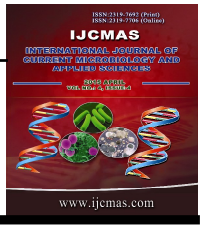
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 568-580

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 568-580**
http://www.ijcmas.com



Original Research Article

Potential Cyanobacteria from Loktak Lake, a Freshwater Lake in North-East Region of India as Source of Phycobiliprotein, a Natural Colourant

Keithellakpam Ojit Singh*, Minerva Shamjetshabam, Oinam Avijeet Singh, Thingujam Indrama and Onkar Nath Tiwari

Freshwater Cyanobacterial and Microalgal Repository, Microbial Resources Division, Institute of Bioresources and Sustainable Development, (An autonomous Institute under the DBT, Govt. of India), Takyelpat, Imphal-795001, Manipur, India
**Corresponding author*

Keywords

Cyanobacteria, Freshwater, Loktak lake, Natural colours, Phycobili-proteins

A B S T R A C T

There is an increasing demand for natural colours which are of use in food, pharmaceuticals, cosmetics, textiles and as printing dyes. Due to the toxic effect of several synthetic dyes, there is an increasing preference to use natural colours for various end uses. In this study, five (05) unialgal cyanobacterial strains of *Nostoc* sp. from freshwater Loktak lake were characterized. In order to determine production of phycobiliproteins in cyanobacterial strains, the quantity of these pigments in 05 heterocystous filamentous cyanobacteria was investigated. The analysis showed that the contents of phycobiliproteins depended not only on the strain, but also on the composition of growth media. Higher pigment content was found in strains which was grown in nitrogen free medium compared to strains with nitrogen medium. The highest content of phycocyanin was found in *Nostoc* strain BTA67 which was grown in both nitrogen free and nitrogen medium (95.42 µg ml^{-1} and 79.00 µg ml^{-1} respectively). The *Nostoc* strain BTA67 also had the highest content of allophycocyanin growing in both type of nutritious media (129.51 µg ml^{-1} and 97.84 µg ml^{-1} respectively). The highest phycoerythrin concentration was characterized the strain *Nostoc* BTA in nitrogen free medium (126.90 µg ml^{-1}) and in presence of nitrogen (95.98 µg ml^{-1}). The results of total phycobiliprotein content of tested cyanobacterial strains showed that the highest content in nitrogen free condition characterized *Nostoc* strain BTA61 (202.56 µg ml^{-1}) and *Nostoc* strain BTA67 (254.44 µg ml^{-1}) and *Anabaena* strain BTA964 (173.29 µg ml^{-1}). Therefore, studied strains of *Anabaena* and *Nostoc* genera represent excellent sources of one or more phycobiliproteins.

Introduction

Phycobiliproteins were used as a natural protein dye in the food industry (C-phycocyanin) and in the cosmetic industry (Santiago-Santos et al., 2004). Natural colourants for food were made from renewable sources. Natural colourants such as phycobiliproteins are gaining importance over synthetic ones as they are non-toxic and non-carcinogenic. Cyanobacteria, as specific group of microorganisms represent a potential source of commercially important chemicals and pharmaceutical products. Among them, phycobiliproteins were very interesting cell constituents with high commercial value. Because of their protein nature, unique colour, fluorescence and other properties a wide range of promising applications of phycobiliproteins are possible (Zhao et al., 1995; Rossano et al., 2003; Sekar and Chandramohan, 2008). Due to the toxic and possible cancer genetic effects of several synthetic dyes, there is an increasing preference to use natural colours such as phycobiliproteins (Sekar and Chandramohan, 2008). These pigments can

be used as natural colourants in food and drug industry and in cosmetic preparation replacing the synthetic dyes (Cohen-Bazire and Bryant, 1982; Soni et al., 2006).

Cyanobacteria are one of the potential organisms, which are useful to mankind in various ways. (Mitsui et al., 1981; Prabhaharan and Subramanian, 1995; Gustafson et al., 1989; Sundararaman et al., 1996; Subramanian and Uma, 1996). At present, cyanobacteria generally remain as potential sources for further investigations as prospective and excellent sources of biologically active constituents produced during primary and especially secondary metabolism (Skulberg, 2000). On account of their immense applied biotechnological potential, they are being explored widely. They are often referred as 'miniature factories' of the biological world and represent an alternative source of a variety of bioactive compounds, lipids/fatty acids, proteins, enzymes, pigments and compounds of pharmaceutical and nutraceutical value (Schaeffer and Krylov, 2000; Rastogi and Sinha, 2009). Phycobiliproteins have been widely used in industry, cosmetics and clinical or research immunological laboratories as labels for antibodies, receptors and other biological molecules (Telford et al., 2001).

Cyanobacterial phycobiliproteins have gained importance in the commercial sector as they have several applications. The primary potential of these molecules are as natural dyes but a number of investigations have shown on their health-promoting properties and broad range of pharmaceutical applications. Thus, one of the application of phycocyanin is to use as food pigment replacing current synthetic pigments. In addition, phycobiliproteins are widely used in clinical and immunological research laboratories (Spolaore et al., 2006).

Pioneering work of the last decades has raised the status of these microbes to a level where they are being viewed with favour in biotechnologically relevant spheres. Intensive research is thus warranted to understand many of the basic aspects pertaining to the production of a metabolite with the concurrent evolution of applied research towards the large production of the products. Filamentous cyanobacteria are particularly attractive for the photoproduction of phycobiliproteins and other chemicals (Borowitzka, 1995). Nevertheless, studies on cyanobacteria from Loktak lake of the state still remain largely unexplored. The aim of the present investigation was to screen five (05) strains of heterocystous filamentous cyanobacteria isolated from Loktak lake for their potential as producers of phycobiliprotein pigments.

Materials and Methods

Cyanobacterial strains

Cyanobacterial strains used in this study were isolated from freshwater Loktak lake, Manipur, India (location of Loktak lake as shown in Fig. 1). The study of morphology of the strains was carried out using trinocular research microscope (NIKON Eclipse 80*i*, Japan) and Carl Zeiss fluorescence microscope, Axio Scope A1 coupled with Carl Zeiss Imaging Systems 32 software AxioVision 4.7.2 followed by taxonomical characterization referring to key given by Desikachary (1959).

Growth conditions

Cyanobacteria strains (log phase of growth) were homogenized and 1 ml of the culture was inoculated into 250 ml cotton-plugged Erlenmeyer flasks containing 100 ml of BG11 medium (with and without nitrogen) (Stanier et al., 1971) and kept in the culture

room. The culture room rack was fitted with photoperiodic automatic model timer coupled with Biotech room temperature controller to provide alternative light and dark phases. The strains were allowed to grow in light intensity of 40 µmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes following light:dark cycles of 14:10 h condition maintained at 28±2ºC. The flasks were stirred twice daily to allow uniform light penetration and circulation of air and nutrients. After 15 days of incubation, 10 ml of samples was taken and centrifuged at 6000 rpm for 10 min. The collected biomass was then washed with buffer 0.05 M phosphate buffer (pH 7.0).

One volume of biomass was then re-suspended in five times of the volume of the same buffer followed by repeated freezing (-20ºC) and thawing (4ºC) shocks for the release of phycobiliproteins. The above procedure was continued till coloured supernatant was obtained from the biomass. The cell debris was removed by centrifugation at 12,000 rpm for 10 min. The absorbance of phycobiliprotein containing cell-free supernatants obtained by the centrifugation was measured at 562 nm, 615 nm and 652 nm using phosphate buffer as a blank. These wavelengths correspond to the absorption maxima of phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) respectively and was determined in spectrophotometer (Shimadzu, UV-1800, Japan) using the following formula given by Bennett and Bogorod (1973).

PC (µg ml^{-1}) = (A_{615}-0.474 x A_{652})/ 5.34
APC (µg ml^{-1}) = (A_{652}-0.208 x A_{615})/ 5.09
PE (µg ml^{-1}) = [A_{562}-{2.41(PC)}-{0.849(APC)}]/9.62

All experiments were performed in triplicates.

Result and Discussion

The strains of cyanobacteria investigated in this study and their morphological characteristics were listed (Table 1; photoplate 1). A total of five (05) cyanobacterial strains comprising four (04) strains of *Nostoc* and one (01) strain of *Anabaena* were isolated from freshwater habitats of Loktak lake. Phycoerythrin content was found to be higher in almost all strains during their growth in nitrogen free medium (Fig. 2). Concentration of PE of strains grown in nitrogen free medium varied from 3.59 µg ml^{-1} (*Anabaena* strain BTA964) to 126.90 µg ml^{-1} (*Nostoc* strain BTA61). Other *Nostoc* strains BTA80, BTA60 and *Nostoc muscorum* BTA 67 were also showed high production of PE in nitrogen free medium with values of 44.79, 35.91 and 29.51 µg ml^{-1} (Fig. 2). Still *Nostoc* sp. BTA61 had showed high PE content (95.98 µg ml^{-1}) whereas *Anabaena* strain BTA964 had least content of PE (1.15 µg ml^{-1}) in the presence of nitrogen (Fig. 2)

PC concentrations in investigated cyanobacteria showed that higher contents of this pigment were found in most strains growing in nitrogen free medium, compared to presence of nitrogen (Fig. 3). The lowest content was characterized *Nostoc* strain BTA80 (25.72 µg ml^{-1}), while the highest content of PC was found in *Anabaena* strain BTA964 (134.79 µg ml^{-1}) when grown in nitrogen free medium. Also high production of PC in nitrogen free condition was detected in *Nostoc muscorum* BTA67, *Nostoc* strain BTA60 and *Nostoc* strain BTA61 with values of 95.42, 63.14 and 61.46 µg ml^{-1}respectively. Growing in presence of nitrogen (Fig. 3), still *Anabaena* strain BTA964 contained the highest PC amount of 91.06 µg ml^{-1} and *Nostoc* strain BTA80 had least content of PC (7.65 µg ml^{-1}).

Allophycocyanin content was higher in almost all strains growing in nitrogen free medium than during their growth in medium with nitrogen (Fig. 4). *Nostoc muscorum* BTA67 had the highest amount of APC (129.51 µg ml^{-1}) and *Nostoc* strain BTA80 had the least (4.87 µg ml^{-1}). Also very high content was found in *Anabaena* strain BTA964 (34.91 µg ml^{-1}). Still *Nostoc muscorum* BTA67 had showed high APC content (97.84 µg ml^{-1}) whereas *Nostoc* strain BTA80 had least content of APC (1.98 µg ml^{-1}) in the presence of nitrogen (Fig 4). Total phycobiliprotein content of the studied cyanobacterial strains showed highest content in nitrogen free medium by *Nostoc muscorum* BTA67 (254.44 µg ml^{-1}), *Nostoc* strain BTA61 (202.56 µg ml^{-1}) and *Anabaena* strain BTA964 (173.29 µg ml^{-1}). The same strains showed the highest content of total phycobiliproteins during their growth in the medium with nitrogen (Fig. 5). The lowest concentration of total phycobiliproteins was found in *Nostoc* strain BTA80 (Fig. 5) both during the growth in nitrogen free medium (75.38 µg ml^{-1}) and in the presence of nitrogen (27.61 µg ml^{-1}). The results showed that qualitative and quantitative content of total and individual phycobilin pigments was different, which clearly shows the existence of specific features in the pigment composition of every examined strain. The analysis of the distribution of phycobiliproteins in all strains showed the presence of all three types of phycobilin pigments-PE, PC and APC in different proportions.

Moreno et al (1995) found that in some strains of *Anabaena* and *Nostoc* genera the prevalent type of phycobiliproteins was C-phycocyanin followed by allophycocyanin with levels of 17 and 11% dry weight respectively while C-phycoerythrin was the major pigment in several *Nostoc* strains showing 10% dry weight. In the present study, all the cyanobacterial strains showed the high content of allophycocyanin (in nitrogen free medium), while the highest content of phycocyanin was found in one *Anabaena* strain in nitrogen free medium and followed by *Nostoc* strains. The highest content of phycoerythrin was characterized in *Nostoc* strain BTA61 in both conditions (free nitrogen and with nitrogen medium)

There were also other information about contents of phycoerythrin, phycocyanin, and allophycocyanin which are found in other cyanobacteria. The study showed that *Fremyella diplosiphon* contains from 35-40 µg ml^{-1} of phycoerythrin while *Calothrix* sp. biomass had 84 µg ml^{-1} of phycocyanin (Santiago-Santos et al., 2004). Colyer et al (2005) found pigment contents of cyanobacteria detected various concentrations of 3 phycobilins in the range of 0.77-19.30 µg ml^{-1} for phycoerythrin, 0.73-18.24 µg ml^{-1} for phycocyanin and 0.20-4.92 µg ml^{-1} for allophycocyanin.

Our findings reported in this paper were comparable to the previous workers (Cohen, 1986; Dainippon Patent, 1980; Dainippon Patent, 1981; Borowitzka, 1994). The results of the present study suggest that some *Nostoc* and *Anabaena* strains had much higher content of phycobiliproteins compared to values found by Jelica et al (2012). Therefore, these filamentous strains represent promising sources of one or more phycobiliproteins.

Nowadays, scientists are motivated to search for more potential species available in nature for exploiting them in a variety of ways to meet the demands. Higher growth rate and nutrient profile of cyanobacteria make them a potentially valuable source of nutrients (Cannell, 1989). Phycobilin pigments was found to be more during early stationary growth phase i.e. cells start excreting metabolites during this stage only. The analysis clearly revealed the need for a

morpho-physiological and molecular approach for cyanobacterial characterization and their utilization in agriculture and industry. Data generated during present investigations could be useful in understanding of a commercial or biotechnological potential of blue-green algae. The results clearly prove that the composition and content of phycobilin pigments are specific characteristics of every individual cyanobacterial strain which is very dependent on growing conditions.

The medium composition influences the normal growth of cyanobacteria and normal development of physiological processes. The conditions of growth particularly nitrogen and carbon sources determines the content of phycobiliproteins in cyanobacteria (Seker and Chandramohan, 2008).

In our study, the highest content of total phycobiliproteins was present in the all strains when cultivated in the conditions without nitrogen. Kaushik (2000) obtained similar results when analyzed the content of phycobiliproteins of 41 strains of cyanobacteria. Hemlata and Fatma (2009) found that *Anabaena* NCCU-9 produced the highest amount of phycobilins in the condition without nitrogen. Loreto et al (2003) showed that the strain *Anabaena* 7120 produced more phycobiliproteins if it was cultivated in the medium without nitrogen in comparison to the growth in presence of nitrogen.

Importance of growth conditions on the pigment composition and production of phycobilins for cyanobacterial species was studied by Patel et al (2005). Prassana et al (2004) pointed out the fact that cyanobacteria can regulate their composition and content of basic unit of phycobilin, tetrapyrrole depending on the conditions or signals from the surroundings such as the availability of nutrients, intensity and quality of light and temperature. In order to find out optimum growth conditions of cyanobacteria. Kumar et al (2011) investigated the effect of light irradiance and temperature on growth rate, biomass composition and pigment production of *Spirulina platensis*. Maximum contents of phycobiliproteins were found in cultures grown at 35ºC i.e. 7.73 % phycocyanin (PC), 3.46% allophycocyanin (APC) and 1.80 % phycoerythrin (PE) and minimum was observed at 20ºC (5.39 % PC, 2.59% APC and 0.64 % PE). But the phycobiliprotein accumulation (except PE) did not show any significant difference at temperatures 30ºC and 35ºC (Kumar et al., 2011).

Cyanobacteria in general possess all the known phycobiliproteins (phycocyanin, phycoerythrin, phycoerythrocyanin). Among them, phycocyanin and phycoerythrin are commercially valuable.

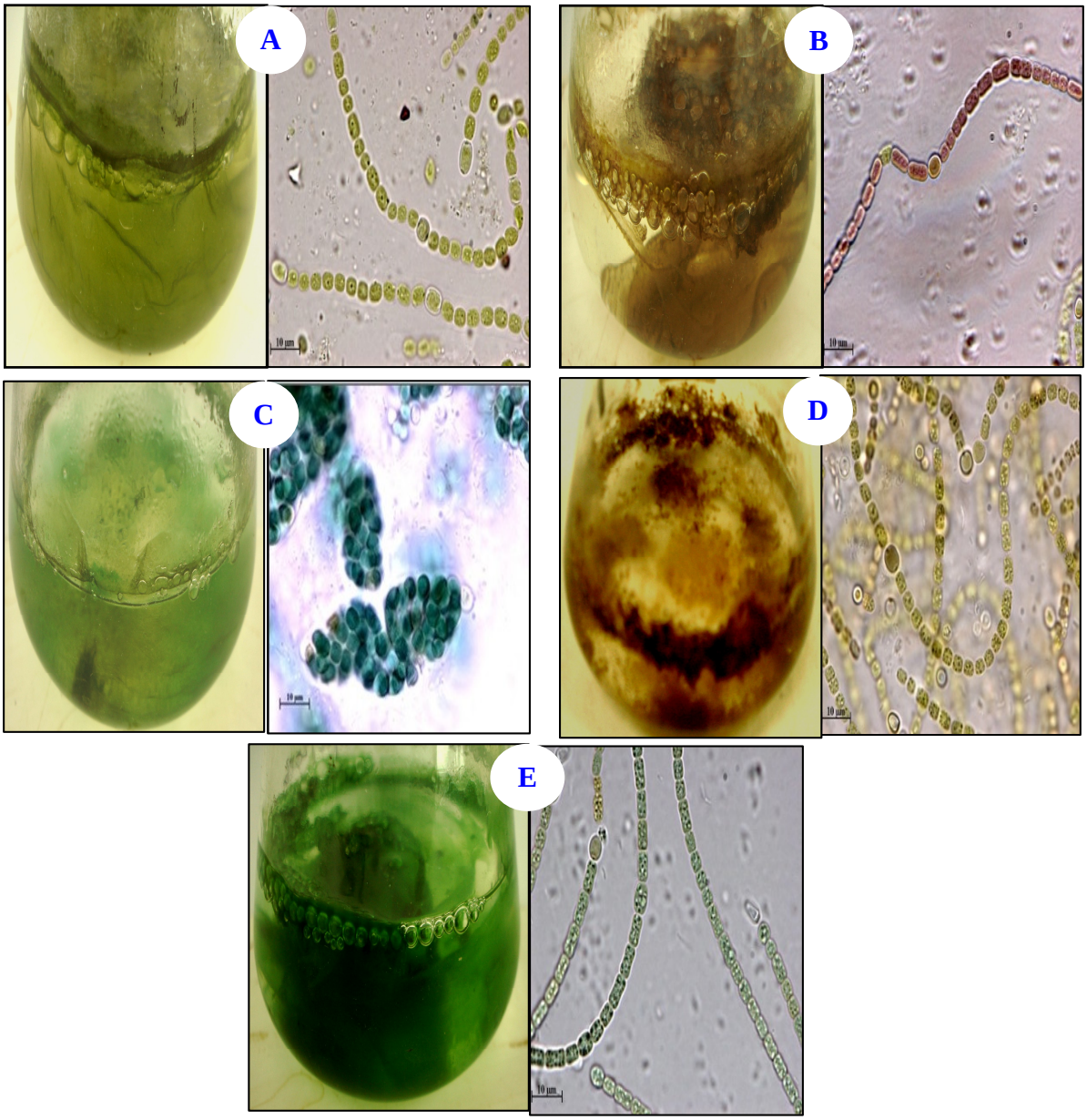
In contrast with marine environment, freshwater sources have been less explored. Our studies will establish the rich cyanobacterial diversity of the region, especially the various niche habitats of Loktak lake and also help conserve and utilize them in bioindustry. Improvement in the phycobilin content with changes of environmental factors (intensity and quality of light, temperature and concentration of nutrients) could be a good basis for the exploitation of studied cyanobacterial strains as a source of biopigments. Since, these isolates were found to contain more of pigments, they are considered as potent candidates for alternative resources of fulfilling gap of colouring agents. Genetic approaches of potential isolates to construct cyanobacterial isolates could be used to improve to natural colouring product from several living microorganisms.

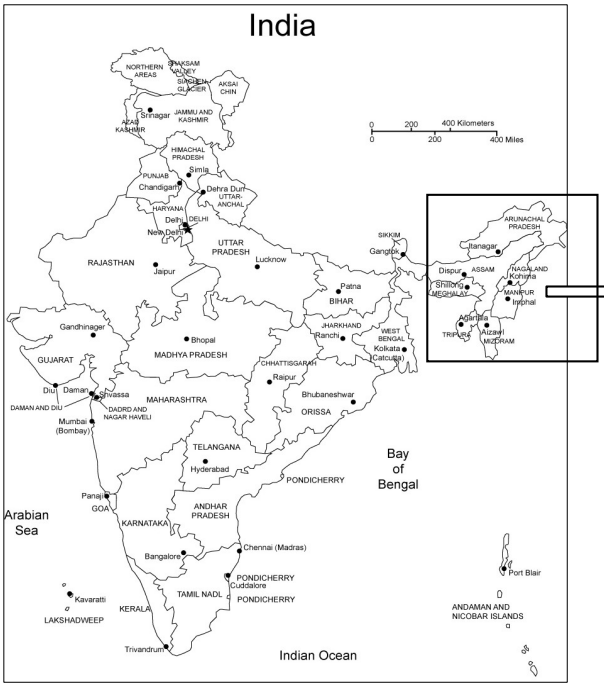
Table.1 Cultural studies and taxonomical characterization of cyanobacterial strains from Loktak lake

Name of the strains	Brief cultures/ taxonomical studies
Nostoc sp. BTA60	Light green, initially bottom attached and later reticulate biomass formation, filamentous, flexuous filament, barrel or spherical cells with both intercalary and terminal heterocysts sub-spherical
Nostoc sp. BTA61	Dark brown, submerged biomass, reticulate and floccose biomass, flexuous filament, cell quadratic with spherical heterocyst
Nostoc commune BTA67	Dark green, bottom attached, floccose biomass, filamentous, filament densely entangled, barrel shape cell spherical shape heterocyst
Nostoc sp. BTA80	Light brown, initially bottom attached and later floating, floccose biomass, filament flexuous, intercalary and terminal spherical heterocyst
Anabaena sp. BTA964	Dark green, submerged, floccose biomass, filament flexuous, hyaline and colourless sheath, barrel shape cell, spherical heterocyst

Photoplate 1

A= Thallus growth and photomicrograph of Nostoc sp. BTA60; B= Thallus growth and photomicrograph of Nostoc sp. BTA61; C= Thallus growth and photomicrograph of Nostoc commune BTA67; D= Thallus growth and photomicrograph of Nostoc sp. BTA80; E= Thallus growth and photomicrograph of Anabaena sp. BTA964





a. Map of India showing North-East region of India

b. Location of Manipur

c. Loktak lake

Fig. 1a,b,c Map of India and location of Loktak lake

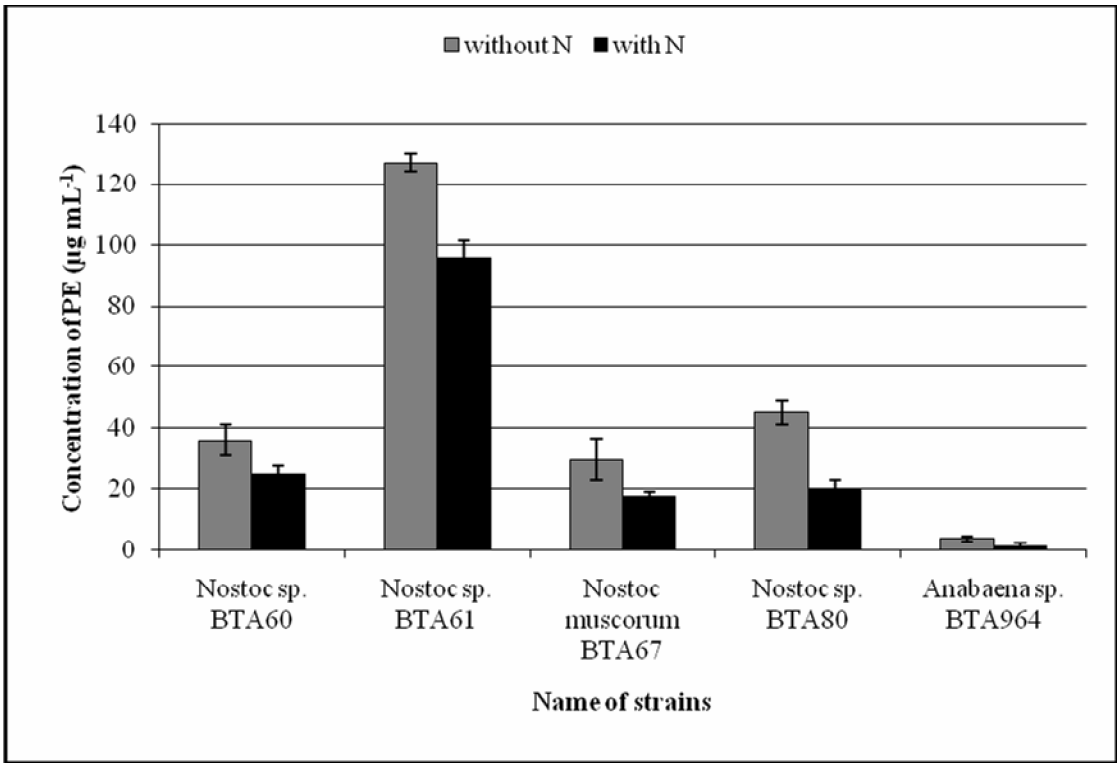


Fig.2 Content of phycoerythrin in cyanobacterial strains of *Nostoc and Anabaena*

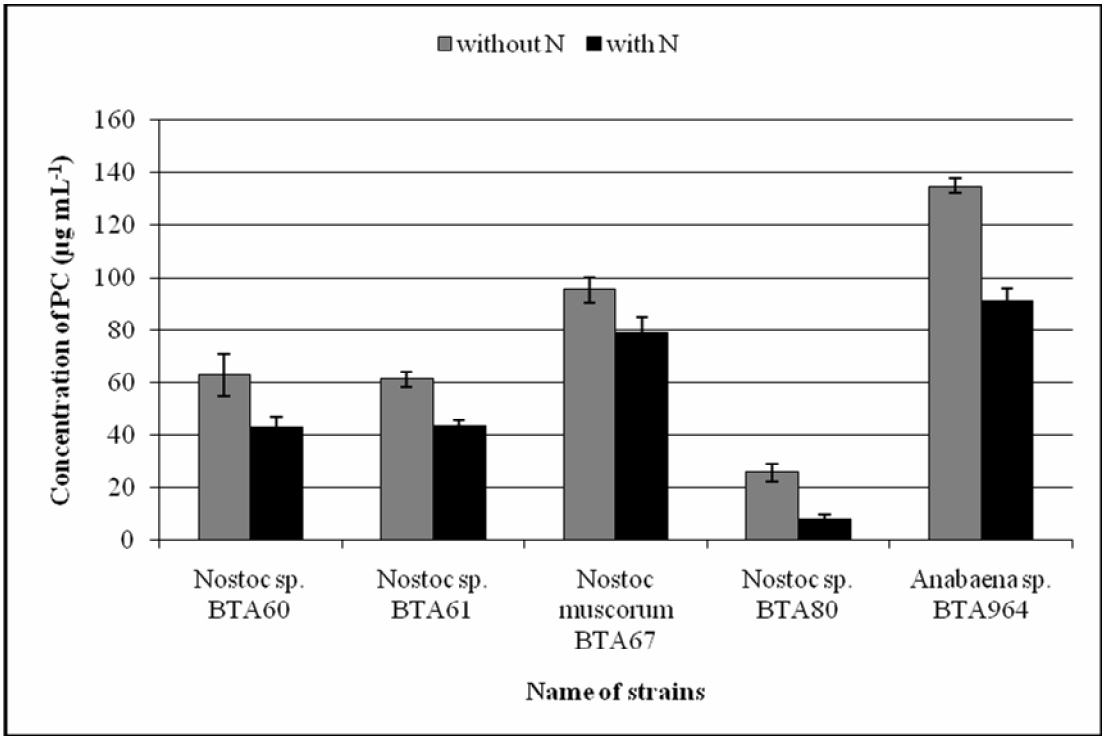


Fig.3 Content of phycocyanin in cyanobacterial strains of *Nostoc and Anabaena*

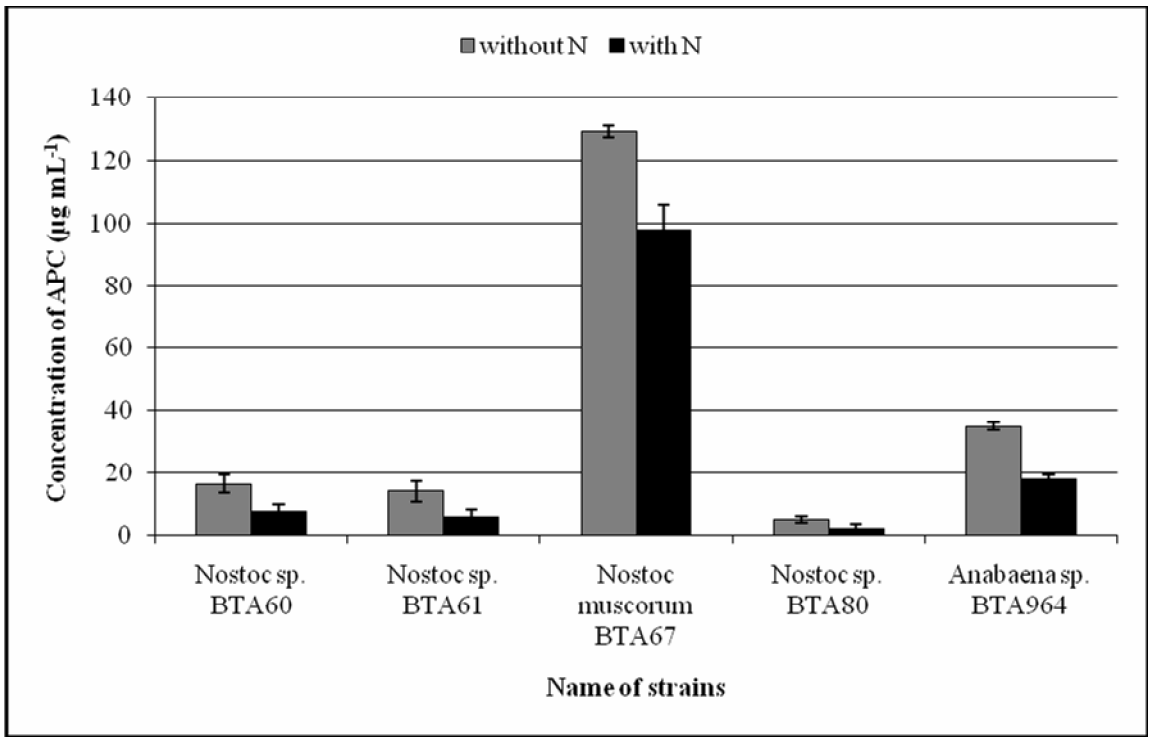


Fig. 4 Content of Allophycocyanin in cyanobacterial strains of *Nostoc and Anabaena*

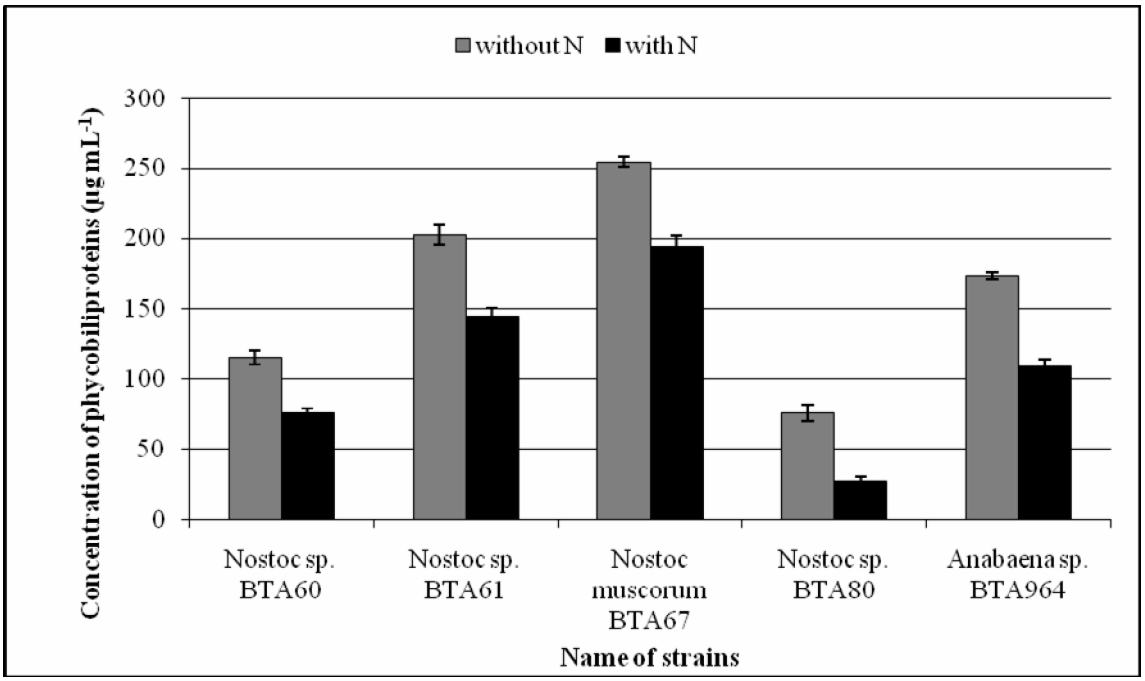


Fig.5 Content of total phycobiliproteins in cyanobacterial strains of *Nostoc and Anabaena*

Basically, many of the metabolites are produced by the organism(s) in low amounts, there is no mass cultivation technology evolved for such potential cyanobacteria and in many cases, the method of industrial extraction is not optimized. Intensive research is thus warranted to understand many of the basic aspects pertaining to the production of a metabolite with the concurrent evolution of applied research towards the large production of the products.

Cyanobacteria are one of the richest resources for novel bioactive compounds and high-value added products including phycobiliproteins. There is increasing interest in the production of these pigments primary because of their food application. In this present study, observation made that niche habitats in Loktak lake, Manipur especially wetlands are promising sources of potent cyanobacterial strains. Cyanobacteria strains such as *Nostoc* strain BTA61, *Nostoc muscorum* BTA67 and *Anabaena* stain BTA964 are very promising producers since they showed the highest content of all three phycobiliproteins. Also, further studies are needed for the need of potent cyanobacteria from this region over the long term and the use of products of biological, non-toxic products from these isolates.

Acknowledgements

The research leading to these results has received funding from the Indian Council of Agricultural Research (ICAR), Government of India (Grant No. NBAIM/ AMAAS /MD(18)/ONT/BG). The authors thank Dr. N. C. Talukdar, Director, IBSD, Imphal, Manipur, India for providing laboratory and required infrastructure. We also thank the labmates of the research group for their help and useful comments on the manuscript.

References

Bennett, A., Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell. Biol*., 58(2): 419-435.

Borowitzka, M.A. (1994). Products from algae. In: Phang, S.M., Lee, K., Borowitzka, M.A., Whitton, B.A. (Eds.), Algal biotechnology in the Asia-Pacific Region. Institute of Advanced Studies, University of Malaya, Kuala Lumpur, pp. 5-15.

Borowitzka, M.A. (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. *J. Appl. Phycol*., 7(1): 3-15.

Cannell, R.J.P. (1989). Algal biotechnology. In: Weetall, H. (Ed.), Applied biochemistry and biotechnology. The Humana Press Inc., New Jersey, pp. 85-105.

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

Cohen-Bazire, G., Bryant, D.A. (1982). Phycobilisomes: composition and structure. In: Carr, N.G., Whitton, B.A. (Eds.), The biology of cyanobacteria, Blackwell Scientific Publications, Oxford, pp. 143-190.

Colyer, C.L., Kinkade, C.S., Viskari, P.J., Landers, J.P. (2005). Analysis of cyanobacterial pigments and proteins by electrophoretic and chromatographic methods. *Anal. Bioanal. Chem*., 382(3): 559-569.

Dainippon Ink and Chemicals Inc. (1980). Production of highly purified alcoholophilic phycocyanin. Japanese Patent 80,77,890.

Dainippon Ink and Chemicals Inc. (1981). Cosmetics containing water soluble

phycocyanin. Japanese Patent 79-138755.

Desikachary, T.V. (1959). Cyanophyta, ICAR, monographs on Algae. Indian Council of Agricultural Research, New Delhi, India.

Gustafson, K.R., Cardellina, J.H., Fuller, R.W., Weislon, O.S., Kiser, R.F., Snader, K.M. (1989). Antiviral sulfolipids from cyanobacteria (blue-green algae). *J. Natl. Cancer. Inst*., 81(16): 1254-1258.

Hemlata, A.E., Fatma, T. (2009). Screening of cyanobacteria for phycobiliproteins and effect of different environmental stress on its yield. *Bull. Environ. Contam. Toxicol*., 83(4): 509-515.

Jelica, B.S., Snezana, B.M., Dajana, J.K., Aleksandra, C.M., Anamarija, I.M., Zorica, B.S. (2012). Filamentous cyanobacteria from Vojvodina region as source of phycobiliprotein pigments as potential natural colorants. *Food Feed Res*., 39(1): 23-31.

Kaushik, B.D. (2000). Screening of cyanobacterial strains for natural colours. *Indian Hydrobiol*., 3: 81-84.

Kumar, M., Jyoti, K., Singh, G.P. (2011). Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. *Braz. J. Microbiol*., 42: 1128-1135.

Loreto, C., Rosales, N., Bermudez, J., Morales, E. (2003). Pigment and protein production of the cyanobacterium *Anabaena* PCC7120 in relation to nitrogen concentration and irradiance. *Gayana Bot*., 60: 83-89.

Mitsui, A., Murray, R., Entenmann, B., Miyazawa, K., Polk, E. (1981). Utilization of marine blue-green algae and macroalgae in warm water mariculture. In: San Pietro, A. (Ed.), Biosaline research-A look to the future. Plenum Press, New York, pp. 215-225.

Patel, A., Sandhya, M., Pawar, R., Ghosh, P.K. (2005). Purification and characterization of C-phycocyanin from cyanobacterial species of marine and freshwater habitat. *Protein Expr. Purif*., 40(2): 248-255.

Prabhaharan, D., Subramanian, G. (1995). Hydrogen photoproduction by marine cyanobacteria *Dicothrix bauriana* BDU 40481. *Physiol. Mol. Biol. Plants*, 1: 45-57.

Prassana, R., Pabby, A., Saxena, S., Singh, P.K. (2004). Modulation of pigment profiles of *Calothrix elenkenii* in response to environmental changes. *J. Plant Physiol*., 161: 1125-1132.

Rastogi, R.P., Sinha, R.P. (2009). Biotechnological and industrial significance of cyanobacterial secondary metabolites. *Biotechnol. Adv*., 27(4): 521-539.

Rossano, R., Ungaro, N., D'Ambrosio, A., Liuzzi, G.M., Riccio, P. (2003). Extracting and purifying R-phycoerythrin from Mediterranean red algae *Corallina elongate*. *J. Biotechnol*., 101: 289-293.

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

Schaeffer, D.J., Krylov, V.S. (2000). Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicol. Environ. Saf*., 45: 208-227.

Sekar, S., Chandramohan, M. (2008). Phycobiliproteins as a commodity: trends in applied research, patents

and commercialization. *J. Appl. Phycol*., 20: 113-136.

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

Soni, B., Beena, K., Ujjval, T., Datta, M. (2006). Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata*-isolated from the rocky shores of BetDwarka, Gujarat, India. *Process Biochem*., 41(9): 2017-2023.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A. (2006). Commercial applications of microalgae. *J. Biosci. Bioeng*., 101(2): 87-96.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales), *Bacteriol. Rev*., 35(2): 171-205.

Subramanian, G., Uma, L. (1996). Cyanobacteria in pollution control. *J. Sci. Ind. Res*., 55: 685-692.

Sundararaman, M., Subramanian, G., Averal, H.I., Akbharsha, M.A. (1996). Evaluation of the bioactivity of marine cyanobacteria on some biochemical parameters of rat serum. *Phytother. Res*., 10: 9-12.

Telford, W.G., Moss, M.W., Morseman, J.P., Allnutt, F.C.T. (2001). Cryptomonad algal phycobiliproteins as fluorochromes for extracellular and intracellular antigen detection by flow cytometry. *Cytometry*, 44(1): 16-23.

Zhao, J., Zhu, J., Jiang, L. (1995). Study on the energy transfer processes in phycobilisomes from blue-green algae by the use of stochastic simulation approach. *Biochim. Biophy. Acta*., 1229: 39-48.