Influence of Growth Medium Composition on Synthesis of Bioactive Compounds and Antioxidant Properties of Selected Strains of *Arthrospira* Cyanobacteria

Tomasz TARKO, Aleksandra DUDA-CHODAK and Małgorzata KOBUS

Department of Fermentation Technology and Technical Microbiology, Food Technology Institute, University of Agriculture in Krakow, Krakow, Poland

Abstract

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We studied how the selection of the growth medium influences the antioxidant properties and synthesis of bioactive compounds (β-carotene, C-phycocyanin, allophycocyanin, and phycoerythrin) in six selected species of cyanobacteria of *Arthrospira* genus. For this purpose, cyanobacteria cultures were cultivated on a typical Zarrouk medium and on a cheaper substitute – RM6 medium. Significant differences were observed in the efficiency of synthesis of the studied compounds depending on the strain of cyanobacteria. The quantitative and qualitative composition of Zarrouk medium was more beneficial for β-carotene synthesis in the cells of all strains of cyanobacteria studied. This medium also allowed for the antioxidant potential of the studied strains to be increased. On the other hand, the RM6 medium, deprived of some mineral ingredients, enabled more efficient synthesis of phycobiliproteins in all studied strains except *A. platensis* SAG 85.79.

Keywords: β-carotene; phycobiliproteins; antioxidant activity; RM6 medium; Zarrouk medium

Abbreviations

A_{562} – absorbance measured at 562 nm; A_{620} – absorbance measured at 620 nm; A_{652} – absorbance measured at 652 nm; ABTS – diammonium salt of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid; APC – allophycocyanin; BHA – butylated hydroxyanisole; BHT – butylated hydroxytoluene; C-PC – C-phycocyanin; DM – dry matter; DW – dry weight; HLC – light-harvesting complexes; OCP – orange carotenoid proteins; PBS – phosphate buffer saline; PE – phycoerythrin; RCP – red carotenoid proteins; Trolox – (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid

The biomass of *Arthrospira* cyanobacteria, known in trade as spirulina, contains approximately 60–70% protein. This is a high-quality protein containing as many as 9 essential amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine, and valine (Belay 2008). Carbohydrates and lipids make up respectively 8–16% and 4–9% of the dry matter (DM) of spirulina (Becker 2007). Cyanobacteria belonging to *Arthrospira* genus are also a rich source of numerous microelements such as Ca, Fe, P, I, Mg, Zn, Se, Cu, Mn, Cr, K, and Na. It was also demonstrated that *Arthrospira platensis* is a very rich source of B-group vitamins, in particular B_{12}, and vitamins D, A, E (Piñero Estrada *et al.* 2001). Additionally, cyanobacteria contain significant amounts of unsaturated fatty acids, including (particularly beneficial to human health) ω-3 and ω-6 (Belay 2008). Very important ingredients of cyanobacterial biomass are pigments (phycocya-

nins, chlorophylls, carotenoids, mainly β-carotene) of strong antioxidant properties (Desmorieux & Decaen 2005).

Carotenoid molecules in the photosynthetic antennas take part in transferring the excitation energy to the reaction centre. The auxiliary pigments are in the light-harvesting complexes (LHC), also called auxiliary antennas. The auxiliary antennas are pigment-protein complexes surrounding the reaction centre, to which they transfer the excitation energy of pigments occurring in them. Another extremely significant function of carotenoids is their protection of photosynthetic systems against photo-oxidation of unsaturated fatty acids in chlorophyll (Kerfeld 2004). The basic carotenoids present in biomass of *Arthrospira* are β-carotene (Figure 1d), β-cryptoxanthin, zeaxanthin, echinenone, oscillaxanthin, and myxoxanthophyll (Mendiola *et al.* 2007). In the cells of cyanobacteria are present also carotenoids bound by specific proteins. They occur in the biomass of cyanobacteria growing in natural environments as well as of those cultivated in laboratories. These complexes are soluble in water and bind neither chlorophyll nor other pigments. The groupings of proteins and carotenoids are called orange carot-

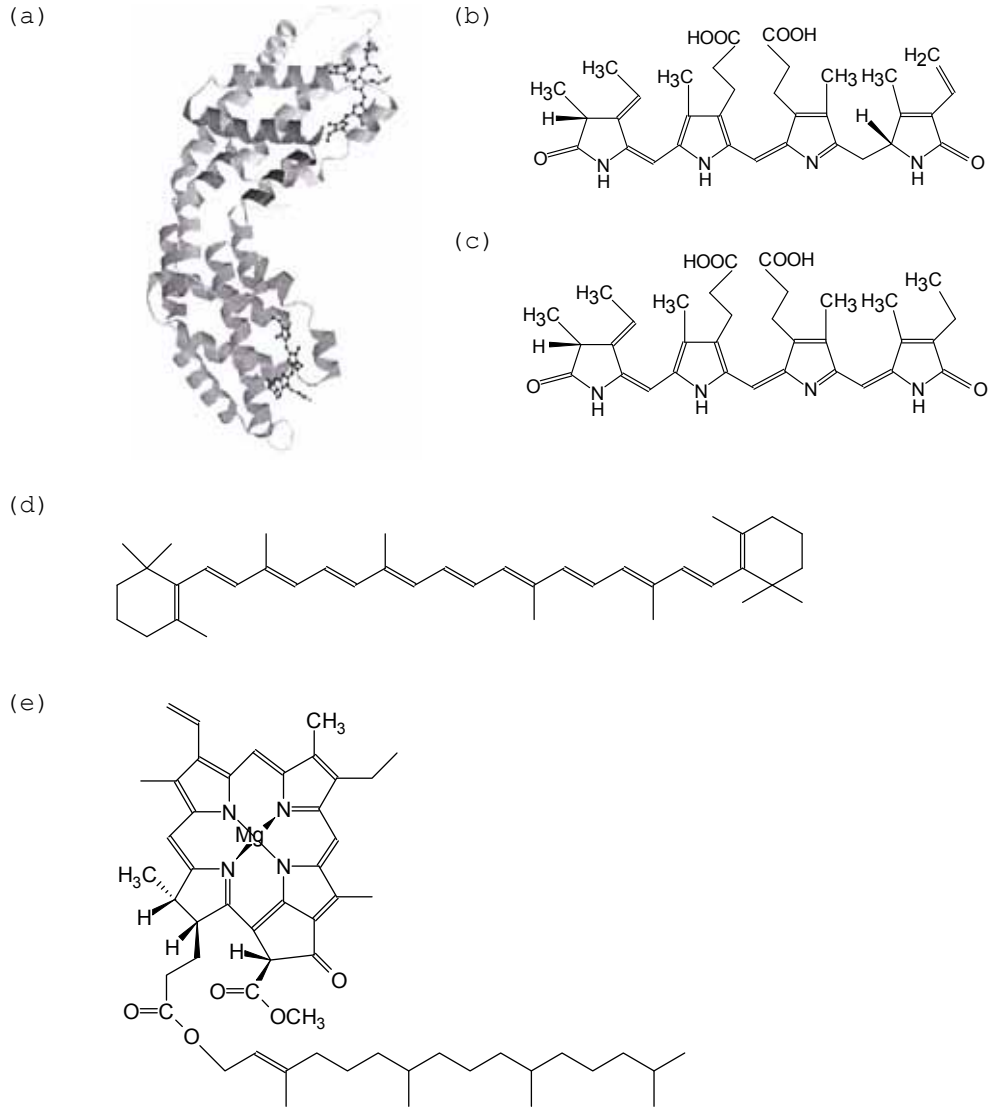

Figure 1. The chemical structure of bioactive compounds present in *Arthrospira* cells (Liu *et al.* 1999; Ferruzzi & Blakeslee 2007; Symonds *et al.* 2009): (a) allophycocyanin (APC) with apparent molecule of incorporated chromophore, (b) phycocyanobilin – the chromophore of phycocyanin (PC) and allophycocyanin, (c) phycoerythrobilin – the chromophore group of phycoerythrin, (d) β-carotene, (e) chlorophyll *a*

enoid proteins (OCP). The probable functions of OCP are: quenching of singlet oxygen and excited chlorophyll (Figure 1e), and inhibiting carotenoid transport within the photosynthetic system (Kerfeld 2004). It was found that the non-active form of OCP, in specific environmental conditions, transforms into an active form of red carotenoid proteins (RCP) (Boulay *et al.* 2008).

Cells of *Arthrospira*, like of all cyanobacteria, contain phycobilisomes, i.e. albuminous structures participating in the photosynthetic process, located on the external surface of the thylakoid membrane. Phycobilisomes are composed (80–85%) of albuminous subunits called phycobiliproteins, which are natural pigments. Phycobiliproteins present in *Arthrospira* biomass include phycoerythrin (PE), containing phycoerythrobilin as a chromophore, and phycocyanin (PC) and allophycocyanin (APC), which have identical chromophore groups (phycocyanobilin) in their structure (Piñero Estrada *et al.* 2001) (Figure 1a–c). All phycobiliproteins are soluble in water, very stable within the physiological range of pH, and have the capacity for fluorescence emission. Their content in cyanobacteria is on average 60% of soluble proteins (Viskari & Colyer 2003). Phycocyanin obtained from *A. platensis* cyanobacterium is used for food dying and as an additive for cosmetics in Japan (Eriksen 2008). Due to its well documented antioxidant properties it is added to nutraceuticals. Small quantities of it are also used in biochemical immunological tests, in microscopy and cytometry, where its fluorescence properties are utilised (Antelo *et al.* 2008).

The antioxidant properties of *Arthrospira* have become in recent times the subject of numerous studies (Manoj *et al.* 1992; Zhi-gang *et al.* 1997; Miranda *et al.* 1998). The authors showed that cyanobacteria are characterised by a high antioxidant potential.

The extracts from cyanobacteria among other things inhibit lipid peroxidation to an extent greater than α-tocopherol and BHA. Moreover, they very effectively scavenge hydroxyl and alkoxy radicals. The content of phycocyanins, and in particular C-phycocyanin, has the greatest influence on the antioxidant potential of cyanobacteria (Romay *et al.* 1998).

The species of *Arthrospira* genus are mixotrophs, which means that they can be nourished autotrophically as well as heterotrophically. The optimum temperatures for growing are 32–45°C. They can also survive temperatures of 60–70°C, but no growth was observed at temperatures below 18°C. Due to that, year-round cultivation of these bacteria in basins is only possible in tropical and subtropical regions (Miklaszewska *et al.* 2008). *Arthrospira* cyanobacteria are counted among alkaliphiles, the optimum pH for their growth being 8.5–10.5. Such high alkalinity of the environment is favourable to maintain microbiological purity during their commercial cultivation. Another important factor is an appropriate chemical composition of the growth media. It was demonstrated that sodium and carbonate ions are essential in high concentrations (Vonshak & Tomaselli 2000). Also the light intensity is essential. Optimum growth takes place at the intensity of 120–200 μmol photons/m^2·s, which is 10–15% of the total solar radiation intensity in the wavelength range of 400–700 nm. When using too intensive illumination in conditions of carbon dioxide deficiency, photoinhibition was observed, probably caused by H_2O_2 accumulation (Miklaszewska *et al.* 2008).

The media used for industrial production of spirulina usually rely on Zarrouk medium (Belay 2008). The quantity of microelements is selected depending on the quality of water used for cultivation (Miklaszewska *et al.* 2008). However, Zarrouk medium is quite expensive and has a complicated composition, which significantly influences the price of the preparations obtained from the biomass of cyanobacteria cultivated on it. That is why more economical media are constantly sought that would permit a reduction of the production costs. Attempts have been made to replace Zarrouk medium with media made on the basis of fertilisers (e.g. superphosphate) or making use of waste raw materials (e.g. molasses) (Raoof *et al.* 2006; Andrade & Costa 2007). However, the studies conducted in this field (Raoof *et al.* 2006; Andrade & Costa 2007) have only concerned the possibility of increasing biomass on non-standard media. But there is a lack of data on how the change in the composition of the growth medium influences the synthesis of the compounds having a significant influence on commercial, nutritive, and therapeutic values of the final product.

The aim of this study was to determine the influence of the growth medium composition on the synthesis of bioactive compounds (β-carotene, phycobiliprotein) and antioxidant properties of cyanobacteria. Six species of *Arthrospira* that were cultivated on standard Zarrouk medium and on the cheaper to prepare RM6 medium were selected for the study.

MATERIAL AND METHODS

Chemicals. Diammonium salt of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS diammonium salt); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2- carboxylic acid (Trolox); a phosphate buffer saline (PBS): 0.01M phosphate buffer, 0.0027M potassium chloride, 0.137M sodium chloride; pH 7.4; Folin-Ciocalteu reagent, vitamin B_{12}. All the chemicals listed, as well as β-carotene standard, were purchased from the Sigma-Aldrich Co. (Darmstadt, Germany). The chemicals Na_2CO_3, K_2S_2O, $NaHCO_3$, $NaNO_3$, K_2SO_4, NaCl, K_2HPO_4, $MgSO_4 \cdot 7\ H_2O$, Na_2EDTA, $CaCl_2$, $FeSO_4 \cdot 7\ H_2O$, Na_2SO_4, Al_2O_3, acetone, hexane, H_3BO_3, $MnCl_2 \cdot 4\ H_2O$, $ZnSO_4 \cdot 7\ H_2O$, Cu_2SO_4, $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, superphosphate $[Ca(H_2PO_4)_2 + CaSO_4]$, KCl and other basic chemicals were obtained from the POCh Company (Gliwice, Poland).

Cyanobacteria. The strains of cyanobacteria used in the study were bought from the collection of cultures Sammlung von Algenkulturen Universität Göttingen (SAG): *Arthrospira laxissima* strain SAG 256.80, *Arthrospira maxima* (strains SAG 49.88 and SAG 84.79), *Arthrospira platensis* (strains SAG 85.79, SAG 86.79, and SAG 257.80). The strains of the same species were of various origin (Lake Chad in Africa, Italy, Peru).

Table 1. The Zarrouk medium composition (Belay 2008)

Component	Concentration (g/l)
$NaHCO_3$	18.000
$NaNO_3$	2.500
K_2SO_4	1.000
NaCl	1.000
K_2HPO_4	0.500
$MgSO_4 \cdot 7\ H_2O$	0.200
Na_2EDTA	0.080
$CaCl_2$	0.040
$FeSO_4 \cdot 7\ H_2O$	0.010
Buffer TE (microelement solution)	1000 ml
– H_3BO_3	2.860
– $MnCl_2 \cdot 4\ H_2O$	1.800
– $ZnSO_4 \cdot 7\ H_2O$	0.220
– Cu_2SO_4	0.080
– $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.020
– Vitamin B_{12}	5×10^{-6}

Table 2. The RM6 medium composition (Raoof *et al.* 2006)

Component	Concentration (g/l)
$NaHCO_3$	8.000
$NaNO_3$	2.500
Superphosphate $[Ca(H_2PO_4)_2 + CaSO_4]$	1.250
KCl	0.898
NaCl	0.500
$MgSO_4 \cdot 7\ H_2O$	0.150
$CaCl_2 \cdot 2\ H_2O$	0.040

The cyanobacteria cultivation was conducted on 2 synthetic media, different in composition, of pH 8.2. Zarrouk medium (composition presented in Table 1) is used as standard in industrial production. RM6 medium (Table 2) was selected as a cheaper equivalent ensuring relatively efficient proliferation of biomass (Raoof *et al.* 2006). The cultivations on both media were conducted in identical conditions: a thermostated box (20 ± 1°C), in illumination (2000–3000 lx) and in cycles 12 h light/12 h dark. Cyanobacteria cells were gathered after 12 days of cultivation, when the amounts of biomass obtained from the examined media were similar (Figure 2).

Before each analysis, approximately 600 ml of the cell suspension was sampled and centrifuged (1055× *g*, 20 min, MPW-350R, MPW Med. instruments, Warsaw, Poland). Then the supernatant was decanted and the residue was rinsed with purified water in order to remove the remains of the medium. The whole was centrifuged again, the supernatant was decanted, and the cell biomass was suspended in a small amount of purified water. In the working suspension of cyanobacteria prepared

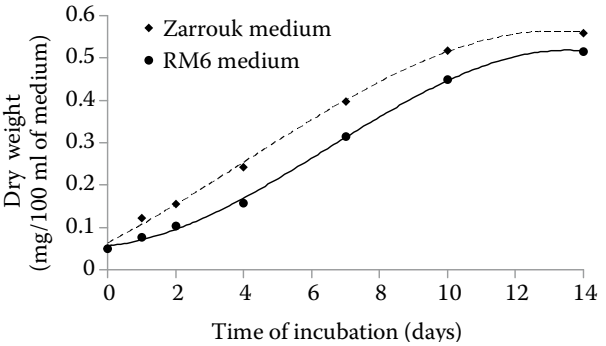

Figure 2. The changes in *Arthrospira platensis* biomass during 14-days cultivation on different media Zarrouk and RM6

in this way, the dry matter content was determined each time using a drying method (105°C, 2 h).

Determination of antioxidant activity by ABTS assay. Two grams of cyanobacteria suspension was weighed and filled up with 25 ml of 80% (vol.) ethanol. The samples were then extracted using a high-speed homogeniser (19 000 r/min, 2 min, UltraTurrax T-25 basic; IKA Werke GmbH, Staufen, Germany), and the homogenate obtained was centrifuged (1055× *g*, 20 min). The obtained supernatant was transferred into a measuring flask and topped up with 80% ethanol to 25 ml. In the extract prepared in this way, the antioxidant activity was determined in accordance with the previously described protocol (Tarko *et al.* 2009).

ABTS radical was generated by chemical reaction between 7mM diammonium salt of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2.45mM potassium persulfate. In order to terminate the reaction and to stabilise the ABTS cation radical, the solution was kept overnight in the dark at ambient temperature. Prior to analysis, the radical solution was diluted with phosphate buffer saline (PBS) to obtain the final absorbance value of A = 0.70 ± 0.02 ($ABTS_{0.7}$) measured at 734 nm.

One hundred-microliter aliquots of the diluted samples or Trolox solution (concentration ranged from 1–10 mg/100 ml) were added to 1 ml of $ABTS_{0.7}$, and the absorbance was measured 6 min after mixing (spectrophotometer BECKMAN DU 650, Beckman Instrument, Inc., Fullerton, USA). Antioxidant capacity was calculated using a standard curve obtained by measuring the absorbance of synthetic vitamin E solutions (Trolox) and expressed in mg of Trolox/100 g of dry mass.

Determination of β-carotene contents. A precisely determined quantity of suspension (approx. 2 g) was extracted with acetone (50 ml) on a magnetic stirrer (150 rpm, 1 h, Wigo, Pruszków, Poland) and centrifuged (1055× *g*, 10 min; MPW-350R). 25 ml of the extract was sampled to the separating funnel, and 15 ml of hexane was added, stirred 1 min, and after separating the phases (~ 15 min), the lower fraction was transferred to another separating funnel. Then 10 ml of hexane was added to it, the mixture was stirred (1 min), and after the phases separation, the lower phase was removed and the upper phase was transferred to the first separating funnel to which 25 ml of distilled water was then added. After the phases separated, the lower phase was removed, and from the upper hexane phase 25 ml was sampled and evaporated in a nitrogen stream to a volume of approximately 1–2 ml.

Subsequently, separation of carotenoids was carried out on a chromatographic column (Allihn funnel), filled successively with anhydrous Na_2SO_4 (approx. 0.7 cm), Al_2O_3 (approx. 7 cm), and anhydrous Na_2SO_4 (approx. 0.5 cm). A condensed extract was introduced into the column, and the elution of pigments was carried out using a mixture of hexane and acetone (100:1). The total fraction of β-carotene (orange ring) was obtained and its absorbance was measured at the wavelength of 450 nm (spectrophotometer BECKMAN DU 650). Based on the calibration curve for β-carotene, the contents of β-carotene in mg/100 g DM of cyanobacteria was read.

Determination of total polyphenol contents. The ethanol extract was prepared analogically as in the determination of the antioxidant activity. The assessment of the total polyphenol content was performed based on the method described by Tarko *et al.* (2009).

Determination of phycobiliprotein contents. 25 ml of phosphate buffer, pH 7, was added to 2 g of cyanobateria and the mixture was homogenised (16 000 r/min, 2 min, Ultra Turrax T-25-basic). The homogenate was frozen at –20°C, and then immediately defrosted at room temperature without light access in order to increase the efficiency of phycobiliprotein extraction. The samples were centrifuged (1055× g, 20 min; MPW-350R), the obtained supernatant was topped up to 25 ml with phosphate buffer, and its absorbance was measured at the wavelengths of 562, 620, and 652 nm (spectrophotometer BECKMAN DU 650), with respect to phosphate buffer as a blind experiment. The wavelengths used are the absorption maxima respectively for phycoerythrin (PE), C-phycocyanin (C-PC), and allophycocyanin (APC). The concentrations of phycobiliproteins (mg/ml) in the extracts were calculated using the following formulae (Patel *et al.* 2005):

$$[\text{C-PC}] = \frac{A_{620} - 0.474 \times A_{652}}{5.34}$$

$$[\text{APC}] = \frac{A_{652} - 0.208 \times A_{620}}{5.09}$$

$$[\text{PE}] = \frac{A_{562} - 2.41 \times [\text{PC}] - 0.849 \times [\text{APC}]}{9.62}$$

The final results were expressed in mg of the individual phycobiliproteins/100 g DM of cyanobacteria.

Statistical analysis. There were a minimum of 3 replications of the whole analysis. The results are shown as the arithmetic mean (± standard deviation). The Kolmogorov-Smirnov test was applied to assess the normality of distribution and a single-factor analysis of variance (ANOVA) with *post hoc* Tukey test was applied to assess the differences between the means (InStat, Version 3.01, GraphPad Software, Inc., San Diego, USA).

RESULTS AND DISCUSSION

Antioxidant activity

It was found that the antioxidant activity of the extracts obtained from cyanobacteria biomass is influenced by the composition of the growth medium as well as the strain used for cultivation (Table 3). The antioxidant capacity fluctuated over a range from 586 mg of Trolox/100 g DM (*A. maxima* SAG 49.88, RM6 medium) to 2716 mg of Trolox/100 g DM (*A. platensis* 85.79, Zarrouk medium). In almost all strains (except *A. platensis* SAG 86.79), the antioxidant activity was significantly higher in cultivations on Zarrouk medium, which suggests that the cheaper RM6 medium significantly reduced the quality of the biomass obtained on it and its pro-health value.

The antioxidant properties of cyanobacteria extracts are strongly dependent on the concentrations of carotenoids, phycobiliproteins, chlorophyll, and some of their decay products, vitamins and unsaturated fatty acids included in the biomass (Wang *et al.* 2007). In this study, the compounds belonging only to 2 of the above groups were determined and no simple dependence was found between the contents of β-carotene and phycobiliproteins in the individual strains and the total antioxidant capacity of the extracts obtained from them. This shows that other compounds are present in the biomass of these cyanobacteria that have key significance for the antioxidant potential.

Phycocyanins and phycobilins from cyanobacteria have the ability to quench hydroxyl radicals, peroxyl radicals and peroxynitrite, thereby inhibiting the occurrence of damage triggered by these compounds (Bhat & Madyastha 2000, 2001; Pinero Estrada *et al.* 2001). Wang *et al.* (2007) assessed, in their studies on the extracts from *A. platensis* cyanobacterium obtained with the help of supercritical carbon dioxide extraction, their antioxidant ability by determining the degree of inhibition of linoleic acid peroxidation. Their studies showed that the activity of the analysed extracts was lower compared to butylated hydroxytoluene (BHT) or Trolox, but significantly higher compared to α-tocopherol.

Total polyphenol contents

The contents of these compounds in the extracts were at the detection method limit or their presence was not found.

β-carotene contents

Statistically significant differences in the contents of β-carotene depending on the studied strain of cyanobacteria were observed. The applied growth medium also had a significant influence (Table 3). In cyanobacteria cultivated on Zarrouk medium the highest concentration of β-carotene was found in *A. laxissima* SAG 256.80 biomass (226 mg/100 g DM). However, the same strain cultivated on RM6 medium contained 15 times less β-carotene. Also in the other strains the use of the cheaper medium caused even a several dozen-fold decrease in β-carotene synthesis. The exception is the strain *A. platensis* SAG 86.79, with which β-carotene concentration is comparable in both cases and very low (7.4 and 5.5 mg/100 g DM respectively for Zarrouk medium and RM6 medium).

The studies carried out by Kobayashi *et al.* (1992) showed that the addition of iron salt to the growth medium causes an increase in astaxanthin production by *Haematococcus pluvialis.* This fact was explained by Tjahjono *et al.* (1994), who discovered that the presence of iron ions favours the generation of hydroxyl radicals ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO^{\cdot}$), which stimulate carotenoid synthesis in a cell. Other studies (Bhosale 2004) prove that the addition of copper salts and zinc salts to the growth medium significantly increases the production of carotenoids by *Rhodotorula* yeast. This is probably caused by the generation of free radicals that stimulate the cells to increase the production of compounds as anti-radical protection. The media used for cyanobacteria cultivation in this study differ significantly in chemical composition and in particular in the presence and quantity of metal ions. Zarrouk medium contains iron ions and

Table 3. Influence of medium type on antioxidant activity of extract and on β-carotene concentration in dry weight obtained from different strain of cyanobacteria (mg of Trolox/100 g of DW)

Strains of cyanobacteria	Antioxidant activity		β-carotene concentration	
	Zarrouk medium	RM6 medium	Zarrouk medium	RM6 medium
A. platensis (SAG 85.79)	2716 ± 54[b]	1605 ± 13[c]	104.2 ± 0.9[b]	14.0 ± 0.1[b]
A. platensis (SAG 86.79)	1037 ± 154[c]	1392 ± 41[d]	7.4 ± 0.4[c]	5.5 ± 0.1[c]
A. platensis (SAG 257.80)	2126 ± 69[a]	985 ± 1[b]	141.8 ± 1.0[a]	17.4 ± 0.0[a]
A. maxima (SAG 49.88)	1836 ± 99[e]	586 ± 7[e]	193.3 ± 2.5[e]	16.6 ± 0.1[e]
A. maxima (SAG 84.79)	1568 ± 55[d]	1263 ± 13	45.8 ± 1.1[d]	1.3 ± 0.0[d]
A. laxissima (SAG 256.80)	2071 ± 58[a]	1255 ± 17[a]	226.0 ± 1.4[f]	14.7 ± 0.2[f]

[a–e]the same letters within a column mean no statistically significant differences between strains of cyanobacteria cultivation on the same medium ($P < 0.05$)

trace elements (manganese, boron, molybdenum, zinc, copper) which are absent in RM6 medium. Probably the shortage of these microelements is the cause of such a significant difference in the quantity of β-carotene synthesised by the same strain, depending on the growth medium applied.

Significant differences between the contents of β-carotene were also observed with the strains belonging to one species. In the case of cyanobacteria cultivated on Zarrouk medium, the strain *A. maxima* SAG 84.79, isolated from Lake Chad in 1963, contained approximately four times higher content of it than the strain SAG 49.88 from Italy, belonging to the same species. Similarly, of the species *A. platensis*, the strain SAG 257.80 (from Lake Laguna Huacachina in Peru) created 20 times more β-carotene than the strain SAG 86.79 isolated from Lake Chad in 1982. Thus, it is clear that, in industrial production, the selection of an appropriate strain of cyanobacteria is most important. In further order the selection of appropriate cultivation conditions should be considered, especially the adequate growth medium, illumination rate, pH, or temperature. Having at disposal a weak microbial producer, even the best optimisation of the process conditions will not be able to compensate for a low production efficiency. Conversely, an efficient microbial producer may allow for achieving a high profit even on a cheap growth medium.

The contents of carotenoids in microalgae may fluctuate in a very broad range: from 10 mg/100 g to over 400 mg/100 g DM (Miki *et al.* 1986), the contents of β-carotene being very variable. Studies carried out on cyanobacteria cells of *Synechococcus* sp. strain PCC7942 showed that β-carotene constituted 52% of the total quantity of carotenoids (Prasanna *et al.* 2010). Rao *et al.* (2010) studied the characteristics of carotenoids of three microorganisms, *Arthrospira platensis*, *Haematococcus pluvialis*, and *Botryococcus braunii*, showing that β-carotene made up respectively 69.5, 1.7, and 1.5% of the total carotenoids content. In the powder made from cyanobacteria *A. platensis*, the content of β-carotene was 211 mg/100 g (Belay 2008).

Phycobiliprotein contents

The analysis of the biomass of *Arthrospira* and extracts made from it led to the conclusion that the phycocyanin, and in particular C-phycocyanin (C-PC), content has the greatest influence on the antioxidant potential (Romay *et al.* 1998). Among phycobiliproteins especially C-PC has found the broadest use in industry, among other things in food and cosmetic dying (Eriksen 2008), and due to the favourable antioxidant properties is it also used as an additive to nutraceuticals (Antelo *et al.* 2008).

Table 4 presents the concentrations of the individual phycobiliproteins in the individual strains of cyanobacteria studied depending on the applied growth medium. Except for the content of C-phycocyanin in strain SAG 85.79, the contents of other phycobiliproteins were always higher in the biomass of cyanobacteria cultivated on RM6 medium than on Zarrouk medium, in some even 5-fold higher. On a typical Zarrouk medium, the best cyanobacterial producer of C-phycocyanin

Table 4. Influence of medium type on phycobiliprotein [C-phycocyanin (C-PC), allophycocyanin (APC) and phycoerythrin (PE)] concentration in dry weight of different strains of cyanobacteria (mg/100 g DM)

Strains of cyanobacteria	Zarrouk medium			RM6 medium		
	C-PC	APC	PE	C-PC	APC	PE
A. platensis (SAG 85.79)	461.4 ± 16.1^{c}	393.2 ± 18.4^{c}	221.2 ± 16.2^{d}	382.6 ± 12.6^{b}	402.4 ± 14.7^{b}	277.6 ± 8.8^{b}
A. platensis (SAG 86.79)	152.9 ± 14.0^{a}	152.7 ± 53.7^{d}	117.6 ± 8.2^{a}	224.2 ± 22.3^{a}	238.7 ± 14.4^{a}	164.1 ± 16.6^{a}
A. platensis (SAG 257.80)	180.0 ± 11.9^{a}	177.1 ± 15.0^{a}	115.5 ± 4.3^{a}	209.7 ± 10.9^{a}	226.4 ± 16.1^{a}	155.4 ± 11.1^{a}
A. maxima (SAG 49.88)	85.8 ± 27.4^{b}	55.1 ± 12.1^{b}	42.9 ± 2.4^{c}	262.0 ± 7.7^{c}	302.1 ± 9.7^{d}	208.0 ± 3.2^{c}
A. maxima (SAG 84.79)	143.4 ± 6.1^{a}	124.3 ± 2.4^{a}	75.4 ± 8.4^{b}	410.6 ± 6.0^{b}	458.1 ± 9.8^{c}	263.3 ± 25.8^{b}
A. laxissima (SAG 256.80)	93.3 ± 3.2^{b}	95.9 ± 31.6^{b}	67.1 ± 9.2^{b,c}	157.4 ± 7.5^{d}	193.3 ± 28.6^{a}	110.4 ± 3.3^{d}

$^{a–d}$same letters within a column mean no statistically significant differences between strains of cyanobacteria cultivation on the same medium ($p<0.05$)

(C-PC), allophycocyanin (APC), and phycoerythrin (PE) was found to be strain SAG 85.79, allowing to obtain in every 100 g DM respectively 461, 393, and 221 mg of these compounds. The other strains synthesised several times lower amounts of phycobiliproteins. However, the selection of another species, *A. maxima* SAG 84.79, allows to obtain comparable, and even higher concentrations (respectively 411, 458, and 263) using the cheaper RM6 medium.

One of the main factors influencing the production of pigments by cyanobacteria is the accessibility of nutritive components containing elements such as sulphur, nitrogen, phosphorus, carbon, and iron. Sendersky *et al.* (2005) identified in the studies on *Synechococcus* PCC 7942 a protein factor (NblC) that initiates the degradation of phycobilisomes if there is a lack of nitrogen compounds, sulfur compounds, or phosphorus compounds in the environment. In the studies presented in this paper, both growth media were a source of these compounds. However, it was observed that on Zarrouk medium the biomass grew more rapidly than on RM6 medium (within the first 9 days even ~2 times higher content of dry matter in the same period). An intense increase in cells might have led to the complete utilisation of some nutritive components causing, as the final result, the disintegration of phycobilisomes and smaller contents of phycobiliproteins per unit of dry matter.

The contents of the individual phycobiliproteins in the strains cultivated on Zarrouk medium decreased in the order C-PC, APC, PE. The cyanobacteria cultivated on RM6 medium contained allophycocyanin in the greatest quantity, then C-phycocyanin, and phycoerythrin in the smallest quantity. The studies conducted on three species of cyanobacteria (*Spirulina* sp., *Phormidium* sp., *Lyngbya* sp.) cultivated in open systems on standard growth medium showed that the contents of these 3 phycobiliproteins decrease in each of these species in the order C-phycocyanin, allophycocyanin, phycoerythrin (Patel *et al.* 2005). The change of this regularity in the case of using RM6 medium may show that their contents are favourable for increasing the production of allophycocyanin or reducing the production of C-phycocyanin, or that both these phenomena take place simultaneously.

Among the three phycobiliproteins analysed in this work, C-phycocyanin has the broadest use in the food and pharmaceutical industries (Eriksen 2008). Apt *et al.* (1995) state that the content of C-phycocyanin in the biomass of *A. platensis*, under optimum growth conditions, may reach as much as 14.8% of cells the dry matter. Graverholt and Eriksen (2007) found in studies on different strains of red algae of *Galdieria sulphuraria* that the contents of C-phycocyanin may fluctuate between 200–2600 mg/100 g DM. Among the cyanobacteria strains analysed in this work, *A. platensis* SAG 85.79 (461 mg/100 g DM) cultivated on Zarrouk medium and *A. maxima* SAG 84.79 (411 mg/100 g DM) cultivated on RM6 medium provided the greatest amounts of C-phycocyanin. These values are significantly lower than the reference values. Thus, the conclusion could be made that regardless of the type of the medium applied, the cultivation conditions were not optimal for the production of this pigment. Further studies on the kinetics of

the bioactive compounds production depending on the strain, cultivation medium, and conditions should be carried out.

The studies conducted by Ranjitha and Kaushik (2005) showed that a high light intensity (13–28 klx) during the cultivation of *Nostoc muscorum* stimulates the production of carotenoids, while the application of a low light intensity (1 klx) favours an increase in the production and accumulation of phycobiliproteins. The light intensity applied during the cultivation of cyanobacteria in this study was 2–3 klx, which is optimal for the growth of their biomass. This fact may explain the relatively low C-phycocyanin content (Prasanna *et al.* 2010).

CONCLUSIONS

This study investigated how a change in the composition of the growth medium influences the synthesis of β-carotene and phycobiliproteins, bioactive compounds of the potential use in the food and pharmaceutical industries. Six species of *Arthrospira* that were cultivated on standard Zarrouk medium and on the cheaper-to-prepare RM6 medium were used in this study. It was found that, depending on the final product desired, first of all the selection of an appropriate strain of cyanobacteria is essential, and then the selection of the medium. Nevertheless, Zarrouk medium is very favourable for such cultivations whose aim is to obtain high concentrations of β-carotene, while RM6 medium is favourable for phycobiliprotein synthesis. Certainly, the studies aimed at obtaining new growth media stimulating the production of bioactive compounds should be continued. Appropriately prepared cyanobacterial biomass is also a potential source of compounds possessing antioxidant activity, which further justifies the search for economical and effective growth media that may be used on the industrial scale.

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Corresponding author:

Tomasz Tarko, Ph.D., University of Agriculture in Krakow, Food Technology Institute,
Department of Fermentation Technology and Technical Microbiology, ul. Balicka 122, 30-149 Krakow, Poland
tel. + 48 12 662 47 92, e-mail: ttarko@ar.krakow.pl