JOURNAL OF NEW RESULTS IN SCIENCE (JNRS)
ISSN: 1304-7981
http://dergipark.gov.tr/jnrs
Research Article

Volume : 6
Issue: 1
Year: 2017
Pages: 47-52

***Received*:** 08.02.2017
***Accepted*:** 15.08.2017

Editors-in-Chief: ***Ebubekir ALTUNTAS***
Area Editor: ***Bilge Hilal CADİRCİ***

Growth and Metabolite Production of *Chroococcus minutus* Under Different Temperature and Light Conditions

Tunay Karan[a], Zekeriya Altuner[a], Ramazan Erenler[b*]

[a]*Department of Biology, Faculty of Art and Science, Gaziosmanpasa University, 60240 Tokat, Turkey. email: tunay.karan@gop.edu.tr; zekeriya.altuner@gop.edu.tr*
[b]*Department of Chemistry, Faculty of Art and Science, Gaziosmanpasa University, 60240 Tokat, Turkey.*
**Corresponding author email: ramazan.erenler@gop.edu.tr*

ABSTRACT: Cyanobacteria consisting of valuable bioactive compounds reveal the pharmaceutical and biological activities. In this work, the sample was collected from freshwater and was isolated under inverted microscope. Identification was carried out by morphologically. The isolated *Chroococcus minutus* was cultivated in Bristol nutrient medium. Growth and norharmane production of *Chroococcus minutus* under temperature and light conditions were presented. The most norharmane production and the cell numbers were observed at 35 °C and irradiation of 1896 lux as 4.017 µg/g and 154.3 µg/g respectively.

Keywords: *Chroococcus minutus, norharmane, HPLC, temperature, light*

1. Introduction

Cyanobacteria classified as oxygenic photoautotroph are widely distributed in nature and play a significant role in primary production and the global carbon cycle (Arrigo, 2005). Bioactive natural products from cyanobacteria (blue-green algae) have yielded a wealthy of new molecules with many basically new chemotypes and unusual potential for biomedical research and application (Blunt et al., 2003). Cyanobacteria have emerged to overcome extreme environmental conditions including UV exposure, desiccation, variable and high salinity and temperature extremes (Dillon and Castenholz, 1999). Cyanobacteria have developed the defense strategies resulting in a significant level of chemical compounds by different metabolic pathways to survive in a competitive environment (Barros et al., 2005). The exploration of cyanobacteria for pharmaceutical purposes has exhibited the important chemical structures for the discovery of new agents and new syntheses of compounds with the biomedicinal application. In addition, cyanobacteria are promising organisms for providing biologically active substances as well as essential compounds for human nutrition (Tringali, 1997). A wide range of algae which are well known to have high contents of minerals, vitamins, antioxidants and fiber have been consumed by many countries. Recently, cultivation process has been preferred rather than the wild harvest to produce important new products on a large scale. However, toxins yielded by freshwater and marine algae represent an increasing hazard to water supplies, recreational beaches, reservoirs, as well as seafood contamination (Cardozo et al., 2007). Freshwater cyanobacteria are exposed to rapid fluctuations in environmental nutrients concentrations and their adaptation is vital for competition, succession, and dominance. It was reported

that *C. minutus* exhibited a fast and high ability to remove nonylphenol via bioaccumulation and biodegradation (He et al., 2016). A survey was executed in Kizilirmak River (Turkey) and 73 species belonging to Cyanobacteria (25), Chlorophyta (23), Bacillariophyta (14), Pyrrophyta (5), Cryptophyta (3), and Euglenophyta (3), were identified. *C. minutus* was one of the dominant species (Demirkalp et al., 2010). Fatty acid composition was determined in *C. minutus* and 9-Octadecenoic (9-18:1) and 9,12,15-Octadecatrienoic (9,12,15-18:3) were found as the major unsaturated fatty acids (Řezanka et al., 2003). The norharmane concentrations were investigated for some cyanobacteria including *C. minutus* and the results showed that *C. minutus* excreted significant amounts of norharmane (52.18 µg/L)(Volk, 2008).

Herein we presented the growth and norharmane production of *Chroococcus minutus* (Kützing) Nageli under temperature and light conditions.

2. Material and Methods

2.1. Sample collection, identification and cultivation

The sample was collected from Yesilirmak River (40º 17 40.19" N, 36º 19 28.81" E). The identification was executed under the light microscope. *C. minutus* was streaked onto agarised Bristol medium (with 1.5% agar). Incubation was kept for 2 weeks at 26 °C ±2 for 12/12 h (light/dark) (Sanyo, MLR-351). The light intensity was 2465 lux (Karan et al., 2016).

2.2. Experimental Conditions

The different temperature and light conditions were executed at 15 °C and 35 °C at the irradiation of 1896 lux and 4300 lux (Sanyo MIR-253). The experiment was carried out at four parameters. 25 ml stock culture was transferred into the 250 ml Erlenmeyer flask for incubation for two weeks. The trial was executed at triplicate (Kuhne et al., 2013).

2.3. Cell counting

Cell number was counted with a hemacytometer. Culture samples (15 ml) were centrifuged at 5000 × g for 10 min. After washing the pellets with distilled water (pH 4), they were dried at 50 °C for 6 h, and then weighed (Pelah et al., 2004).

2.4. Norharmane Analysis

The methanol extract of *C. minutus* was used for HPLC analysis. The detection of norharmane was determined by HPLC using the C18 120 A reverse phase column (4.6 × 150 mm, 3 µm particle size). The flow rate was adjusted to 1 ml/min (Volk, 2008).

Statistical analyses

All experiments were executed with three times. The statistical analysis was carried out by ANOVA and using the SPSS software (SPSS Inc., version 20).

3. Results and Discussion

3.1. Morphological identification

Morphological features of *C. minutus* were ovoid or spherical. They were observed as singular or as a cluster of 2 to 4 cells (Figure 1). Diameters of large enveloped cells were measured as 6 – 15 µm, and of non-enveloped cells were measured as 4 – 10 µm with colorless (John et al., 2002).

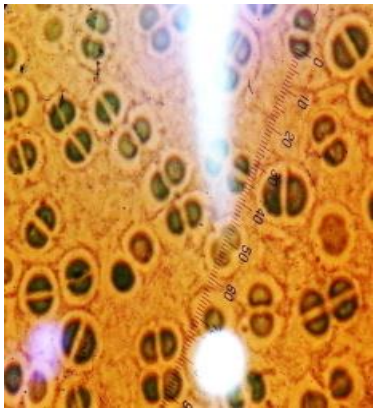

Figure 1. Imagine of *C. minutus* under light microscope

15 °C and irradiation of 1896 lux, 15 °C and irradiation of 4300 lux, 35 °C and irradiation of 1896 lux, 35 °C and irradiation of 4300 lux were applied for temperature and light conditions. 26 °C and 2465 lux irradiation was used as a control stock culture. Cell number at this condition was 124×10^4 cells/ml and norharmane production was 8.82 µg/g (Karan et al., 2016). HPLC chromatogram was given at Figure 2.

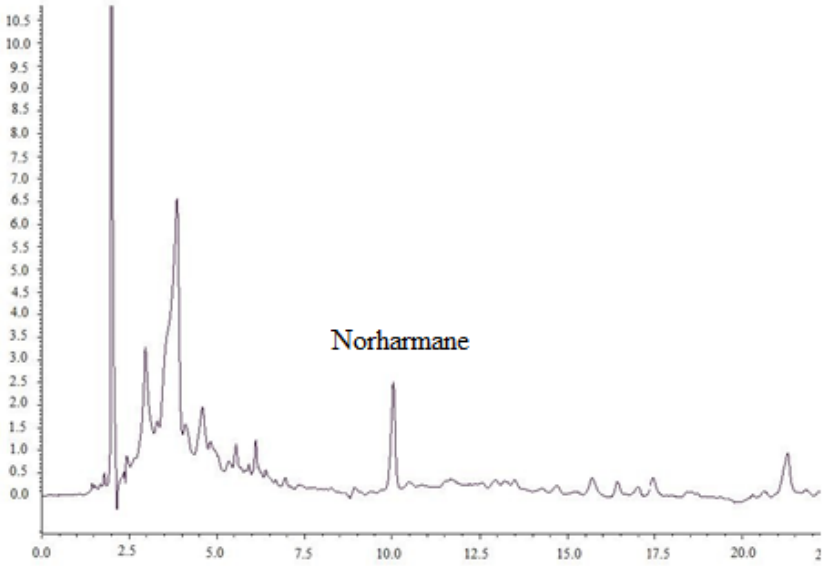

Figure 2. HPLC chromatogram of *C. minutus* methanol extract

Amounts of norharmane (µg/g) were calculated according to the Gauss method by drawing a calibration curve over the absorbance value in the 247 nm wavelength of the standard. Based on the results, the amount of norharmane at 15 °C and 1896 lux, 15 °C and 4300 lux, 35 °C and 1896 lux, 35 °C and 4300 lux were 0.107, 0.040, 4.017, 0.55 µg/g, respectively (Table 1). The cell numbers were 64.00, 74.667, 154.33, 98.33 respectively (Table 2). The statistical evaluation revealed the $p<0.05$ indicating the significant difference of mean and $p<0.01$ showing the highly significant difference of mean.

Table 1. Norharmane production of *C. minutus* at different temperature and light conditions (µg/g).

Cyanobacteria	Temp.	Irradiation						
		1896 lux		4300 lux		Total		Sig. (p)
		mean	sd	mean	sd	mean	sd	
C. minutus	15 °C	0.107	0.006	0.040	0.002	0.074	0.037	0.0001
	35 °C	4.017	0.001	0.554	0.003	2.286	1.897	0.0001
	Total	2.062	2.142	0.297	0.282	1.180	1.723	0.0998
	Sig. (p)	0.0001		0.0001		0.0355		

The most norharmane production was observed at the 35 °C and 1896 lux irradiation as a 4.017 µg/g.

Table 2. Cell numbers of *C. minutus* under the temperature and light conditions (µg/g).

Cyanobacteria	Temp.	Irradiation						
		1896 lux		4300 lux		Total		Sig. (p)
		mean	sd	mean	sd	mean	sd	
C. minutus	15 ºC	64.000	2.646	74.667	3.055	69.333	6.377	0.0103
	35 ºC	154.333	2.517	98.333	6.506	126.333	30.988	0.0002
	Total	109.167	49.531	86.500	13.737	97.833	36.620	0.3232
	Sig. (p)	0.0001		0.0047		0.0057		

The most cell numbers were observed at the 35 °C and irradiation of 1896 lux as a 154.3 µg/g. The growth at 35 °C was rather higher than that of the 15 °C. The suitable temperature for toxin production of most cyanobacteria haven been reported as 20-25 °C (Codd and Poon, 1988). However, increased temperature stimulated the toxin/antifungal

enzyme production in some cyanobacteria (Radhakrishnan et al., 2009). Cyanobacteria are dominant to the phytoplankton. The optimum temperature of *Anabaena*, *Aphanizomenon*, *Microcystis* and *Oscillatoria* was determined to be 25 °C (Robarts and Zohary, 1987). The temperature effect on growth of *Aphanizomenon flos*-aquae was determined between 23 °C and 29 °C (Tsujimura et al., 2001). *Spirulina platensis* tolerated the temperature between 20 °C and 40 °C and the best growth temperature was 23±2 °C. The growth continued until the temperature approach to 50 °C (Muruga et al., 2014). The light plays a significant role in cultivation of algae and cyanobacteria. The light is the energy source used in photosynthesis to convert the nutrient into the algal biomass. The light also affects the photosynthesis rate. While the light intensity increases, photosynthesis rate also increases but after a certain point of light intensity, it inhibits the photosynthesis (Muller-Feuga et al., 2003). We also presented the optimum temperature of *C. minutus* and it revealed the same trend with the literature as cited.

4. Conclusion

The cyanobacteria *Chroococcus minutus* was isolated, identified and cultivated. The highest growth and norharmane production were depicted at various temperature and light conditions. The most norharmane production and the highest cell numbers were determined at higher temperature (35 °C) and 1896 lux irradiation as 4.017 µg/g and 154.3 µg/g respectively. Therefore, most efficient growth condition and metabolite production of *Chroococcus minutus* were affirmed. *C. minutus* could be a significant source of norharmane. Norharmane and other secondary metabolites should be isolated to determine the fully phytochemical properties of *C. minutus*.

Acknowledgement

The authors acknowledge the financial assistance provided by Gaziosmanpasa University Research Council (No: 2013/128).

References

Arrigo, K.R., 2005. Marine microorganisms and global nutrient cycles. *Nature,* 437: 349-355.

Barros, M.P., Pinto, E., Sigaud-Kutner, T.C.S., Cardozo, K.H.M., Colepicolo, P., 2005. Rhythmicity and oxidative/nitrosative stress in algae. *Biol Rhythm Res,* 36: 67-82.

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T., Prinsep, M.R., 2003. Marine natural products. *Nat Prod Rep*, 20: 1-48.

Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcao, V.R., Tonon, A.P., et al, 2007. Metabolites from algae with economical impact. *Comp Biochem Phys C,* 146: 60-78.

Codd, G.A., Poon, G.K. 1988. Cyanobacterial toxins. Proceedings of the phytochemistry soci-ety of Europe. In *Biochemistry of the algae and cyanobacteria*, ed. Rogers, J., Gallon, J.R., Oxford, Oxford University Press, pp. 283-296.

Demirkalp, F.Y., Saygi, Y., Caglar, S.S., Gunduz, E., Kilinc, S., 2010. Limnological Assesment on the Brakish Shallow Liman Lake from Kizilirmak Delta (Turkey). *J Anim Vet Adv,* 9: 2132-2139.

Dillon, J.G., Castenholz, R.W., 1999. Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *J Phycol,* 35: 673-681.

He, N., Sun, X., Zhong, Y., Sun, K., Liu, W., Duan, S., 2016. Removal and Biodegradation of Nonylphenol by Four Freshwater Microalgae. *International Journal of Environmental Research and Public Health,* 13: 1239.

John, D.M., Whitton, B.A., Brook, A.J. 2002. *The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae*. Cambridge University Press, UK, :pp. 40-41.

Karan, T., Dastan, T., Baral, I., Altuner, Z., 2016. Effects of Differential Time Applications on Some Cyanobacterial Norharman Production Rates. *Cumhuriyet Uni Facul Sci*, 37: 398-404.

Kuhne, S., Lakatos, M., Foltz, S., Muffler, K., Ulber, R., 2013. Characterization of terrestrial cyanobacteria to increase process efficiency in low energy consuming production processes. *Sustain Chem Process*: 1-6.

Muller-Feuga, A., Moal, J., Kaas, R. 2003. The Microalgae of Aquaculture. In *Live Feeds in Marine Aquaculture*, ed. Støttrup, J.G., McEvoy, L.A., Blackwell Publishing, Oxford, UK, pp. 206-252.

Muruga, B.N., Wagacha, J.M., Kabaru, J.M., Amugune, N., Duboise, S.M., 2014. Effect of physicochemical conditions on growth rates of cyanobacteria species isolated from Lake Magadi, a soda lake in Kenya. *J Sci Res*, 2: 41-50.

Pelah, D., Sintov, A., Cohen, E., 2004. The effect of salt stress on the production of canthaxanthin and astaxanthin by Chlorella zofingiensis grown under limited light intensity. *World J Microb Biot*, 20: 483-486.

Radhakrishnan, B., Prasanna, R., Jaiswal, P., Nayak, S., Dureja, P., 2009. Modulation of biocidal activity of Calothrix sp. and Anabaena sp. by environmental factors. *Biologia*, 64: 881-889.

Řezanka, T., Dor, I., Prell, A., Dembitsky, V., 2003. Fatty acid composition of six freshwater wild cyanobacterial species. *Folia microbiologica*, 48: 71-75.

Robarts, R.D., Zohary, T., 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N Z J Mar Freshwater Res*, 21: 391-399.

Tringali, C., 1997. Bioactive metabolites from marine algae: Recent results. *Curr Org Chem*, 1: 375-394.

Tsujimura, S., Ishikawa, K., Tsukada, H., 2001. Effect of temperature on growth of the cyanobacterium Aphanizomenon flos-aquae in Lake Biwa and Lake Yogo. *Phycological Res*, 49: 275-280.

Volk, R.B., 2008. Screening of microalgae for species excreting norharmane, a manifold biologically active indole alkaloid. *Microbiol Res*, 163: 307-313.