Materials of Conferences

FUNCTIONAL ACTIVITY OF DUNALIELLA CELLS UNDER COMBINED LOW-TEMPERATURE AND UV IMPACT

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This work presents the results of photosynthetic oxygen discharge by control cells and cell that have been grown up by insufflation of air mixture into photo-reactors under hardening temperature (5°C) and exposed to further influence of low positive temperatures and various doses of UV-light. It is defined, that cells, that have been grown up in intensive culture under hardening temperature show high functional steadiness to further low-temperature and varios doses of UV-light impact. The resume has been done, that this steadiness of seaweed is related to intercellular metabolism and conjugated stability.

Plants reaction to low-temperature stress consists in various alterations of metabolic and physiological processes that are supposed to adapt vegetative organism to changing conditions. Vegetative cell possesses a powerful protective system to oxidation stress that is developed within the cells of heat-loving plants under the impact of low positive temperatures[4, 6]. Low-temperature stress leads to alterations in number of protective ferments and their activity, as well as non-enzymatic elements, such as carotenoids, flavonoids, α-tocopherol, ascorbate, etc.[5] Accumulation of anti-oxidants can be labeled as a display of general non-specific protective reaction of cell to low-temperature stress [4-6].

It is also known, that plants survival in unfavourable conditions is directile linked to their adaptive abilities. Facts are known while vegetative organism adapts to one factor obtains steadiness to another one. Along with that conjugated quality can turn out to be quite distant from the initial adaptive reaction. The study of conjugated steadiness are carried out with different sets of stress factor couples [2] and obtained data is sometimes discrepant.

The goal of this work is to study photosynthetic activity of control cells that have been grown up under low hardening temperatures (5°C) and exposed to further impact of low positive temperatures and various doses of UV-light in order to define the steadiness limits of Dunaliella seaweed population.

Methods and materials

Green single-cellular seaweed Dunaliella salina IPPAS D-294 that has been discharged out of salted Apsheron lakes and registered as a culture served as an object of our research. The weeds were grown under the temperature 27 °C in glass photo-reactors of 250 ml at the single-cellular weed cultures growth facility. The mineral environment contained (g/l): NaCI – 87,5; KNO_3 – 5,0; KH_2PO_4 – 1,25; $MgSO_4$ – 50; $FeSO_4$ – 0,009 and also a solution of micro-elements, 1ml/l. Suspension of cells in photo-reactors was lightened up with white light (16 Vt/m^2) all the time and continuously blown by the mixture (oxygen + 1,5 CO_2) under temperature 27 °C for control and 5 °C – for experimental suspensions. The source of UV-light was a mercury lamp of high pressure.

The rate of culture growth was defined by periodic calculation of the cells number in Goryaev chamber with a microscope or in a non-felometric way and by the alteration of suspension optical density.

The speed of oxygen discharge by cells was defined on polarographic facility with the implementation of platinum Clark electrode while lightening suspension in thermostabilised cell (40 °C) with white light of satiating intensity (100 Vt/m^2).

Results and discussions

Growing up cells in intensive culture with giving air mixture of temperature 5 °C (temperature of hardening) into experimental photoreactors led to the decrease in bio-productivity of 5-10 %. However, hardened suspensions were characterized by higher indexes of functional activity under the further stressors impact in comparison with the control cells.

Figure 1 provides us with the indexes of photosynthetic oxygen discharge by control (curve 1) and experimental cells, that were grown up under hardening temperature of 5 °C from the duration of further low positive (10 °C) temperature impact. As it is seen from the picture, control suspensions that were exposed to further impact of low positive temperature of 10 °C for 30 minutes decreased their photosynthetic oxygen discharge by up to 80 %. After two hours of low-temperature processing functional cells activity decreases significantly and equals 34 % (curve 1). Experimental cells were also exposed to further low-temperature (10 °C) impact. Functional activity of cells suspension after 30 minutes of incubation under the temperature of 10 °C equaled 90 %, and after 60 minutes of impact – decreased down to 74 %.The level of experimental cells functional activity stayed the same under the further period of low-temperature processing increase (curve 2).

Figure 2 shows us the results of the dependence of photosynthetic oxygen discharge by control cells (curve 1) and those that were grown up under the hardening temperature of 5 °C on further low positive temperature impact duration (5°C). Thus, after 30 minutes of low positive temperature impact photosynthetic oxygen discharge of the control cells decreased down to 75 %. As the duration period increased, the steadiness of the suspension functional activity decreased and after two hours it equaled

22 %. Experimental cells that were grown up under the conditions of cold hardening were also exposed to low-temperature impact (curve 2). As shown by the picture, experimental cells that were grown up in intensive culture with giving air mixture of temperature of 5 °C into the photoreactors show high steadiness to further low positive temperature (5 °C) impact. Thus, after 30 minutes of cold impact photosynthetic oxygen discharge equaled about 80-85 %, and after 60 minutes of incubation – 70 %. An increase in further cold impact duration did not decrease the functional activity of the cells.

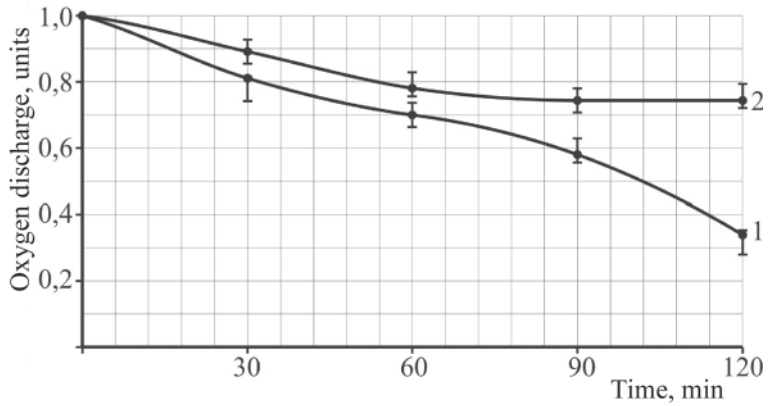

Fig. 1. Steadiness of photosynthetic activity of control cells(1) and those that were grown up under the hardening tempera-ture(5 °C) (2) in dependence on further low-temperature (10 °C) impact duration

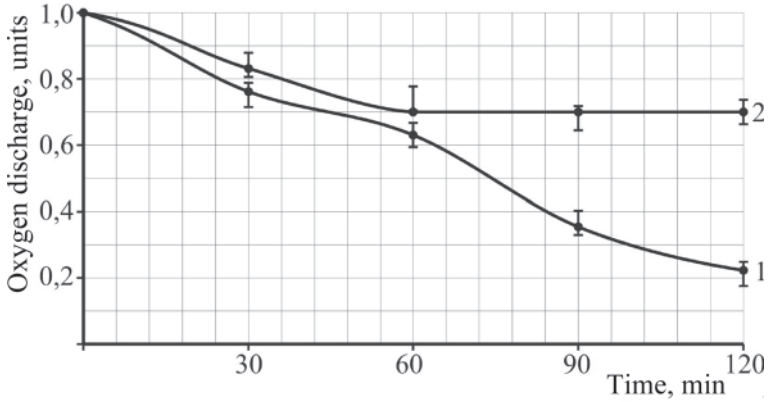

Fig. 2. Steadiness of photosynthetic activity of control cells(1) and those that were grown up under the hardening temperature(5 °C) (2) in dependence on further low-temperature (5 °C) impact duration

So, low further temperatures of 10 and 5 °C cause significant decrease in photosynthetic activity of the control cells trough all the 2-hour impact period down to 34 and 22 % correspondingly. Experimental suspensions that were grown up under the hardening temperature of 5 °C in intensive culture, show high steadiness to further impact of low positive temperatures of 10 and 5 °C. Evident stability of 74 and 70 % of the experimental cells stays the same during all the two-hours impact duration and is described by a stable plateau.

Research on how various doses of UV-light influence functional activity of control and experimental cells suspension was carried out as well.

Figure 3 shows us the dependence of photosynthetic oxygen discharge by control cells (curve 1) and cells, that have been grown up under low hardening temperature of 5 °C (curve 2).

As picture shows, under optimum temperature(40 °C) in polarographic cell control cells show high photosynthetic activity potential. Irradiation of control cells by acute doses of UV-light in various duration period showed that its dose-effect curve has more dramatic decrease that that of the experimental cells. Thus, under the irradiation dose of 10 seconds photosynthetic oxygen discharge equaled 80 %. Under these conditions steadiness of experimental cells functional activ-

ity was 90 %. As the dose of UV-light irradiation increased, the difference between functional activity of control and experimental cells equaled 20–25 %. Probably, hardening temperature of 5 °C increases the activity of anti-oxidant protective system and also increases cells resistance to acute doses of UV-light. Similar results were obtained by us earlier [1], when an increase in population resistance to chronic UV-light doses while growing up seaweed in intensive culture with giving hardening air mixture of 15, 10, and 5 °C into photoreactors.

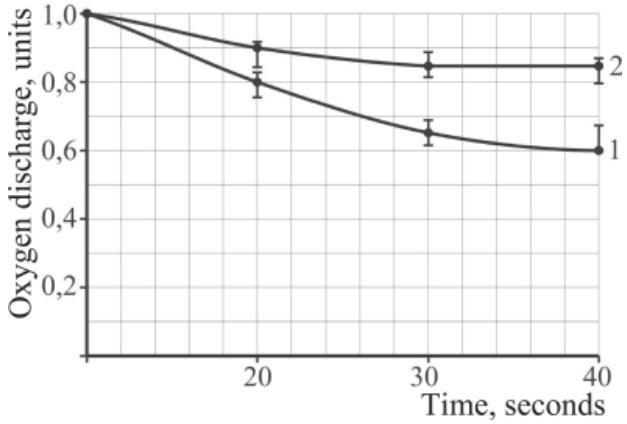

Fig. 3. Steadiness of photosynthetic activity of control cells(1) and those that were grown up under the hardening tempera-ture(5 °C) (2) in dependence on further UV-light impact duration

Most interesting, from our point of view, are the results obtained under the consecutive impact of two stress factors (low-temperature and UV-light) on photosynthetic oxygen discharge by seaweed suspension.

Figure 4 shows us the results of various acute UV-light doses irradiation (curve 1) and both low-temperature (5 °C) processing and various acute UV-light doses irradiation (curve 2) impact on photosynthetic oxygen discharge by cells. As it is seen from the picture, after 20–40 minutes of UV-light impact a decrease in photosynthetic activity equals 80–70 %, and preliminary low-temperature processing (30 minutes) significantly strengthen negative effect of UV-light (60–55 %). Obtained data testifies that consecutive impact of low temperature and UV-light significantly undermine functional cells activity.

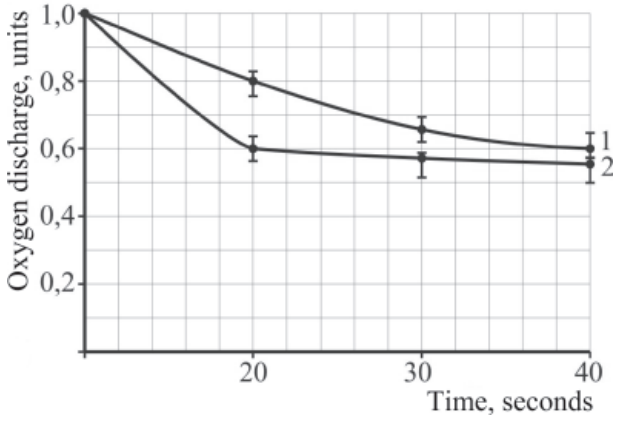

Fig. 4. Steadiness of photosynthetic activity of irradiated bu UV-light control cells(1) and those that were processed (30 minutes) under low temperature (5 °C) and UV-light

Thus, we can conclude that while growing up seaweed in intensive culture and hardening temperature a steadiness to both high doses of UV-light and further low temperature impact is developed within it. This data can be interpreted in accordance with the theory of «conjugated steadiness» [2]. Seaweed

that were grown up under the control conditions and exposed to consecutive impact of low positive temperature and UV-impact show a significant decrease in their functional activity.

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INFLUENCE OF SURFACE MODIFICATION OF ORTOPEDIC IMPLANTS BY NANOCOMPOSITE CARBON NITRIDE ON THEIR PROPERTIES

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The research task of this work was to study in vivo the effect of porous titanium surface modification by nanocomposite carbon nitride films on osteointegration properties of implants.

Materials and methods

Porous titanium implants with porosity θ = 40 % were made from granules of titanium sponge. The system of randomly distributed interconnected pores includes microscale pores of 2–5 μm and macrochannels (100–400) μm. The proportion of pores communicating to the surface accounted for ~75 % of the total number of pores. Diamond-like carbon nitride film (20–50) nm thick was deposited by the method of pulsed arc sputtering of graphite in nitrogen atmosphere. Porous titanium (PTi) and porous titanium with $CN_{0,25}$ film (PTi($CN_{0,25}$)) implants were saturated by the adherent fraction of autologous bone marrow extracted from the wing of iliac bone. The experiment was performed on 18 adult White giant rabbits of herd breeding, weighing 4–5 kg. The implants were implanted in rabbit right tibia and femoral condyles. Histological examination of bone tissue formed inside of porous was performed after removing titanium matrix in a solution of hydrofluoric acid and ethylene glycol. Mechanical testing of neogenic bone tissue (tensile strength) was performed on a universal testing machine FP 100/1 by original method. The designed method enables to determine the integral strength of neogenic bone tissue on the implant – host bone interface. Relative tensile strength (σ_{rel}) was determined as the ratio (σ_{NBT}/σ_{NB}), where σ_{NBT} was ultimate tensile strength of neogenic bone tissue and σ_{NB} – ultimate tensile strength of the native compact bone.

Results

According to histological studies, at 4 weeks after surgery the bone tissue of different maturity degrees takes about 30 % of the cross-sectional implants surface, therefore, all the pores interconnected with implant surface are filled completely with bone tissue. Histological reaction around foreign body (presence of multinucleated giant cells, osteoclastic resorption near the implant) was not found. Implant pores are filled with bone tissue due to both ingrowth mature bone trabecules from site of parent bed and through formation of the young bone tissue out of osteogenous progenitor cells.

At 16 weeks in the implants pores there formed the more mature bone structures. In center of implants the areas with immature bone tissue are still remaining. On the implant periphery it can be noticed integration of parent bed bone tissue and newly formed bone trabecules. Analysis of specimens in 52 weeks showed absence of fibrous capsule, no giant cell reaction. In the implantation site of PTi the mineralized bone structures are saved. In the peripheral areas there are signs of reconstruction bone tissue with hardening effects which are expressed more intensely than in PTi ($CN_{0,25}$). In place of introduction PTi ($CN_{0,25}$) the cortical plate is restored. Bone tissue integrates 1/3 implant diameter. Central bone tissue is thickened. The results of examination of neogenic bone tissue quality are shown on Fig. 1. Modification of porous titanium by $CN_{0.25}$ films improve quality of bone tissue which formed in the pores of implants.

The results of neogenic bone tissue tensile strength evaluation are presented in Fig. 2. As seen from Fig. 2, the bonding strength of the implant with the host bone is quite high after 4 weeks already. Relative tensile strength (σ_{rel}) of PTi($CN_{0.25}$) is higher than PTi implants. In 16 weeks the average strength of a neogenic tissue in the implant – host bone interface increased in a row: σ_{rel}(PTi) < σ_{rel} (PTi ($CN_{0,25}$)). At 52 weeks all implants showed the ultimate tensile strength values close to those of the contralateral limb compact bone. In two cases the failure occurred not at the interface of implant-host