Research Inventy: International Journal of Engineering And Science
Vol.8, Issue 2 (May 2018), PP -60-63
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

C-Phycocyanin Extraction and Purification from *Spirulina* Platensis

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Abstract-*C-Phycocyanin is a natural blue dye used in food and pharmaceutical industry. Phycocyanin (PC) is used to capture light energy for photosynthesis, unique to cyanobacteria. In the present study, a simple and efficient method to extract C-phycocyanin from Spirulina platensis biomass is used. The extractions were carried out by using distilled water, sodium phosphate buffer and sonication. Sodium phosphate buffer is most efficient method for extraction of C-PC in which yield is 0.60±0.04 mg/ml with purity ratio 0.160 ±0.04. Purification of phycocyanin by dialysis was maximum in comparison of crude extract and ammonium sulfate precipitation. In SDS-PAGE analysis, the purified PC showed the presence of two subunit α and β.*

Keywords: *Spirulina; Biomass; Extraction; Purification; C-Phycocyanin.*

DATE OF SUBMISSION: 08-06-2018 DATE OF ACCEPTANCE: 23-06-2018

I. Introduction

A cyanobacterium is an ancient group of photosynthetic prokaryotes that are thought to be the first organisms to carry out oxygenic photosynthesis. Phycocyanin (PC) is used to capture light energy for photosynthesis, unique to cyanobacteria. [1]. The Cyanobacterium, Spirulina platensis has been commercialized in several countries for its use as a health food and for therapeutic purposes due to its valuable constituents, particularly proteins and vitamins [2]. This microalga has great potential in the production of food and related nutritional materials, such as colouring agents, vitamins, γ-linolenic acid and enzymes [3]. C-phycocyanin is the major component of the phycobiliprotein family. It is not only used as a nutritive ingredient and natural dye in foods (chewing gums, dairy products, ice sherbets, jellies etc) and cosmetics in Japan, Thailand and China, but also used as a potential therapeutic agent in oxidative diseases and as a fluorescent marker in biomedical research[4,5]. The extraction of phycobiliproteins involves cell rupture and release of these proteins from within the cell. The cell walls of cryptophytes are easily disrupted, but those of cyanobacteria are extremely resistant [6]. Thus, the use of variations in the osmotic pressure, abrasive conditions, chemical treatment, freezing-thawing and sonication, amongst other disruption methods, are necessary. Mechanical cell disintegration methods are currently preferred for large-scale operations since a complete disintegration of the biomass is desired, with high product and activity yields [7]. The optimization of extraction from dried biomass was studied by Moraes [8] and Silveira [9]. The reported methods to extract C-PC from wet biomass include freezing and thawing, sonication, homogenization, lysozyme treatment and acid treatment [10-12]. The aim of this research paper was extraction and purification of C-phycocyanin from Spirulina platensis, so as to obtain C-phycocyanin extracts with greater concentration and purity.

II. Material And Methods

2.1 Growth and maintenance of culture

Spirulina platensis was procured from IARI, New Delhi, India Spirulina *platensis* was maintained in chemically defined Zarrouk's medium [13] at pH 9.8 illuminated under white lamp and aerated with atmospheric dry air 5ml/min.

2.2 Protein extracts preparation

Three different extraction methods i.e distilled water, sodium phosphate buffer and sonication methods were used to optimize the isolation of C-phycocyanin (C-PC). Briefly, C-PC was extracted from Spirulina platensis biomass 1:25 (w/v) in distilled water at 42°C for 24 hr. In sonication method, 1:25 (w/v) Spirulina biomass in distilled water was irradiated at 40 kHz for 40 min. The resultant slurry from both the methods was centrifuged at 10,000g for 18 min at 4°C to remove the cell debris. The precipitate was discarded and the supernatant crud extract was collected. The pH of the crud extract was adjusted to pH 7.0.

2.3 Electrophoresis in polyacrylamide gel (SDS-PAGE)

Electrophoresis of dialyzed sample as well as gel filtration fractions in polyacrylamide gel was carried out in a vertical chamber using 12% polyacrylamide gel with SDS (SDSPAGE) [14]. The gel prepared was 1.0 mm thick containing 0.1% (w/v) SDS. Samples were mixed with equal volume of sample buffer containing 2.5 % (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2 mercaptoethanol 0.002% (w/v) bromophenol blue and 60 mM Tris-HCl (pH 6.7), and boiled for 12 min. Electrophoresis was carried out at room temperature and the gel was stained by 0.1% Coomassie Brilliant G250 solution. Molecular marker was protein marker broad range, obtained from Bangalore GeNei, India.

2.4 Spectroscopic measurement

UV–Vis absorbance spectra were recorded on T60 UV-VIS Spectrophotometer (PERSEE, China).The purity of C-PC was evaluated according to the absorbance ratios (A620/A280) [15]. The amount of C-PC in the sample was calculated using simultaneous equations of Bennett and Bogorad [16].

2.5 Protein determination

The total Protein contents were determined by the method [17]. The results were treated by one-way analysis of variance (ANOVA) using Statistical 6.0 [18]. All analyses were performed considering a level of 95% of confidence.

III. Results And Discussion

Spirulina is used as a high quality protein mainly for phycocyanin [19], which is an important cyanobacterial accessory pigment having a number of industrial applications. Extraction of phycocyanin, was completed in three major steps: Distilled water (Step I), Sodium phosphate buffer (Step II) and Sonication (Step III) "Table1".

Table 1: Different methods for extraction of C-PC from *Spirulina*

Extraction methods	C-PC (mg/ml)	Purity Ratio (A615/280)
Distilled water	0.55±0.03	0.148 ±0.06
Sodium phosphate buffer	0.60±0.04	0.160 ±0.04
Sonication	0.25±0.04	0.08 ±0.03

Purification of phycocyanin, was completed in three major steps: crude extract (Step I), ammonium sulfate precipitation (Step II) and dialyses (Step III) "Table 2".

Table 2: The purity, yield and the recovery of C-PC in different stages

Steps	Volume (ml)	PC (µg/ml)	Purity (A620/A280)	Recovery (%)
Crude extract	100	39.0	0.39	100
Ammonium sulphate ppt	10	125.2	1.52	82
Dialysis	10	610.4	2.90	41

After ammonium sulphate precipitation the purity of phycocyanin was 1.52 and after dialyses purity were 2.90. So from crude extract purification to dialysis, purity increased nearly seven times. Purity was also reconfirmed by the presence of single bands of α-subunit (16 kDa) and β-subunit (17 kDa) during native gel electrophoresis in which M is molecular marker and lane 1 PC "Fig 1".

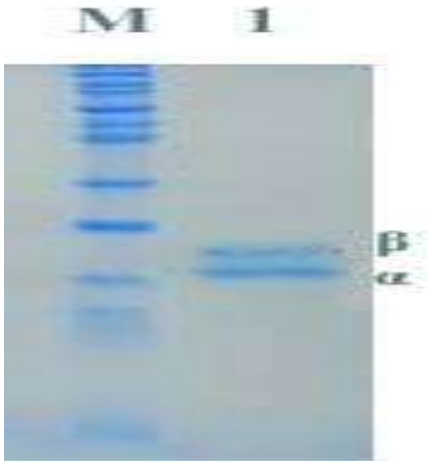

Fig. 1. SDS-PAGE of purified phycocyanin

3.1 Determination of Molecular Weight

The extracted and purified C-PC was used to determine the molecular weight by SDS-PAGE [20]. In the figure samples are as1- Protein marker, 2- 40% Ammonium sulphate precipitate, 3-Dialyzed sample and 4-Crude extract. After loading samples than running the purified fractions at each step, the result showed the presence of C-PC "Fig. 2".

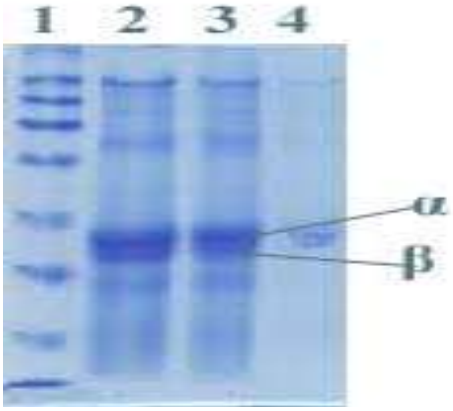

Fig.2 : 12% polyacrylamide gel electrophoresis (SDS-PAGE) of different fractions of C-PC.

3.2 Statistics

All of the analyzed values were presented as the mean ± S.D., and all of the experimental data were analyzed using the statistics software SPSS 16.0. Tests of the significance of differences were analyzed by ANOVA (analysis of variance).

IV. Conclusions

In this research work, extraction process associated with relatively stable C-PC from *Spirulina* platensis as in high purity. This process also has the advantage of using fewer steps and having a shorter process time, which may have contributed to the improvement in the stability and the purity of the C-PC. Therefore, these results suggest application of this combined process for expanding the uses of C-PC for future pharmacological and medical applications.

Acknowledgments

We are thankful to Mewar University, Rajasthan for allowing the present study at the institute.

References

[1]. H. Pinaki and S. K. Gargi , Isolation and purification of phycocyanin from cyanobacteria of a mangrove forest, *Journal of the Korean Society for Applied Biological Chemistry*, 60(6), 2017, 631–636.
[2]. M.F. McCarty, Clinical potential of Spirulina as a source of phycocyanobilin. *J. Med Food*, 10(4), 2007, 566-70.
[3]. R. Rippka, J. Deruelles, J. B. Waterbury, M. Herdman, R. Y. Stanier, *J. Chem. Microbiol.*, 1,1979, 111.
[4]. C. Romay, A. Aspee, M. Pizarro, E. A Lissi, *Free Radical Biol. Med. 28*, 2000, 1051.
[5]. V. B. Bhat and K. M. Madyastha, *Biochem. Biophys. Res. Commun.* 20, 2000, 275.
[6]. H. W. Siegelman, and H. J. Kycia, Algal biliproteins. In: J.A. Hellebust and J. S. Craigie, (Handbook of Phycological Methods. Cambridge University Press, Cambridge ,1978).
[7]. P. Gacesa and J. Hubble, Tecnología de las Enzimas, (Editorial Acribia, Zaragoza 1900).
[8]. C. C. Moares, J. F. M. Burkert and S. J. Kalil, C-Phycocyanin Extraction Process for Large-Scale Use, *Journal of Food Biochemistry*, 34 (1), 2010,133.
[9]. S. T. Silveira, J. F. M Burkert, J. A. V Costa, C. A. V. Burkert and S. J. Kalil, Optimization of Phycocyanin Extraction from *Spirulina platensis* Using Factorial Design. *Bioresource Technology*, 98 (8), 2007,1629.
[10]. R. Bermejo, M. A. Felipe, E. M. Talavera and J. M. Alvarez-Pez, Expanded Bed Adsorption Chromatography for Recovery of Phycocyanins from the Microalga *Spirulina platensis*. *Chromatographia*, 63(1-2), 2006, 59.
[11]. B. Soni, U. Trivedi and D. Madamwar, A Novel Method of Single Step Hydrophobic Interaction Chromatography for the Purification of Phycocyanin from *Phormidium fragile* and its Characterization for Antioxidant Property. *Bioresource Technology*, 99(1), 2008,188.
[12]. R. Sarada, M. G. Pillai and G. A. Ravishankar, Phycocyanin from *Spirulina* sp.: Influence of Processing of Biomass on Phycocyanin Yield, Analysis of Efficacy of Extraction Methods and Stability Studies on Phycocyanin, *Process Biochemestry*, 34 (8), 1999,795.
[13]. C. Zarrouk, Contribution a I' etude d' une cyanobacterie: Influence de divers facteurs physiques et chimiques et la photosynthese de Spirulina maxima (Setchell et Gardener) Geitler Ph.D.thesis, University of paris, France, 1966.
[14]. U. K. Laemmli. Cleavage of structural protein during assembly of the head of bacteriophageT4, *Nature*, 227, 1970, 680-685.
[15]. J. Abalde, L. Betancourt, E. Torres, A. Cid and C. Barwell, "Purification and characterization of phycocyanin from the marine cyanobacterium Synechococcus sp. IO9201," *Plant Sci*, 136, 1998, 109-20.
[16]. A. Bennett and L. Bogorad, "Complementary chromatic adaptation in filamentous blue-green algae," *J. Cell. Biol*, 58, 1973, 419-435.
[17]. H. O. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall,"Protein measurement with the Folin phenol reagent, *J.Biol. Chem*, 193, 1951, 265-275.
[18]. Statsoft Inc., Statistica for Windows, Version 6.0, 2300 East 14th Street, Tulsa, OK, 74104, USA, 1998.
[19]. N. T. Eriksen, Production of phycocyanin a pigment with applications in biology, biotechnology, foods and medicine, *Applied Microbiology and Biotechnology*, 80, 2008, 1–14.
[20]. P. K. Suresh, B. G Rajendra, B. P Rimal and D. S Keshav, Extraction and purification of C-phycocyanin from dry Spirulina powder and evaluating its antioxidant, anticoagulation and prevention of DNA damage activity, *Journal of Applied Pharmaceutical Science*, 3 (08), 2013,149-153.

Shubham Nagar " C-Phycocyanin Extraction and Purification from Spirulina Platensis" Research Inventy: International Journal of Engineering And Science, vol. 08, no. 02, 2018, pp. 60–63.