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Recent Developments in Production of C-Phycocyanin and Its Application Through Biotechnological Perspective

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Abstract — Microalgae have found commercial application as natural resources of valuable metabolites like protein, lipids, carbohydrates, pigments, carotenoids or vitamin and chlorophyll. Among these, C-Phycocyanin is a blue pigment in cyanobacteria, rhodophytes and cryophytes with fluorescent and antioxidant properties. C-phycocyanin has many applications in food, health and therapeutical purposes. Now-a-days increased awareness of the toxic effect of synthetic compounds and community preference towards the use of natural products have led to focus on microalgae as a source of natural colorant. Recently, phycocyanin is being considered to be used to treat against Ebola virus disease by its super anti-inflammatory, anti-oxidant and various medicinal virtues. This paper presents recent developments about the new sources and production of C-phycocyanin, extraction and purification techniques in biotechnological perspective. Besides, this paper focuses on present and promising applications of C-phycocyanin in food industry, also for diagnostics purposes and neutraceuticals at large.

Keywords: microalgae; spirulina plantesis; phycocyanin; phycobiliprotein; pigment.

I. Introduction

Use of synthetic color compound in food industry has increased a lot due to augmented awareness of the toxic effect of synthetic compounds. Communal preference towards the use of natural products has led to more focus on microalgae as a natural source of colorant. Microalgae have also found commercial application as natural resources of valuable metabolite like protein, lipids, carbohydrates, pigments, carotenoids or vitamin and chlorophyll.

Among these, C-Phycocyanin is a blue pigment in cyanobacteria, rhodophytes and cryophytes with fluorescent and antioxidant properties. Isolation of C-Phycocyanin (C-PC) from different source of microalgae has fluorescent blue color which can be used in food industry as a dye for Jam, Jellies, and Ice-cream and in cosmetics industry for sun protection cream, hair color, lipsticks and eyeliner [1-3]. C-PC has significant properties like antioxidant, antinflammatary, hepatoprotective and radicle scavenging therefore used as preventive medicine in most of the diseases. C-PC evidenced as a fluroscent marker in diagnostic histochemistry and replacement for organic flurochrome [2-4]. Recently, It also proved as antiviral agent in Ebola virus disease [5].

C-PC is a blue colored accessory photosynthetic pigment of the phycobiliprotein family which are mainly found in the stroma of chloroplast of cyanobacteria, rhodophytes, glaucophyta and some cryptomaonads [2]. Phycocyanin is a pigment-protein complex from light harvesting phycobiliprotein family along with allophycocyanin and phycoerythrin. Phycobilins are made up of chromophore-bearing polypeptides containing α and β subunits having molecular weight of about 20kDa. The major classes of phycobiliproteins are phycocyanin (PC- blue pigment), Allophycocyanin (APC- Bluish green pigment), Phycoerythrin (PE- Red pigment), exhibiting maximum absorbance at 620nm, 650nm and 565nm, respectively [6]. Phycobilins have no phytol chain are attached to water soluble protein.

II. Sources and Cultivation Methods of C-Phycocyanin

Mostly *Spirulina plantesis* is a good source of protein and vitamins. It is less expensive and rich source of pigment about 30 % of its biomass. Production of C-Phycocyanin from various microalgae species have been studied by various researcher as *Spirulina plantesis* [1-4], *Spirulina Maxima* [7], *Spirulina fusiform* [8], *Galdieria sulphuraria* [3], *Anabaena circinalis* [9], *Arthronema africanum* [10], *Anabaena sp. Phormidium sp. Nostoc. Sp*.[11,15], *Coccochloris elabens* [12], *Gracilaria chilensis* [13], *Lyptolyngbya sp.* [14].

Various methods reported for cultivation of microalgae for phycocyanin production as

A) Photo-Trophic

The worldwide production of *spirulina* has been increased from 1970 in Lake Texcoco by Sosa Texcoco S. A. (Mexican City, Mexico).

In this method utilization of light as sole energy source which is converted into chemical energy through photosynthetic reactions. The major advantage of photoautotrophic cultivation is that the microalgae utilize carbon dioxide as sole carbon source for cell growth. Contamination problem is less severe than other type of cultivation. Therefore, outdoor scale up microalgae cultivation system, such as open ponds and raceway ponds, are usually operated under photoautotrophic condition. In this method light source can be sun or xenon lamp or Light emiiting diodes (LEDs) [16,17].

B) Mixotrophic

It involves a metabolic process, in which photosynthesis is the main energy source, although both organic compounds and carbon dioxide are essential. It has been proves experimentally that in mixotrophic culture, the addition of organic substrate resulted in the increase in the growth rate, as well as in the final biomass concentration (Andrade & Costa, 2007). In the exponential mixotrophic phase, the specific growth rate did not depend on light, since the algae were able to use glucose or any other organic carbon source and it act as an energy source, a fact verified by increasing the specific rate of glucose consumption on reducing the light intensity**.** When the organic substrate (Glucose, sodium acetate) was completely consumed after that, an autotrophic phase followed [18-20]. Light source is sun or Xenon lamp or LEDs.

C) Heterotrophic

It involves the utilization of only organic compounds as carbon and energy source. Heterotrophic production has also been successfully used for algal biomass and metabolites. This type of cultivation can avoid the problem associated with limited light that hinder high cell density in large scale photobioreactors during photoautotrophic condition. Heterotrophic condition gives much higher lipid productivity, is nearly 20 times higher than that obtained under photoautotrophic cultivation. However, the sugar based heterotrophic system frequently suffers from problems with contamination [3 and 21].

D) Photoheterotrophic

Microalgae require light when using organic compound as the carbon source. The main difference between mixotrophic and photoheterotrophic cultivation is that the latter requires light as the energy source, while mixotrophic cultivation use organic compound to serve this purpose. Hence, photo heterotrophic cultivation needs both sugars and light at the same time [22].

E) Recombinant

Recombinant protein production is an alternative method for heterotrophic cultivation of C-PC. Complete synthesis of recombinant phycobiliprotein depends on co-expression of the α and β chain as well as parallel synthesis and insertion of the correct phycobilin chromophores. Recombinant C-PC in which *cpc*A and *cpc*B genes were fused to His_6 tags for affinity chromatographic purification has been produced in photoautotrophic *Anabaena sp.* which naturally synthesizes and inserts phycobilins into C-PC [23].

III. Extractions, Isolation and Purification of C-PC

C-PC extractions are reported by various researcher to obtain better yield. The process of extraction involves physical, chemical and enzymatic method to break up the cell wall and cell disruption and then separation of crude C-PC from unwanted pigments and cell debries after that purification of crude extract followed by dried in a lyophilizer and confirmation of product. Extraction of C-PC depend on nature of organism and biomass which is wet, oven dry (low temp. 40-50^{o}C) or Lyophilised biomass . Cell Disruption to release phycocyanin followes first order kinetics reaction which indicates no time lag between cell wall disruption and release of pigment from the cell. Physical methods like Repeated freezing and thawing, ultrasonication and homogenization with high pressure are commonly used. Chemical Method like for extraction used different concentration of HCL or organic solvents. C-PC extraction by treatment with Lysozyme. Method varies from organism and type of biomass, environmental condition and pH and temperature of the system. C-PC is stable at pH 5 and temp. 44^{o}C. For large scale it is better to use wet biomass to save energy and extraction with homogenizer with pressure and repeated freezing and thawing [24-29].

A) Isolation and Purification of C-PC

After extraction separation of phycocyanin from cell debris and other pigment like chlorophyll and carotenoids. Which require centrifugation, filtration with small pore size cloth or whatmann filter paper. The purity of C-PC is generally evaluated on the absorbance ratio of A620/A280. C-PC of purity 0.7 is considered as food grade, till 3.9 as a reactive grade and greater than 4.0 as an analytical grade [30]. Various methods have been investigated by researcher for separation and purification of C-PC. In previous year chromatographic methods were used for purification like ammonium Sulphate precipitation combined with ion exchange chromatography and gel filtration which shows purity till 5.0. Recovery of C-PC by expanded bed adsorption chromatography by using hydroxypetite column

which includes crude extract passed through expanded bed adsorption followed by hydroxypetite chromatography after that elution of fraction with different molarity ammonium Sulphate, which loaded to the anion exchange column. Eluted column with Nacl and blue fraction measured for purity which is approximately. 5.0 [24]. Recently Purification of C-PC by aqueous two phase extraction using polyethylene glycol is reported which is shows highest purity 6.69. They digest crude extract with polyethylene glycol after that sample loaded on ion exchange chromatography where fractions collected with 0.3 M Nacl and check absorbance at ratio A620/A280.

SDS-Page is carried out to confirm 2 bands of Phycocyanin (α and β) with Molecular Weight. CD spectra were used to examine structural intact- ness of the purified C-PC [30-33].

IV. Application of C-Phycocyanin

Applications of C-PC in different areas are focused by scientist as follows.

A) C-Phycocyanin as a Fluorescent Dye

C-PC is stable protein, non-toxic and does not change its spectral characteristics in staining of tissues and DNA . C-PC is an antenna pigment contains multiple chromophore prosthetic group which are responsible for fluorescent properties of these proteins. Therefore are often used in research as chemical tag or fluroscence marker in immunodiagnostics. Phycobiliprotein by binding with blood cells, nuclei and genomic DNA [4,6 and 34].

B) Uses In Food Additives And Health Food

Spirulina plantesis gaining worldwide attractiveness as a food supplement. Blue color pigment used in health like candies, ice-cream, soup, pasta, chewing gums, dairy products and non-alcochoic beverages and also addition into fish food [35-37]. *Spirulina* which is major source of phycocynin recommended as a whole food to a patient for stimulation of immune defence system and also has anti-oxidant, anti-inflammatory, antiviral, anticancer and cholesterol lowering effects due to C-PC. Recently Researcher proved that role of C-PC enriched with *Spirulina* is effective in treatment in Ebola virus disease. Study shows that Phycocyanin inhibit the transmission of affected cell to another healthy cell through its ability to bind oligomannoses-8/9 on the Ebola Virus surface glycoprotein. Hence if it is not able to bind the cell may not spread into healthy cell [5].

C) C-PC In Nutraceutical And Pharmaceutical

C-PC support the stem cell found in bone marrow which produces white blood cells that make up the cellular immune system and red blood cells that oxygenate the body. Cytotoxic T lymphocytes and NK cell function more effectively with intake of phycocyanin. Therefore can be used in as anti-cancer agent. C-PC has anti-inflammatory action which inhibits transmission of infected cells by free radical scavenging or including alkoxyl, hydroxyl and peroxy radicals or suppressing inducible nitric oxide synthase expression. It also inhibit liver microsomal lipid peroxidation, pro-inflammatory cytokine formation such as TNFα, Suppresses Cyclo-oxygenase expression, decreses prostaglandin production and helps body to protecting in Infection. This finding shows that C-PC play crucial role as a neutraceuticls and Pharmaceutical drug [5].

V. Conclusions

This paper discussed recent developments in production of C-PC and various applications in biotechnological perspective. Different sources and Cultivation Methods of C-phycocyanin such as Photo-trophic, Mixotrophic, Heterotrophic, Photoheterotrophic etc. were deliberated in brief. The process of extraction, isolation and purification of C-PC were studied in depth. Applications of C-PC in different areas such as in food additives, health food, Nutraceuticals and Pharmaceuticals are highlighted.

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