Review Article

Biotechnological applications of cyanobacterial phycobiliproteins

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A B S T R A C T

Cyanobacteria, a group of morphologically diverse and widely distributed photosynthetic prokaryotes, produce phycobiliproteins as accessory photosynthetic pigments, constituting important components of light-harvesting complexes of photosynthetic machinery in these organisms. Phycobiliproteins are water-soluble, brilliantly coloured and highly fluorescent protein-pigment complexes which include phycocyanin (PC, blue pigment), allophycocyanin (APC, bluish-green pigment) and phycoerythrin (PE, red pigment). Commercially, they are high-value natural products with actual and/or potential biotechnological applications in nutraceuticals and pharmaceuticals, food and cosmetic industries as well as in biomedical research and clinical diagnostics. Specially, the use of phycobiliproteins as non-toxic and non-carcinogenic natural food colorants is gaining importance worldwide in view of the potential toxicity and carcinogenicity of the synthetic food colorants.

Keywords
Cyanobacteria; Phycobiliproteins; Phycocyanin; Allophycocyanin; Phycoerythrin; Food colorants; Pharmacological activities.

Introduction

Cyanobacteria (Blue-green algae) are a morphologically diverse and widely distributed group of photosynthetic prokaryotes which exhibit oxygenic (O\textsubscript{2}-evolving) photosynthesis similar to plants (Stanier and Cohen-Bazire, 1977). They are among the oldest life forms on earth which evolved around 2.4 billion years ago (Fischer, 2008). Comprising about 150 genera with more than 2000 species, they exhibit remarkable diversity in their morphology, ranging from simple unicellular and colonial to complex filamentous forms with or without branching (Van den Hoek et al., 1995). Owing to their remarkable adaptability to varying environmental conditions, they successfully colonize almost all kinds of terrestrial and aquatic ecosystems (Tandeau de Marsac and Houmard, 1993). Many cyanobacteria grow and thrive in extreme environments, such as ice-based cold habitats (Zakhia et al., 2008; Pandey et al., 1995), geothermal habitats (Ward and Castenholz, 2000), hypersaline habitats (Oren, 2000) and deserts (Wynn-Williams, 2000). The ability of many cyanobacteria to perform both photosynthesis and nitrogen fixation together with their efficient nutrient uptake mechanisms and adaptation to low light intensity makes them highly productive
Cyanobacteria are ecologically and economically important organisms. Ecologically, they contribute significantly to the primary production of various ecosystems, especially freshwater and marine ecosystems, and play significant role in carbon, oxygen and nitrogen cycling (Tomitani et al., 2006; Waterbury et al., 1979). The biotechnological applications of cyanobacteria in diverse areas, such as agriculture, aquaculture, pollution control (bioremediation), bioenergy and biofuels, and nutraceuticals have been well-documented (Patterson, 1996; Abed et al., 2009; Pandey, 2010). Moreover, they are known to produce a wide range of pharmacologically important bioactive compounds (e.g. antibacterial, antifungal, antiviral, anticancerous, muscle relaxants) and commercially important high-value products, such as polyunsaturated fatty acids (PUFA) and phycobiliproteins (Pandey et al., 2007; Tan, 2007; Skulberg, 2000; Barrios-Llerena et al., 2007; Sekar and Chandramohan, 2008; Eriksen, 2008).

Phycobiliproteins (PBPs), a group of accessory photosynthetic pigments, are water-soluble fluorescent pigment-protein complexes which constitute important components of the light-harvesting complexes of photosynthetic machinery. In addition to cyanobacteria, they are found in eukaryotic algae, such as red algae and cryptomonads (Grossman et al., 1993). The major classes of phycobiliproteins are phycocyanin (PC, blue pigment), allophycocyanin (APC, bluish green pigment) and phycoerythrin (PE, red pigment), exhibiting maximum absorbance (A_max) at 620nm, 650nm and 565 nm, respectively. Phycobiliproteins are organized into supramolecular complexes, called phycobilisomes (PBS), which are attached to the cytoplasmic surface of thylakoid membranes in cyanobacteria (Grossman et al., 1993). In cyanobacteria, phycobiliproteins may comprise up to 40% of total soluble protein content. Phycobiliproteins possess a wide spectrum of actual and/or potential biotechnological applications, for instance, nutraceuticals and pharmaceuticals, food industry, cosmetics, biomedical research and clinical diagnostics. Screening of 297 patents on phycobiliproteins searched from global patent databases, revealed 51 patents on phycobiliprotein production, 30 patents on their application in food industry, medicine, cosmetics and other fields, and 216 patents on their fluorescent based applications (Sekar and Chandramohan, 2008). Although numerous reports have revealed the potential of various cyanobacterial species for commercial production of phycobiliproteins, the species of *Spirulina (Arthrospira)* are largely exploited at commercial scale. (Takano et al., 1995; Chen et al., 1996; Chaneva et al., 2007). Because of the diverse applications of cyanobacterial phycobiliproteins, mass culture of promising cyanobacterial species, and the extraction and purification of phycobiliproteins have achieved industrial dimensions in many countries. Many private companies have established research and development wing focussing on commercial exploitation of phycobiliproteins (Thajuddin and Subramanian, 2005).

**Biotechnological Applications of Phycobiliproteins**

**Nutraceuticals and pharmaceuticals**

The term nutraceutical was first coined by Stephen DeFelice in 1989 from the word ‘nutrition’ and ‘pharmaceutical’ that can
be defined as a food or part of a food that provides medical or health benefits, including the prevention and/or treatment of a disease (Brower, 1998; Zeisel, 1999). A large number of cyanobacteria-derived natural products have been proved to be pharmacologically important as potential chemotherapeutic agents (Namikoshi and Rinehart, 1996; Skulberg, 2000; Singh et al., 2005). Phycobiliproteins, particularly phycocyanin, from various cyanobacterial species have been reported to exhibit a variety of pharmacological activities, such as antioxidant, anticancerous, neuroprotective, anti-inflammatory, hepatoprotective and hypocholesterolemic (Rimbau et al., 1999; Liu et al., 2000; Romay et al., 1998, 2003; Nagaoka et al., 2005; Cherung et al., 2007). Reactive oxygen species (ROS), such as singlet oxygen ($^1$O$_2$), superoxide radical (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$) are continuously produced in aerobic organisms as result of cellular metabolism, and removed enzymatically and non-enzymatically by cellular antioxidant defence system. However, overproduction of ROS leads to oxidative stress. Being highly reactive, ROS oxidise biomolecules, such as proteins, lipids and DNA. In humans, they are implicated in several pathophysiological conditions or diseases, such as cancer, diabetes, arthritis, inflammation, genotoxicity, liver damage, neurodegeneration, arteriosclerosis and ageing (Kehrer, 1993; Kohen and Nyska, 2002; Wiseman and Halliwell, 1996). Most of the synthetic antioxidants, which are in use, are known to have side effects, such as carcinogenesis and liver damage. Antioxidants from natural sources are gaining importance as safe and effective alternative to synthetic antioxidants. The antioxidant and radical scavenging activities of phycocyanin from different cyanobacteria are well documented (Romay et al., 1998; Bhat and Madyastha, 2000; Benedetti et al., 2004; Bermejo et al., 2008; Soni et al., 2008). A variety of impaired physiologically conditions have reportedly been relieved in experimental animals by phycocyanin administration (Romay et al., 1998; Riss et al., 2007; Rimbau et al., 1999; Sathyasaikumar et al., 2007; Liu et al., 2000). Phycocyanin has been reported to inhibit cell proliferation (Liu et al., 2000) and induce apoptosis in cancerous cell lines (Roy et al., 2007; Subhashini et al., 2004). Liu et al. (2000) observed the inhibitory effect of phycocyanin from the cyanobacterium Spirulina platensis on the growth of human leukemia K562 cells in a dose-and time-dependent manner. The importance of filamentous cyanobacterium Spirulina platensis (Arthrospira) in human nutrition and health is well-known. It is used as food, feed, dietary supplement and functional food due to its richness in various nutritional components (Cohen, 1997). C-phycocyanin derived from Spirulina platensis exhibited hypocholesterolemic activity in rats, reducing the serum cholesterol concentrations (Nagaoka et al., 2005). Phycocyanin also exerts hepatoprotective and anti-inflammatory effects in a human hepatitis animal model. It reduced alanine amino transferase (ALT), aspartate amino transferase (AST) and malondialdehyde (MDA) in the serum (Gonzalez et al., 2003).

**Natural colorants for food and cosmetics**

The use of phycobiliproteins as non-toxic and non-carcinogenic natural food colorants is gaining importance because of the potential toxicity and carcinogenicity of the currently used synthetic food colorants (Cohen, 1986; Mille-Claire et
al., 1993; Chaneva et al., 2007). They can be used as natural colorants for food, cosmetic and drug industry, replacing the synthetic colorants (Cohen, 1986). Phycocyanin is extensively used as a colorant rather than phycoerythrin. However, phycoerythrin is mostly used in fluorescent applications. Phycocyanin is used as a natural colorant in food items, such as chewing gum, ice cream, dairy products, soft drinks (e.g. Pepsi® blue), soft candies and jellies as well as in cosmetics, such as lipsticks, eyeliners and eye shadows (Santiago-Santos et al., 2004; Jespersen et al., 2005; Sekar and Chandramohan, 2008). C-phycocyanin from Spirulina platensis is marketed as a food and cosmetics colorant in Japan (Prasanna et al., 2007). Linablue, a phycocyanin product, is produced commercially by Dainippon Ink and Chemicals, Tokyo, Japan to be used as food colorant and cosmetics (Dainippon Ink and Chemicals, 1985). The use of any natural colorant in food items requires a detailed knowledge of its colour stability, particularly towards heat, light and pH. A few studies have addressed the colour stability (Jespersen et al., 2005; Mishra et al., 2008; Chaiklahan et al., 2012) and rheological properties (Batista et al., 2006) of phycocyanin. Phycocyanin is unstable to heat and light in aqueous solution. It is insoluble in acidic solution (pH 3) and denatures at temperature above 45 °C at pH 5 and 7, leading to colour change (Jespersen et al., 2005). The use of phycocyanin as a natural colorant is limited by its lower stability to heat and light (Jespersen et al., 2005). In a recent study, phycocyanin was considered a more versatile blue colorant than gardenia and indigo, providing a bright blue colour in jelly gum and coated soft candy, despite its lower stability towards heat and light (Jespersen et al., 2005).

Fluorescent agent

Phycobiliproteins are brilliantly coloured and highly fluorescent pigments. The colours of the phycobiliproteins arise from the presence of chromophores- bilins, which are covalently attached to cysteine residues of the apoproteins. The bilins are linear or open-chain tetrapyrroles derived biosynthetically from heme via biliverdin (Beale and Cornejo, 1991). The visible absorption spectra of individual phycobiliproteins arise from the particular bilins attached to the protein and modulated by the conformation, environment and interchromophore interactions (Glazer, 1994). Due to their unique fluorescent properties, phycobiliproteins find applications in flow cytometry, fluorescent immunoassays and fluorescence microscopy for diagnostics and biomedical research (Glazer, 1994). Moreover, they can be used as protein markers for electrophoretic techniques (Araoz et al., 1998). High molar extinction coefficients and fluorescence quantum yields, large Stokes shift, and immunity from quenching by naturally occurring biological substances are some of the properties of phycobiliproteins which make them interesting and unique fluorophores or fluorochromes for varied applications in fluorescence-based detection systems (Sekar and Chandramohan, 2008; Kronick and Grossman, 1983). The apoprotein chains of phycobiliproteins contain amino and carboxyl groups that can form bonds with other molecules (Glazer and Stryer, 1984; Glazer, 1994; Sun et al., 2003). Phycobiliproteins conjugated to molecules possessing biological specificity, such as immunoglobulins, avidin and biotin are excellent reagents for two color fluorescence analysis of single cells using fluorescence activated cell sorter (FACS).
(Oi et al., 1982). The stabilized phycobilisomes, designated as PBXL-3L, can be used as a fluorochromes for extracellular antigen detection by flow cytometry (Telford et al., 2001). Phycoerythrin is the most widely used fluorescent probes, since it is isolated as $\alpha_6\beta_6$ hexamers (Glazer, 1994) and has fluorescence quantum yields of 82–98% (Oi et al., 1982). Phycobiliproteins bound to monoclonal and polyclonal antibodies are valuable fluorescent markers for cell sorting and investigation of the surface cell antigens (Sun et al., 2003).

Discussion

Cyanobacteria are one of the richest resources of novel bioactive compounds and high-value products, including phycobiliproteins. The content and composition of phycobiliproteins in cyanobacteria vary with species, and are influenced by nutrient availability and environmental factors, such as light, temperature, water and pH. The yield of phycobiliproteins can be maximized by controlling or optimizing the nutrient and environmental factors. The interesting and attracting features which make cyanobacteria a suitable bioresource for the commercial production of phycobiliproteins include rapid growth rate, simple growth requirements, amenability to controlled laboratory culture and ubiquity. The number of cyanobacterial species that are presently used for the commercial production of phycobiliproteins is very small. Bioprospecting of cyanobacteria from unexplored habitats and their screening for the production of phycobiliproteins is imperative and desired to meet the ever-increasing demand. Cyanobacterial biomass production at commercial scale can be achieved in open pond system or in closed photobioreactor, each with its own merits and demerits.

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