

***Фізіолого-біохімічні, генетико-біотехнологічні та екологічні аспекти адаптації організмів до факторів середовища***

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якого в умовах *in situ* наприкінці першого вегетаційного сезону становили 100 %, а другого – 61 %. Це вище за результати, отримані іншими дослідниками за використання матеріалу колекцій *ex situ*.

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**LIPID ACCUMULATION IN THREE EXTREMOPHILIC ALGAL SPECIES UNDER DIFFERENT CULTURE CONDITIONS**

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Some species of extremophilic algae are cultivated industrially, e.g., *Dunaliella salina* Teodoresco, to manufacture mixed carotenoids (mainly beta-carotene) and *Haematococcus pluvialis* Flotow, to manufacture astaxanthin for food and feed. In their cells, secondary

## ***Фізіолого-біохімічні, генетико-біотехнологічні та екологічні аспекти адаптації організмів до факторів середовища***

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carotenoids accumulate outside the thylakoid membranes in oil bodies inside the chloroplast stroma or the cytosol and do not participate in photosynthetic light-harvesting. It is known, that carotenoid and triacylglycerol deposition correlates in these algae. In *D. salina*, inhibition of triacylglycerol biosynthesis (by sethoxydim or cerulenin) caused the suppression of beta-carotene overproduction under sulfate starvation and high light [4]. Algal lipids are of use as bioactive ingredients containing valuable polyunsaturated fatty acids and as biodiesel sources. Studying culture condition effects onto carotenoid and lipid content in algae is of practical importance and theoretical one as well. It is necessary for finding the mechanistic explanation for the co-regulation of carotenoid and triacylglycerides accumulation in algal cells.

This work is aimed at quantification of cell yield, total lipid and carotenoid contents in the cells of three algal species, carotenogenic *D. salina* and *H. pluvialis*, and non-carotenogenic *Dunaliella viridis* Teodoresco, under different salinity and irradiance, nitrate and phosphate starvation or supplementation, and bicarbonate addition.

*Dunaliella* species were grown in Artari medium and *H. pluvialis* in Bold Basal Medium. All experimental conditions were set at two levels: the salinity of 1 and 4 M by NaCl (except for freshwater *H. pluvialis*), the irradiance of 2 and 8 klx, KNO<sub>3</sub> or NaNO<sub>3</sub> – 0 and 80 mg/L, K<sub>2</sub>HPO<sub>4</sub> – 0 and 10 mg/L, NaHCO<sub>3</sub> – 0 and 200 mg/L. A full factorial experiment was carried out. On the 48<sup>th</sup> day, final cell concentrations, total cellular lipids, and carotenoids were quantified. Cells were counted using Goryaev's hemocytometer. Lipids and carotenoids were extracted according to Bligh and Dyer [1]. Carotenoid content was determined photometrically by extract optical density at 450 nm. Total lipid content was measured with phosphovanillin reagent [5]. The experiment was repeated in triplicate, the data checked for normality with Shapiro – Wilk test, analyzed by ANOVA and Pearson correlation test. Mean values and standard errors of mean are given in the text in brackets.

In both *Dunaliella* species, nitrogen and phosphorus starvation and high salinity inhibited culture growth. In *H. pluvialis*, the culture growth rate decreased at nitrogen but not phosphorus deficiency under the intense illumination. The growth ceased at bicarbonate addition at

## **Фізіолого-біохімічні, генетико-біотехнологічні та екологічні аспекти адаптації організмів до факторів середовища**

---

low light. In *D. salina*, more cellular lipids accumulated in the cultures grown under the combination of nitrogen deficiency, elevated salinity, and bicarbonate supplementation ( $737.4 \pm 140.1$  pg per cell). In *D. viridis*, bicarbonate addition stimulated cellular lipid accumulation too, but, on the contrary, the highest lipid content ( $80.5 \pm 2.5$  pg per cell) was obtained in the cells under low salinity and the deficiency of phosphorus. Illumination did not influence lipid content in the *Dunaliella* species. The cells of *H. pluvialis* accumulated more lipids under bicarbonate supplementation, especially at an intense light (up to  $4.3 \pm 0.8$  ng per cell). The same conditions as for the highest cellular lipid content promoted the highest levels of carotenoid accumulation. In the three algal species studied, cellular carotenoid and lipid contents positively correlated. *H. pluvialis* showed a good direct linear correlation ( $r = 0.80$ ,  $p \leq 0.05$ ). Even in *D. viridis*, which is considered unable to accumulate secondary carotenoids, cellular contents of lipids and carotenoids positively correlated ( $r = 0.85$ ,  $p \leq 0.05$ ). In *D. salina*, the correlation was impaired by salinity: at high salinity level, the content of carotenoids was lower than it could be expected from the lipid content ( $r = 0.42$  by all experimental variants;  $r = 0.71$  if high salinity variants were excluded). The correlation between the yield of cells and cellular lipid and carotenoid accumulation was reverse, which agreed with the extensive literature.

Thus, in the multifactorial experiment, we found species-specific combinations of culture conditions that are the most favorable for lipid accumulation in the cells of three algal species: nitrogen deficiency, high salinity, and bicarbonate supplementation in *D. salina*; phosphorus deficiency, low salinity, and bicarbonate supplementation in *D. viridis*; bicarbonate supplementation and high light in *H. pluvialis*. For an explanation of the correlation and dependence of beta-carotene and triglycerides accumulation in *D. salina*, Rabbani et al. [4] proposed a hypothesis of sequestering beta-carotene in lipid droplet sink thus preventing inhibition of its biosynthetic machinery with the final product. This hypothesis is not sufficient to explain the relatively low carotenoid against the highest lipid content at high salinity in *D. salina* and the difference between high irradiation effect in *H. pluvialis* and *Dunaliella* species. The

***Фізіолого-біохімічні, генетико-біотехнологічні та екологічні  
аспекти адаптації організмів до факторів середовища***

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biological role of secondary beta-carotene deposits in cellular oil bodies remains unclear. We propose another hypothesis to explain the interrelation between lipid and carotenoid accumulation in these algae. When biomass synthesis is halted by some nutrient deficiency (nitrogen or phosphorus), the cells continue to photosynthesize and accumulate storage triglycerides in the form of oil bodies. Bicarbonate stimulates lipid accumulation as being an additional source of inorganic carbon. In freshwater algae, intense light stimulates photosynthesis and lipid deposition. Under high salinity, carbon assimilation rate could be limited not by irradiance but by lower gas solubility in saline waters. In hypersaline water algae, high salinity induces carbonic anhydrase [2] that facilitates added bicarbonate assimilation. That might lead to enhanced lipid accumulation. Lipid storage is a necessary metabolic prerequisite of carotenoid deposition. The function of secondary carotenoids in extremophilic algae might be protection against storage lipid peroxidation. Some products of lipid or carotenoid oxidation might represent another signal level of carotenoid synthesis regulation under environmental stress. The concentration of secondary carotenoids in lipid deposits might depend on the intensity of stress response. The most severe stress might cause oxidative copolymerization of lipids and carotenoids discharged to the outer surface of the plasma membrane. As a result, the rigid sporopollenin cell wall might form around the cells [3], letting them survive stress as cysts, zygotes, or aplanospores. Additional research is necessary to prove the hypothesis proposed. The three algal species studied could be used to manufacture lipids and carotenoids in the two-stage cultivation mode. The first stage must envisage the maximum culture growth rate, and the second stage – lipid accumulation under the culture conditions defined in this work.

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**Фізіолого-біохімічні, генетико-біотехнологічні та екологічні  
аспекти адаптації організмів до факторів середовища**

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**ВИВЧЕННЯ ГЕНОТОКСИЧНОГО ВПЛИВУ  
АРОМАТИЧНОЇ ЗАПРАВКИ «STRAWBERRY» НА  
ПРОРОСТАННЯ НАСІННЯ ЦИБУЛІ РІПЧАСТОЇ**

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Відомо, що куріння, як шкідлива для здоров'я звичка, призводить до незворотних наслідків і смертельних захворювань. Багато людей залежні від куріння та вдаються до методів, щоб позбутися цієї залежності. В якості альтернативи класичним сигаретам, на початку 2000-х років з'явилися електронні сигарети, так звані, вейпи. У ході досліджень, фахівці прийшли до висновку, що ці сигарети найближчим часом можуть замінити звичайну [5].

Електронна сигарета працює за принципом інгалятора. Складається е-виріб з декількох основних частин: змінного картриджа, мікробатарейки і парогенератора. Картридж містить заправну рідину. Саме від її складу залежить смак і "міцність" сигарети. Різноманітність картриджів задовольнить навіть дуже