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Production and Purity of Phycobiliproteins from Selected Marine and Freshwater Cyanobacteria Subjected to Different Drying Methods

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Abstract

Phycobiliproteins, light-harvesting pigments found in cyanobacteria, red algae and cryptomonad are gaining importance in food, nutraceutical and pharmaceutical industries. Thus, a sustainable source of phycobiliproteins production is an essential consideration in meeting the increasing demand for these natural pigments. The present work aimed to compare the concentration and purity of phycobiliproteins from marine (*Geitlerinema* sp. and *Synechococcus* sp.) and freshwater (*Oscillatoria* sp. and *Spirulina* sp.) cyanobacteria, when subjected to different drying methods viz. sun-drying, oven-drying and freeze-drying. Results showed that the three different drying methods influenced the concentration (mg.mL⁻¹) was significantly higher (P < 0.05) in marine *Geitlerinema* sp. followed by *Oscillatoria* sp. *Synechococcus* sp. and *Spirulina* sp., respectively, compared to sun-drying and freeze-drying methods. Phycoerythrin and allophycocyanin concentrations were also significantly higher (P < 0.05) in marine *geitlerinema* sp. when compared to other cyanobacteria subjected to oven drying. In addition, results from oven-dried marine periphytic *Geitlerinema* sp. showed that total phycobiliproteins production and purity ratio of phycocyanin were significantly higher (P < 0.05) in comparison to sun-drying or freeze-drying *Spirulina* sp., *Synechococcus* sp. and freshwater *Oscillatoria* sp.

Keywords: cyanobacteria, phycobiliproteins, phycocyanin, phycoerythrin, drying conditions

Introduction

Cyanobacteria and algae contain valuable pigments such as carotenoids and phycobiliproteins, in addition, they are the immense sources of several metabolites such as alkaloids, carbohydrates, flavonoids, pigments, phenols, saponins, steroids, tannins, terpenes, and vitamins which can be utilised in biotechnology and industrial fields (Guihéneuf et al., 2016). The potential use of cyanobacteria in agriculture, aquaculture, nutraceuticals, bioenergy and pollution control (bioremediation) is well recognised (Abed et al., 2009). The unique spectral features such as strong absorbance and fluorescence, proteinaceous nature and, some imperative properties like hepato-protective, antioxidants, antiinflammatory and anti-ageing activity of phycobiliproteins enable their use in foods, cosmetics, pharmaceutical and biomedical industries (Sonani et al., 2016). Moreover, phycobiliproteins are used as colouring agents in cosmetics, dairy products, ice creams, jellies, diagnostics, biomedical research and oxidative stress-induced diseases as they are proteinaceous and possess uniaue colour, fluorescence and antioxidant properties (Pandey, 2013). Phycocyanin from Spiruling plays an important role in inducing apoptosis on HeLa cells (Li et al., 2009), enhancing wound healing (Madhyastha et al., 2008), retardation of platelet aggregation (Chiu et al., 2006) and eradication of cancer cells in vitro (Li et al., 2010).

Phycobiliproteins in cyanobacteria contribute 50 % of the total cellular proteins, and these include phycocyanin (blue pigment), phycoerythrin (red pigment) and allophycocyanin (Madhyastha et al., 2008) based on inherent colour and absorbance properties. Phycocyanin is an accessory piqment of cyanobacteria, but not all cyanobacteria are bluish due to the presence of phycoerythrin which gives them a red or pink colouration. Phycocyanin (blue) and phycoerythrin (red) are the two major natural pigments commercially used from cyanobacteria.

Cyanobacteria are a potential source for the commercial production of phycocyanin. In cyanobacteria, a significant proportion of the total protein is contributed by the phycobiliproteins, which are located in granules attached to the photosynthetic membrane (Santiago-Santos et al., 2004). They can use light spectra between absorption peaks of chlorophyll a and carotenoids with the help of accessory pigments (phycocyanin, phycoerythrin and allophycocyanin) (Santiago-Santos et al., 2004). The contents of pigments depend on the species and cultivation conditions (Begum et al., 2016). Currently, phycocyanin and phycoerythrin have been extracted from Spirulina sp. (Hemlata, 2009), Calothrix 7601 (Prasanna et al., 2004) and marine cyanobacterium Porphyridium cruentum (Roman et al., 2002).

The drying of cyanobacterial biomass before extraction of phycobiliproteins is an important step as bacterial degradation of the wet biomass could affect the pigment production. In general, drying methods can reduce the quantity and purity of phycocyanin. Thus, a method that could preserve the original material, but minimise thermal damages such as freeze-drying is preferred. During the freeze-drying process, water is removed via vacuum pumping from the material while it is frozen (Nireesha et al., 2013).

Processing of cyanobacterial biomass by sun-drying is perhaps the cheapest drying method engaged (Prakash et al., 1997). However, sun-drying technique is a lengthy process and requires large drying surface. In addition, there is a risk of loss of some bio-reactive products. Low-pressure shelf drying is another lowcost drying technology that has been investigated (Prakash et al., 1997) but has low efficiency. Sarada et al. (1999) reported 50 % loss of phycocyanin when Spiruling was dried using spray or convective dryers. Drum-drying (Prakash et al., 1997), spray-drying (Desmorieux and Decaen, 2006), fluidised bed drying (Leach et al., 1998), freeze-drying (Millamena et al., 1990). and refractance window dehydration technologies are commonly used as drying methods which are efficient but costly.

There is great market demand for phycobiliproteins at present, but the scarcity of information in terms of species selection for high-quality pigment and drying methods of algal biomass hampers its commercial production. Therefore, this study aimed to compare the production and purity of phycobiliproteins from selected marine and freshwater cyanobacteria using different drying methods.

Materials and Methods

Cyanobacteria culture conditions

Two marine (Geitlerinema sp., Synechococcus sp.) and two freshwater (Oscillatoria sp., Spirulina sp.) cyanobacteria were used in this study to determine whether there are differences between marine and freshwater algae in terms of phycobiliproteins contents. All the cyanobacteria were isolated from Malaysian water bodies, and pure cultures were maintained in Microalgae Culture Unit, Universiti Putra Malaysia. Marine periphytic Geitlerinema sp. and Synechococcus sp. were cultured in Conway medium (Tompkins et al., 1995), whereas freshwater Oscillatoria sp. was cultured in Bold's basal medium (Nichols and Bold, 1965) and Spirulina sp. in Zarrouk's medium (Aiba and Ogawa, 1977). All the species were cultured in 20 L glass tanks, aerated and kept under a shade with an average light intensity of 140 µmol photons m⁻² s⁻¹. All treatments were carried out in triplicates. The biomass of cultured species was calculated according to Lavens and Sorgeloos (1996). In addition, the specific growth rate (μ day⁻¹) of the cultured species were calculated according to Clesceri et al. (1989).

Identification of cyanobacteria

All four isolates of marine and freshwater cyanobacteria were identified using the conventional method. Isolates were identified to generic level (Bellinger, 1992). The isolate which showed the highest production and purity was confirmed by molecular identification. Molecular identification was done according to Nübel et al. (1997) with minor modifications.

Harvesting

The cultures were harvested at their stationary phase by centrifugation at 6,000 ×g for 15 min. The cell pellets were washed three times with distilled water.

Drying and extraction methods

The harvested biomass of different cyanobacteria was subjected to different drying methods such as oven-drying (40 °C), freeze-drying and sun-drying. Harvested biomass was dried overnight in the oven according to Sarada et al., (1999). Samples were dried in freeze dryer until it was fully dry. In case of sundrying, biomass was kept outside under shade until dry. The dried powders were then ground using mortar and pestle and sieved (120 μ mesh). Dried powder (40 mg) was then soaked in 10 mL phosphate buffer (0.1 M; pH 7.0) and vortexed to mix and stored at 4 °C for 24 h for phycobiliproteins extraction. The

the phycobiliproteins containing supernatant was centrifuged at 6000 rpm for 10 min. The supernatant was collected and absorbance was read at different wavelengths (562, 615 and 652 nm) using phosphate buffer as blank. All experiments of drying and extraction were carried out in triplicates.

Spectrophotometric estimation of phycobiliproteins

The amount of phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) in the sample was calculated using simultaneous equations (Bennett and Bogorad, 1973) and the extinction coefficients (Siegelman and Kycia, 1978) as follows:

Phycocyanin (PC) mg.mL⁻¹ = $\{A615 - (0.474 \times A652)\} / 5.34$

Allophycocyanin (APC) mg.mL⁻¹ = $\{A652 - (0.208 \times A615)\} / 5.09$

Phycoerythrin (PE) mg.mL⁻¹ = $\{A562 - (2.41 \times PC) - (0.849 \times APC)\} / 9.62$

Total phycocyanin, phycoerythrin and allophycocyanin (mg.g⁻¹) were calculated according to Silveira et al. (2007):

Pigment concentration \times V/ DB

where, V = solvent volume and DB = dried biomass.

Purification factor

Phycocyanin, phycoerythrin and allophycocyanin were extracted and the purity was monitored spectrophotometrically by the A620/A280, A565/A280 and A650/A280 ratio (Bennett and Bogorad, 1973).

Statistical analysis

Data were analysed using both one way and two-way ANOVA. Duncan multiple range test at P < 0.05 level of probability was used to determine significant differences among different cyanobacteria and drying methods. All the data which were expressed in percentages were arcsine-transformed to satisfy the condition of homogeneity of variance (Zar, 1984). Statistical analyses were accomplished using the statistical analysis system (SAS, 2002) computer software.

Results

All the four isolates were identified according to their morphological feature and confirmed as *Geitlerinema*

sp., Synechococcus sp., Oscillatoria sp. and Spirulina sp. Geitlerinema sp., which showed maximum production and purity of phycobiliproteins and were further confirmed by molecular identification (Figs. 1, 2). Based on BLAST comparison, the sequence from the isolated marine periphytic cyanobacteria possessed 99 % sequence identity to reference *Geitlerinema* sp. from NCBI (National Center for Biotechnology Information) GenBank (Accession number: HQ197684).



Fig. 1. Gel electrophoresis of PCR product amplified using 16S rRNA forward and reverse primers. Lane 1 represents the 100bp DNA ladder (Fermentas) and Lane 2 PCR product of marine periphytic cyanobacteria.

The growth performance of experimental cyanobacteria species cultured in 20 L glass tanks showed that *Spirulina* sp. had the lowest biomass (P < 0.05) compared to the others (Table 1). Its specific growth rate was also the lowest, but not significantly different from *Synechococcus* sp.

Marine (*Geitlerinema* sp. and *Synechococcus* sp.) and freshwater cyanobacteria (*Oscillatoria* sp. and *Spirulina* sp.) were subjected to different drying methods (sun-drying, oven-drying and freeze-drying) and screened to compare the concentration and purity of phycobiliproteins (PC, PE and APC). Results showed that biomass dried in the oven under controlled temperature had significantly (P < 0.05) higher concentration (mg.mL⁻¹ of culture volume) of PC, PE and APC in marine *Geitlerinema* sp. (0.55 ± 0.1; 0.08 ± 0.2; 0.20 ± 0.1) compared to sun-dried (0.34 ± 0.0; 0.04 ± 0.2; 0.14 ± 0.1) and freeze-dried (0.20 ± 0.1; 0.06 ± 0.1; 0.10 ± 0.2) biomass (Figs. 3a, 3b, 3c).

In case of total phycobiliproteins production under oven drying method, marine *Geitlerinema* sp. (208.1 \pm 3.14 mg.g⁻¹) showed significantly higher content (*P* < 0.05) followed by freshwater *Oscillatoria* sp. (182.0 \pm 1.0 mg.g⁻¹), *Spirulina* sp. (116.1 \pm 1.3 mg.g⁻¹) and *Synechococcus* sp. (141.5 \pm 2.2 mg.g⁻¹) (Table 2).

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Sbjct	127	GCTGCTAATACCCCATA	ATGCCGAAAGGTGAAAA	GAAATTTGCCTTGAGAG	GGCTCGCGT	186	
Query	120	CCGAT	TAGCTAGTTGO	TGAGGTAAGAGCTTACC	AAGGCGACGATCGGTAG	CTGGTCTGAG	179
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Ouerv	180	AGGAT	GAGCAGCCACZ	CTGGGACTGAGACACGG	CCCAGACTCCTACGGGA	GGCAGCAGTG	239
Sbict	247	AGGAT	GAGCAGCCACA		CCCAGACTCCTACGGGA	GGCAGCAGTG	306
Ouerv	240	GGGAA	TTTTCCGCAAT	GGGCGAAAGCCTGACGG	AGCAACGCCGCGTGGGG	GAAGAAGGCC	299
Sbict	307	GGGAA	TTTTCCGCAAT	GGGCGAAAGCCTGACGG	AGCAACGCCGCGTGGGG	GAAGAAGGCC	366
Ouerv	300	TTTGG	GTTGTAAACTO	CTTTTCTCAGGGAAGAA	GAACTGACGGTACCTGA	GGAATCAGCC	359
Sbjct	367	11111 TTTGG	GTTGTAAACTO	CTTTTCTCAGGGAAGAA	GAACTGACGGTACCTGA	GGAATCAGCC	426
Query	360	TCGGC	TAACTCCGTGC	CAGCAGCCGCGGTAATA	CGGAGGAGGCAAGCGTT	ATCCGGAATT	419
Sbjct	427	TCGGCTAACTCCGTGCC		CAGCAGCCGCGGTAATA	CGGAGGAGGCAAGCGTT	111111111 ATCCGGAATT	486
Query	420	ATTGG	GCGTAAAGCGI	TCGTAGGCGGCGTTTCA	AGTCTGCTGTCAAAGGC	CGAGGCTCAA	479
Sbjct	487	ATTGG	GCGTAAAGCG1	TCGTAGGCGGCGTTTCA	AGTCTGCTGTCAAAGGC	CGAGGCTCAA	546
Query	480	CTTCG	GAAAGGCAGTO	GAAACTGAAAAGCTAGA	GGTCGGTAGGGGCAGAG	GGAATTCCCA	539
Sbjct	547	CTTCG	GAAAGGCAGTO	GAAACTGAAAAGCTAGA	GGTCGGTAGGGGCAGAG	GGAATTCCCA	606
Query	540	GTGTA	GCGGTGAAATG	CGTAGATATTGGGAAGA	ACACCGGTGGCGAAAGC	GCTCTGCTGG	599
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Sbjct	667	GCCGAACCTGACGCTGAGGGACGAAAGCTAGGGGAGCGAATGGGATTAGATACCCC-		IIIII III TACCCC-AGT	725		
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	726	1111 NGTC	720				

Fig. 2. Partial nucleotide sequence of the 16S rRNA gene from marine periphytic cyanobacteria aligned with *Geitlerinema* sp. (Accession number: HQ197684) partial sequence available in NCBI GenBank database.

Table 1. Mean values (\pm standard error of the means, n = 3) of biomass and specific growth rates (SGR) of experimental cyanobacteria species. Mean values in columns with different superscripts are significantly different at P < 0.05.

Species	Biomass(g.L-1)	SGR(µ.day⁻¹)
Geitlerinema sp.	0.88 ± 0.91ª	0.45 ± 0.01ª
Synechococcus sp.	0.78 ± 0.31ª	0.35 ± 0.03^{b}
Oscillatoria sp.	0.88 ± 0.11^{a}	0.45 ± 0.01ª
Spirulina sp.	0.48 ± 0.21ª	0.30 ± 0.02^{b}





In addition, the purity ratio of PC, PE and APC were also significantly higher (P < 0.05) under oven drying condition in marine *Geitlerinema* sp. (1.2 ± 0.1; 0.6 ± 0.1; 0.5 ± 0.2) compared to freshwater *Oscillatoria* sp. (0.7 ± 0.2; 0.4 ± 0.2; 0.5 ± 0.3); Spirulina sp. (0.5 ± 0.3; 0.3 ± 0.4; 0.3 ± 0.3) and Synechococcus sp. (0.4 ± 0.2; 0.3 ± 0.2; 0.3 ± 0.2), and also to other drying methods such as sun-drying and freeze-drying (Figs. 4a, 4b, 4c).

Discussion

Phycobiliproteins (PBPs) are a family of accessory light-harvesting macromolecules organised in supramolecular complexes, called phycobilisomes (PBSs) that function as components of the photosynthetic apparatus in cyanobacteria and some eukaryotic algae. Modern research and development in the synthesis and function of PBSs have expanded the potential applications of PBPs in biotechnology, diagnostic, food and medicine (Vinod et al., 2011). They are extensively commercialised for fluorescent application in clinical and immunological analysis. Malaysia is a tropical country and it has an excessive diversity in its cyanobacterial resources that can be exploited commercially. Nevertheless, cyanobacterial phycobiliproteins are limited and need more attention to this aspect. In the United States, the price of phycobiliproteins products range from USD3 to USD25 mg.g⁻¹ for native pigment but they can reach USD1,500 mg.g⁻¹ for certain cross-linked pigments with antibodies or other fluorescent molecules. In the near future, the price is likely to increase by 20 % annually (Sekar and Chandramohan, 2008).

In view of the great demand for phycocyanin at commercial level, it is therefore important to develop a simple drying method to store maximum amount of phycocyanin in the biomass during the drying process. In the present study, different drying methods of selected cyanobacterial biomass were evaluated in order to minimise the loss of phycobiliproteins during the drying process. In addition, the purity ratio of phycobiliproteins

Table 2. Total production of phycobiliproteins (mg.g⁻¹) from *Geitlerinema* sp., freshwater *Oscillatoria* sp., *Spirulina* sp., and *Synechococcus* sp. under different drying methods.

Quanchastarial spanias	Drying methods				
Cyanobacterial species	Sun-drying	Oven-drying	Freeze-drying		
Geitlerinema sp.	121.9 ± 1.1 ^{b**}	208.1±3.1ª*	85.1±1.5°**		
Oscillatoria sp.	135.3 ± 1.9°*	182.0 ± 1.0ª**	148.1±0.8 ^{b*}		
Spirulina sp.	59.8 ± 2.1°***	116.1±1.3ª****	66.8±1.2 ^{b****}		
Synechococcus sp.	58.9 ± 0.7°***	141.5 ± 2.2ª***	80.2 ± 2.5 ^{b***}		

Mean values with superscripts ^{a, b, c} are significantly different from others in row (P < 0.05) and mean values with *, **, *** and **** are significantly different from others in column (P < 0.05).



Fig. 4. Purity ratio of phycocyanin (a), phycoerythrin (b) and allophycocyanin (c) extract from *Geitlerinema* sp., freshwater *Oscillatoria* sp., *Spirulina* sp., and *Synechococcus* sp. under different drying methods.

extracted from the selected cyanobacterial biomass subjected to different drying methods was evaluated.

The first step in many extraction processes is the removal of water from the sample or drying. Drying minimises the microbial growth and deterioration by chemical reactions of the wet biomass and helps to conserve the desirable qualities. As reported by other studies, dried biomass is suitable and efficient for extraction of phycocyanin from Spirulina with high purity ratio. Temperature is an important factor and plays a significant role in the drying process of cyanobacterial biomass and extraction of phycocyanin. Considerable loss of phycocyanin concentration was observed by Sarada and coresearchers (Sarada et al., 1999) when wet biomass was dried between 60-150 °C. This could be due to phycocyanin's peripheral position in phycobilisomes on the thylakoid membrane and attributable to phycobilisomes sensitivity to temperature (Sarada et al., 1999). Phycocyanin is extremely sensitive to factors such as temperature, light, and pH. These factors can lead to excessive loss of phycocyanin (Martelli et al., 2014). High drying temperature (>60 °C) decreased the amount of the phycocyanin extractable from Spirulina platensis (Oliveira et al., 2008). Güroy et al. (2017) reported that biomass drying at 80 °C results in loss of phycocyanin. In the present study, biomass dried in oven under low temperature below 60 °C showed maximum production of phycobiliproteins with high purity ratio of phycocyanin. Drying method under shade by air circulation can be used for largescale extraction of PC (Doke, 2005). In another study by Doke (2005), Spirulina biomass dried at 25 °C under shade by air circulation had maximum phycocyanin (80 mg.g⁻¹) production and relatively high purity ratio. Similarly, in the present study, Geitlerinema sp. biomass dried in oven under low temperature showed maximum production of phycobiliproteins with purity ratio of relatively high phycocyanin, phycoerythrin and allophycocynin in comparison to the other cyanobacterial species. In addition, phycocyanin extracted from oven-dried biomass also showed high purity ratio (1.14) than those reported in the literature for cell extract (0.97, 0.95) (Zhang and Chen, 1999).

The purity of phycocyanin plays a significant role in commercial applications. A purity of 0.7 is considered as food-grade, 3.9 as reactive grade and greater than 4.0 as analytical grade (Rito-Palmares et al., 2001). Different workers have applied different purification process in order to get high purity ratio. The purity of phycocyanin was reported as 1.8, 4.9 and 3.5 in Spirulina platensis, Synechococcus sp. 109201 and Calothrix sp. by using chitosan adsorption and aqueous two-phase extraction, hydrophobic and ionexchange chromatography, 0-Sepharose and hydrophobic interaction chromatography, respectively (Abalde et al., 1998; Patil et. al., 2006). A study by Seo et al. (2013) demonstrated that phycocyanin from Spirulina platensis isolated using high-pressure process and hexane separation method had a purity of 0.909, which was better than that of the phycocyanin standard 0.904. In a subsequent study, they also reported that purity of 0.909 phycocyanin from *Spirulina platensis* was used in experiments to test for its anti-cancerous effect. However, in the present study phycocyanin purity of 1.2 was found in the crude extract of periphytic *Geitlerinema* sp. from oven-dried biomass which can be further used for different applications. In our study, we used this phycocyanin to test for anticancer activity on HepG2 cell line and found good inhibitory activity (Begum, 2014).

Production of phycobiliproteins from freshwater Oscillatoria sp., Spirulina sp., periphytic Geitlerinema sp. and blue-green Synechococcus sp. biomass dried under different conditions do greatly affect the pigment and purity ratio of phycocyanin. The present study showed that biomass dried in the oven at 40 °C had maximum production of phycocyanin, phycoerythrin and allophycocyanin. Hence, it can be summarised that to obtain maximum production and purity ratio of phycobiliproteins from the biomass, oven-drying method is the most suitable and efficient. The oven-drying method can be used commercially as it is simple, efficient and convenient.

Conclusion

The information from this study is important and valuable in selecting the potential cyanobacterial species and defining the most favourable drying conditions for maximum production of phycocyanin. This is because if a drying method works well for one species, it may not work well for other cyanobacterial species. During the screening of cyanobacteria for phycobiliproteins, *Geitlerinema* sp. produced maximum phycobiliproteins. Further research is required to determine how the spectral composition of light and extraction process controls pigments biosynthesis pathways.

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References

Abalde, J., Betancour, L., Torres, E., Cid, A., Barwell, C. 1998. Purification and characterization of phycocyanin from marine cyanobacterium *Synechococcus* sp. 109201. Plant Science 136: 109– 120. <u>http://doi.org/10.1016/S0168-9452(98)00113-7</u>

- Abed, R.M.M., Dobretsov, S., Sudesh, K. 2009. Applications of cyanobacteria in biotechnology. Journal of Applied Microbiology 106:1-12. <u>http://doi.org/10.1111/j.1365-2672.2008.03918.x</u>
- Aiba, S., Ogawa, T. 1977. Assessment of growth yield of a blue-green alga: Spirulina platensis, in axenic and continuous culture. Journal of General Microbiology 102:179–182. http://doi.org/10.1099/00221287-102-1-179
- Begum, H. 2014. Production and characterization of phycobiliproteins from cyanobacteria and its effects on HepG2 cancer cell lines. PhD Thesis, Universiti Putra Malaysia, Malaysia. 210 pp.
- Begum, H., Yusoff, F.M., Banerjee, S., Khatoon, H., Shariff, M. 2016. Availability and utilization of pigments from microalgae. Critical Reviews in Food Science and Nutrition 56: 2209–2222. <u>http://doi.org/10.1080/10408398.2013.764841</u>
- Bellinger, E.G. 1992. A key to common algae. Freshwater, estuarine and some coastal species. Institute of Water and Environmental Management, London. 659 pp.
- Bennett, A., Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. Journal of Cell Biology 58:419-435. <u>http://doi.org/10.1083/jcb.58.2.419</u>
- Chiu, H.F., Yang, S.P., Kuo, Y.L., Lai, Y.S., Chou, T.C. 2006. Mechanisms involved in the antiplatelet effect of C-phycocyanin. British Journal of Nutrition 95:435-440. <u>http://doi.org/10.1079/bjn20051643</u>
- Clesceri, L.S., Greenberg, A.E., Trussel, R.R. 1989. Standards methods for the methods for the examination of water and wastewater. 17th Edition. American Public Health Association, Washington, DC. 1268 pp.
- Desmorieux, H., Decaen, N. 2006. Convective drying of *Spirulina* in thin layer. Journal of Food Engineering 77:64–70. http://dx.doi.org/10.1016/j.jfoodeng.2005.05.060
- Doke, J.M. 2005. An improved and efficient method for the extraction of phycocyanin from *Spirulina* sp. International Journal of Food Engineering 1:1-11. <u>http://dx.doi.org/10.2202/1556-3758.1037</u>
- Guihéneuf, F., Khan, A., Tran, L. S P. 2016. Genetic engineering: a promising tool to engender physiological, biochemical, and molecular stress resilience in green microalgae. Frontiers in Plant Science 7:400. <u>https://doi.org/10.3389/fpls.2016.00400</u>
- Güroy, B., Karadal, O., Mantoğlu, S., Cebeci, I.O. 2017. Effects of different drying methods on C-phycocyanin content of *Spirulina platensis* powder. Ege Journal of Fisheries and Aquatic Sciences 34:129-132. <u>https://doi.org/10.12714/egejfas.2017.34.2.02</u>
- Hemlata, T.F. 2009. Screening of cyanobacteria for phycobiliproteins and effect of different environmental stress on its yield. Bulletin of Environmental Contamination and Toxicology 83:509–515. <u>http://dx.doi.org/10.1007/s00128-009-9837-y</u>
- Lavens, P., Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper No. 361. FAO, Rome. 295 pp.
- Leach, G., Oliveira, G., Morais, R. 1998. Spray-drying of *Dunaliella salina* to produce a b-carotene rich powder. Journal of Industrial Microbiology and Biotechnology 20:82–85. <u>https://doi.org/10.1038</u>/<u>/sj.jim.2900485</u>
- Li, B., Chu, X., Gao, M., and Zhang, X. 2009. Study on the molecular mechanism of C-phycocyanin from *Spirulina platensis* induced apoptosis in HeLa cells. Chinese Pharmacological Bulletin 25:1045– 1050.
- Li, B., Chu, X., Gao, M., Li, W. 2010. Apoptotic mechanism of MCF-7 breast cells in vivo and in vitro induced by photodynamic therapy with C-phycocyanin. Acta Biochimica et Biophysica Sinica 42:80–89. <u>http://dx.doi.org/10.1093/abbs/gmp104</u>
- Madhyastha, H.K., Radha, K.S., Nakajima, Y., Omura, S., Maruyama, M. 2008. uPA dependent and independent mechanisms of wound

healing by C-phycocyanin. Journal of Cellular and Molecular Medicine 12:2691–2703. <u>http://dx.doi.org/10.1111/j.1582-4934.2008.00272.x</u>

- Martelli, G., Folli, C., Visai, L., Daglia, M., Ferrari, D. 2014. Thermal stability improvement of blue colorant C-Phycocyanin from Spirulina platensis for food industry applications. Process Biochemistry 49:154–159. <u>https://doi.org/10.1016/j.procbio.2013.10.008</u>
- Millamena, O.M., Aujero, E.J., Borlongan, I.G. 1990. Techniques on algae harvesting and preservation for use in culture and as larval food. Aquacultural Engineering 9:295–304. <u>https://doi.org/10.1016/0144-8609(90)90022-R</u>
- Nichols, H.W., Bold, H.C. 1965. Growth media-fresh water. In: Hand book of physiological methods, Stein, J.R. (Ed.), Cambridge University Press, USA, pp. 7–24.
- Nireesha, G.R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M.N., Lavakumar, V. 2013. Lyophilization / Freeze drying - An review. International Journal of Novel Trends in Pharmaceutical Sciences 3:87-98.
- Nübel, U., Garcia-Pichel, F., Muyzer, G. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied and Environmental Microbiology 63:3327–3332. <u>https://doi.org/10.1128/AEM.63.8.3327– 3332.1997</u>
- Oliveira, E.G., Rosa, G.S., Moraes, M.A., Pinto, L.A.A. 2008. Phycocyanin content of *Spirulina platensis* dried in spouted bed and thin layer. Journal of Food Process Engineering, 31:34-50. <u>https://doi.org</u> /10.1111/j.1745-4530.2007.00143.x
- Pandey, V.D., Pandey, A., Sharma, V. 2013. Biotechnological applications of cyanobacterial phycobiliproteins. International Journal of Current Microbiology and Applied Sciences 2:89–97.
- Patil, G., Chethana, S., Sridevi, A.S., Raghavarao, K.S.M.S. 2006. Method to obtain C-phycocyanin of high purity. Journal of Chromatography 1127:76-81. <u>https://doi.org/10.1016/j.chroma.2006.05.073</u>
- Prakash, J., Pushparaj, B., Carlozzi, P., Torzillo, G., Montaini, E., Materassi, R. 1997. Microalgal biomass drying by a simple solar device. International Journal of Solar Energy 18:303–311. <u>https://doi.org/10.1080/01425919708914325</u>
- Prasanna, R., Pabby, A., Saxena, S., Singh, P.K. 2004. Modulation of pigment profiles of *Calothrix elenkenii* in response to environmental changes. Journal of Plant Physiology 161: 1125–1132. <u>https://doi.org</u> /10.1016/j.jplph.2003.09.001
- Rito-Palmares, M., Nunez, L., Amador, D. 2001. Practical application of aqueous two phase systems for the development of prototype process for phycocyanin recovery from *Spirulina maxima*. Journal of Chemical Technology & Biotechnology 76:1273–1280. <u>https://doi.org</u> /10.1002/jctb.507
- Roman, B.R, Alvarez-Pez, J.M., Fernandez, A., Grima, E.M. 2002. Recovery of pure B- Phycoerythrin from the microalga *Porphyridium cruentum*. Journal of Biotechnology 93:73-85. <u>https://doi.org</u> /10.1016/S0168-1656(01)00385-6
- Santiago-Santos, M.C., Ponce-Noyola, T., Olvera-Ram'irez, R., Ortega-López, J., Cañizares-Villanueva, R.O. 2004. Extraction and purification of phycocyanin from *Calothrix* sp. Process Biochemistry 39:2047–2052. <u>https://doi.org/10.1016/j.procbio.2003.10.007</u>
- Sarada, R., Manoj, G., Pillai, G., Ravishankar, A. 1999. Phycocyanin from Spirulina sp. influence of processing of biomass on phycocyanin yield, analysis of efficiency of extraction methods and stability studies on phycocyanin. Process Biochemistry 34:795-801. https://doi.org/10.1016/S0032-9592(98)00153-8
- SAS. 2002. Statistical analysis system, version 9.1. SAS Institute Inc., Cary, NC, USA.
- Sekar, S., Chandramohan, M. 2008. Phycobiliprotein as a commodity: Trends in applied research, patents and commercialization. Journal

of Applied Phycology 20:113-136. <u>https://doi.org/10.1007/s10811-007-9188-1</u>

- Seo, Y.C., Choi, W.S., Park, J.H., Park, J.O., Jung, K.H., Lee, H.Y. 2013. Stable isolation of phycocyanin from *Spirulina platensis* associated with high-pressure extraction process. International Journal of Molecular Sciences 14:1778–1787. <u>https://doi.org/10.3390</u> /<u>iims14011778</u>
- Siegelman, H.W., Kycia, J.H. 1978. Algal biliproteins. In: Handbook of phycological methods. Volume 2. Physiological and biochemical methods, Hellebust, J.A., Craigie, J.S. (Eds.), Cambridge University Press, pp. 71-79.
- Silveira, S.T., Burkert, J.F.M., Costa, J.A.V., Burkert, C.A.V., Kalil, S.J. 2007. Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. Bioresource Technology 98:1629– 1634. <u>https://doi.org/10.1016/i.biortech.2006.05.050</u>
- Sonani, R.R., Rastogi, R.P., Patel, R., Madamwar, D. 2016. Recent advances in production, purification and applications of phycobiliproteins. World Journal of Biological Chemistry 26:100–109. <u>https://doi.org/10.4331/wjbc.v7.i1.100</u>
- Tompkins, J., Deville, M.M., Day, J.G., Turner, M.F. 1995. Culture collection of algae and protozoa. Catalogue of strains. Ambleside, UK. 208 pp.
- Vinod, K.R., Kannaujiya, Kesheri, M., Singh G., Sinha, R.P. 2011. Biotechnological potentials of phycobiliproteins. International Journal of Pharma and Bio Sciences 2: 446–454

Zar, J.H. 1984. Biostatistical analysis, Prentice-Hall, New Jersey. 718 pp.

Zhang, Y.M., Chen, F. 1999. A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnology Techniques 13: 601–603. <u>https://doi.org</u> /10.1023/A:1008914405302