Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Extraction and partial purification of C-Phycocyanin from the marine cyanobacterium, *Arthrospira maxima*

Sreejith Kottuparambil[1]* and Roshni Lilly Thankamony[2]

[1]*Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia.*

[2]*Advanced Membranes and Porous Materials Center, Division of Physical Sciences and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia.*

***Correspondence**: Email: sreejithkottuparambil@gmail.com

Abstract

Phycocyanin (PC) is one of the water-soluble accessory pigments found in cyanobacteria. This versatile pigment–protein compound has been widely used as nutritional ingredients, natural dyes and florescent markers. This study demonstrates extraction and partial purification of C-PC from the marine filamentous cyanobacterium, *Arthrospira maxima*. The crude extract was partially purified by ammonium sulfate precipitation method and Chitosan/activated charcoal method. The later yielded food grade C-PC with purity ratio (*R*) of 1.25. The partially purified C-PC showed absorbance maxima at 620 nm and fluorescence emission maxima at 652 nm, values widely reported for C-PC. Our study suggests *A. maxima* as a promising source for large-scale production of C-PC for various applications.

Keywords: Arthrospira, Cyanobacteria, phycobiliproteins, phycocyanin, purity ratio.

Abbreviations: PBP, Phycobiliproteins; PC, phycocyanin; PE, phycoerythrin; *R*, purity ratio.

Introduction

Bioactive metabolites from microalgae have gained immense attention in recent times due to their wide spectrum of biotechnological and pharmaceutical potentials. Among them, Phycobiliproteins (PBP) are of particular interest, with extensive use in food, cosmetics, pharmaceutical and biomedical industries. PBP are brightly colored, highly fluorescent pigments found in the photosynthetic light-harvesting antenna complexes of red algae, cyanobacteria, and cryptomonads (Glazer, 1994). They include phycoerythrin (PE), C-phycocyanin (C-PC), and allophycocyanin (A-PC). In cyanobacteria, PBP are located in the supramolecular phycobilisomes on the external surface of the thylakoid membrane, acting as major photosynthetic accessory pigments, along with other vital biological functions such as photoprotection (Horváth et al., 2013; Sonani et al., 2016). C-PC is a blue pigment with high biotechnological potential as nutraceutical for pharmaceutical/biomedical research and as natural dye in food and cosmetic industries (Pan-utai et al., 2018). It also acts as antioxidant against free radicals and shows anti-inflammatory and potent cancer chemopreventive activities (Xia et al., 2016).

The structure of C-PC comprises of a protein and chromophore, the protein moiety consists of alpha and beta sub-units of molecular weights in the range of 18,000 and 20,000 Da, respectively (Chethana et al., 2015). It exhibits high stability in the pH range of 5–8 and absorbs light at a wavelength of approximately 620 nm and fluoresces at approximately 640 nm (Bennett and Bogorad, 1973). The purity and integrity of C-PC depends on the extraction procedure. The absorbance ratio of A_{620}/A_{280} is an indicator of purity of C-PC wherein a purity (*R*) of 0.7 is considered as food grade, 3.9 as reactive grade and greater than 4.0 as analytical grade (Rito-Palomares et al., 2001).

Arthrospira maxima is a marine filamentous cyanobacterium that belongs to the gram-negative bacteria, capable of performing photosynthesis with oxygen production. *Arthrospira*, commonly referred as *Spirulina* has been extensively used as a food additive due to its high content of proteins (60-71% of dry matter), carbohydrates (13-16%), lipids (6-7%) and essential nutrients like carotenoids, vitamins, and minerals, thereby being an attractive natural nutraceutical (Kottuparambil et al., 2013). Genus *Arthrospira* is also known for its high PC content (Chen and Wong, 2008; Rodríguez-Sánchez et al., 2012). C-phycocyanin is the major component of the phycobiliprotein family in *Arthrospira* and makes up from 7 to 20% of protein (Liao et al., 2011). *A. maxima* has marked antioxidant activity *in vivo* and *in vitro*, as well as anti-inflammatory and anticancer activities in certain experimental models (Martínez-

Palma et al., 2017). Due to its high growth rate in laboratory cultures, *A. maxima* is a promising candidate for microalgal biomass utilization for value added bioactive molecules. The present study describes a simple extraction and partial purification method for C-PC from *A. maxima*. The spectroscopic characterization of the extracted C-PC has been presented.

Materials and methods

Culture of A. maxima

A. maxima, obtained form Korean Marine Microalgae Culture Center (KMMCC; strain No. CY-023), was grown photoautotropically using Spirulina medium (Kottuparambil et al., 2013) containing 160 mM sodium bicarbonate at 20 °C under white fluorescent irradiation of 60 µmol photons m^{-2} s^{-1} with a 16:8 h L:D photoperiod. Sterile techniques were exercised for all cultural attempts to minimize the contaminating bacterial growth. Exponentially growing cultures of *A. maxima* were harvested and resuspended in fresh medium for extraction of C-PC. The morphology of the exponentially growing coiled trichomes of *A. maxima* is shown in the Figure 1.

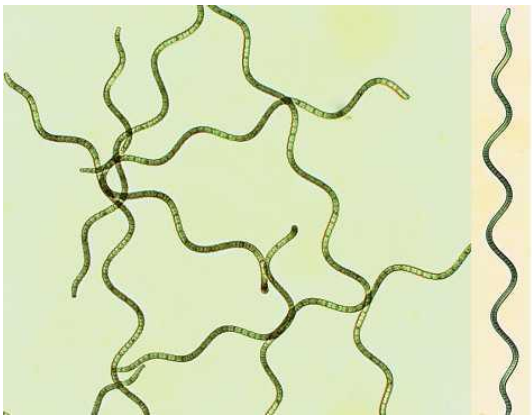

Figure 1: Morphology of trichomes of *Arthrospira maxima* (40 X magnification).

Extraction of C-PC

Fresh filaments of *A. maxima* were harvested by centrifugation at 4000 RPM at room temperature for 15 min and resuspended in phosphate buffer (0.75 M; pH 7) after washing with distilled water. The cells were frozen at −20 °C for 4 hours and subsequently thawed to room temperature for 1 hour and this freeze-thaw cycle was repeated for 5 times. The slurry was centrifuged at 10,000 rpm for 10 min at 4 °C. The deep blue colored supernatant was collected as the crude extract of phycocyanin (Figure 2).

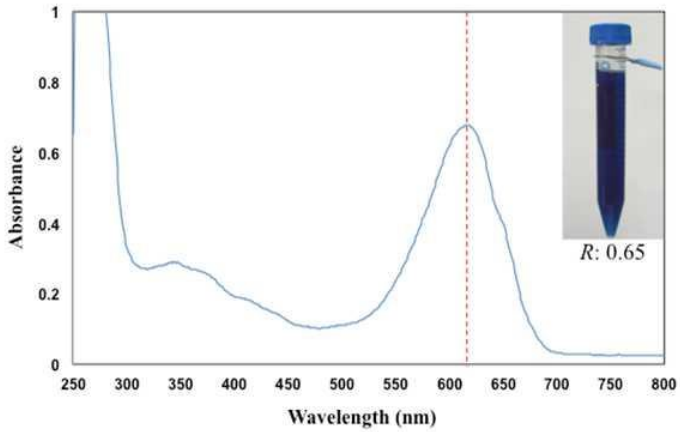

Figure 2: UV-Vis absorption spectrum of *A. maxima* crude extract of C-PC.

Purification of C-PC

We compared two chemical methods for C-PC purification in the crude extract from *A. maxima*. In the first step, finely powdered ammonium sulfate was gradually added to obtain 20% saturation with continuous stirring for 30 minutes (Soni et al. 2006). This solution was kept for 8 hours and centrifuged at 10000 rpm for 15 minutes. The supernatant was collected and added ammonium sulfate at 70 % saturation. After 8 hours incubation, the precipitate was obtained by similar centrifugation at 10000 rpm for 15 minutes. The pellets were dissolved in phosphate buffer (0.75 M).

Alternatively, chitosan solution was added to the crude *A. maxima* extract at 2% w/w, and the pH was adjusted to 6.9 (Liao et al., 2011). This solution was stirred for 5 minutes and subsequently centrifuged at 5000 rpm at 4 °C for 10 minutes. To the collected supernatant, activated charcoal powder (10 mg/ ml of crude extract) was added with stirring for 5 min. After centrifugation (8,000 rpm, 10 min, 4 °C), the supernatant was dissolved in phosphate buffer (0.75 M).

The purity of the extracts was measured spectrophotometrically as the ratio of absorbance at 680 and 280 nm (A_{620}/A_{280}) (Soni et al., 2006) (Figure 3).

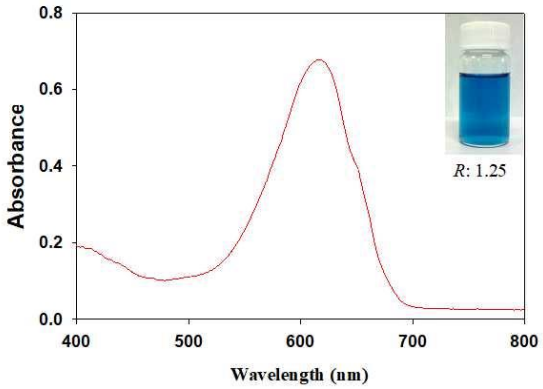

Figure 3: UV-Vis absorption spectrum of partially purified C-PC from *A. maxima* showing absorbance maximum at 620 nm

Characterization of C PC

The absorbance characteristics of the crude and partially purified C-PC extracts were measured on a S–3100 UV-Vis spectrophotometer (Scinco, Korea). The absorbance spectrum between 250 nm and 800 nm was obtained. The fluorescence excitation and emission spectra were obtained using a microplate fluorescence reader (Spectramax Gemini EM, Molecular Devices, USA) (Figure 4).

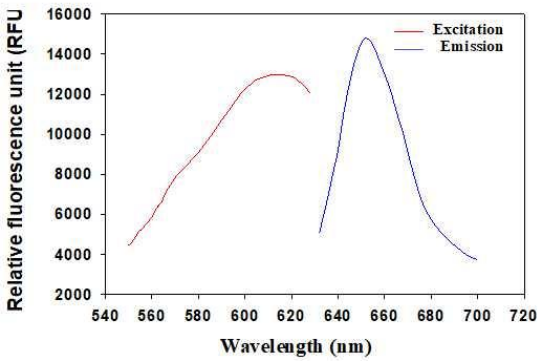

Figure 4: Excitation and fluorescence spectra of partially purified C-PC from *A. maxima*.

Results and Discussion

Physical rupture of cells by freeze and thaw has been accepted as a simple method to extract C-PC from cyanobacterial cells. The mechanism behind the freeze–thaw extraction is that it causes cells to

swell and ultimately break, due to sharp ice crystals formed during the freezing process and then contract during thawing (Soni et al., 2006). Horváth et al suggested that (Horváth et al., 2013) two freeze–thaw cycles yielded the maximum PC content from filamentous cyanobacteria, but the amount was not greatly increased after the second cycle. In general, the yield of C-PC relies on the strain specificity in the phycobiliprotein content and the culture conditions of the cyanobacterial strains used.

The crude extract of *A. maxima* appeared bright blue in color indicating the high yield of phycocyanin pigment. Previous reports have suggested the genus *Arthrospira* as an excellent source of high-grade phycocyanin (Kissoudi et al. 2018; Vernès et al., 2015). Spectrophotometric analysis of the crude extract showed a clear peak at 615 nm (Figure 2). This confirms the presence of C-PC (Su et al., 2010). However, the crude extract also contained high amounts of proteins and other biomolecules, as indicated by the high absorption peak below 300 nm. The purity ratio (A_{620}/A_{280}) of the crude extract was 0.65.

However, after ammonium sulfate purification, the purity of the extract was improved to 0.75, indicating the yield of food grade C-PC. The fraction of proteins was effectively removed by the 20% ammonium sulfate precipitation and C-PC was obtained by the 70% saturated ammonium sulfate precipitation (Zhang and Chen, 1999). Kumar et al. (Kumar et al., 2014) has achieved a purity of 1.5 for phycocyanin from *A. platensis* after precipitation with 65 % ammonium sulfate.

Our study shows the efficacy of Chitosan/activated charcoal method to significantly improve the purity of crude C-PC extracts. This is a simple and cost effective method and we obtained a purity ratio of 1.24. This value ranks the extract as high quality food grade C-PC. Apart from use as natural dye in foods such as diary products, jellies and chewing gums, this C-PC can be applicable as a therapeutic agent in oxidative diseases and as a fluorescent marker in research (Rodríguez-Sánchez et al., 2012). However, for analytical and biomedical applications of C-PC, high degree of purity is required. Previous studies have reported that aqueous two-phase systems (Rito-Palomares et al., 2001) and ion-exchange chromatography (Liao et al., 2011; Mishra et al., 2011) are effective methods to purify C-PC to meet commercial standard requirements. However, in general, extraction of bioactive molecules such as C-PC from cyanobacteria is challenging because of difficulties to eliminate debris and some contaminants during the process and due to unfavorable impact of intracellular pollutants.

The partially purified C-PC was subsequently characterized for their spectroscopic properties. At room temperature, the extract showed a single maximum absorbance at 620 nm (Figure 3). This is the characteristic absorbance of C-PC (Minkova et al., 2003). The absence of shoulder peak at 650 nm indicates the absence of A-PC. Moreover, no absorbance peak was found at 540 nm, indicating the absence of PE. The fluorometric analysis of the extract revealed an excitation wavelength of 616 nm and an emission maximum of 652 nm (when excited at 616 nm) was observed. These values are in agreement with the values reported for C-PC extracted from *A. platensis* (Moreno et al., 1997) and *A. fusiformis* (Minkova et al., 2003).

Conclusion

The spectroscopic characterization of *A. maxima* extract revealed the abundant presence of C-PC and no APC and PE. *A. maxima* gives large amount of biomass in short time due to its higher growth rate and the high saline medium used in this study prevents the growth of other contaminant bacteria in the culture, giving unialgal biomass which is an important prerequisite for algal biomass utilization. High yield of C-PC was obtained with an easy and cost effective method and partial purification yielded high food grade C-PC. Our study confirms the potential use of *A. maxima* as a source of commercial production of C-PC for various applications.

Acknowledgements

We are grateful to the Institute of Green Environmental Research, Incheon National University, South Korea for support and laboratory facilities. We thank King Abdullah University of Science and Technology (KAUST) for financial support.

References

Bennett, A., Bogorad, L., 1973. Complementary chromatic adaptation in a filamentous blue-green alga. The Journal of cell biology 58, 419-435.

Chen, T., Wong, Y.-S., 2008. In vitro antioxidant and antiproliferative activities of selenium-containing phycocyanin from selenium-enriched Spirulina platensis. Journal of agricultural and food chemistry 56, 4352-4358.
Chethana, S., Nayak, C.A., Madhusudhan, M., Raghavarao, K., 2015. Single step aqueous two-phase extraction for downstream processing of C-phycocyanin from Spirulina platensis. Journal of food science and technology 52, 2415-2421.
Glazer, A.N., 1994. Phycobiliproteins—a family of valuable, widely used fluorophores. J Appl Phycol 6, 105-112.
Horváth, H., Kovács, A.W., Riddick, C., Présing, M., 2013. Extraction methods for phycocyanin determination in freshwater filamentous cyanobacteria and their application in a shallow lake. Eur J Phycol 48, 278-286.
Kissoudi, M., Sarakatsianos, I., Samanidou, V., 2018. Isolation and purification of food-grade C-phycocyanin from Arthrospira platensis and its determination in confectionery by HPLC with diode array detection. Journal of separation science 41, 975-981.
Kottuparambil, S., Lee, S., Han, T., 2013. Single and interactive effects of the antifouling booster herbicides diuron and Irgarol 1051 on photosynthesis in the marine cyanobacterium, Arthrospira maxima. Toxicology and Environmental Health Sciences 5, 71-81.
Kumar, D., Dhar, D.W., Pabbi, S., Kumar, N., Walia, S., 2014. Extraction and purification of C-phycocyanin from Spirulina platensis (CCC540). Indian journal of plant physiology 19, 184-188.
Liao, X., Zhang, B., Wang, X., Yan, H., Zhang, X., 2011. Purification of C-phycocyanin from Spirulina platensis by single-step ion-exchange chromatography. Chromatographia 73, 291-296.
Martínez-Palma, N.Y., Dávila-Ortiz, G., Jiménez-Martínez, C., Madrigal-Bujaidar, E., Álvarez-González, I., 2017. Chemopreventive and antioxidant effect of polyphenol free Spirulina maxima and its hydrolyzed protein content: Investigation on azoxymethane treated mice. Pharmacognosy magazine 13, S164.
Minkova, K., Tchernov, A., Tchorbadjieva, M., Fournadjieva, S., Antova, R., Busheva, M.C., 2003. Purification of C-phycocyanin from Spirulina (Arthrospira) fusiformis. Journal of biotechnology 102, 55-59.
Mishra, S.K., Shrivastav, A., Mishra, S., 2011. Preparation of highly purified C-phycoerythrin from marine cyanobacterium Pseudanabaena sp. Protein expression and purification 80, 234-238.
Moreno, A., Bermejo, R., Talavera, E., Alvarez-Pez, J., Sanz-Aparicio, J., Romero-Garrido, A., 1997. Purification, Crystallization and Preliminary X-ray Diffraction Studies of C-Phycocyanin and Allophycocyanin from Spirulina platensis. Acta Crystallographica Section D 53, 321-326.
Pan-utai, W., Kahapana, W., Iamtham, S., 2018. Extraction of C-phycocyanin from Arthrospira (Spirulina) and its thermal stability with citric acid. J Appl Phycol 30, 231-242.
Rito-Palomares, M., Nunez, L., Amador, D., 2001. Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from Spirulina maxima. Journal of Chemical Technology & Biotechnology 76, 1273-1280.
Rodríguez-Sánchez, R., Ortiz-Butrón, R., Blas-Valdivia, V., Hernández-García, A., Cano-Europa, E., 2012. Phycobiliproteins or C-phycocyanin of Arthrospira (Spirulina) maxima protect against HgCl2-caused oxidative stress and renal damage. Food chemistry 135, 2359-2365.
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 7, 100.
Soni, B., Kalavadia, B., Trivedi, U., Madamwar, D., 2006. Extraction, purification and characterization of phycocyanin from Oscillatoria quadripunctulata—Isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochemistry 41, 2017-2023.
Su, H.-N., Xie, B.-B., Chen, X.-L., Wang, J.-X., Zhang, X.-Y., Zhou, B.-C., Zhang, Y.-Z., 2010. Efficient separation and purification of allophycocyanin from Spirulina (Arthrospira) platensis. J Appl Phycol 22, 65-70.
Vernès, L., Granvillain, P., Chemat, F., Vian, M., 2015. Phycocyanin from Arthrospira platensis. Production, extraction and analysis. Current Biotechnology 4, 481-491.
Xia, D., Liu, B., Xin, W., Liu, T., Sun, J., Liu, N., Qin, S., Du, Z., 2016. Protective effects of C-phycocyanin on alcohol-induced subacute liver injury in mice. J Appl Phycol 28, 765-772.
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.