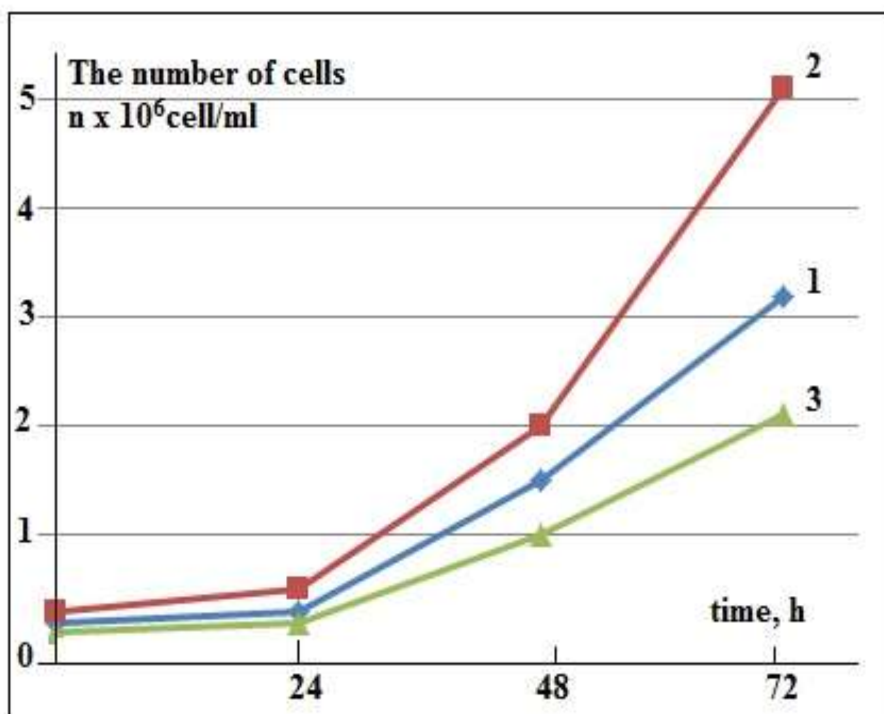

Figure.2 The profilation kinetics of *Dunaliella Salina* IPPAS D-24 micro-algae culture at cultivating in various air content regime1- 5^0C; 2 - 10^0C; 3-27^0C gas mixture. The salinity of medium- 1,5 M NaCl, temperature-27^0C, intensity of light 24 vt/m^2

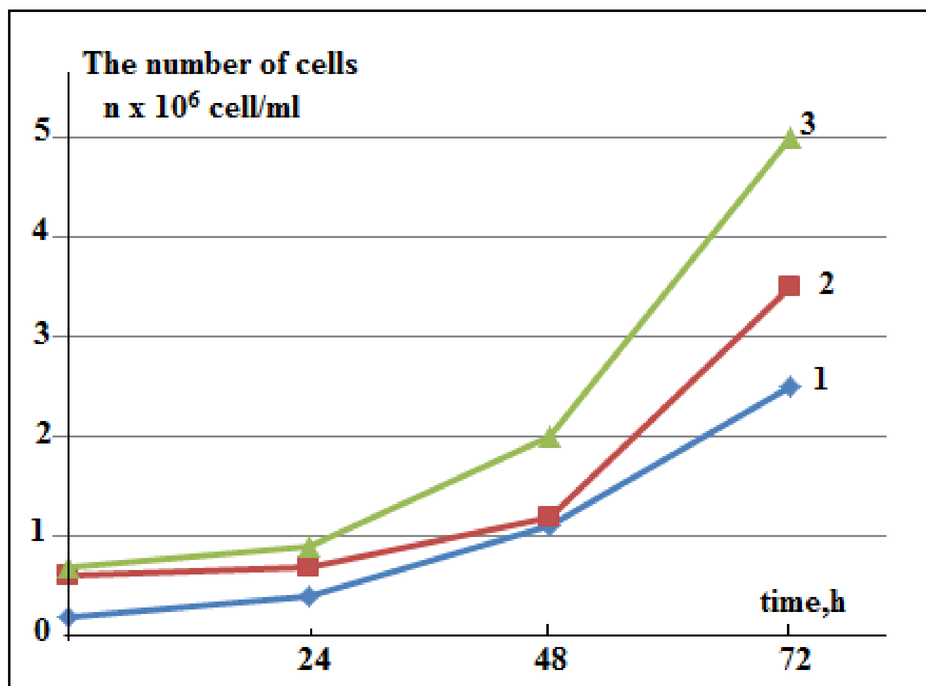

Table 2 shows the results of catalase activity in intensive cultured micro-algae cells in medium Abdullaev-Semenenko nutrients with NaCl salt. The study of the volume of oxygen released in 5, 10, 15 and 20 minutes shows that the catalase activity of cultured cells in 1,5 M NaCl concentration has higher rates compared to cultured cells in 1.0M and 3.0M NaCl concentration. Analysis of the results shows that non-enzymatic protection systems (carotenoids, etc.) are more active in fermentative protection systems in order to ensure the sustainability of cells adapted to increase the salinity by 2 times.

Table 3 shows the change in catalase activity of D cells intensively cultivated in different temperature air mixture modes. The results of Table 3 show that when the temperature of the air mixture is 5°C, catalase enzyme activity is higher in 20 minutes exposition. As temperature of air mixture increases, catalase activity decreases. In the low time exposures (5, 10, 15 minutes), the activity of the catalase in air mixture with various temperatures is not significantly different.

Thus, the analysis of the obtained results shows that the catalase activity of the cells is stimulated in terms of the amount of oxygen allocated over a different period of time (5,10,15 and 20 minutes), as the temperature of the air mixture decreases. Cell catalase activity has higher rates during intensive cultivation in temperature 5°C and 10°C air mixture modes compared to optimum temperature (27^0C). It is clear that in the extreme ecological environment, cellular defense systems are activated, resulting in the adaptation of cells to changing environments. The mechanism of relation between the activation of the oxidation processes and the increase in the catalase activity by the presence of active oxygen in membrane lipids of the cells under the influence of stress factors are investigated.

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