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BIOCHEMICAL CHARACTERISTICS OF A NEWLY ISOLATED STRAIN *COELASTRELLA* SP. BGV CULTIVATED AT DIFFERENT TEMPERATURES AND LIGHT INTENSITIES

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Abstract: The adaptive capacity of microalgae to varying temperature is of key importance for their mass outdoor cultivation because daily and seasonal temperature fluctuations are significant. Our aim was to study the influence of temperature and light intensity on the growth and cellular metabolites of a newly isolated *Coelastrella* sp. strain BGV. The alga was cultivated at wide temperature range (20 – 44ºC) and two light intensities (unilateral – 8000 lux and bilateral illumination – 2 x 8000 lux). Optimal temperature for algal growth was 25ºC and 35ºC at low and high light intensity, respectively. Regardless of the illumination, the extreme temperatures (40, 42 and 44ºC) were a stress factor and led to algal growth retardation. *Coelastrella* sp. BGV required lower light intensity for biomass accumulation (7.7 $g.l^{-1}$ dry weight at 25ºC) and temperatures above 35ºC for enhanced protein synthesis. Carbohydrates reached their maximum at 30ºC – 50.7% and 45.7% of DW. Lipids were the most affected by the temperature and light intensity metabolites. They varied from 7.3% to 40% of DW at unilateral illumination and from 10,3% to 37% for bilateral illumination. According to gas-chromatographic analysis the main fatty acids were myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), alpha-linolenic (α-18:3) and their proportion clearly varied according to different growth conditions.

Carotenoid production increased with the raising of temperature. *Coelastrella* sp. BGV showed high productivity and preserved good qualitative composition of the biomass in the range 20-35°C. The strain could be recommended as a promising for intensive mass outdoor cultivation.

INTRODUCTION

The microalgae from genus *Coelastrella* are one of the known species with prospects to be utilized in microalgal biotechnology. The genus was first described by Chodat (1922), but only during the past few years scientists began studying its biochemical characteristics. So far it is known that *Coelastrella* are able to produce carotenoids, both primary (essential for the photosynthetic apparatus) and secondary, after exposure to specific stimuli (Abe et al., 2007). Known *Coelastrella* carotenoids include β-carotene, lutein, free astaxanthin, canthaxanthin and phytofluene (Iyer et al., 2015). The importance of carotenoids is highlighted by the fact that they are natural antioxidants which makes them crucial for the protection against oxidative stress (Guedes et al., 2011). *Coelastrella* algae are also able to accumulate 15-16% total lipids, which could be enhanced to 24% via nitrogen starvation or 37% via addition of 3% NaCl to the media (Karpagam et al., 2015). Abe et al. (2007) note that *Coelastrella* is able to produce important fatty acids like C16:0, C18:1, C18:2, α-18:3. These facts could highlight the fact that *Coelastrella* are potential algae to be implemented in mass cultivation for the needs of nutrition, forage, pharmaceutics and cosmetics.

For the needs of mass cultivation in Bulgaria, it is important to work and characterize locally isolated strains of microalgae, like the strain *Coelastrella* BGV which is the topic of this manuscript. The source of the microalgal probe was a metal tub found in the village Varvara, Bulgaria, with a sample taken by Prof. Georgi Petkov (Dr.Sc), for which we are thankful to him. The isolation was conducted by streaking cells across agar plates according to a method described by Andersen and Kawachi (2005). Identification of the strain as being of the genus Coelastrella was done by Associate Professor Blagoy Uzunov (PhD) from Sofia University "St. Kliment Ohridski", and we are grateful to him for that. The strain-containing cultures are being held in solid and liquid form at the algal collection of the Laboratory of Experimental Algology, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences.

MATERIALS AND METHODS

Monoalgal cultures of *Coelastrella* sp. strain BGV were grown autotrophically on a block for intense laboratory cultivation at set temperatures (20 – 44ºC) at two light intensities: unilateral – 8000 lux (132 μmol m^{-2} s^{-1}), marked as LL, low light and bilateral illumination – 2 x 8000 lux (2 x 132 μmol m^{-2} s^{-1}), marked as HL,

high light. The carbon source was provided by bubbling with 2% enriched with CO_2 air (v/v). The cultivation was conducted for 96 h. The growth was estimated daily by gravimetrical analysis of dried algal biomass. Biochemical analysis was conducted at the end of the experiment.

Pigment content was determined according to McKinney (1941). Protein content was determined according to the method of Lowry (1952). Carbohydrate content was estimated according to the method of Dubois et al. (1956). Total lipids were measured as described by Petkov (1990) after the biomass was treated with a mixture of chloroform: methanol (2:1, v/v). The total lipids were subsequently converted to fatty acid methyl esters (FAME) by heating in methanol containing 6% m/m anhydrous HCl at 60 °C for 1.5 h. The FAME were extracted with hexane and purified by TLC on silica gel with hexane – diethyl ether (10:1 v/v). Gas chromatography of FAME was carried out on a column 2.5% SE52/10% DEGS at 195 °C on a Perkin-Elmer gas chromatograph with flame ionization detector.

RESULTS AND DISCUSSION

The growth of *Coelastrella* sp. strain BGV with bilateral and unilateral illumination is shown respectively on Fig 1 and Fig 2. The experiments clearly show that regardless of the illumination, the growth of the alga stops completely when cultivated at 40, 42, 44° C. Growth is possible in the range of 20-35ºC which confirms that *Coelastrella* sp. strain BGV is highly adaptable and thus is suitable for outdoor cultivation. The optimal temperature of growth at unilateral illumination is estimated to be 25ºC, while at bilateral illumination is shown to be 35ºC.

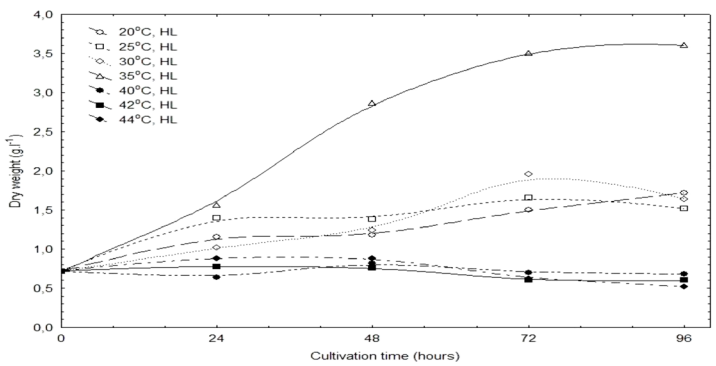

Fig 1. Cultivation of *Coelastrella* sp. strain BGV at different temperatures and 2x 8 000 lux bilateral illumination (HL – high light).

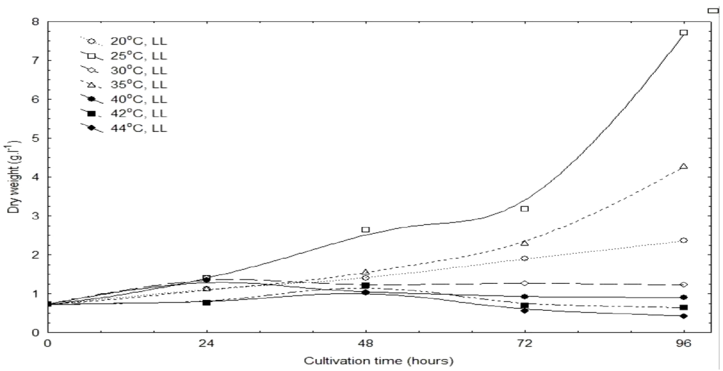

Fig 2. Cultivation of *Coelastrella* sp. strain BGV at different temperatures and 8 000 lux unilateral illumination (LL – low light)

Changes in the biochemical composition are detected and shown on Table 1.

Table 1. Biochemical changes (metabolite content presented as % of dry weight) in the cells of *Coelastrella* sp. strain BGV with regards to illumination and temperature.

Temperature, °C	Proteins, %	Carbohydrates, %	Lipids, %	Chl *a*, %	Chl *b*, %	Carotenoids, %
2 x 8000 lx						
20°C	29, 8	39, 1	25, 5	1.3 ± 0.01	0.5 ± 0.01	0.5 ± 0.01
25°C	24, 1	29, 8	37, 0	0.9 ± 0.04	0.4 ± 0.06	0.3 ± 0.01
30°C	18, 3	50, 7	25, 0	1.2 ± 0.0	0.5 ± 0.01	0.5 ± 0.01
35°C	34, 7	28, 7	18, 0	0.9 ± 0.0	0.3 ± 0.01	0.3 ± 0.01
40°C	54, 8	28, 3	10, 3	-	-	-
42°C	38, 5	35, 0	21, 0	-	-	-
44°C	29, 9	11, 8	23, 5	-	-	-
8000 lx						
20°C	28, 2	31, 8	27, 1	1.8 ± 0.1	0.6 ± 0.05	0.6 ± 0.03
25°C	27, 6	28, 9	16, 9	3.1 ± 0.9	1.1 ± 0.3	0.7 ± 0.2
30°C	24, 4	45, 7	21, 3	2.0 ± 0.07	0.9 ± 0.03	0.8 ± 0.03
35°C	26, 0	24, 8	7, 3	2.0 ± 1.2	0.9 ± 0.6	0.7 ± 0.4
40°C	57, 9	16, 1	8, 2	-	-	-
42°C	46, 2	15, 9	14, 3	-	-	-
44°C	20, 5	20, 4	40, 0	-	-	-

We have estimated that at 40ºC the protein content reaches the highest level. At this temperature and bilateral illumination the content is 54%. If the illumination is unilateral, the protein content is 57%, a record high for this strain.

The temperature which leads to production of the highest carbohydrate content is estimated to be 30˚C both for bilateral (50.7%) and unilateral (45.7%) illumination, while extreme temperatures lead to a significant decrease of carbohydrates – at 44ºC the content is 11.8% for bilateral illumination and at 42ºC it is 15.9% for unilateral illumination – these are so far the lowest numbers recorded.

Chlorophyll and carotenoids changes for bilateral illumination are inconclusive. There are some notable and measurable differences for unilateral illumination. The overall pigment content for 20ºC is noted to be the lowest. The peak of chlorophyll *a* and *b* concentration is at 25ºC, after which it begins to decrease. At the three temperature extremes (40, 42 and 44ºC) the pigment content cannot be measured – macroscopically the cultures look white.

The lipid content varies in a wide range. For bilateral illumination we have measured the highest lipid content (37%) at 25ºC, while the lowest content is 10.3% at 40ºC. Unexpectedly, unilateral illumination at 40ºC produces a very high lipid content (40%). More experiments may be needed to estimate why the total lipid content varies so much and whether this is a stable trend.

It is noteworthy that at the optimal temperatures (25ºC, LL and 35ºC, HL) regardless of light intensity carbohydrate and lipid contents are almost the same (Table 1). The bilateral illumination stimulates protein production and unilateral stimulates the pigment synthesis.

The protein content in the cells of *Coelastrella* sp. BGV at optimal temperatures of growth (34.7% and 27.6% of DW, Table 1) is lower in comparison with that detected for *Chlorella vulgaris* R-06/2 (39 – 53%) and a mass outdoor cultivated strain *Scenedesmus incrassatulus* R-83 (40.8 – 52.5%) (Gacheva & Pilarski, 2008; Livansky et al., 1995) but carbohydrates and lipids are produced to higher extent in *Coelastrella* sp. (Table 1). Our results for accumulation of chlorophylls and carotenoids in *Coelastrella* sp. BGV are comparable with the data for the green microalgae *Haematococcus pluvialis* (Shah et al., 2016) and previously discussed *Chlorella* and *Scenedesmus* strains.

According to gas-chromatographic analysis the main fatty acids of *Coelastrella* sp. BGV are myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), alpha-linolenic (α-18:3) (data shown on Table 2). Our results correspond with these determined by Abe et al. (2007). An unidentified so far fatty acid has been also detected. According to our preliminary calculations we presume this could be a fatty acid with 16 carbon atoms and more than one double bond (probably 16:2 or 16:3). Further analysis on the fatty acid composition of this alga will give more detail information.

Table 2. Fatty acid composition and content (%, m/m) of *Coelastrella* sp. BGV, cultivated at different temperatures and light intensities.

Fatty acid	Cultivation conditions					
	20°C		25°C		30°C	35°C
	8 klx	2 x 8 klx	8 klx	2 x 8 klx	8 klx	8 klx
14:00	2, 6	1, 5	1, 1	0, 3	1, 8	1, 4
16:00	20, 3	33, 3	26, 7	27, 8	15, 2	53, 5
18:00	tr.	tr.	tr.	tr.	tr.	tr.
18:01	26, 0	29, 2	33, 1	23, 7	1, 4	23, 6
18:02	15, 6	10, 0	13, 0	28, 8	24, 2	18, 2
α-18:3	35, 5	26, 0	26, 1	19, 4	57, 4	3, 3

Fatty acid content is also affected by the cultivation conditions. Myristic and stearic acids have the lowest levels. Palmitic acid varies from 15.2% at 30 °C, 8 000 lx to 53.5% at 35°C (Fig. 3).

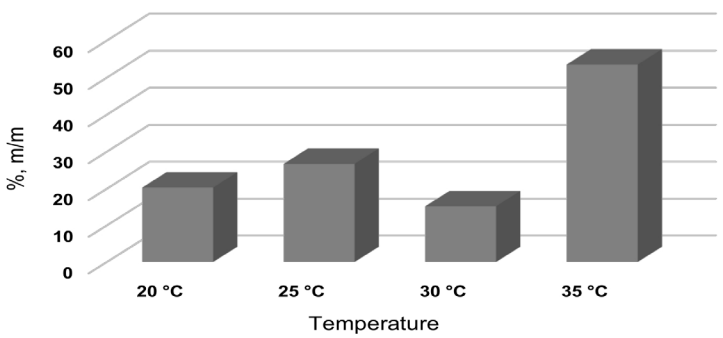

Fig. 3. Palmitic acid content in the cells of *Coelastrella* sp. BGV, cultivated at different temperatures and 8 000 lux unilateral illumination

A significant decrease in oleic acid content is observed at 30°C, unilateral illumination. The quantity of linoleic acid is vastly affected by the light intensity, it increases twice at 25°C, bilateral illumination and decreases at 20°C. Alpha-linolenic acid shows the most prominent change from 3.3% to 57.4% (Fig. 4).

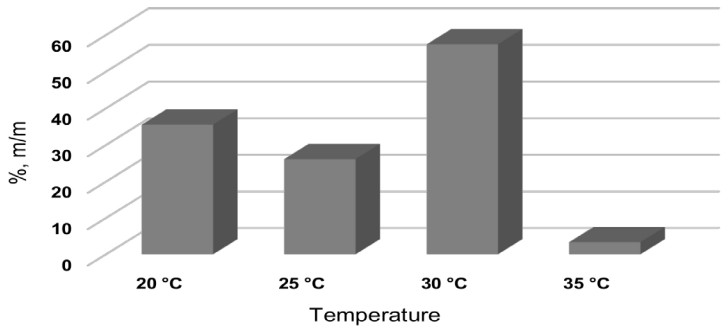

Fig. 4. Alpha-linolenic acid content in the cells of *Coelastrella* sp. BGV, cultivated at different temperatures and 8 000 lux unilateral illumination

The significant change in the content of palmitic, oleic and alpha-linolenic acids in a very small temperature range (30 – 35°C) is worth more extensive research. Given that this is at the optimal temperature range for cultivation, it could be said that these changes are not linked to large extent to a stress factor, but could be considered as a characteristic of the species.

The importance of dietary fats in human health, with emphasis on polyunsaturated fatty acids, is well known (Borowitzka, 1988). The presence of considerable amount of unsaturated linoleic and alpha-linolenic fatty acids in the cells of *Coelastrella* sp. BGV is an advantage of the strain that makes the biomass a valuable dietary supplement as well as a promising food additive for animals.

CONCLUSIONS

Our results show that the extreme temperatures (40, 42 and 44ºC) are able to completely inhibit the growth of *Coelastrella* sp. BGV, but stable growth is achievable in the range of 20 - 35ºC. This shows that the strain *Coelastrella* sp. BGV is adaptable and usable for cultivation in open ponds where the temperature varies.

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REFERENCES

1. Abe, K., Hattori, H., & Hirano, M. 2007. Accumulation and antioxidant activity of secondary carotenoids in the aerial microalga *Coelastrella striolata* var. *multistriata*. *Food chemistry*, 100(2): 656-661.
2. Andersen, R. A., Kawachi, M. 2005. Traditional microalgae isolation techniques. *Algal culturing techniques*: 83: 90-101.
3. Borowitzka, M. A.1988. Microalgae as sources of essential fatty acids. *Australian J. Biotechnol.*, 1: 58-62.
4. Chodat, R. 1922. Matériaux pour l'histoire des algues de la Suisse. *Bulletin de la Société Botanique de Geneve, série 2* 13: 66-114.
5. DuBois, M., K. A. Gilles, J. K. Hamilton, P.A. Rebers & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28 (3): 350–356.
6. Gacheva, G & P. Pilarski. 2008. The resistance of a new strain *Chlorella* sp. R-06/2, isolated from an extreme habitat, to environmental stress factors. *Gen. Appl. Plant Physiol.,* Special Issue 34 (3-4): 347-360.
7. Guedes, A. C., H. M. Amaro & F. X. Malcata. 2011. Microalgae as sources of carotenoids. *Marine drugs*, 9 (4): 625-644.
8. Iyer, G., V. Nagle, Y. V. Gupte, S. Desai, M. Iyer, N. Moramkar & V. Sawant. 2015. Characterization of high carotenoid producing *Coelastrella oocystiformis* and its anti-cancer potential. *Int. J. Curr. Microbiol. App. Sci*, 4(10): 527-536.
9. Karpagam, R., K. J. Raj, B. Ashokkumar & P. Varalakshmi. 2015. Characterization and fatty acid profiling in two fresh water microalgae for biodiesel production: lipid enhancement methods and media optimization using response surface methodology. *Bioresource technology*, 188: 177-184.
10. Livansky, K., M. Kajan & P. Pilarski. 1995. Productivity, respiration and chemical composition of the green alga *Scenedesmus incrassatulus* grown in outdoor cultivation units with and without baffles. Archiv für Hydrobiologie 106 (Algol. studies 76): 111-128.
11. Lowry, O., N. Rosenbrough, A. Z. Farr & R. J.Randball. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265–275.
12. Mackinney, G. 1941. Criteria for purity of chlorophyll preparations. *J. Biol. Chem.*, 132: 91–96.
13. Petkov, G. 1990. Lipids of photoautotrophically cultivated microalgae. Ph. D. Thesis, IPP, BAS,119 pp. (In Bulgarian).
14. Shah, M. M. R., Y. Liang, J. J. Cheng & M. Daroch. 2016. Astaxanthin-producing green microalga *Haematococcus pluvialis*: From single cell to high value commercial products. *Front. Plant Sci.* 7: Article 531.