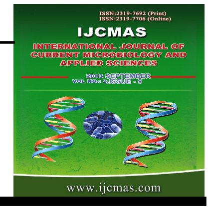
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 9 (2013) pp. 89-97**
http://www.ijcmas.com



Review Article

Biotechnological applications of cyanobacterial phycobiliproteins

V.D. Pandey*, Anita Pandey and Vibhu Sharma

Department of Botany, Government Post-Graduate College
Rishikesh-249201(Uttarakhand), India
**Corresponding author e-mail: vdpandey2@yahoo.co.in*

A B S T R A C T

Keywords

Cyanobacteria; Phyco-biliproteins; Phycocyanin; Allophyco-cyanin; Phycoerythrin; Food colorants; Pharmacological activities.

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.

Introduction

Cyanobacteria (Blue-green algae) are a morphologically diverse and widely distributed group of photosynthetic prokaryotes which exhibit oxygenic (O_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 and efficient biological system. 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; Cherng *et al*., 2007). Reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_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 ^{o}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.

Acknowledgement

The financial assistance provided by Uttarakhand State Council for Science and Technology, Dehradun for the study on cyanobacterial phycobiliproteins is gratefully acknowledged.

References

Abed, R.M.M., S. Dobretsov and Sudesh, K. 2009. Applications of cyanobacteria in biotechnology. J. Appl. Microbiol. 106: 1-12.

Araoz, R., M. Lebert and Hader, D.P. 1998. Electrophoretic applications of phycobiliproteins. Electrophoresis 19: 215-219.

Barrios-Llerena, M.E., A.M. Burja and Wright, P.C. 2007. Genetic analysis of polyketide synthase and peptide synthetase genes in cyanobacteria as a mining tool for secondary metabolites. J. Ind. Microbiol. Biotechnol. 34: 443-456.

Batista, A.P., A. Raymundo, I. Sousa and Empis, J. 2006. Rheological characterization of coloured oil-in-water food emulsions with lutein and phycocyanin added to the oil and aqueous phases. Food Hydrocoll. 20: 44-52.

Beale, S.I., and Cornejo, J. 1991. Biosynthesis of phycobilins: ferredoxin-mediated reduction of biliverdin catalyzed by extracts of *Cyanidium caldarium*. J. Biol. Chem. 266(33): 22328-22332.

Benedetti, S., F. Benvenutti, S. Pagliarani, S. Francogli, S. Scoglio and Canestrari, F. 2004. Antioxidant properties of a novel phycocyanin extract from the blue-green alga

Aphanizomenon flos-aquae. Life Sci.75: 2353-2362.

Bermejo, P., E. Piñero and Villar, Á.M. 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chem. 110: 436-445.

Bhat, V.B., and Madyastha, K.M. 2000. C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. Biochem. Biophys. Res. Comm. 275: 20-25.

Brower, V., 1998. Nutraceuticals: poised for a healthy slice of the healthcare market? Nat. Biotechnol.16: 728-731.

Chaiklahan, R., N. Chirasuwan and Bunnag, B. 2012. Stability of phycocyanin extracted from *Spirulina* sp.: influence of temperature, pH and preservatives. Process Biochem. 47: 659-664.

Chaneva, G., S. Furnadzhieva, K. Minkova and Lukavsky, J. 2007. Effect of light and temperature on the cyanobacterium *Arthronema africanum*- a prospective phycobiliprotein-producing strain. J. Appl. Phycol. 19: 537-544.

Chen, F., Y. Zhang and Guo, S. 1996. Growth and phycocyanin formation of *Spirulina platensis* in photohetrotrophic culture. Biotechnol. Lett. 18: 603-608.

Cherng, S-C., S-N. Cheng, A.Tarn and Chou, T-C. 2007. Anti-inflammatory activity of c-phycocyanin in lipopolysaccharide-stimulated RAW 264.7 macrophages. Life Sci. 81: 1431-1435.

Cohen, Z., 1986. Products from microalgae. In: Richmond, A. (Ed.), CRC Handbook of Microalgae Mass Culture, CRC Press, Boca Raton, Florida, USA, pp.421-454.

Cohen, Z., 1997. The chemicals of *Spirulina*: In: Vonshak, A. (Ed.), *Spirulina platensis* (*Arthrospira*): Physiology, Cell Biology and Biotechnology, Taylor and Francis Ltd. pp. 175-204.

Dainippon Ink and Chemicals.1985. Linablue A (Natural blue colorant of *Spirulina* origin),Technical Information, Dainippon Ink and Chemicals, Tokyo, Japan.

Eriksen, N.T., 2008. Production of phycocyanin- a pigment with applications in biology, biotechnology, foods and medicine. Appl. Microbiol. Biotechnol. 80: 1-14.

Fischer, W. W., 2008. Life before the rise of oxygen. Nature 455: 1051-1052.

Glazer, A.N., 1994. Phycobiliproteins- a family of valuable, widely used fluorophores. J. Appl. Phycol. 6:105-112.

Glazer, A.N., and Stryer, L. 1984. Phycofluor probes. Trends Biochem. Sci. 9: 423-427.

Gonzalez, R., A. Gonzalez and Remirez, D. 2003. Protective effects of phycocyanin on galactosamine - induced hepatitis in rats. Biotecnol. Aplicada. 20: 107-110.

Grossman, A., M.R. Schaefer, G.G. Chiang and Collier J.L. 1993. The phycobilisomes, a light-harvesting complex responsive to environmental conditions. Microbiol. Rev. 57(3): 725-749.

Jespersen, L., L.D. Strømdahl, K. Olsen and Skibsted, L.H. 2005. Heat and light stability of three natural blue colorants for use in confectionery and beverages. Eur. Food Res. Technol. 220: 261-266.

Kehrer, J.P. 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol. 23(1): 21-48.

Kohen, R., and Nyska, A. 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic Pathol. 30: 620-650.

Kronick, M.N., and Grossman, P.D. 1983. Immunoassay techniques with fluorescent phycobiliprotein conjugates. Clin. Chem. 29(9):1582-1588.

Liu, Y., L. Xu, N. Cheng, L. Lin and Zhang, C. 2000. Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells. J. Appl. Phycol. 12: 125-130.

Mille-Claire, C., D. Mille, Y. De-Roeck-Holtzhauer and Roeck, H. 1993. Orange- red colorant comprising a type1 R-phycoerythrin, its manufacture and use. French Patent application 2690452A1,FR92-05333(19920423).

Mishra, S.K., A. Shrivastav and Mishra, S. 2008. Effects of preservatives for food grade C-PC from *Spirulina platensis*. Process Biochem. 43: 339-345.

Nagaoka, S., K. Shimizu, H. Kaneko, F. Shibayama, K. Morikawa, Y. Kanamaru, A. Otsuka, T. Hirahashi and Kato, T. 2005. A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats. J. Nutr. 135: 2425-2430.

Namikoshi, M., and Rinehart, K.L.1996. Bioactive compounds produced by cyanobacteria. J. Indust. Microbiol. 17: 373-384.

Oi, V.T., A.N. Glazer and Stryer, L. 1982. Fluorescent phycobiliproteins conjugates for analysis of cells and molecules. J. Cell Biol. 93: 981-986.

Oren, A., 2000. Salts and brines. In: Whitton, B.A. and Potts, M. (Eds.), The Ecology of Cyanobacteria: Their Diversity in Time and Space, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 281-306.

Pandey, K.D., A.K. Kashyap and Gupta, R. K. 1995. Nutrient status, algal and cyanobacterial flora of six streams of Schirmacher Oasis, Antarctica. Hydrobiologia 299: 83-91.

Pandey, V.D. 2010. Bioremediation of heavy metals by microalgae. In: Das M.K. (Ed.), Algal Biotechnology, Daya Publishing House, Delhi, pp. 287-296.

Pandey, V.D., R.K. Gupta and Singh, S.K. 2007. Cyanobacteria as a source of pharmaceutical compounds. In: Gupta, R. K. and Pandey, V.D.(Eds.), Advances in Applied Phycology, Daya Publishing House, Delhi, pp. 250-260.

Patterson, G.M.L., 1996. Biotechnological applications of cyanobacteria. JSIR 55: 669-684.

Prasanna, R., A. Sood, A. Suresh and Kaushik, B.D. 2007. Potentials and applications of algal pigments in biology and industry. Acta Bot. Hung. 49: 131-156.

Rimbau, V., A. Camins, C. Romay, R. González and Pallàs, M. 1999. Protective effects of C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus. Neurosci Lett. 276: 75-78.

Riss, J., K. Décordé, T. Sutra, M. Delage, J-C. Baccou, N. Jouy, J-P. Brune, M. Oréal, J-P. Cristol and Rouanet, J-M. 2007. Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. J. Agric. Food. Chem. 55: 7962-7967.

Romay, C., J. Armesto, D. Remirez, R. González, N. Ledon and García, I. 1998. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. Inflamm. Res. 47: 36-41.

Romay, C., R. González, N. Ledon, D. Remirez and Rimbau, V. 2003. C-Phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr. Protein and Peptide Sci. 4: 207-216.

Roy, K.R., K.M. Arunasree, N.P. Reddy, B. Dheeraj, G.V. Reddy and Reddanna, P. 2007. Alteration of mitochondrial membrane potential by *Spirulina platensis* C-phycocyanin induces apoptosis in the doxorubicin-resistant human hepatocellular-carcinoma cell line HepG2. Biotechnol. Appl. Biochem. 47: 159-167.

Santiago-Santos, M.C., P.T. Nayalo, R. Olvera-Ramirez, J. Ortega-Lopez and Canizares-Villanueva, R.O. 2004. Extraction and purification of phycocyanin from *Calothrix* spp. Process Biochem. 39 (12): 2047-2052.

Sathyasaikumar, K.V., I. Swapna, P.V.B. Reddy, C.R.K. Murthy, K.R. Roy, D. Gupta, B. Senthilkumaran and Reddanna, P. 2007. Co-administration of C-phycocyanin ameliorates thioacetamide-induced hepatic encephalopathy in Wistar rats. J. Neurol. Sci. 252: 67-75.

Sekar, S., and Chandramohan, M. 2008. Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J. Appl. Phycol. 20:113-136.

Singh, S., K.N. Bhusan and Banerjee, U.C. 2005. Bioactive compounds from cyanobacteria and microalgae: an overview. Crit. Rev. Biotechnol. 25(3): 73-95.

Skulberg, O.M., 2000. Microalgae as a source of bioactive molecules-experience from cyanophyte research. J. Appl. Phycol. 12: 341-348.

Soni, B., U. Trivedi and Madamwar, D. 2008. A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property. Bioresour. Technol. 99: 188-194.

Stanier, R. Y., and Cohen-Bazire, G. 1977. Phototrophic prokaryotes: the cyanobacteria. Ann. Rev. Microbiol. 31: 225-274.

Subhashini, J., V.K. Mahipal, M.C. Reddy, M.M. Reddy, A. Rachamallu and Reddanna, P. 2004. Molecular mechanisms in C-phycocyanin induced apoptosis in human chronic myeloid leukemia cell line- K562. Biochem. Pharmacol. 68: 453-462.

Sun, L., S. Wang, L. Chen and Gong, X. 2003. Promising fluorescent probes from phycobiliproteins. IEEE J Sel Top Quantum Electron 9: 177-188.

Takano, H., T. Arai, M. Hirano and Matsunaga, T. 1995. Effect of intensity and quality of light on phycocyanin production by a marine cyanobacterium *Synechococcus* sp.NKBG042902. Appl. Microbiol. Biotechnol. 43: 1014-1018.

Tan, L.T., 2007. Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 68: 954-979.

Tandeau de Marsac, N., and Houmard J.1993. Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol. Lett. 104: 119-189.

Telford, W.G., M.W. Moss, J.P. Moreseman and Allnutt F.C. 2001. Cyanobacterial stabilized phycobilisomes as fluorochromes for extracellular antigen detection by flow cytometry. J. Immunol. Meth. 254: 13-30.

Thajuddin, N., and Subramanian, G. 2005. Cyanobacterial biodiversity and potential applications in biotechnology. Curr. Sci. 89: 47-57.

Tomitani, A., A.H. Knoll, C.M. Cavanaugh and Ohno, T. 2006. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. PNAS 103(14): 5442-5447.

Van Den Hoek, C., D.G. Mann and Johns, H.M. 1995. Algae: An Introduction to Phycolgy, Cambridge University Press, Cambridge, pp. 623.

Ward, D. M., and Castenholz, R.W. 2000. Cyanobacteria in geothermal habitats. In: Whitton, B. A. and Potts, M (Eds.), The Ecology of Cyanobacteria:Their Diversity in Time and Space, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 37-59.

Waterbury, J. B., S.W. Watson, R.R.L. Guillard and Brand, L.E. 1979. Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 227: 293-294.

Wiseman, H., and Halliwell, B.1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem. J. 313: 17-29.

Wynn-Williams, D.D., 2000. Cyanobacteria in deserts-Life at the limit: In: Whitton, B. A. and Potts, M (Eds.), The Ecology of Cyanobacteria: Their diversity in Time and Space, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 341-366.

Zakhia, F., A-D. Jungblunt, A. Taton, W.F. Vincent and Wilmotte, A. 2008. Cyanobacteria in cold ecosystems. In: Margesin, R., Schinner, F., Marx, J-C. and Gerday, C.(Eds.), Psychrophiles: From Biodiversity to Biotechnology, Springer-Verlag, Berlin Heidelberg, pp. 121-135.

Zeisel, S.H., 1999. Regulation of nutraceuticals. Science 285: 1853-1855.