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Summary of the doctoral dissertation:

Targeted biosynthesis and selected aspects of biological activity of phycobiliproteins

Phycobiliproteins - colored proteins produced by cyanobacteria are an important element of the photosynthetic apparatus of these phototrophic microorganisms. In the structure of phycobiliproteins, the apoprotein frame and the prosthetic groups associated with it - chromophore groups are distinguished. Phycobiliprotein chromophores - phycobilins, are open-chain tetrapyrroles that are not bound to a metal ion. There are four types of phycobilins: phycocyanobilin, phycoerythrobilin, phycourobilin, and phycoviolobilin. The type and number of phycobilins associated with the apoprotein determines the differentiation of these proteins into red phycoerythrin, mauve-pink phycoerythrocyanin, blue phycocyanin, and blue allophycocyanin. The phycobiliproteins found in cyanobacterial cells form phycobilisomes - supermolecules consisting of repeating phycobiliprotein monomers that can contain 200 to 500 chromophores. The presence of phycobilisomes improves the process of energy transfer into photosystems, and phycobiliprotein chromophores enable cyanobacteria to obtain additional quanta of light energy, giving them an advantage over organisms that produce only chlorophylls. Distinct color and lack of toxicity make phycobiliproteins attractive natural dyes, which, apart from the food and cosmetics industry, are used in molecular biology as fluorescent markers. In turn, the neuroprotective and antioxidant properties of phycocyanin indicate the possible use of this colorful protein in the treatment of Alzheimer's or Parkinson's disease. Other studies confirm the anti-cancer and anti-inflammatory properties of phycobiliproteins, as well as the ability to reduce metal ions. Phycobiliproteins also play an important role as an intracellular source of nitrogen. In conditions of limited access to this element, it is replenished through the degradation of phycobiliproteins.

The metabolism of cyanobacteria is regulated by many factors, and the process of producing phycobiliproteins also affects the properties of these proteins. Factors affecting the biosynthesis of phycobiliproteins during the cultivation of cyanobacteria are primarily the quality and intensity of lighting, temperature, reaction and composition of the culture medium.

The aim of the research was to determine the effect of selected modulators on the production of phycobiliproteins, as well as to determine the range of biological activity and the interaction of proteins obtained in this way with gold ions.

Experimental work were carried out as general task, including: assessment of the sensitivity of halophilic and freshwater cyanobacteria to selected stressors: intensity and color of light and chemical modulators, determinization of the range of changes in the content of phycobiliproteins in cyanobacterial cells under the influence of the above-mentioned physico-chemical factors; determination of the range

of biological activity of phycobiliproteins produced by the species *Arthrospira platensis*, treated as a model organism due to its specific sensitivity to selected stimuli and its growing use in the process of obtaining phycobiliproteins on an industrial scale; determination of the range of interactions of phycobiliproteins produced by *Arthrospira platensis* with selected metal ions.

The results of the conducted research prove the possibility of controlling the biosynthesis of phycobiliproteins by cyanobacteria through the use of chemical and physical modulators. It was established that changes in the content and composition of phycobiliproteins under the influence of selected modulators result from the different sensitivity of halophilic and freshwater cyanobacteria to these factors. In the course of experiments in which aminophosphonic compounds were used as physiological stressors of cyanobacteria, it was shown that (i) freshwater cyanobacteria are many times more sensitive than halophilic cyanobacteria and that (ii) one of the tested halophilic species - *Arthrospira platensis* - is extremely susceptible to the effects of modulators as regulators of phycobiliprotein biosynthesis. The production of protein pigments by this species was stimulated with the three phosphonates tested: glyphosate, glyphosine and ATMP, given at lower concentrations. On the other hand, the presence of DTPMP - a phosphonic derivative complexing metal ions, inhibited the production of colored proteins by all tested halophilic species. Freshwater species were characterized by many times higher sensitivity to the presence of the tested phosphonic compounds than halophilic species. The most significant reduction in the production of phycobiliproteins in response to the presence of aminophosphonic compounds was observed in the case of *Anabaena torulosa*, while the species *Chroococcidiopsis thermalis* showed the least susceptibility to this type of derivatives.

Interesting results in terms of changes of the content of phycobiliproteins were also obtained by analyzing the effect of organic boron compounds on this aspect of cyanobacterial metabolism. As in the case of phosphonic compounds, freshwater species were much more sensitive to boronates. Another similarity was the statement that a particular beneficiary of the presence of boronic derivatives turned out to be the halophilic cyanobacteria of the species *Arthrospira platensis*, which intensified the biosynthesis of protein dyes under the influence of phenylboronic acid and benzoxaborole.

In order to determine more precisely impact of chosen modulators on the key processes determining the development of phototrophic bacteria, an original determination of the specific efficiency of phycobiliprotein production (Y_{PBP}) was introduced. The Y_{PBP} value makes it possible to determine whether the effect of the stressor, resulting in increased production of phycobiliproteins, is associated with the overproduction of these protein pigments - which is a specific effect, or with intensive cell growth, which is expressed by the increasing concentration of chlorophyll. The use of the Y_{PBP} parameter turned out to be extremely helpful in determining the possibility of directing the biosynthesis of phycobiliproteins by freshwater and halophilic cyanobacteria by introducing specific modulators into the environment. It was conclusively confirmed that glyphosine, glyphosate, benzoxaborole and

5-fluoro-substituted benzoxaborole strongly stimulated the production of phycobiliproteins by *Arthrospira platensis.* Therefore, this species of halophilic cyanobacteria was selected for the next stage.

Recognizing the fundamental role of matter and energy conversion processes for the development of organisms, changes in the energy status of *A. platensis* cells developing on a laboratory scale in the presence of glyphosate, glyphosine, benzoxaborole and 5-fluoro-substituted benzoxaborole were determined. The energy status (AEC) of *A. platensis* cells under control conditions was characterized by the predominance of catabolic processes, while in the presence of phosphonic compounds selected anabolic processes were promoted, including the biosynthesis of photosynthesis protein pigments. This may also confirm the ability of *A. platensis* to use glyphosine as a source of elements used to create new chemical connections. Benzoxaborole (0.30 mM) promoted the production of phycobiliproteins and chlorophylls, and also intensified anabolic processes. The AEC value of cells developing in the presence of 5-fluoro-substituted benzoxaborole (0.30 mM) was the highest, although the development of this species was comparable to the control. Interestingly, the chlorophyll content was lower when *A. platensis* was grown in the presence of B2 and B3 (3.00 mM), while higher concentrations of phycobiliproteins were noted in these cultures. These results indicate a significant participation of phycobiliproteins in the assimilation of light energy and confirm the possibility of functional compensation of chlorophyll deficiency by protein pigments. It was also noted that the cells of the tested cyanosis were characterized by significantly lower AEC values when DMSO was present in the medium, and the presence of boronates eliminated the negative effect of this solvent.

Analyzing the impact of physical factors, it was found that the use of blue light in the *A. platensis* culture in a photobioreactor resulted in the stimulation of the production of phycobiliproteins. The use of sequences of red and blue light consecutive week after week in a fourteen-day culture allowed to obtain the highest concentration of phycobiliproteins. This was also confirmed by the increased yield values of Y_{PBP} established under the conditions of this experiment. Importantly, the use of the tested chemical modulators, also on a semi-pilot scale, confirmed the positive effect of glyphosate and benzoxaborole on the production of phycobiliproteins. It is worth emphasizing that the semi-pilot scale culture in the presence of compound B2 had a positive effect on the phycobiliprotein content compared to experiments in conical flasks, which proves that the scale-up of the culture was successfully carried out. The efficiency of the process of obtaining phycobiliproteins in the presence of benzoxaborole was significantly higher than in the case of the control culture.

It should be noted that the effect of modulators as stress factors in *A. platensis* culture affected the secondary structure of biosynthesized phycobiliproteins. Although in each case α-helical structures dominated, which seems to be natural for water-soluble proteins, a varied share of β structures was noted. The greatest changes were observed in the case of phycobiliproteins synthesized in the presence

of blue light and glyphosine in both concentrations, where the β structures accounted for about 30% of the total amount of secondary structures, which is the highest value in the tested samples.

Phycobiliproteins, especially blue phycocyanin, have antioxidant properties. The study determined the effect of culture conditions on the ability of the produced phycobiliproteins to reduce free radicals, taking into account the type and content of the obtained dyes. It was found that the phycobiliproteins obtained from cells of *Arthrospira platensis* growing under blue light, in a sequence of red and blue light consecutive week after week in a 14-day culture and in the presence of a boron derivative: 5-fluoro-substituted benzoxaborole (B3 3.00 mM), were characterized by higher antioxidant activity.

Assuming that the changed physicochemical properties of the tested colored proteins may affect their biological activity, the sensitivity of selected microscopic fungi and photoautotrophs - freshwater cyanobacteria and eukaryotic microalgae - to phycobiliproteins obtained from *Arthrospira platensis* cells cultured under various conditions was also determined. The response of microorganisms to the presence of phycobiliproteins was markedly different. In the case of eukaryotic microalgae, a marked inhibition of growth was noted, while freshwater cyanobacteria showed less sensitivity to the presence of colored proteins. It should be noted that most of the tested species of filamentous fungi showed no sensitivity to the presence of the tested proteins, which was not surprising, considering the known ability of these heterotrophs to secrete lytic enzymes into the medium. What was interesting, however, was that the presence of phycobiliproteins in the medium stimulated *Trichoderma koningii* to produce an increased number of spores.

Bearing in mind the interesting effects of interactions between proteins and transition metal ions, experiments involving phycobiliproteins and Au^{3+} gold ions were conducted. It was noticed that the tested proteins changed their physicochemical properties in the presence of gold (III) ions in solution. UV-Vis spectrophotometric measurements confirmed the disappearance of the bands corresponding to the maximum absorption of phycobiliproteins in the presence of gold ions, which was accompanied by the precipitation of these proteins from the solution in the form of a violet-red precipitate. This may indicate the formation of metallic gold particles bound to the surface of the phycobiliproteins. This phenomenon was confirmed on the basis of SEM microscopic observations.