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Short Communication

Content of phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin in some cyanobacterial strains: Applications

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Cyanobacteria are unique prokaryotes, which are capable to perform oxygenic photosynthesis. Within these organisms, phycobilisomes (PBS) act as an antenna of the photosynthetic pigment apparatus. Phycobilisomes contain several phycobiliproteins (PBP): phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC) and phycoerythrocyanin (PEC). The application of phycobiliproteins in the biotechnology, food industry and medicine during the last years is rapidly increasing. The aim of our study was to assess the qualitative and quantitative content of phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin in 14 cyanobacterial strains kept in Plovdiv Algal Culture Collection (PACC) and 4 strains purchased from the Culture Collection of Autotrophic Organisms (CCALA). Our data demonstrated that three strains of *Microcoleus autumnalis* (PACC 5505, PACC 5522 and PACC 5527) have high potential to produce phycoerythrins (0.132, 0.201 and 0.136 mg/mL, respectively). Similarly, one *Microcoleus autumnalis* strain (PACC 5522) and one strain of *Leptolyngbya boryana* (CCALA 084) are suitable for biotechnological production of phycocyanins (0.051 and 0.264 mg/mL, respectively) as well as allophycocyanins (0.102 and 0.171 mg/mL, respectively). In addition, the data about the pigment content could be used as a biochemical marker for taxonomic purposes within the group.

Keywords: APC / C-PC / C-PE / PEC / Cyanobacteria / Phycobiliproteins

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1 Introduction

Cyanobacteria are an ancient group of photosynthetic prokaryotes with cosmopolitan distribution. They inhabit marine and freshwater habitats, soils, thermal springs and the snow cover of the poles [1–4]. As primary biomass producers, cyanobacteria are at the base of the food chain. Some cyanobacteria (order Nostocales) perform fixation of atmospheric nitrogen, which makes them also preferred partners in symbiosis [5, 6].

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Abbreviations: APC, allophycocyanins; BBM, Bold’s Basal Medium; BG, Blue-Green Medium; CCALA, Culture Collection of Autotrophic Organisms; C-PC, cyanobacterial phycocyanins; C-PE, cyanobacterial phycoerythrins; PACC, Plovdiv Algal Culture Collection; PBP, phycobiliproteins; PBS, phycobilisomes; PC, phycocyanins; PDT, photodynamic therapy; PE, phycoerythrins; PEC, phycoerythrocyanin; Z, Zehnder Medium

These unique organisms produce as secondary metabolites a number of biologically active substances with antifungal, antibacterial, antitumor, immunosuppressive, immunostimulating and toxic effects [7–9]. This determined their high potential as sources of substances used in the food and pharmaceutical industries, medicine and biotechnology. Bio-hydrogen production by cyanobacteria, for example, attracts a great commercial interest due to its potential as an alternative, reliable and renewable energy source [10].

Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis. In cyanobacteria, phycobilisomes (PBS) act as antenna of the photosynthetic pigment apparatus. They contain phycobiliproteins (PBP) and form complexes containing 200 to 500 phycobilin chromophores covalently bound to the proteins. There are over ten various PBP known, which falls into one of the four groups: phycoerythrins (PE), phycocyanins (PC), allophycocyanins (APC), and phycoerythrocyanins (PEC) [11]. In red algae, the phycobilisomes are anchored to the stromal side of thylakoid membranes of chloroplasts. The light energy is captured by phycoerythrin and then passed on to the reaction centre chlorophyll pair, most of the time via the

phycobiliproteins phycocyanin and allophycocyanin. Allophycocyanin is a pigment, which allows the energy trapped by phycobiliproteins to be transferred to the chlorophyll [12].

Application of PBP in the biotechnological processes, food industry and medicine is increasing [13–15]. It was found that these pigments are ideal for highly sensitive studies in flow cytometry and immunoassays as well as for the photodynamic therapy (PDT) [16, 17]. According to the literature, purified phycobiliproteins have antioxidant, anticancer, anti-inflammatory, immunomodulatory, hepatoprotective, nephroprotective and neuroprotective effects [14, 15, 18].

The aim of our study is to assess the qualitative and quantitative content of phycobiliproteins in 14 cyanobacterial strains (kept in PACC) and 4 strains purchased from the Culture Collection of Autotrophic Organisms (CCALA), belonging to 3 orders, 6 families and 10 genera. This study evaluates the potential of these cyanobacterial strains for the production of phycobiliproteins in quantities suitable for biotechnological production. On the other hand, the data allows us to conclude whether the content and composition of these pigments can help to solve taxonomic cases among the group.

2 Materials and methods

2.1 Cyanobacterial cultures and cultivation conditions

In the present study, 14 cyanobacterial strains kept in the Plovdiv Algal Culture Collection (PACC) and 4 strains purchased from the Culture Collection of Autotrophic Organisms (CCALA) were used (Table 1). Three types of culture media – Bold's Basal Medium (BBM), Zehnder Medium (Z) and Blue-Green Medium (BG) were used to grow the strains.

The cyanobacterial strains were cultivated extensively (at least 1 month) or intensively (14 days). For both types of cultivation, all three media were used. Extensive cultivation was performed at room temperature (20–25°C) and scattered sunlight, using the three nutrient media in two variants – liquid and biphasic. The intensive cultivation was carried out in a cultivation block. Each strain was cultured on the three broth media (BBM, Z, BG) in Ackermann tubes. The cultivation regime is selected so as to bring the conditions of development of the species studied during the summer months into natural habitats as close as possible: temperature 26–27°C, photoperiod 15/9 (light: dark), light intensity 75 $\mu\text{mol photons}/(\text{m}^2 \text{ s})$, purge with 100 L air per hour per liter of suspension.

The growth rate of cyanobacterial cultures was determined spectrophotometrically (M107 Visible Spectrophotometer, Spectronic Camspec Ltd., Leeds, UK) by measuring the optical density at 750 nm as previously described [19].

2.2 Taxonomical determination

The taxonomic position of the studied strains was verified and updated according to changes occurring in recent years in the cyanobacterial taxonomy [20]. A standard Magnum-T light microscope connected to a high definition digital camera (Medline

Scientific, Chalgrove, Oxon, UK) was used for the morphological analysis. Micrographs of the studied strains have been taken to document the morphological traits (data not shown).

2.3 Treatment of samples, extraction of pigments (C-PE, C-PC, APC and PEC)

Cyanobacterial samples with wet weight of 120–140 mg were used. The cyanobacterial bulk was treated with liquid nitrogen and ground with a mortar to completely break the cell wall. The extraction of pigments was performed according Pereira et al. [21] with slight modifications. Briefly, 2 mL of phosphate buffer (pH = 7) was added to the samples and they were centrifuged in micro-centrifuge at 1000 rpm for 20 min.

2.4 Determination of pigments concentration

The supernatant obtained was measured in a spectrophotometer (M107 Visible Spectrophotometer, Spectronic Camspec Ltd., Leeds, UK) at the following wavelength: 455, 565, 572, 592, 612, 618, 645, 647, 652 and 730 nm. Phosphate buffer was used as a blank for the measurements. Calculation of pigment concentration was done according Kussar & Alberte [22] and Kaplan et al. [23] by using absorbance values recorded for the different samples at different wavelengths.

2.5 Statistical analysis

Results are presented as mean values $\pm$ standard deviation (SD) from two individual experiments with at least three replicates. Since the probability distribution of the phycobiliproteins within the analyzed strains are not known in advance, we have used non-parametric tests. The Kolmogorov–Smirnov test was used to analyze the distribution, and Kruskal–Wallis test to determine the levels of difference between the investigated strains. Values of $p < 0.05$ were considered significant. Statistical tests were performed with StatView software (SAS Institute Inc., Cary, NC, USA).

3 Results and discussion

The production of phycobiliproteins by most cyanobacterial strains involved in the present study has not been a subject of other scientific interests so far. Thus, data generated during the study contribute to enriching our knowledge of these cyanobacteria as a resource for biotech industries. Similar studies have been conducted with some cyanobacterial strains from the genera *Scytonema*, *Synechocystis*, *Gloeocapsa*, *Calothrix*, *Lynbya*, *Spirulina*, *Anabaena* and *Nostoc* [24–28]. It was found that the cell content of PE, PC and APC depends from their taxonomic position and culture conditions. Variations in pH, temperature, light intensity, light/dark cycle, nitrogen content of the nutrient medium can influence the production of phycobiliproteins [25, 26, 28].

Table 1. Data on the origin, taxonomy of the strains and their storage in the PACC and CCALA

Number	Strain	Identification code	Isolated by	Locality
Order Oscillatoriales				
1	<i>Oscillatoria</i> sp.	PACC 8167	Vodeničarov	Bulgaria
2	<i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek & J.R.Johansen	PACC 5505	Schwabe	England, Surtsay, S-crater Nr. 237
3	<i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek & J.R.Johansen	PACC 5511	Steubing	Germany
4	<i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek & J.R.Johansen	PACC 5517	Sprecht	Germany
5	<i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek & J.R.Johansen	PACC 5522	Schwartz-Kraepelin	Germany, moss culture
6	<i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek & J.R.Johansen	PACC 5527	Clasen	Germany, Siegburg, weir Wahnbach
7	<i>Wilmottia murayi</i> (West & G.S.West) Strunecký, Elster & Komárek	CCALA 843	Kovacik	Antarctis, King George island, whale skeleton
Order Synechococcales				
8	<i>Phormidesmis molle</i> (Gomont) Turicchia, Ventura, Komárková & Komárek	PACC 5088	Hegewald	Germany, Jülich
9	<i>Phormidesmis molle</i> (Gomont) Turicchia, Ventura, Komárková & Komárek	PACC 8140	Vodeničarov	Bulgaria, Pestera, swamp
10	<i>Nodosilinea bijugata</i> (Kongisser) Perkerson & Kovácik	PACC 8602	Angelieva	Bulgaria, river Topolnica
11	<i>Leptolyngbya boryana</i> (Gomont) Anagnostidis & Komárek	CCALA 084	Marvin	Germany, Hamburg, Elbe, backwater
12	<i>Leptolyngbya boryana</i> (Gomont) Anagnostidis & Komárek	CCALA 078	Hindak	Cuba, El Dique, reservoir
Order Nostocales				
13	<i>Anabaena</i> sp. Bory ex Bornet & Flahault	PACC 8632	Angelieva	Bulgaria, Plovdiv
14	<i>Nostoc punctiforme</i> Hariot	PACC 8646	Angelieva	Bulgaria, Plovdiv, Sachattepe
15	<i>Nostoc linckia</i> Bornet ex Bornet & Flahault	PACC 5085	Hegewald,	Hungary, Szeged
16	<i>Nostoc</i> sp. Vaucher ex Bornet & Flahault	PACC 8205	Vodeničarov	Bulgaria, Rhodopes, Čair lake, fish pond
17	<i>Desmonostoc muscorum</i> (C.Agardh ex Bornet & Flahault) Hrouzek & Ventura	CCALA 125	Lukesova	Czech Republic, Chelice, soil
18	<i>Calothrix elenkinii</i> Kossinskaja	PACC 8754	Angelieva	Bulgaria, Pleven

Considering this, in our study we varied the cultivation methods (extensively/intensively), as well as the growth media (Z, BG-11 and BBM). We have found that the liquid media was the most suitable for the development of these cyanobacterial cultures. Z-medium (pH = 8.5) was the most suitable for all strains, followed by BBM and BG-11 media. Compared to BBM and BG-11, the Z-medium has lower nitrogen content. The higher production of phycobiliproteins in this growth medium correlate with previously reported data that low-nitrogen media are more suitable for cultivation of cyanobacteria for production of phycobiliproteins [25, 26].

The optimal temperature was between 23 and 25°C, while the extensive cultivation showed to be preferable than the intensive one. Here are shown the obtained data only for the optimal conditions (extensive cultivation, liquid Z-medium, pH = 8.5).

After the extraction of the pigments and the determination of their content, we have summarized the obtained results (Fig. 1). In fifteen out of the eighteen cyanobacterial strains studied, al-
lophycocyanin concentration was found to be higher compared with the phycocyanin. An exception was *Wilmottia murayi*, where

Figure 1. Content of al-
lophycocyanin (APC), phycocyanin (PC), phycoerythrin (PE) and phycoerythrocyanin (PEC) in the cyanobacterial strains involved in the present study. Cyanobacteria were cultured extensively in liquid Z-medium (pH = 8.5), at room temperature (20–25°C) and scattered sunlight. Data are shown as mean values ± standard deviation (SD) from two individual experiments with at least three replicates.

the production of phycocyanin was twice higher than that of allophycocyanin; another exception was found in the two strains of *Leptolyngbya boryana*, where one of them (*Leptolyngbya boryana* CCALA 084) produced a significant amount of both pigments PC and APC (0.264 and 0.171 mg/mL, respectively) and showed significant potential for use in biotechnological production. *Microcoleus autumnalis* PACC 5522 was another cyanobacterial strain that produced phycocyanin and allophycocyanin at considerably high concentrations (0.051 and 0.102 mg/mL, respectively). Generally, the chart that presents the amount of allophycocyanin produced by the strains follows the profile of the chart that shows the quantitative content of phycocyanin (Fig. 1). The quantity of pigments, produced from our strains, exceeded what has been reported so far. In similar studies with *Synechocystis* sp. and *Geitlerinema sulphureum* at optimal conditions for development of these strains, the phycocyanin concentrations reported were 0.023 and 0.071 mg/mL, respectively [29, 30].

In all Nostocales strains (Table 1), the amount of phycoerythrin dominated the amounts of phycocyanin and allophycocyanin. For example, the strain of *Anabaena* sp. PACC 8632 produced PE at a concentration of 0.069 mg/mL and those from the genus *Nostoc* between 0.018 and 0.035 mg/mL (Fig. 1). At the same time, PC and APC production for *Anabaena* sp. PACC 8632 was 0.024 and 0.025 mg/mL, and for the *Nostoc* species between 0.008–0.012 mg/mL PC and 0.008–0.020 mg/mL APC, respectively. Representatives of the genera *Anabaena* and *Calothrix* had higher production of phycobiliproteins in comparison with the strains of genus *Nostoc*, which in general, produce low concentrations of all three pigments. Similar amounts of PC and APC were previously reported for *Anabaena* and *Nostoc* strains [25].

In our study, the order Synechococcales (Table 1) is presented by 5 strains that belong to three genera – *Phormidesmis*, *Nodosilinea* and *Leptolyngbya*. *Phormidesmis* and *Nodosilinea* strains produced a similar amount of pigments with dominance of phycoerythrin and showed very similar profiles in terms of the amount of pigments produced (Fig. 1). *Leptolyngbya* strains that belong to the same species – *Leptolyngbya boryana*, showed significant differences in the amount of phycobiliproteins produced. Despite the variations in the amount of pigments, the profile of the two *Leptolyngbya boryana* strains was similar, probably due to the common taxonomic position. On the other hand, the difference in the phycobiliproteins production of the two *Leptolyngbya boryana* strains was the reason to conclude that the production of phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin is strictly individual and most likely related to the characteristics of each cyanobacterial strain. Taking into account the same growth conditions of the strains, this conclusion appeared to be necessary.

Oscillatoriales strains (Table 1), included in this study, belong to the genera *Oscillatoria*, *Microcoleus* and *Wilmottia*. Interestingly, variations in the quantitative distribution of the four pigments were observed not only among the representatives of different genera, but also within one species. Indicative in this case were the results of the pigments production among the genus *Microcoleus*. All *Microcoleus autumnalis* strains (PACC 5505, PACC 5511, PACC 5517, PACC 5522 and PACC 5527) are characterized by a common profile of the phycobiliproteins production, both

quantitatively and qualitatively. Our data demonstrated that four strains of triplicates *Microcoleus autumnalis* (PACC 5505, PACC 5511, PACC 5522 and PACC 5527) have high potential to produce phycoerythrin (0.132, 0.106, 0.201 and 0.136 mg/mL, respectively). Also, two of the *Microcoleus autumnalis* strains (PACC 5522 and PACC 5527) were among the best producers of phycoerythrocyanin (0.0286 mg/mL and 0.0333 mg/mL, respectively) (Fig. 1). These data are additional argument in support of the above conclusion that the production of phycobiliproteins is strictly strain specific. Generally, the representatives of order Oscillatoriales in the present study (genus *Oscillatoria*, genus *Microcoleus*) produce a greater amount of phycobiliproteins than those of the order Synechococcales and Nostocales. Genus *Microcoleus* as well as certain strains (*Leptolyngbya boryana* CCALA 084 and *Microcoleus autumnalis* PACC 5522) showed specificity of the phycobiliproteins production.

The market price per milligram of the studied pigments is about 244 USD for PE and 535 USD for PC (Sigma-Aldrich). According to the results obtained in this study, it can be speculated that for the case of *Leptolyngbya boryana* CCALA 084, 0.5 mg of PC may be obtained from a biomass of 250 mg (wet weight); the market price of this amount comes to 270 USD.

Currently, the classification and taxonomy of cyanobacteria is in process of revision and re-evaluation [20]. Cyanobacteria have always been a difficult group for classification due to their wide variety, simple morphology and lack of sufficient discriminative features. Polyphasic taxonomy, which combines morphological, ultrastructural, biochemical and molecular-genetic traits, is considered by the scientists as the most adequate system. The biochemical characteristics, including pigment content, are considered as stable and appropriate for the taxonomy and classification of cyanobacteria [31]. Therefore, we believe that the data obtained in the present study, showing a correlation between the amount of phycobiliproteins produced and the taxonomic position at the order and genus level, would serve also as an additional taxonomic criterion.

4 Concluding remarks

This study showed for the first time the potential of 18 cyanobacterial strains to produce phycobiliproteins. Following the qualitative and quantitative characterization of the phycocyanin, allophycocyanin and phycoerythrin content, it was found that four of the strains studied, produced high amount of phycobiliproteins and may have biotechnological application. Data demonstrated that two strains of *Microcoleus autumnalis* (PACC 5511 and PACC 5527) have produced phycoerythrins (0.106 and 0.136 mg/mL, respectively). Similarly, one *Microcoleus autumnalis* strain (PACC 5522) and one strain of *Leptolyngbya boryana* (CCALA 084) are suitable for biotechnological production of phycocyanins (0.051 and 0.264 mg/mL, respectively) as well as allophycocyanins (0.102 and 0.171 mg/mL, respectively). In this view, further studies, related to the cultivation condition optimization and production of phycobiliproteins from these strains should be considered obligatory as well as the possibility of their mass cultivation and exploitation. In addition, the data about the pigment content could be used as a biochemical marker for taxonomic purposes within the group.

Practical application

Phycobilisomes are antennal complexes located on the thylacoid membrane of cyanobacteria, and they play an important role in the process of photosynthesis. Phycobiliproteins (phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin), which are the building structures of phycobilisomes in cyanobacteria, have an increasing application in the food, pharmaceutical, cosmetic, medical and biotech industries. Cyanobacteria are a potential source of phycobiliproteins, but they are still poorly studied. The assessment of the potential of 18 cyanobacterial strains for production of phycobiliproteins showed that three *Microcoleus autumnalis* strains (PACC 5505, PACC 5522 and PACC 5527) and one *Leptolyngbya boryana* strain (CCALA 084) produced phycobiliproteins at concentrations suitable for their biotechnological production. There is a real possibility for massive cultivation and exploitation of the above-mentioned cyanobacterial strains in order to produce these pigments. Moreover, the composition of the pigments and their amount may also serve as an additional biochemical marker for solving taxonomic cases within the division Cyanobacteria.

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