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METHODS FOR EXTRACTION AND PURIFICATION OF PHYCOBILIPROTEINS

M. Y. PYLYPENKO*, L. P. KUZMINA
National Aviation University, Kyiv

Phycobiliproteins are pigments of protein nature present in cyanobacteria, red algae and cryptomonads. They are widely used in biotechnology, foods, pharmacology, cosmetics, etc. Different methods for extraction and purification of phycobiliproteins have been studied for many years, but no standard technique exists. This review highlights an overview on the basic methods used for extraction and purification of phycobiliproteins.

Key words: *phycobiliproteins, extraction, purification, phycoerythrin, phycocyanin, allophycocyanin.*

Introduction. Phycobiliproteins are family of water-soluble, brilliantly colored, light harvesting and highly fluorescent proteins. They are commonly presented in cyanobacteria, rhodophytes, and cryptomonads. Phycobiliproteins generally can be classified into three main types according to their structure and spectral properties: phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). Another classification is based on their colors: red – PE and blue – PC. Furthermore, one more classification is based on absorbance wavelength, the phycobiliproteins naturally occurring in blue-green and red algae are divided into four groups: PE (λ_{max} = 490-570 nm), PC (λ_{max}= 610-625 nm), APC (λ_{max} = 650-660 nm), and phycoerythrocyanin (PEC, λ_{max} = 560-600 nm), while the phycobiliproteins naturally occurring in cryptomonads are divided into 2 types: PE (λ_{max} = 540-570 nm), and PC λ_{max} = 610-625 nm) [1].

*Scientific supervisor – candidate of medical sciences, O. A. Vasylchenko

Phycobiliproteins are assembled into an organized cellular structure called phycobilisomes (PBSs) that are attached to the external surface of the thylakoid membrane. The structure of PBS is shown in Fig. 1. The PBSs helps to optimize the capture of light and transfer of energy due to its geometrical arrangement [2].

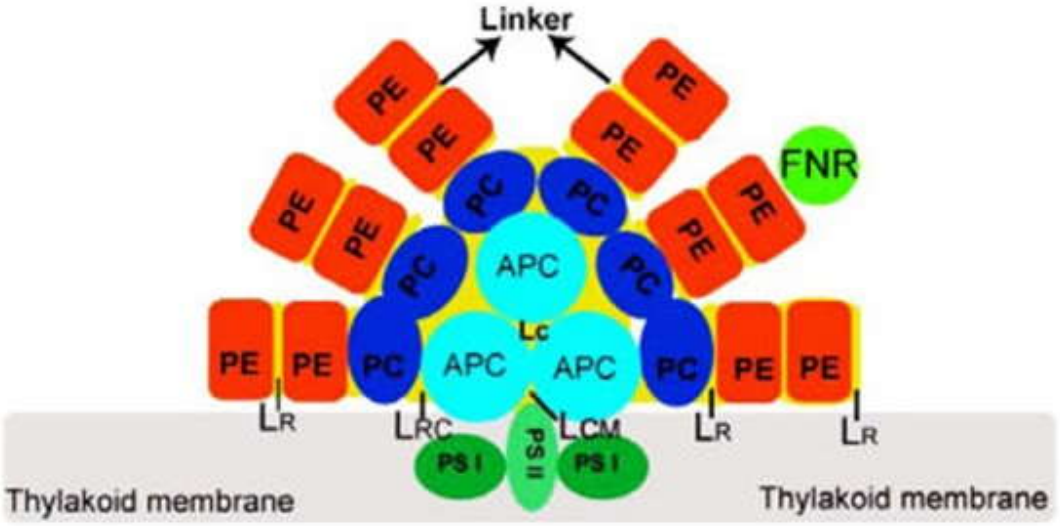

Fig.1. Structure of the phycobilisome [3].

Phycobiliproteins are widely used as colorants in food, cosmetics, textile and pharmaceutical industries due to its natural origin, absence of toxic effects, and stability (does not change with pH, stable under light, but sensitive to heat). Another important application of phycobiliproteins is as fluorescent probes. They have high fluorescence, good storage stability, high absorbance coefficient and emission, high photostability and can bind to antibodies and other proteins without changing its spectral characteristics. PC have antioxidant, anti-inflammatory, neuroprotective and hepatoprotective activity, which greatly increases the interest to it [4]. The applying of phycobiliproteins in such spheres requires the high level of purity and makes prices for it up to US $ 1500 mg^{-1} for highly purified molecular markers [5]. Thus, a better understanding of existing methods of extraction and purification of phycobiliproteins and the search for new ones along with

effective biomass production and harvesting methods can reduce the price and make it more accessible for use.

The main material. The maximum recovery of phycobiliproteins from algae is possible using suitable for the strain downstream processes (extraction and purification). The extraction of phycobiliproteins is still difficult due to small size of the cyanobacteria and resistant multilayer cell wall. Various methods have been used, but no standard technique exists. The method may be suitable for one organism and may not work for another [6].

The phycobiliproteins are isolated as intracellular protein-pigment complexes under appropriate control of pH. They may be extracted from the wet or dry biomass. The extraction of phycobiliproteins from wet biomass is more preferable because it helps to avoid loss in pigments during drying processes and does not require additional costs on this process. The recovery of phycobiliproteins from biomass is carried out through cell disruption, extraction, purification and characterization of phycobiliproteins as products, and various techniques are applied for each step [7].

Physical and chemical methods are used for cell disruption. One or a combination of the techniques is applied for treating of the biomass. Physical methods includes cavitation, sonication, nitrogen lysis, high-pressure exposure, homogenization, osmotic shock or freezing and thawing at different temperatures. Chemical techniques are represented by usage of enzymes, acids, alkali or detergents [8]. Crude extract is collected and further is exposed to precipitation, aqueous two-phase extraction, ultrafiltration, gel filtration or chromatography column. The phycobiliproteins should be purified from desalted extract by using ion exchange chromatography, absorption chromatography. Finally, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used to characterize the yield of the phycobiliproteins [9].

Spirulina spp. are widely used to obtain phycobiliproteins. Summarized methods for extraction, isolation and purification of it are given in figure 2. At first, simple chromatography and crystallization were applied, but this techniques lacked specificity

and whole phycobilisome is separated. Pigment separation became possible with thermal shock-based separation technique, but the purity and the yield of the process is still low.

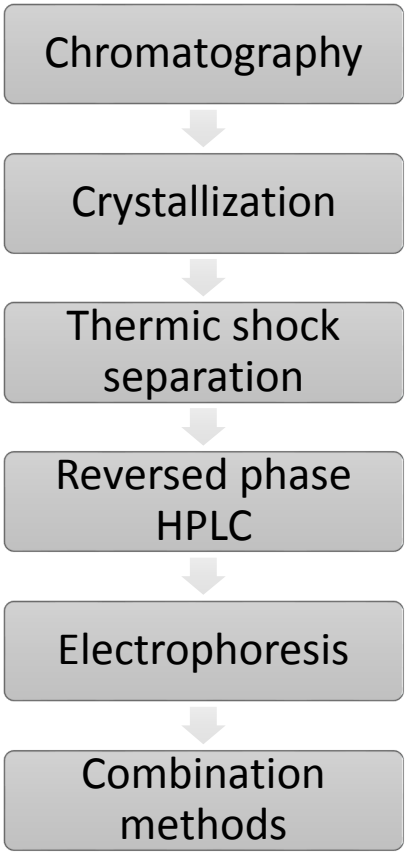

Fig. 2. Summarized methods for extraction, isolation and purification of phycobiliproteins from *Spirulina spp.*

It was possible to achieve up to 85 % of C-PC and APC with the use of reversed phase high-performance liquid chromatography. The mixture of C-PC and APC with the yield of 90-100 % is achieved by applying electrophoresis-based technique. The pure C-PC with the yield of ≈ 45 % is achieved with polyacrylamide/dodecyl sulphate gel, pre-treating the samples by precipitation with ammonium sulphate followed by further separation in chromatographic columns by Sephadex. Subsequently, different combinations of methods were used: HPLC coupled with a flame ionization detector (the phycobiliproteins was extracted with sodium phosphate neutral buffer, and then further purified them via dialysis and gel filtration chromatography) – yielding C-PC with purity

of 4,98; chromatography combined with expanded bed adsorption, anion interchange, and hydroxyapatite columns – yielding C-PC with purity of 3,2; hydrophobic interaction chromatography with ammonium sulphate and liquid nitrogen precipitation pre-treatments – yielding C-PC with purity of 4,5. Nowadays, one of the most widely used methods is ionic exchange chromatography, which involves pretreating vegetable samples of *Spirulina platensis* with two aqueous phases, leading to a purity of 6,69 [10].

CONCLUSIONS

Choosing the extraction method for phycobiliproteins should be taken into account the small size of the cells, multilayer cell wall, and appropriate pH control. Various techniques are used for extraction and purification, but no standard method exists. Ionic exchange chromatography with two aqueous phases enables to get phycobiliproteins with more purity (up to 6,69) comparatively to applying HPLC coupled with a flame ionization detector (purity up to 4,98). However, getting superiorly purified products can reduce its output. Thus, it is important to search new methods for phycobiliproteins isolation and purification to maximize their yield.

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МЕТОДИ ЕКСТРАКЦІЇ ТА ОЧИЩЕННЯ ФІКОБІЛІПРОТЕЇНІВ

М. Я. ПИЛИПЕНКО*, Л. П. КУЗЬМІНА

Національний авіаційний університет, м. Київ

Фікобіліпротеїни мають широкий спектр застосування. Різні методи екстракції та очищення фікобіліпротеїнів були вивчені протягом багатьох років, але стандартної методики не існує. У статті описані основні методи, які використовуються для екстракції та очищення фікобіліпротеїнів.

Ключові слова: *фікобіліпротеїни, екстракція, очищення, фікоеритрин, фікоціанін, аллофікоціанін.*

МЕТОДЫ ЭКСТРАКЦИИ И ОЧИСТКИ ФИКОБИЛИПРОТЕИНОВ

М. Я. ПИЛИПЕНКО*, Л. П. КУЗЬМИНА

Национальный авиационный университет, г. Киев

Фикобилипротеины имеют широкий спектр применения. Различные методы экстракции и очистки фикобилипротеинов были изучены в течение многихлет, но стандартной методики не существует. В статье описаны основные методы, используемые для экстракции и очистки фикобилипротеинов.

Ключевые слова: *фикобилипротеины, экстракция, очистка, фикоэритрин, фикоцианин, аллофикоцианин.*