

Cyanobacteria are oxygenic phototrophs containing chl 'a' and accessory pigments, inhabiting most of the earth's biotopes. Recently, there has been a renewed interest in the potential of cyanobacteria as producers of high value metabolites and novel bioactive compounds. The PB making up the PBS constitutes the major light harvesting pigments in almost all cyanobacteria. Phycobiliproteins are known to play a pivotal role in assisting the organism to survive in extreme and stressful environmental conditions. Apart from their characteristic physiological role, PB are attracting attention due to their unique physical and spectroscopic properties, which make them suitable for highly sensitive fluorescence based applications.

The present investigation aims to study some relevant photobiochemistry and molecular biology of a family of proteins called phycobiliproteins (PB) present in the cyanobacteria. Unique characteristic organization of oxygen evolving photosynthetic system in a prokaryotic genome makes cyanobacteria an excellent candidate for biochemical studies and molecular biological investigations. A successful attempt was made for establishing an improved method for the isolation and purification of selected filamentous cyanobacteria, which is mandatory for the above mentioned studies. Many different chemical agents and antibiotics have been previously employed for obtaining axenic cultures of cyanobacteria (Palinska and Krumbein, 1998), but cyanobacteria being prokaryotes are sensitive to most of these agents, which has resulted in comparatively, small amount of cyanobacteria available in axenic form. The attempts towards making the selected strains axenic, reveal that low concentration (0.05 mg/ml) of detergent treatment may prove to be an apt solution towards obtaining axenic cultures.

In response to light modulations, cyanobacteria are known to exhibit alterations in the pigment content to modify their light harvesting capabilities and make optimum use of the quantity and quality of light available. The study of the effect of light intensity on PB content confirms the reports of Tandeau de Marsac and Houmard (1988), that an inverse correlation between light intensity and pigment content. The pigment content decreases under high light and increase under low light, thus adapting to variations in light intensity. High light intensity leads to destruction of PBS assembly, which was revealed by the decreased number of linkers in the protein profile of PB extracted. *A. indica* and *P. tenue* seem to have a higher resistance to light stress as no major shift in λ_{\max} of PC was observed, as contrasted with ~ 8 nm shift in PB extract of *L. limnetica* grown under high light intensity.

Chromatic adaptation studies reveal that *A. indica* and *P. tenue* are non-chromatic adapting strains, since, *A. indica* does not contain PE and *P. tenue* does not show any variation in PE content even when grown under green light. *L. limnetica* though is a clear example of chromatically adapting strain as a considerable rise in PE content was observed, when culture was exposed to green light. Green light does seem to have a negative effect on *A. indica* as well as *P. tenue*, wherein, a blue shift is observed in the λ_{\max} of PC, indicating possible damage to PB assembly. This is supported by another observation of absence of 30 kD polypeptide, reported to be associated with PC assembly, in the protein profile of PB extract from *P. tenue* grown under green light. The two amide peak positions in the FT-IR spectras showed no shift in any of the samples indicating that there may not be any changes in the 2° structure composition due to light quality and quantity variation.

Phycobiliproteins constitute ~50% of the soluble proteins of the cyanobacterial cell resulting in nitrogen availability having a pronounced effect on the synthesis and degradation of PBS. Thus a complete lack of nitrate in the medium does show a decrease in growth rate and PB degradation in all the three strains. Comparatively, higher nitrate content does augment the growth rate due to optimum protein production, which in turn enhances the metabolism. A striking revelation during this study was that, the double concentration of nitrate, as compared to that in optimal medium proves to be limiting concentration for PB production in *P. tenue* and *A. indica*. No such limiting factor was observed in *L. limnetica*. Thus, these limiting factors may vary from organism to organism. Also, PE showed increase in content when grown in 3000 ppm nitrate in the late log phase, indicating that the concentration of a particular nutrient and growth stage may have regulatory effect on the metabolism of the organism. Though linkers decrease in “no nitrate” condition, the α and β subunits of PB are not much affected as observed in the protein profile, this leads to the inference that during degradation, due to limiting nitrate concentration, PBS structure is first disintegrated and the subunits may be affected in the later stages of acute deficiency.

pH is one of the essential, external factors affecting the metabolic activities of aquatic organisms. The study regarding the effect of pH changes shows that freshwater strain *A. indica* has an affinity for alkaline medium and two marine strains thrive better in neutral to near neutral pH. PBS composition may not be exclusively affected by pH. Since it affects the overall growth, PB production is also consequently affected. *P. tenue* seems to be more sensitive towards pH changes than the other investigated strains, as a conspicuous decrease in group III linkers is observed under alkaline pH.

Genes encoding phycobilisome components have been isolated and studied from all three strains of cyanobacteria. Unfortunately, there is still a paucity of documented sequence of PB for thin oscillatorian strains, from marine origin, which could be due to the lack of availability of specific primers and difficulties in obtaining axenic cultures. No significant similarity was observed in the PC amplification products in the two marine strains, *L. limnetica* and *P. tenue*, though they are morphologically similar and even amplification products were obtained with the same pair of primers. This result supports the belief that a complex correlation exists between genotypic and phenotypic characters. A comparative study with sequence of different cyanobacterial strains showed that *A. indica* is closely related to the different members of the same genera, closest being *A. platensis* FACHBOUQOS6 (97% identity).

Sequence alignment studies show 76% identity between freshwater strain *A. indica* and marine *L. limnetica*, which supports the hypothesis that all PB genes arose from duplication of a single ancestral gene (Troxler et al., 1981).

A comparative study of the marine cyanobacterial strains along with one fresh water strain was initiated for understanding the role of environment on the PB production. Studies on the phenotypic factors and genetic variability of PB genes aids in better understanding of the PB production in terms of quality as well as quantity of each strain studied. Hence, one can manipulate both, physiological as well as genetic factors in order to obtain desired PB in required quantity, which can be further exploited for their biotechnological applications.

Future scope of work

The present study explores the physiological factors affecting PB production and provides an insight into the genetic make up of PB operon. Production of PB with predetermined bilin composition suited to particular application still remains an unsolved query. Molecular mechanism involved in the physiological adaptations during environmental variations, still remains to be elucidated. There is also little information on how the factors control the biosynthesis of PB and thus, this too remains a fertile area of future investigation.