

IMPACT OF ECOLOGICAL FACTORS ON THE MICROALGA *Dunaliella salina* IPPAS D-294 UNDER DIFFERENT SALINITY CONDITIONS

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Abstract. The influence of temperature and illumination on the grown characteristics and functional activity of the photosynthetic apparatus of the unicellular green alga *Dunaliella salina* (strain IPPAS D-294) grown at different salinity was studied, has been studied. Changes in the culture were monitored by cell counting and measuring the millisecond component of delayed emission of photosystem II chlorophyll. It was established that the optimal conditions for vegetative grown (maximum cell density) are achieved at moderate salinity (1.5 M NaCl), a temperature of 27-28°C and an illumination of 30 W/m². The results demonstrate that increasing salinity to 3.0 M NaCl, while reducing the overall grown rate, contributes to expanding the temperature tolerance range and preserving the functional activity of photosystem II under heat stress (up to 40°C).

Keywords: *Dunaliella salina*, photosynthesis, delayed light emission, salinity, temperature.

1. Introduction

Investigating the mechanisms of living organisms resistance to extreme environmental factors is a priority in plant science. In this context, hyperhalophilic microorganisms, capable of functioning under conditions lethal to most life forms, are of particular interest. Understanding the combined effects of abiotic stressors (temperature, pH, insolation, salinity) is essential not only for fundamental science uncovering of the limits biosphere endurance and the evolutionary mechanisms of adapting to extreme environmental conditions but also for applied biotechnological tasks (Paliwal *et al.*, 2017). In addition, other abiotic stress factors such as heavy metals can significantly affect pigment composition and photosynthetic activity in microalgae (Abdullayeva, 2019). Ultraviolet radiation is also an important environmental factor influencing primary photosynthetic processes and antioxidant activity in *Dunaliella* cells (Alizade, 2009). The latter include predicting ecosystem responses to global climate change, developing technologies for the bioremediation of saline soils and the industrial production of valuable biomolecules (Vasiliev & Lobakova, 2019).

The object of this study is the unicellular green microalga *Dunaliella salina*, a unique eukaryotic organism that dominates hypersaline water bodies (salt lakes, lagoons) worldwide, including the unique salt lakes of the Absheron Peninsula (Aliyeva, 2018). This species serves as a model organism for studying osmotic regulation and stress

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adaptation due to its remarkable ability to tolerate NaCl concentrations ranging from 0.5 M to full saturation (approximately 5.5 M).

The physiological response of microalgae to cultivation conditions is rarely determined by a single factor; more often, a complex synergistic effect is observed (Borovkov *et al.*, 2020; Mishra *et al.*, 2008). For instance, the temperature optimum can shift depending on the environmental salinity and the light requirement can vary with CO₂ concentration (Gururani *et al.*, 2015). In the majority of existing studies, only one parameter is manipulated while stabilizing the others, which does not always allow for the recreation of a realistic ecological scenario (Trenkenshu, 2019). The aim of the present work is to identify the patterns of the combined effects of temperature, light intensity and various concentration of sodium chloride on grown processes and photochemical response of photosystem II (PSII) reaction centers in *Dunaliella salina* strain IPPAS D-294.

2. Materials and methods

A culture of the halophilic microalga *Dunaliella salina*, strain IPPAS D-294 was used as the object of study. This strain is characterized by high β -carotene productivity and broad ecological plasticity (Vasiliev & Lobakova, 2019).



Figure 1. Unicellular microalga *Dunaliella salina* strain IPPAS D-294

D. salina cells (Figure 1) lack a rigid cell wall, which provides them with the ability to rapidly change volume in response to osmotic pressure fluctuations (Oren, 2014). The primary mechanism of osmotic regulation is the synthesis and accumulation of intracellular glycerol, the concentration of which changes in proportion to external salinity. Cultivation was carried out at different NaCl concentrations (group P1 - 1.5 M; group P2 - 3.0 M). A temperature gradient was established in the range of 15 to 40°C. Illumination varied from 10 to 60 W/m².

Two main parameters were used to assess the physiological state of the culture:

1. Cell population dynamics. Cell counting was performed using a Goryaev chamber (hemocytometer) under a light microscope (Borovkov *et al.*, 2020).

2. Functional activity of the photosynthetic apparatus. Functional activity of the cell was evaluated using the millisecond component of delayed light emission (ms-DLE) of PSII chlorophyll measured in a set-up early reported in (Matorin & Rubin, 2012). The wavelength of the actinic light initiating the photochemical reaction was $\lambda > 650$ nm and the intensity was ~ 150 W/m². This technique is a highly sensitive *in vivo* indicator of the state of thylakoid membranes, electron transport rate and membrane energization. The

fast and slow components of the induction curves, reflecting different stages of photochemical reactions, were analyzed.

Statistical processing of results. To determine statistically significant differences between experimental results, a Student's t-test was used. All calculations were performed in IBM SPSS Statistics 26.0 using data from five replicates ($n=5$). Differences were considered statistically significant at a significance level of $p \leq 0.05$ or lower.

3. Results and discussion

Temperature is a critical factor determining the rate of enzymatic reactions and membrane fluidity (Borovkov *et al.*, 2020). We investigated the effect of temperature on the cell density of the *D. salina* culture at two salt concentrations. The data presented in Figure 2 shows a clear dependence of grown on temperature, with a well-defined optimum.

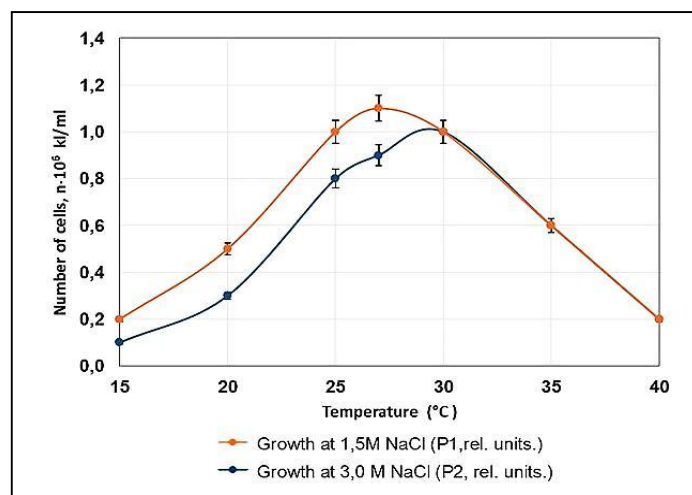


Figure 2. Dependence of *Dunaliella salina* IPPAS D-294 cell culture grown on temperature (P1 - cells grown in 1.5 M NaCl; P2 - cells grown in 3.0 M NaCl). Illumination: 24 W/m², ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$)

An analysis of the curves reveals significant differences in adaptation strategies: Under moderate salinity (1.5 M NaCl, curve P1), more intensive grown is observed. The maximum cell density reaches 1.1×10^6 cells/mL in the temperature range of 27-28°C. Under high salinity (3.0 M NaCl, curve P2), the overall rate of cell division is lower (maximum 1.0×10^6 cells/mL); however, the temperature optimum shifts to a higher range (slightly above 30°C). It is important to note that at temperatures below 20°C and above 35°C, grown processes are suppressed in both groups. Nevertheless, the high-salinity culture demonstrates a more gradual decline in performance during heat stress. This confirms the hypothesis that a high salt concentration, by stimulating the accumulation of osmolytes (glycerol) and the synthesis of heat shock proteins, significantly enhances the thermal tolerance of the organism (Hasanova, 2017).

Photosynthesis is driven by light, but an excess of light can induce the inactivation of photosynthetic machinery and oxidative stress. Figure 3 illustrates the dependence of cell count on light intensity at a fixed temperature of 27°C.

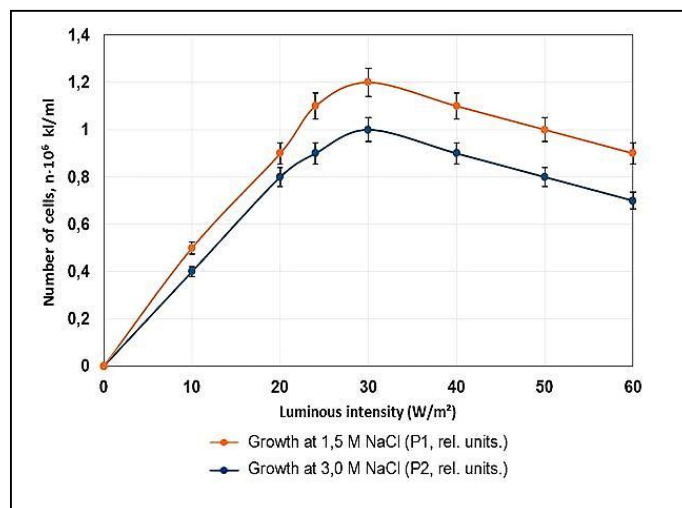


Figure 3. Dependence of *Dunaliella salina* IPPAS D-294 cell culture grown on light intensity (P1 - 1.5 M NaCl; P2 - 3.0 M NaCl). Temperature: 27°C, ($p \leq 0,001$, $p \leq 0,01$ and $p \leq 0,05$)

In the range of low light intensities (up to 20 W/m²), almost linear biomass grown is observed, indicating that the process is limited by light energy deficiency. The optimum illumination for both salt concentrations lies around 30 W/m². With a further increase in the quantum flux (over 40-50 W/m²), a decrease in cell number occurs, likely associated with photo-oxidative damage to photosystems and the activation of protective mechanisms. Notably, the culture grown at 1.5 M NaCl achieves higher density values across the entire illumination range, indicating lower energy expenditures on maintaining homeostasis.

We analyzed the parameters of ms-DLE, which arises as a result of turnover of photochemical reactions in the PSII reaction center. Figure 4 shows the results of the effect of temperature on the components of ms-DLE of *Dunaliella salina* cells grown at a salt concentration of 1.5 M NaCl.

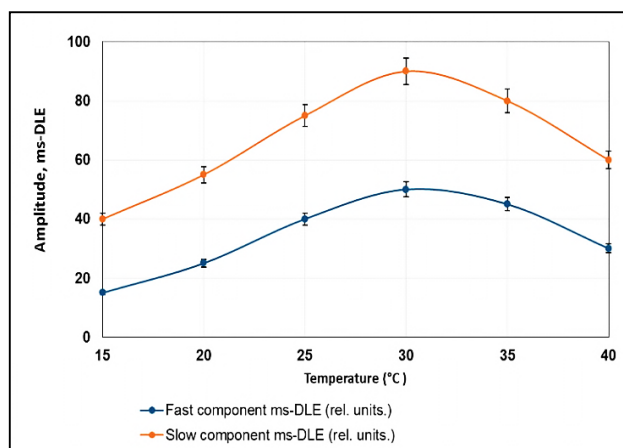


Figure 4. Dependence of the fast and slow components of ms-DLE induction curves of *Dunaliella salina* cells grown in 1.5 M NaCl on temperature, ($p \leq 0,001$, $p \leq 0,01$ and $p \leq 0,05$)

Both the fast and slow components increase as the temperature rises from 15 to 30°C, reflecting the activation of electron transport. However, after passing the peak at

30°C, a sharp drop in the signal is observed, indicating a disruption of the functional integrity of thylakoid membranes and the inactivation of reaction centers under heat stress (Matorin & Rubin, 2012). A different pattern is observed for cells grown at high salinity (3.0 M NaCl), as shown in Figure 5.

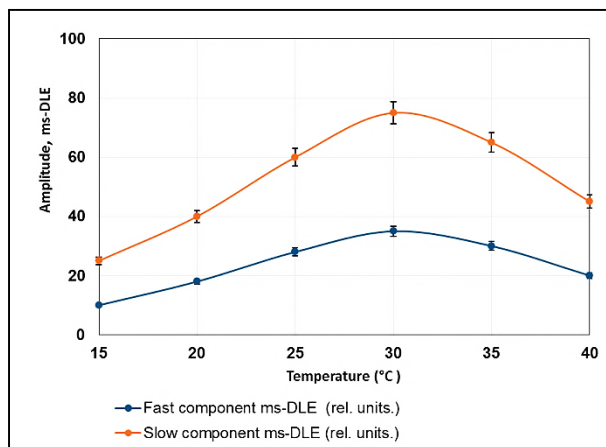


Figure 5. Dependence of the fast and slow components of ms-DLE induction curves of *Dunaliella salina* cells grown in 3.0 M NaCl on temperature ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$)

Although the general shape of the ms-DLE curves obtained from the cells grown at different temperatures is preserved (grown to an optimum followed by a decline), it is crucial to note the relative stability of the parameters in the high-temperature region (35–40°C) compared to the low-salinity culture. The amplitude of the slow component, associated with membrane energization, remains at a sufficiently high level. This indicates that “salt” adaptation protects the photosynthetic apparatus from thermal inactivation (Gao *et al.*, 2016). The obtained results allow us to formulate the concept of a physiological trade-off in *Dunaliella salina*.

Under moderate salinity conditions (1.5 M NaCl), the cell directs its primary energy resources toward biosynthesis and division processes. The environmental osmotic pressure does not require extreme expenditures on the operation of ion pumps and glycerol synthesis. This ensures maximum productivity.

Under high salinity conditions (3.0 M NaCl), a significant portion of ATP energy is consumed to maintain ion homeostasis (pumping out Na^+ ions) and to synthesize massive amounts of glycerol and protective pigments. This naturally reduces the rate of cell division (growth slows down); however, the resulting protective system makes the cell very resilient. The high content of intracellular glycerol and specific proteins stabilizes the lipid bilayer of membranes and enzyme complexes, allowing the alga to maintain photosynthetic activity at temperatures that would be lethal or critical for cells from a less saline environment (Gao *et al.*, 2016).

Similar protective mechanisms involving membrane stability and antioxidant systems under stress conditions have been reported for other plant species (Morsy *et al.*, 2012).

The geographical location of salt lakes (e.g., Lake Masazir in Azerbaijan; Figure 6) frequently coincides with zones of high insolation and extreme temperatures (Aliyeva, 2018; Oren, 2014). Therefore, the identified cross-tolerance (where salt increases heat resistance) represents an evolutionary advantage (Hasanova, 2017).



Figure 6. Lake Masazir a hypersaline water body serving as a natural habitat for *Dunaliella salina*

4. Conclusion

The results of the study reveal the complex nature of the interaction of ecological factors in regulating the vital activity of *Dunaliella salina* IPPAS D-294. It was established that the optimal conditions for biomass accumulation are: NaCl concentration of 1.5 M, temperature of 27-28°C and illumination of 30 W/m². Increasing environmental salinity to 3.0 M leads to a decrease in grown rates but induces cross-tolerance mechanisms. Cells adapted to high salt exhibit a shift in their temperature optimum toward higher temperatures and preserve the functional activity of the photosynthetic apparatus under heat stress (40°C) better than cells from a moderately saline environment.

The ms-DLE data confirm that high salinity contributes to the stabilization of thylakoid membranes, preventing the disruption of electron transport at extreme temperatures.

The practical significance of this study lies in the ability to manage cultivation parameters: to obtain maximum biomass, moderate salinity should be maintained, whereas to increase culture survival during hot seasons (survival at elevated temperatures) or to induce the synthesis of secondary metabolites, it is advisable to increase the salinity of the medium.

References

- Abdullayeva, G.S. (2019). Influence of elevated concentrations of heavy metal salts on pigment composition and photosynthetic activity of *Dunaliella salina*. *Bulletin of Baku University, Natural Sciences Series*, 1, 45-52.
- Aliyeva, F.K. (2018). Algoflora of salt lakes of the Absheron Peninsula. *Proceedings of the Azerbaijan National Academy of Sciences*, 73(2), 34-40.
- Alizade, G.I. (2009). Effect of UV-C and UV-B radiation on primary photosynthetic processes and catalase activity in *Dunaliella* cells. *Modern Problems of Science and Education*, 4, 18-25.
- Borovkov, A.B., Gudvilovich, I.N. & Avsiyan, A.L. (2020). Effect of temperature and salinity on the grown and biochemical composition of *Dunaliella salina*. *Aquaculture International*, 28(5), 1833-1845.
- Gao, S., Wang, Y. & Chen, F. (2016). Responses of *Dunaliella salina* to stepwise vs. one-step salt stress. *Algal Research*, 19, 91-99.
- Gururani, M.A., Venkatesh, J. & Tran, L.S.P. (2015). Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, 8(9), 1304-1320.

- Hasanova, R.A. (2017). Physiological and biochemical basis of microalgae adaptation to stress. *Proceedings of the Azerbaijan National Academy of Sciences*, 72(1), 50-57.
- Matorin, D.N., Rubin, A.B. (2012). *Chlorophyll Fluorescence of Higher Plants and Algae*. Moscow, 256. (In Russian).
- Mishra, A., Mandoli, A. & Jha, B. (2008). Physiological characterization and stress-induced metabolic responses of *Dunaliella salina* isolated from salt pan. *Journal of Industrial Microbiology and Biotechnology*, 35(10), 1093.
- Morsy, A.A., Ali Salama, K.H., Kamel, H.A. & Fahim Mansour, M.M. (2012). Effect of heavy metals on plasma membrane lipids and antioxidant enzymes of *Zygophyllum* species. *Eurasian Journal of Biosciences*, 6.
- Oren, A. (2014). The ecology of *Dunaliella* in high-salt environments. *Journal of Biological Research-Thessaloniki*, 21(1), 23.
- Paliwal, C., Mitra, M., Bhayani, K., Bharadwaj, S.V., Ghosh, T., Dubey, S. & Mishra, S. (2017). Abiotic stresses as tools for metabolites in microalgae. *Bioresource Technology*, 244, 1216-1226.
- Trenkenshu, R.P. (2019). Calculation of the specific growth rate of microalgae. *Marine Biological Journal*, 4(1), 100-108.
- Vasiliev, A.A., Lobakova, E.S. (2019). Cultivation of microalga *Dunaliella salina* for β -carotene production. *Biotechnology*, 35(4), 60-68.