PHYCOBILIPROTEIN ACCUMULATION IN CYANOBACTERIUM *Nostoc linckia* AND MODIFICATION OF ANTIOXIDANT ACTIVITY

Ana VALUTA[*], Liliana CEPOI[*], Ludmila RUDI[*], Ion BULHAC[**], Paulina BOUROSH[***], Olga BOLOGA[**]

[*]*Institute of Microbiology and Biotechnology, Academy of Sciences of Moldova, Chisinau, Moldova*
[**]*Institute of Chemistry, Academy of Sciences of Moldova, Chisinau, Moldova*
[***]*Institute of Applied Physics, Academy of Sciences of Moldova, Chisinau, Moldova*
Corresponding author: Ana Valuta, Institute of Microbiology and Biotechnology, 1, Academiei Str., 2028 MD, Chisinau, Moldova, phone:+373(22)725306, fax: +373(22)725754, e-mail: annavaluta@yahoo.com

Abstract. The article deals with iron(III) coordination compounds with Schiff bases as ligands and their impact on phycobiliprotein accumulation by cyanobacterium *Nostoc linckia*. Stimulatory effect depends on the applied dose and in case of three compounds, the concentration 20 mg/L was determined as one with moderate intensity. Lower concentrations resulted in an increase of the phycobiliprotein synthesis. There was found a significant positive correlation between phycobiliprotein content and ABTS (2.2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay values displayed by aqueous extracts from *Nostoc linckia* biomass cultivated in nutrient medium with these coordination compounds. Hence, it is possible to modify the antioxidant activity of *Nostoc* biomass by applying low concentrations of chemical stimuli.

Keywords*:* phycobiliproteins, cyanobacteria, *Nostoc linckia*, antioxidant activity, ABTS assay, correlation

INTRODUCTION

The intensive search for new sources of natural antioxidants with potential for treatment and prevention of human diseases and to meet other needs is currently a key topic in many laboratories and industries. In recent years, biological synthesis of the antioxidants as an alternative to chemical synthesis has attracted great attention, mainly due to safety concerns and the possibility to design highly efficient complex preparations with antioxidant and antiradical activities. Interrelations between phycobiotechnology, microbial biotechnology and coordination chemistry offer vast opportunities in this direction.

Cyanobacteria possess several protective and repair mechanisms comprising of enzymatic and non-enzymatic antioxidants as natural arsenal to alleviate the damages caused by reactive oxygen species (ROS) [8]. The phototrophic nature of these organisms means that they not only need to manage the oxidative stress generated by oxygen reduction in the same way as heterotrophic organisms, but also that produced during photosynthetic electron transport [11]. Strong antioxidant activity of cyanobacteria is also due to their cosmopolitan distribution in diverse ecological niches, where they were equipped evolutionary with more mechanisms to maintain viability than many other biological organisms. Phycobiliproteins as non-enzymatic antioxidants absorb and dissipate excitation energy as heat and efficiently transfer the absorbed energy to photosynthetic reaction centers, in order to reduce the production of singlet oxygen $^1O_2$ (ROS) [24]. In cyanobacteria, more than 99% of UV-B is absorbed by chlorophyll-binding proteins and the phycobilisome light-harvesting complexes, which are attached to the outer surface of the thylakoid membrane.

These water-soluble protein complexes are brilliantly colored, highly absorbent and fluorescent due to the presence of covalently attached chromophores called bilins, linear tetrapyrroles derived from heme. Based on their structure and spectral properties, phycobilin pigments are classified into three major types: phycoerythrin, phycocyanin and allosteric phycocyanin. In general, all these compounds are antioxidants that reduce the intensity of oxidative stress by maintaining the redox environment or modulating the intracellular communication [7, 14, 23]. The responses and mechanisms employed by cyanobacteria may be implemented to design strategies for obtaining valuable widely used fluorophores for further development of technologies relevant to produce the antioxidant preparations.

The yield of phycobiliproteins can be maximized by controlling or optimizing the nutrient and environmental factors [12, 20, 21]. Metal coordination compounds with different ligands draw more and more attention to biotechnologists due to their ability to model biosynthetic processes within living cells, even at very low concentrations [3]. As xenobiotics to living organisms, these compounds can generate an oxidative stress of different intensity, which is often associated with an initial increase in antioxidant activity of cells. In this way, transition metal coordination compounds may serve as modulators for directed synthesis of antioxidant components in the objects of biotechnological interest.

Iron is an essential component in many protein complexes involved in photosynthesis, serving as a redox co-factor. The cyanobacterial photosynthetic system Photosystem I (PSI) and the intermediary electron transport complex cytochrome b_6f both contain considerable amounts of iron and are thus synthesized in lower amounts during times of iron scarcity [5]. In cyanobacteria, the biosynthesis of the phycobiliprotein and phytochrome chromophore precursor phycocyanobilin is catalyzed by the ferredoxin-dependent enzyme phycocyanobilin:ferredoxin oxidoreductase (PcyA), which mediates an atypical four-electron reduction of biliverdin IX [4]. However, the redox properties that makes iron a valuable co-factor also lead to oxidative interactions, resulting in the formation of harmful radicals. Therefore, iron accumulation in cells should be tightly regulated, a

process in which ferritin family proteins play an important role.

Commercially, phycobiliproteins are high-value natural products with potential biotechnological applications in nutraceuticals and pharmaceuticals, food and cosmetic industries as well as in biomedical research and clinical diagnostics. The use of phycobiliproteins as non-toxic and non-carcinogenic natural food colorants is gaining importance worldwide in view of the potential toxicity and carcinogenicity of the synthetic food colorants [15, 17-19]. As of now the annual market of phycocyanin is around 5-10 million US dollars [19]. Various studies have reported that phycobiliproteins appear to enhance immunity, suppress tumor development, slow aging and the progression of a range of neurodegenerative disorders [2, 13, 23, 25, 26]. The identification of extracted phycobilin pigments and their application as additional biomarkers is a promising approach for the routine monitoring of cyanobacteria in coastal, densely inhabited, and less-populated aquatic environments [22].

Rapid growth rate, simple growth requirements, amenability to controlled laboratory culture and ubiquity make cyanobacteria a suitable bioresource for the commercial production of phycobiliproteins. The number of cyanobacterial species currently used for the commercial production of phycobiliproteins is very small. Major producers of phycobiliproteins are the cyanobacterium *Spirulina* and the rhodophyte *Porphyridium* [9, 10]. A promising source of phycobiliproteins is *Nostoc linckia*, a multicellular filamentous cyanobacterium in the phylum *Cyanobacteria*. In general, this cyanobacterial strain proved to be a valuable source of bioactive substances with antioxidant and antiradical activities [1].

The goal of our experiments was assessing the phycobiliprotein accumulation and antioxidant activity of aqueous extract from cyanobacterium *Nostoc linckia* under experimental conditions with iron(III) coordination compounds with Schiff base ligands.

MATERIALS AND METHODS

The object of the study was cyanobacterial strain *Nostoc linckia (Roth) Born et Flah CNM-CB-03* deposited in the National Collection of Nonpathogenic Microorganisms of the Republic of Moldova. Optimized Gromov 6 nutrient medium with the following composition was used:

- macroelements (g/L) - KNO_3 - 0.5, K_2HPO_4 - 0.45, $NaHCO_3$ - 0.05, $MgSO_4 \cdot 7H_2O$ - 0.1, $CaCl_2$ - 0.11;
- microelements (mg/L) - $ZnSO_4 \cdot 7H_2O$ - 0.05, $MnSO_4$ - 2, H_3BO_3 - 0.85, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ - 2.25, $FeSO_4 \cdot 7H_2O$ - 4, $Co(NO_3)_2 \cdot H_2O$ - 0,009, EDTA - 4.75.

Nostoc linckia was grown in Erlenmeyer flasks with pH 6.8 - 7.2, temperature 25 - 27 °C, light intensity 37-55 μmoles of photons/m^2/s, periodic slow stirring. The amount of inoculum was 0.2 g/L in recalculation to absolutely dry biomass.

Chemical stimulators. We used four coordination compounds of iron with Schiff bases as ligands, obtained from condensation of 2,6-diacetylpyridine with isonicotinic acid hydrazide (H_2L^1) and nicotinic acid hidrazide (H_2L^2) with the following formulaes: $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$; $[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$; $[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$; $[Fe(H_2L^2)(H_2O)_2](ClO_4)_3 \cdot H_2O$. These complexes were synthesized in the laboratory of Coordination Chemistry of the Institute of Chemistry, Academy of Sciences of Moldova. Each compound was added to the nutrient medium in 5 different concentrations from 1 to 20 mg/L. In Table 1 we have also indicated molar concentrations of the iron compounds used in the experiment.

The molecular or ionic-level structure of substances and crystalline one, including coordination compounds, is one of the key determinants of physical and chemical properties, spectral, and biological activity, etc. Therefore, its knowledge for tested complexes is necessary and extremely valuable information to better understand their mode of action on the activity of cyanobacterium *Nostoc linckia*. The structure determined with single-crystal X-ray diffraction method in the Institute of Applied Physics, Academy of Sciences of Moldova, demonstrating that these compounds are ionic and contain common complex cations. Structure of one of them - $[Fe(H_2L^2)(H_2O)_2]^+$ is given in Figure 1.

Determination of *Nostoc* productivity. Absolutely dry biomass of *Nostoc linckia* was determined spectrophotometrically using formula:

$$\mathbf{ADB\ (g/l) = 0.88 \times E_{590} \times \eta},$$

where E_{590} - the optical density of the sample at wavelength 590 nm ($0 \leq E \leq 0.2$), η - dilution of the sample.

Preparation of sample extracts. To prevent the accumulation of free radicals, the biomass was collected at day 10 of the cultivation cycle, when culture of *Nostoc linckia* enters into stationary phase. *Nostoc* biomass was first separated from the culture fluid by centrifugation for 5 min at 1500 g. Then, biomass was standardized with distilled water in 5:1 ratio (mg biomass: ml water). To facilitate the extraction, standardized biomass was subjected to freezing at -20 °C and thawing for at least 8 times. The aqueous extracts were subjected to phycobiliproteins evaluation.

Table 1. Molar concentrations of iron(III) Schiff base complexes

Coordination compounds	M, g/mol	1 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L
		$C_M \cdot 10^{-6}$, mol/L				
$[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$	706.343	1.41	7.08	14.15	21.23	28.31
$[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$	834.701	1.20	5.99	11.98	17.94	23.96
$[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$	769.397	1.30	6.50	13.00	19.49	25.99
$Fe(H_2L^2)(H_2O)_2](ClO_4)_3 \cdot H_2O$	809.673	1.23	6.17	12.35	18.52	24.70

Phycobiliproteins evaluation. Phycobiliprotein concentration was determined in supernatant (aqueous extract) by spectrophotometry analysis. The absorbency of extracts was measured at wavelengths 565, 620 and 650 nm using T60 Visible Spectrophotometer, and concentrations were calculated using equations as reported by Gantt and Lipschultz [6].

Antioxidant activity by the $ABTS^{+}$ radical cation assay (ABTS method). The total antioxidant activity of extracts was measured by the $ABTS^{+}$ (2.2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay [16]. $ABTS^{+}$ was generated by oxidation of ABTS with potassium persulphate. ABTS was dissolved in deionized water to a 7mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-16 h) in the dark before use. Prior to assay, the $ABTS^{+}$ stock solution was diluted with ethanol to an absorbency of 0.700 ± 0.020 at 734 nm. Then 1 ml of diluted $ABTS^{+}$ solution was mixed with 10 µl of the test sample (1.0 mg/ml), and the absorbency was measured at 734 nm after 6 min. The reduction of the absorbency (inhibition %) for ABTS reagent was calculated using the following equation:

Inhibition% = $(Abs_{t=0} - Abs_{t=6\ min})/Abs_{t=0}$ x 100;

where $Abs_{t=0\ min}$ was the absorbency of ABTS reagent at 0 min and $Abs_{t=6\ min}$ was the absorbency of ABTS reagent after 6 min.

Statistical analysis. Each trial was performed three repetitions. The results were processed by applying the descriptive statistical indices, using Excel possibilities.

RESULTS

Nostoc linckia growth process and the synthesis of primary and secondary metabolites are clearly dependent on culture medium composition. Iron(III) Schiff base complexes were included into nutrient medium on the 1st day of cultivation cycle in concentrations from 1 to 20 mg/L. Biomass was harvested on the 10th day of the life cycle in which two parameters were monitored, namely phycobiliprotein concentration and antioxidant activity of aqueous extracts.

The results from PBPs quantitative study in the presence of $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$ and correlation with antioxidant activity of aqueous extracts from *Nostoc linckia* biomass are shown in Figure 2. The highest values were determined when adding this compound in concentration of 15 mg/L, which caused an increase in phycobiliprotein synthesis by 132%, compared to control biomass. The amount of total phycobiliproteins in control biomass was estimated at 28 mg/g biomass. The compound concentration of 5 mg/L was found to enhance the phycobiliprotein content by 60%. In terms of antioxidant activity, these aqueous extracts from *Nostoc* biomass inhibited ABTS radical up to 80% and 70%, respectively. There were no significant changes

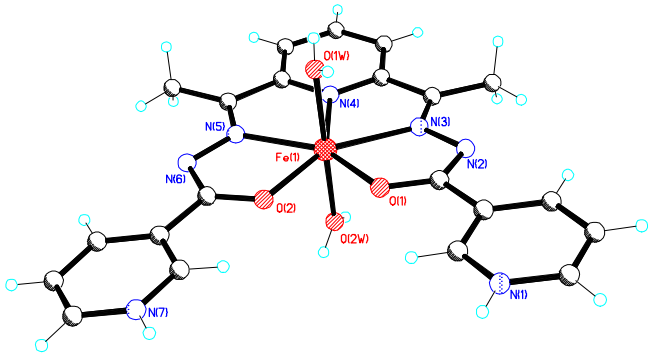

Figure 1. Structure of complex cation in $[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$

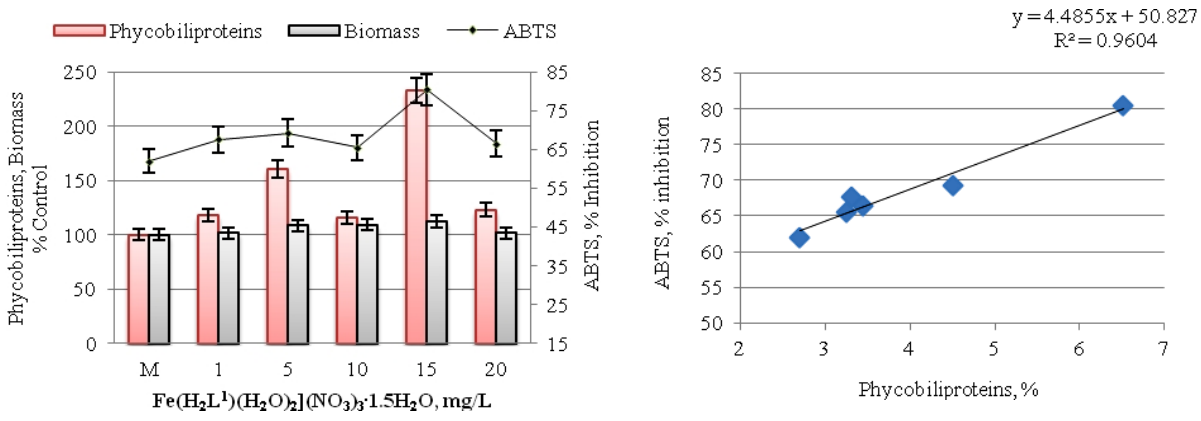

Figure 2. Total phycobiliprotein content (% dry weight) and correlation with ABTS values of the aqueous extracts from *Nostoc* biomass in the presence of $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$.

in PBPs synthesis under cultivation conditions with other concentrations of the coordination compound. Increasing the amount of pigment was accompanied by an insignificant accumulation of *Nostoc linckia* biomass. The ABTS radical inhibition is tightly dependent on phycobiliprotein content in the aqueous extracts, in the case when cyanobacterial strain *Nostoc linckia* was inoculated into nutrient medium supplemented with $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$ in different concentrations. This is also indicated through the value of the coefficient of determination, 0.96.

Data in Figure 3 present the total amount of the phycobiliprotein pigments and ABTS radical scavenging ability of aqueous extracts from *Nostoc* biomass under influence of coordination compound $[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$. The effect of increasing the phycobiliprotein synthesis was determined at all applied concentrations of the coordination compound. The compound concentrations ranging from 1 mg/L to 15 mg/L have induced an increase of phycobiliprotein content estimated between 40-70% compared to control sample. This coordination compound in concentration of 20 mg/L proved to be a powerful stimulant of phycobilingenesis, it was 2.7 times greater than the control. In this case, ABTS radical scavenging activity of aqueous extract reached 92%. Biomass accumulation corresponded to control level in this experimental variant. We also recorded an increasing of the antioxidant activity of aqueous extracts, which inhibited about 80% of ABTS cation radical under cultivation conditions with the lowest concentrations, namely 1 mg/L and 5 mg/L. ABTS values displayed by the extracts from *Nostoc linckia* biomass, cultivated on nutrient medium containing the coordination compound in doses of 10 mg/L and 15 mg/L, were equivalent to the control sample. Assessing the correlation between PBP content and values of ABTS antioxidant assay has established the coefficient of determination (R^2) of 0.75 (Fig. 3).

We found the coordination compound $[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$ stimulating the phycobiliprotein synthesis, when it was especially applied in small concentrations of 1, 5 and 10 mg/L to *Nostoc* cultivation (Fig. 4). The stimulatory effect is directly proportional to the increasing concentration of compound between the above-mentioned limits and started with values of about 40% compared to the control sample. The dose of 10 mg/L doubled the content of phycobiliproteins in *Nostoc* biomass. This was the maximum phycobiliprotein content determined in the experiments with the given compound. We have not noticed any inhibitory effect on phycobiliprotein synthesis. *Nostoc* growth was weakly inhibited under these cultivation conditions.

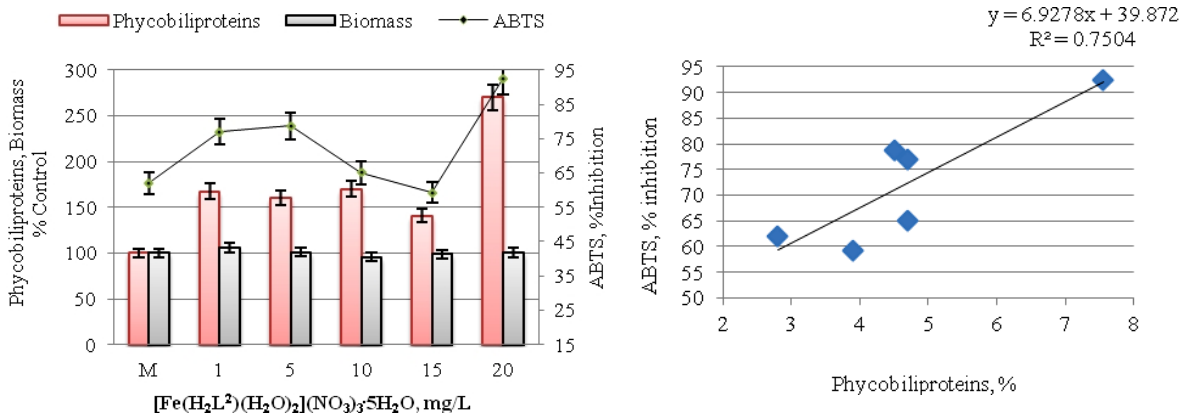

Figure 3. Total phycobiliprotein content (% dry weight) and correlation with ABTS values of the aqueous extracts from *Nostoc* biomass in the presence of $[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$

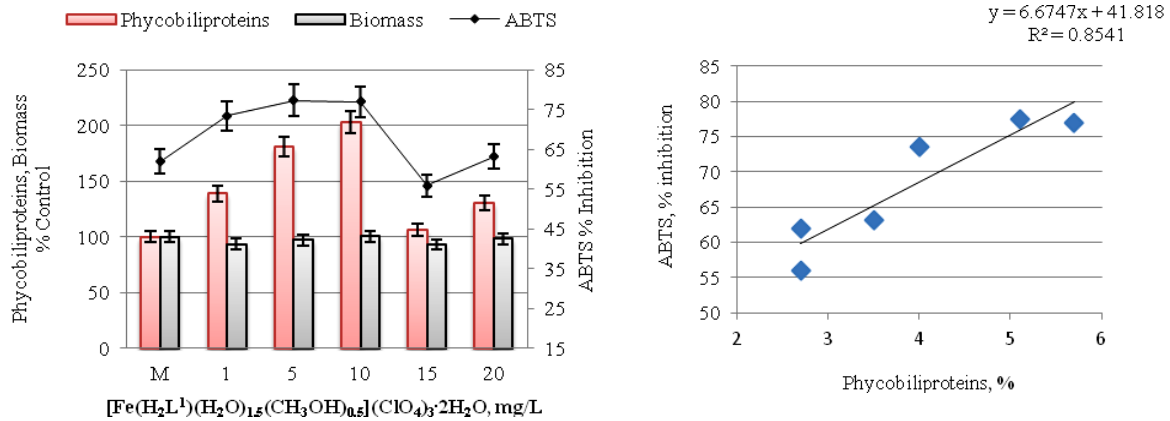

Figure 4. Total phycobiliprotein content (% dry weight) and correlation with ABTS values of the aqueous extracts from *Nostoc* biomass in the presence of $[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$

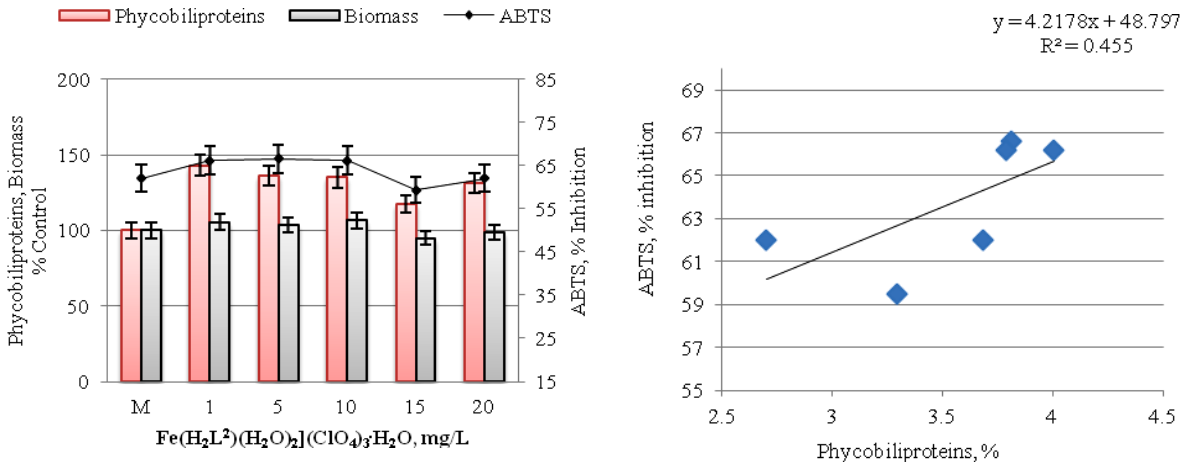

Figure 5. Total phycobiliprotein content (% dry weight) and correlation with ABTS values of the aqueous extracts from *Nostoc* biomass in the presence of $[Fe(H_2L^2)(H_2O)_2](ClO_4)_3 \cdot H_2O$

The maximum ABTS radical-scavenging activity reached 77%, which was recorded in case of the aqueous extracts while applying the compound in concentrations of 5 and 10 mg/L. Correlation between content of PBPs and ABTS assay values was strong. For 85% of cases, increasing the phycobiliproteins through induced synthesis is accompanied by higher values of antioxidant test.

The compound $[Fe(H_2L^2)(H_2O)_2](ClO_4)_3 \cdot H_2O$ has enhanced the phycobiliprotein content in *Nostoc* biomass without registering the maximum values (Fig. 5). We found the same stimulatory effect for all applied concentrations. The content of PBPs exceeded by 30-40% compared to standard biomass. The compound concentration of 20 mg/L was estimated as less significant, as in the case of last two iron complexes with Schiff base ligands. Biomass productivity was similar to control.

The increasing PBP content in *Nostoc* biomass with about 30% did not alter the antioxidant assay values. The antioxidant activity of aqueous extracts ranged within the limits of control 62%, when *Nostoc* biomass was collected under experimental conditions with this coordination compound. In this case, the correlation between phycobiliprotein content and ABTS assay values displayed the following coefficient of determination $R^2 = 0.455$.

DISCUSSIONS

The iron(III) coordination compounds with Schiff base ligands were added to the nutrient medium in various concentrations, to induce an oxidative stress in exposed cyanobacterium *Nostoc linckia*. That responds by modifying the synthesis of protective factors, including phycobiliproteins (PBPs). Phycobiliproteins act as free-radical scavengers or chain breaking antioxidants, which undergo electron transfer and hydrogen transfer reactions [7]. In fact, when stress factor becomes critical as the primary response reaction, some strains of cyanobacteria, including *Nostoc linckia*, have developed avoidance as a first line of defense mechanisms. This includes migration in the surface layers of nutrient medium under laboratory conditions or synthesis of extracellular polysaccharides that can act as natural metal chelators. Moreover, the exopolysaccharides can reduce the metal mainly through chemical functional groups and store it in the form of nanoparticles. This provides much insight for understanding the mechanisms responsible for metal ion transport and maintaining homeostatic levels.

Our results demonstrate visible changes in the amount of phycobiliproteins in biomass. In the case of $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$, increasing the amount of pigment was accompanied by an insignificant accumulation of *Nostoc linckia* biomass. Under these conditions, the compound did not cause an oxidative stress. The increase of phycobiliprotein content can be a reaction involved in the activation of light energy assimilation. At the same time, strong correlation between phycobiliprotein content and antioxidant activity shows the dominance of pigments in the extract and the presence of other minor components with antioxidant activity.

In the case of $[Fe(H_2L^2)(H_2O)_2](NO_3)_3 \cdot 5H_2O$, the highest content of phycobiliproteins was observed under the concentration of 20 mg/L (250% M). Biomass accumulation corresponded to control level in this experimental variant, so we could not state that phycobiliprotein function was just photosynthetic. The correlation between phycobiliprotein content and antioxidant activity of aqueous extracts is lower in this case, that proves the presence of other compounds with antioxidant activity. We can assume that high content of phycobiliproteins under maximum concentration of the compound is a reaction of antioxidant protection, since it did not stimulated the productivity of cyanobacterium *Nostoc linckia*.

The $[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$ weakly inhibited *Nostoc* growth and development. Its concentrations 1, 5 and 10 mg/L increased both the content of phycobiliproteins and antioxidant activity of aqueous extracts. We can assume that culture of *Nostoc* intensifies the synthesis of phycobilins as antioxidant

components under these conditions. In the variants with higher concentrations of the compound, the level of phycobilins was the same as in control. This, amid a slightly decreased level of productivity, can be a proof of the inclusion of other antioxidant protection systems than those based on the excessive accumulation of these pigments.

In the case of $[Fe(H_2L^2)(H_2O)_2](ClO_4)_3 \cdot H_2O$ we have the lowest level of correlation between phycobiliprotein content and antioxidant activity of the extracts, but also the lowest content of phycobilins in the biomass of the experimental samples. Under conditions of biomass productivity, as well as antioxidant activity of aqueous extracts similar to control, we can assume that this compound is the most neutral for culture of *Nostoc linckia*.

In conclusion, we can affirm the stimulatory effect on phycobiliprotein synthesis in the experiments with supplementing the growing medium with iron(III) *Schiff* base complexes. Stimulating effect depends on the applied dose and in case of three compounds, the concentration 20 mg/L was determined as one with moderate intensity. The antioxidant activity determined the existence of a dependent correlation between the content of PBPs and ABTS assay values shown by the aqueous extracts from experimental biomass of *Nostoc linckia*. Hence, it is possible to alter the antioxidant activity of *Nostoc* biomass by applying low concentrations of chemical stimuli.

In our variants the highest level of correlation between the studied parameters was observed in the cases of stimulation of *Nostoc* growth (compound $[Fe(H_2L^1)(H_2O)_2](NO_3)_3 \cdot 1.5H_2O$) or inhibition of this process ($[Fe(H_2L^1)(H_2O)_{1.5}(CH_3OH)_{0.5}](ClO_4)_3 \cdot 2H_2O$). The more neutral is the action of external factors, the lower is the correlation between phycobiliprotein content and antioxidant activity of the aqueous extracts. This conclusion suggests dual functions of phycobilin pigments for algal culture - both photosynthetic, and protective. The intensity of these functional aspects is determined by the nature and action of external factors influencing the culture.

REFERENCES:

[1] Cepoi, L., Rudi, L., Miscu, V., Cojocari, A., Chiriac, T., Sadovnic, D., (2009): Antioxidative activity of ethanol extracts from *Spirulina platensis* and *Nostoc linckia* measured by various methods. Analele Universității din Oradea, Fascicula Biologie, XVI(2): 43-48.

[2] Chang, C.J., Yang, Y.H., Liang, Y.C., Chiu, C.J., Chu, K.H., Chou, H.N., Chiang, B.L., (2011): A novel phycobiliprotein alleviates allergic airway inflammation by modulating immune responses. American Journal of Respiratory and Critical Care Medicine, 183: 15-25.

[3] Clapco, S., Bivol, C., Ciloci, A., Stratan, M., Coropceanu, E., Tiurin, J., Rija, A., Labliuc, S., Bulhac, I., (2013): The effect of some metal complexes of oxime ligands on proteolytic activity of *Fusarium gibbosum* CNMN FD 12 strain. Analele Universității din Oradea, Fascicula Biologie, XX(1): 53-58.

[4] Frankenberg, N., Lagarias, J.C., (2003): Phycocyanobilin:Ferredoxin Oxidoreductase of *Anabaena* sp. PCC 7120. The Journal of Biological Chemistry, 278(11): 9219-9226.

[5] Fraser, J.M., Tulk, S.E., Jeans, J.A., Campbell, D.A., Bibby, T.S., Cockshutt, A.M., (2013): Photophysiological and Photosynthetic Complex Changes during Iron Starvation in Synechocystis sp. PCC 6803 and Synechococcus elongatus PCC 7942. PLOS ONE, 8(3): 1-11.

[6] Gantt, E., Lipschultz, C.A., (1974): Phycobilisomes of *Porphyridium cruentum* pigment analysis. Biochemistry, 13: 14-20.

[7] Ji, L.L., Cui, G.Y, Zhao, X.X., (2011): Antioxidant activities of phycobiliproteins isolated from wild *Nostoc commune*. Advances in Biomedical Engineering, 3-5: 34-43.

[8] Richa, K.M., Sinha, R.P., (2011): Antioxidants as natural arsenal against multiple stresses in cyanobacteria. International Journal of Pharma and Bio Sciences, 2(2): 168-187.

[9] Gurpreet Kaur, Khattar, J.I.S., Singh, D.P., Singh, Y., Nadda, J., (2009): Microalgae: A source of natural colours. pp. 129-150. In Khattar, J.I.S., ., Singh, D.P., Gurpreet Kaur (eds.): Algal Biology and Biotechnology. I.K. International Publishing House Pvt. Ltd.

[10] Kumar, D., Dhar, D.W., Pabbi, S., Kumar, N., Walia, S., (2014): Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). Indian Journal of Plant Physiology, 19(2): 184–188.

[11] Latifi, A., Ruiz, M., Zhang, C.C., (2009): Oxidative stress in cyanobacteria. FEMS Microbiology Reviews, 33: 258-278.

[12] Maurya, S.S., Maurya, J.N., Pandey, V.D., (2014): Factors regulating phycobiliprotein production in cyanobacteria. International Journal of Current Microbiology and Applied Sciences, 3(5): 764-771.

[13] McCarty, M.F, Barroso-Aranda, J, Contreras, F., (2010): Oral phycocyanobilin may diminish the pathogenicity of activated brain microglia in neurodegenerative disorders. Medical Hypotheses, 74(3): 601-605.

[14] Olvera-Ramirez, R., Estrada-Perez, A., Alcalde-Vazquez, R., Muñoz, J.L., (2013): Antioxidant effect of phycobiliproteins on proteins and DNA exposed to a reactive oxygen species generating system. The FASEB Journal, 27 (Meeting Abstract Supplement) 890.15.

[15] Pandey, V.D., Pandey, A., Sharma, V., (2013): Biotechnological applications of cyanobacterial phycobiliproteins. International Journal of Current Microbiology and Applied Sciences, 2(9): 89-97.

[16] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26: 1231-1237.

[17] Richa, Kannaujiya, V.K., Kesheri, M., Singh, G., Sinha, R.P., (2011): Biotechnological potentials of phycobiliproteins. International Journal of Pharma and Bio Sciences, 2(4): 446-454.

[18] Richa, Rastogi, R.P., Kumari, S., Singh, K.L., Kannaujiya, V.K., Singh, G., Kesheri, M., Sinha, R.P., (2011): Biotechnological potential of mycosporine-like amino acids and phycobiliproteins of cyanobacterial origin. Biotechnology, Bioinformatics and Bioengineering, I(2): 159-171.

[19] Sekar, S., Chandramohan, M., (2008): Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J. Appl. Phycol, 20: 113-136.

[20] Sharma, G., Kumar, M., Ali, M.I., Jasuja, N.D., (2014): Effect of carbon content, salinity and pH on *Spirulina*

platensis for phycocyanin, allophycocyanin and phycoerythrin accumulation. Journal of Microbial & Biochemical Technology, 6(4): 202-206.

[21] Simeunović, J., Bešlin, K., Svirčev, Z., Kovač, D., Babić, O., (2013): Impact of nitrogen and drought on phycobiliprotein content in terrestrial cyanobacterial strains. Journal of Applied Phycology, 25: 597–607.

[22] Sobiechowska-Sasim, M., Stoń-Egiert, J., Kosakowska, A., (2014): Quantitative analysis of extracted phycobilin pigments in cyanobacteria-an assessment of spectrophotometric and spectrofluorometric methods. Journal of Applied Phycology, 26: 2065–2074.

[23] Sonani, R.R., Singh, N.K., Kumar, J., Thakar, D., Madamwar, D., (2014): Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya* sp. A09DM: An antioxidant and anti-aging potential of phycoerythrin in *Caenorhabditis elegans*. Process Biochemistry, 49(10): 1757–1766.

[24] Wada, N., Sakamoto, T., Matsugo, S., (2013): Multiple roles of photosynthetic and sunscreen pigments in cyanobacteria focusing on the oxidative stress. Metabolites, 3: 463-483.

[25] Wang, Y., Cai, C., Li, B., Liu, C., He, P., (2009): Photodynamic effect of two kinds of phycobiliproteins on human liver cancer cell line SMMC-7721 *in vitro*. Sheng Wu Gong Cheng Xue Bao, 25(9): 1417-1423.

[26] Zhang, L.X., Cai, C.E., Guo, T.T., Gu, J.W., Xu, H.L., Zhou, Y., Wang, Y., Liu, C.C., He P.M., (2011): Anti-cancer effects of polysaccharide and phycocyanin from *Porphyra yezoensis*. Journal of Marine Science and Technology, 19(4): 377-382.

Received: 20 November 2014
Accepted: 11 January 2015
Published Online: 16 January 2015
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House