Name of Scholar: Hemlata

Name of supervisor: Prof. Tasneem Fatma

Department: Department of Biosciences

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ABSTRACT

The phycobiliproteins (PBPs) are antennae-protein pigments involved in light harvesting in cyanobacteria (blue-green algae, procaryotic), rhodophytes (red algae, eukaryotic) and cryptomonads. Three main classes of phycobiliproteins exist: allophycocyanin (APC, bluish green), phycocyanin (PC, blue) and phycoerythrin (PE, red) having A_{max} of 650–655 nm, 615–640 nm and 565–575 nm respectively.

In the last 10–15 years, there has been increasing interest in the potential uses of cyanobacterial phycobiliproteins in the commercial sector, as they have several applications. Thus, the first and most important application of phycocyanin is as food pigment, replacing current synthetic pigments, Dainippon Ink & Chemicals (Sakura) has developed a product called "Lina" blue which is used in chewing gum, ice sherbets, popsicles, candies, soft drinks, dairy products, but a number of investigations have shown on their health-promoting properties and broad range of pharmaceutical applications. In addition, phycobiliproteins are widely used in clinical and immunological research laboratories. Indeed, their properties make them very powerful and highly sensitive fluorescent reagents, so they can serve as labels for antibodies, receptors and other biological molecules in a fluorescence-activated cell sorter, in immune-labelling experiments, fluorescence microscopy and diagnostics.

Cyanobacterium *Spirulina platensis* and the red alga *Phorphyridium cruentum* are exploited commercially for phycocyanin and phycoerythrin production respectively (Roman et al. 2002). Several multinational companies exploit these phycobiliproteins as a commercial

commodity for the medical and biotechnology research industry. Their global market was estimated at more than US$ 50 million in 1997. But present the prices of phycobiliproteins products are 3 to 25/mg US $ for native pigment and can reach 1500/mg US$ for certain cross linked phycobiliproteins (with antibodies or other fluorescent molecules).

The success in the production of phycobiliproteins depends on the nature of organisms, its growth characteristics, availability of mass cultivation technology, the extent of accumulation of phycobiliproteins as well as efficacy of downstream processing. Further research is needed to develop fast growing organisms adapted to suit conditions in mass cultivation like resistant to photo bleaching, contaminants, variation in temperature.

However, a successful extraction and purification of phycobiliproteins is the one of the most promising aspect. Therefore in present study screening of 18 cyanobacterial strains was done for maximum phycobiliproteins production. This was followed by optimization of efficient extraction protocol for phycobiliproteins- (phycocyanin and phycoerythrin) along with purification and characterization.

1. *Anabaena* NCCU-9 has produced maximum phycobiliprotein (91.54 mg/g total phycobiliproteins /dry weight).
2. Sodium phosphate buffer (10 mM; pH-7) appeared as best buffer yielding 100.5 mg/g phycobiliproteins.
3. Maximum extraction of phycobiliprotein (114.5 mg/g) was achieved at pH 7.5.
4. Supplementation of 0.15M sodium chloride (NaCl) enhanced the total phycobiliproteins extraction capacity from 114.5 to 131.8 mg/g.
5. Repeated freezing & thawing method gave maximum phycobiliprotein (128.2 mg/g).

6. Freezing at –20 ºC and thawing at 4ºC appeared as optimal temperature for highest phycobiliproteins extraction (128 mg/g).

7. Ammonium sulphate was turned out to be the best precipitating agent giving a precipitate of 0.333 g/10 ml phycobiliproteins.

8. *Anabaena* phycocyanin showed gradual increase in its purity (0.87 - 4.45) after passing through fractional precipitation, dialysis, Sephadex G-25 and sephadex G-100 column.

9. Absorbance and fluorescence studies of purified phycocyanin have confirmed that purified phycocyanin of *Anabaena* was C-Phycocyanin.

10. A single band of 18.5 kDa corresponds to its alpha subunit of pure phycocyanin was observed on SDS PAGE.

11. *Michrochaete* phycoerythrin also showed gradual increase in its purity (1.5 – 3.2) after passing through fractional precipitation, dialysis and DEAE-Cellulose column.

12. Three bands of phycoerythrin corresponds to 21.8, 35.4 and 38 kDa were observed corresponds to α, β and γ subunits respectively.