ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

Cyanobacterial Phycoerythrin with Special Reference to *Microchaete* sp. CCU342

Hemlata[1], Fareha Bano[2], Tasneem Fatma[3]

Ph.D., Department of Biosciences, Jamia Millia Islamia (Central University), New Delhi-110025, India[1&2]

Professor, Department of Biosciences, Jamia Millia Islamia (Central University), New Delhi-110025, India[3]

ABSTRACT: Phycoerythrin (PE) is a light harvesting red protein, found only in cyanophyta, rhodophyta, glaucophyta and cryptophyta. Till now, red algae are well characterized and exploited commercially for PE. High cost of PE (50-3600 US$ /mg), has compelled the scientists to look for its alternate sources. During present cyanobacterial screening, *Microchaete* yielded maximum PE (26.32 mg/g), which was further purified on DEAE-cellulose column using sodium acetate buffer (pH-5.5). In final step, purity value of analytical grade PE (5.12) was obtained that showed 21.8 kDa band corresponding to α-subunit of B-PE. 30ºC temperature, 12.5 µmol photons/m^{-2}/s light, green wavelength, pH-8, 16:8 photoperiod and 50 mM salinity were the best conditions for maximum PE production. Pesticides (malathion and chlorpyriphos) and heavy metal (Cd^{2+}, Cr^{6+}) showed PE reduction whereas ($Cu^{2+,}$ Zn^{2+}, Pb^{2+}) showed PE enhancement at lower concentrations.

Keywords: Screening · *Microchaete* · Phycoerythrin · Purity value · Environmental stress

I. INTRODUCTION

Phycobiliproteins are primary group of photosynthetic coloured proteins. Based on the presence of different chromophores they are classified into 3 groups (i) Phycoerythin, PE (ii) Phycocyanin, PC and phycoerythrocyanin, PEC (iii) Allophycocyanin, APC. Stacks of phycobiliproteins are organized inside phycobilisomes, which are anchored on the outer surface of the thylakoid membrane and are located adjacent to the photo reaction centre of PS II [1]. The pink colored PE have application in food that do not require heating and thus is used in desserts, ice cream, sweets, cakes, decoration, milk shakes etc. [2]. Red algal PE has also been used in cosmetics such as eye shadow, face make up and lipstick, in the form of powders or creams [3]. PE is good for human health due to its free radical scavenging, anti inflammatory and anticancer nature [4]. For curing carcinosis and leukaemia, a new type of PE laser photosensitizer is developed that plays a crucial role in photodynamic therapy [5]. PE can be easily cross linked with antibody, immunoglobulins, avidin and other proteins by conventional molecule tagging technique and could be developed as fluorescence probes without losing its fluorescent properties [6]. PE is mainly extracted and purified from red algae- *Palmaria palmate*, *Gracilaria verrucosa*, *Porphyridium cruentum*, *Carollina elongate*, *Polysiphonia urceolata*, *Porphyra yezoensis* but most of them contained huge amount of polysaccharides, which form gel (carbohydrate) and thereby making the purification task most difficult and tedious [7]. There are very few reports of PE purification from cyanobacterium *Lyngbya* [8]. Therefore during present study cyanobacterial strains were screened for PE. The best strain was chosen for purification, characterization and environmental stress response studies.

II. MATERIALS AND METHODS

Cyanobacteria were procured from National Center for Conservation and Utilization of Blue Green Algae, IARI, New Delhi. Cultures were grown for 27 days in BG-11 medium [9] at 30±1ºC under of 25 µmol/photons/m^2 illumination, 12:12 light/dark photoperiod and pH-8. For PE screening, cyanobacterial biomass was harvested by centrifugation and washed thoroughly using distilled water to remove adhering salts then dried overnight at 50°C. PE was extracted by repeated freezing and thawing using 0.1 M potassium phosphate buffer (pH-7.1) till pellet color faded and absorbance

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

was recorded at 562, 615 and 652 nm against 0.1 M phosphate buffer as a blank [10]. For purifying PE slightly modified protocol was adopted [11]. Pink colour elutes were collected drop by drop in eppendorf tubes. For characterizing PE, each coloured fractions of PE was analyzed by absorption and fluorescence spectroscopy at each step and also by calculating their purity ratio of A_{max}/A_{280}, which is considered as a good indication of the purity level. Absorbance at 555 nm and 280 nm indicated the concentration of B-PE and proteins respectively. SDS-PAGE was carried out using 15 % polyacrylamide gel with medium range protein marker [12]. Impact of various environmental conditions like temperature, irradiances, coloured wavelength, photoperiods, pH, salinity, pesticides and heavy metals stress were studied on PE in best strain.

Stastical analysis of the data was done by one way analysis of variance (ANOVA). Dunnett's multiple comparison test was used in experimental setup with control in which significant difference at a level of significance of 0.01, 0.001 and 0.0001 ($p>0.01$, $p>0.001$, $p>0.0001$) and 'ns' for non significant are represented, while post tests for linear trend method was used in experiment setup without control (Graph Pad software, San Diego, CA, USA).

III. RESULTS AND DISCUSSIONS

PE has immense potential applications in pharmaceutical and food industry. But due to limited distribution and difficulties in obtaining high purity, their application has become challenging endeavour. In order to find out alternate good quality bio resource of PE, present work was done. The findings are discussed below----

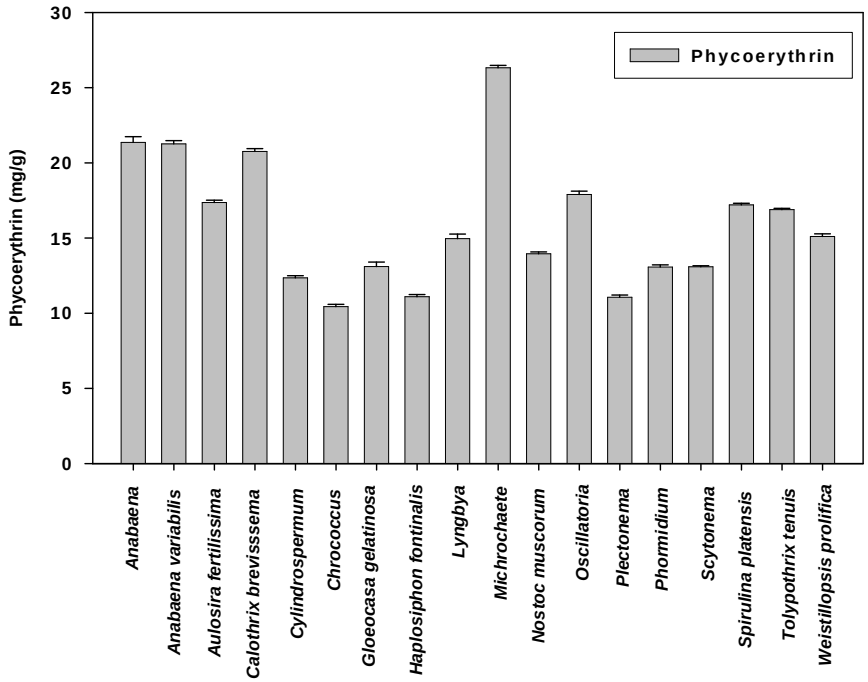

Fig. 1 Screening of cyanobacteria for PE (Error bars = ±SD)

During screening, cyanobacterial PE yield ranged from 10.0 mg/g (*Chrococcus* CCU207) to 26.32 mg/g (*Microchaete* CCU342) (Fig. 1). *Microchaete sp*. was selected for further studies e.g. PE purification, characterization and environmental impact assessment.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

Purification Steps	Volume (ml)	PE (mg/ml)	Purity index		(A_{555}/A_{280})	Yield (%)
			OD_{555}	OD_{280}		
Crude extract	100	34.33	2.184	1.56	1.4	100
Ammonium sulphate	10	31.54	1.10	0.37	2.93	86.3
Dialysis	10	25.28	0.69	0.218	3.2	74.2
50 mM Ac- buffer	5	23.37	0.58	0.147	3.94	66.7
250 mM Ac-buffer	7.5	22.51	0.55	0.119	4.6	61.5
350 mM Ac-buffer	7.1	27.65	0.455	0.089	5.1	42.3

Table 1 Stepwise purification of PE from *Microchaete* CCU342 with purity index

Purity ratio is an indicator of degree of purification. A purity of 0.7 is considered as food grade, 3.9 as reactive grade and greater than 4.0 as analytical grade. In present study successive steps of PE purification increased purity value from 1.4 to 5.1 (Table 1). Less than our purity value were reported in cyanobacteria–*Synechococcus* (3.37) (Kim et al. 2011); *Lyngbya* (3.7), *Phormidium* (3.9) and *Halomicronema* (4.0) (Parmar et al. 2011). However higher purity values has been reported in red algae, e.g. *Polysiphonia urceolata* (5.6) [7] and *Porphyra yezoensis* (6.67) [13].

In present study *Microchaete sp*. Crude and purified PE fraction of *Microchaete* showed absorption maximum peak at 555 nm (Fig. 2a & 2b) and fluorescence emission at 575 nm (Fig. 3a & 3b). Absence of peaks at 620 nm and 652 nm suggested that there was no contamination of PC or APC.

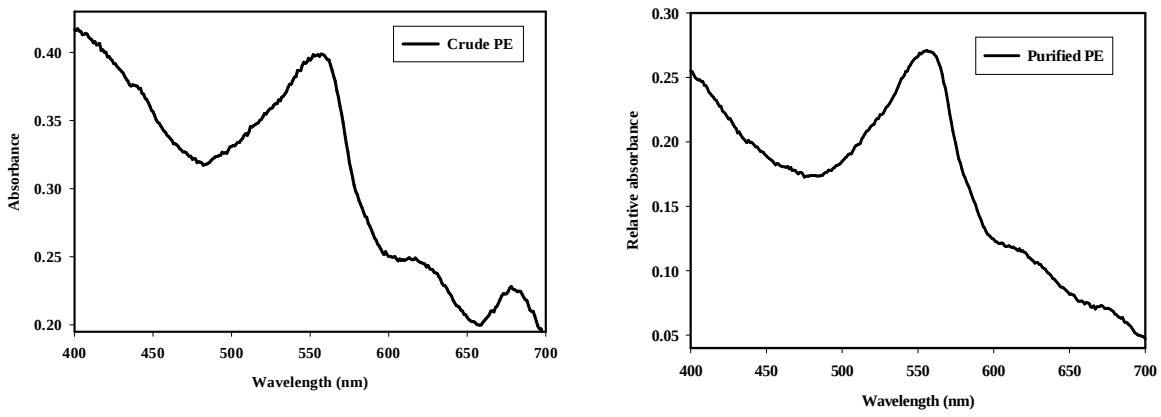

Fig. 2 Absorption spectra of crude and purified PE from *Microchaete* CCU342

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

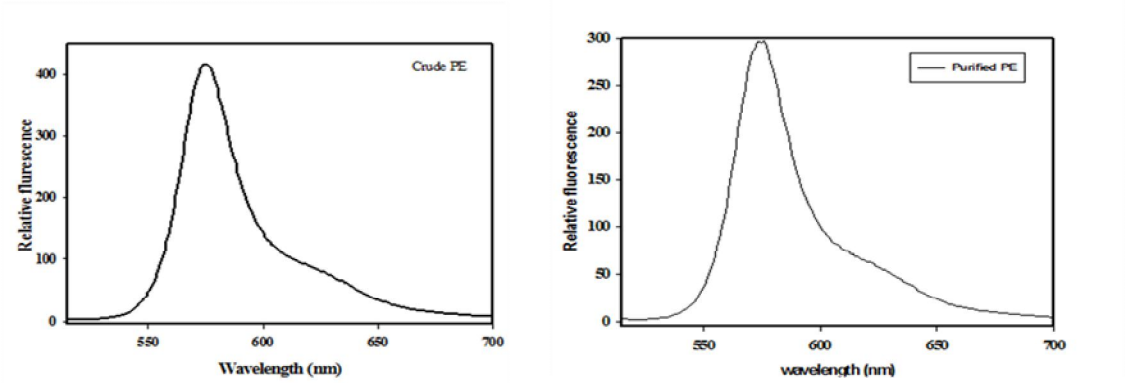

Fig. 3 Fluorescence spectra of crude and purified PE from *Microchaete* CCU342

SDS-PAGE analysis of the purified PE showed 21.8 kDa band representing α subunit in *Microchaete* CCU342 (Fig. 4). PE generally exists in hexameric state $(\alpha\ \beta)_6$, but its subunits may vary from 15-22 kDa. Two subunits of PE are reported in *Aphanocapsa* strain 6701 (20 and 22 kDa) and *Nostoc muscorum* (19.4 and 16.9 kDa) [14]; [15]. Whereas three subunits (18, 20 and 32 kDa) are reported from cyanobacterium *Lyngbya arboricola* [8]. Four subunits were reported from *Lyngbya* AO9DM (18, 18.5, 20 and 23 kDa); *Phormidium* A27DM (18, 19, 19.5 and 21) and *Halomicronema* A32DM (18, 18.5, 20 and 22) reported by [16], but old culture of same strains revealed a single band of 14 kDa in *Lyngbya* and *Halomicronema*. Recently a truncated PE as 14 kDa subunit was reported in *Phormidium tenue* [17] and a new type of PE–SCH (17.9 and 19kDa) in *Synechococcus* [18].

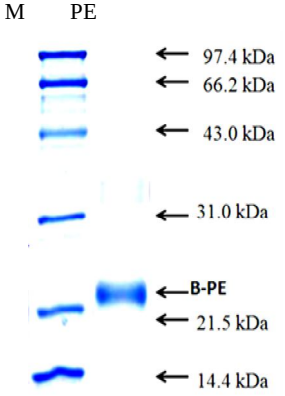

Fig. 4 SDS-PAGE analysis of purified PE from the cyanobacterium *Microchaete*.
(M- Protein molecular weight marker, PE-purified PE)

In present study, optimum temperature for PE was 25ºC with 31.906 mg/g PE yield (Table 2). Analysis of variance revealed $r^2 = 0.405$, $p<0.0001$, post test for linear trend (n=5). Ranjitha and Kaushik, [19] also observed maximum PE at 25ºC in cyanobacterium *Nostoc muscorum*. In red algae *Leptosomia simplex* [20], *Grateleloupa cortica* and *Grateleloupa lithophyta* [21] was noted highest PE at 28 ºC. In *Porphyra cortica* higher PE content were found at 10 ºC but, in *Porphyra linearis* and *Porphyra umbilicus* at 15 ºC [22].

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

12.5 µmol.photons/m^2/sec was the best irradiance for PE production (30.79 mg/g) in *Microchaete's* CCU342 (Table 2). Analysis of variance by post tests for linear trend revealed r^2=0.988, p<0.0001, (n=4) that was significant. In cyanobacteria light irradiance preferences vary like, 10 µmol.photons/m^2/sec in *Nostoc* UAM-206 [23]; 20 µmol.photons/m^2/s in *Westiellopsis iyengari* [24] and 25 µmol.photons/m^2/sec in *Nostoc muscorum* [19] and *Spirulina subsalsa* [25]. While high light intensities 40 and 90 µmol.photons/m^2/s produced maximum PE in red algae *Gracilaria tenuistipitata* [26] and *Porphyridium cruentum* [27] respectively.

The order of suitable chromatic regime for the PE production was found to be Green>Blue>White>Yellow>Red (Table 2). Analysis of variance revealed r^2=0.000389, p<0.1203, post tests for linear trend (n=5) that was not significant. *Microchaete* CCU342, showed 35.8% increase in green light and exhibited complementary chromatic adaptation of group-III, in which cyanobacteria can modulate both PE and PC level and the rate of synthesis of PE is greater in green light and of PC is higher in Red light [28]. Similar trend was also observed by Mishra et al. [29] in *Pseudoanabaena* where green light enhanced the PE content by 1.6 fold than white light. Bell and Fu [30] found up to 2 fold increase in *Trichodesmium* GBR TRL1101. Tomasseli et al [25] also found green coloured wavelength enhanced the production of PE in *Spirulina subsalsa.* In red algae *Rhodella reticulate* PE enhancement was up to 27% under green light [31]. Schmitt and Federspeil [32] found that the operon encoding PE, cpeBA is transcriptionally activated in green light and is expressed at very low levels in red light.

16:8 light: dark regime was the optimal photo-period for the production of PE in *Microchaete* CCU342 (Table 2). Analysis of variance revealed r^2= 0.975, p<0.0001, post test for linear trends (n=4). Longest photoperiod was also found optimal for PE production in *Calothrix elenkenii* [33] and *Nostoc muscorum* [34]. While, shorter (10:14) photoperiod gave high PE in red algae *Hypnea musciformis*,[35].

In *Microchaete* CCU342, the maximum PE was attained at pH-8 (Table 2). Analysis of variance revealed r^2= 0.213, p<0.0001, post test for linear trends (n=7). In red algae *Porphyridium cruentum* maximum PE was found at pH-8 [27]. In cyanobacterium *Nostoc muscorum* UAM-206 the PE content decreased at pH-7 (39.45 %) while enhancement in PE content (37 %) at pH-9 [23].

Conditions	PE (mg/g)
Temperature (ºC)	
20 ºC	25.00***
25 ºC	31.90***
30 ºC	29.68***
35 ºC	23.20***
40 ºC	20.05***
Light intensity (µ mol photons$m^{-2}sec^{-1}$)	
12.5	30.79***
25.0	26.08***
36.5	21.60***
50.0	15.15***
Light colour (Wavelength)	
Red	20.87***
Green	45.28***
Yellow	22.2 ***
White	33.41***
Blue	26.18***
pH	
4	19.12***
5	20.52***

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

6	26.0 ***
7	31.65***
8	35.82***
9	29.18***
10	16.06***
Photoperiod (Light: dark)	
16:8	36.04***
12:12	31.6 ***
10:14	28.63***
8:16	24.92***

Probability of error is represented by asterisk (*) for * p<0.01,** p<0.001, *** p<0.0001

Table 2 Effect of different environmental stress on PE content (mg/g) from *Michrochaete* CCU342

In *Microchaete* PE increased till 50 mM (p<0.0001) salt concentrationas compared to control and thereafter it decreased (Table 3). This result is also in agreement with the study of Kumar et al. [36], who found that hypersalinity (45 ppt) enhanced (70 %) PE in red algae *Grcacilaria cortica*. Some enhancement in PE was also observed in *Grcacilaria crassa* and *Grcacilaria edulis* at 35 ppt salinity [37].

NaCl (mM)	**PE (mg/g)**
Control	28.25
10	31.08***
50	37.42***
100	26.29***
150	22.43***
200	18.65***
250	10.08***

Probability of error is represented by asterisk (*) for * p<0.01,** p<0.001, *** p<0.0001

Table 3 Effect of salt (NaCl) stress on PE from *Michrochaete* CCU342

Chlorpyriphos and malathion pesticide showed 88% (p<0.0001) and 100% (p<0.0001) reduction in PE at the concentration of 0.015% respectively (Table 4). The drastic decrease in PE with rapid bleaching was more pronounced under malathion. The inhibitory effect of pesticides on PE was reported in cyanobacteria *Anabaena fertilissima, Aulosira fertillisma, Westiellopsis prolica* [38], *Plectonema boryanum* [39]. *Anabaena dolilolum Bhar* [40] and *Anabaena sphaeroids* [41]. This reduction in PE may be due to accumulation of toxicants in the thylakoid membrane, that change membrane fluidity [42]. Such decrease in PE may be ascribed to the inhibition of pigment synthesis or accelerated degradation of the pigment due to increased active oxygen species (AOS) at various sites of the photosynthetic electron transport chain [43].

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

Pesticide (%)	Cholorpyriphos PE (mg/g)	Malathion PE (mg/g)
Control	30.4	30.4
0.003 %	27.11***	24.33***
0.006 %	24.05***	21.72***
0.009 %	21.37***	14.34***
0.012 %	9.32***	5.27***
0.015 %	3.68***	----

Probability of error is represented by asterisk (*) for * p<0.01,** p<0.001, *** p<0.0001

Table 4 Effect of different pesticides on PE from *Michrochaete* CCU342

In present study, heavy metals resulted PE reduction in *Microchaete* (Table 5). The order of toxicity at 1.5 mM metal was chromium>cadmium>copper> zinc> lead (significant value (p<0.0001). Copper, zinc and lead showed slight PE increase in lower metal concentrations but inhibited at higher concentration. Li et al. [44], Ahmad and Aftab [45] also reported enhanced PE in lower concentration of Cu^{2+} and Zn^{2+} in *Porphyridium haitanesis* and *Nostoc muscorum*. Under Pb^{2+} stress PE reduction was observed in *Spirulina platensis and Nostoc muscorum* [46,45], *Anabaena-flos-aquae* [47], but Zaccaro et al. [48] found 4 times PE boost in *Microchaete tenera* and suggested it may be due to organism's effort to increase organic nitrogen reserve as PE under stress. Cd^{2+} stress reduce PE in *Anabaena-flos-aquae* [49, 50]. PE decline at higher concentrations of metal ions could be due to an enhanced need of metabolic energy for responses associated with adaptive mechanism of various enzymes [51]. Heavy metal induced changes in the arrangement and structure in thylakoid membrane also changes PE position and block electron transport causing inactivation of the PSII reaction centre [52].

Concentration (mM)	Copper PE (mg/g)	Zinc PE (mg/g)	Lead PE (mg/g)	Cadmium PE (mg/g)	Chromium PE (mg/g)
Control	27.83	27.83	27.83	27.83	27.83
0.05	30.55***	33.48***	32.54***	25.40***	23.59***
0.10	29.22***	36.10***	34.37***	22.31***	17.40***
0.50	25.64***	39.23***	34.80***	19.38***	13.28***
1.00	20.02***	24.64***	26.10***	16.42***	9.47***
1.50	15.22***	19.36***	21.07***	10.02***	5.26***

Probability of error is represented by asterisk (*) for * p<0.01,** p<0.001, *** p<0.0001

Table 5 Effect of different heavy metals on PE content (mg/g) from *Michrochaete* CCU342

IV. CONCLUSION

From the present study it can be concluded that *Microchaete* CCU342 may possibly be a good source of cyanobacterial PE with fast growth and purity value 5.1. It's purified PE resembled with B-PE.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

V. ACKNOWLEDGEMENTS

The authors are thankful to I.A.R.I., New Delhi for providing cyanobacterial strains and to the University Grants Commission, India for providing financial support.

REFERENCES

[1] Glazer AN, "Phycobiliproteins a family of valuable widely used fluorophores". J of Appl Phycol, vol. 6, pp. 105-112, 1994.
[2] D'Agnolo E, Rizzo R, Paoletti S, Muurano E, "R-Phycoerthrin from the red algae *Gracilaria longa"*. Phytochemistry vol. 35, pp. 693- 696, 1994.
[3] Arad MS, Yaron A, "Natural pigments from red microalgae for use in foods and cosmetics". Trend Food Sci Technol, vol. 3-4, pp. 92- 96, 1992.
[5] Huang B, Wang GC, Zeng CK, Li ZG, "The experimental research of R-phycoerythrin subunits on cancer treatment –A new photosensitizer in PDT". Cancer Biotherm Radiopharm, vol. 17, pp. 35-42, 2002.
[6] Telford WG, Moss MW, Moreseman JP, "Cyanobacterial stabilised phycobilisomes as fluorochromes for extracellular antigen detection by flow cytometry". J Immunol Meth, vol. 254, pp. 13-30, 2001.
[7] Niu JF, Wang GC, Tseng CK, " Methods for large scale isolation and purification of R-PE from red algae *Polysiphonia urceolata* Grev. Prot Express Purification 49:23-31, 2006.
[8] Tripathi SN, Kapoor S, Shrivastava A, "Extraction and purification of an unusual PE in a terrestrial desiccation tolerant cyanobacterium *Lyngbya arboricola"*. J Appl Phycol, vol. 19, pp. 441-447, 2007.
[4] Tseng CK, "Algal biotechnology industries and research activities in China". J of Appl Physiol, vol. 13, pp. 375-380, 2001.
[9] Stanier RY, Kunisawa R, Mandel MD, Cohen-Bazire, G "Purification and properties of unicellular blue green algae (order *chrococcales*)". Bacterial Reviews, vol. 35, pp. 171, 1971.
[10] Siegelman HW and Kycia JH, "Algal biliproteins In: Hellebust JA, Craige JS (ed) Hand Book of Phycological Methods- physiological and biochemical methods, Cambridge University Press, Cambridge pp. 72-79, 1978.
[11] Roman RB, Alva´rez-Pez JM, Acie´n FG, Molina E, " Recovery of pure B-PE from the microalga *Porphyridium cruentum"*. J Biotechnol, vol. 93, pp.73–85, 2002.
[12] Laemmli UK, "Cleavage of structural protein during assembly of the head of bacteriophage T4". Nature, vol. 227, pp. 680-685, 1970.
[13] Niu JF, Chen ZF, Wang GC, Zhou BC, "Purification of PE from *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta) using expended bed adsorption". J Appl Phycol, vol. 22, pp. 25-31, 2010.
[14] Glazer AN and Cohen-Bazire G, "Subunit Structure of the Phycobiliproteins of Blue Green Algae". Proc Nat Acad Sci, vol. 68, pp. 1398-1401, 1971.
[15] Ranjitha K and Kaushik BD, " Purification of phycobiliproteins from *Nostocmuscorum"*. Journal of Scientific and Indusrtrial Research, vol. 64, pp. 372-375, 2005b.
[16] Parmar A, Singh NK, Kaushal A, Sonawala S, Madamwar D, " Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. Bioresourcse Technology, vol. 102, pp. 1795-1802, 2011.
[17] Soni BR, Hasan MI, Parmar A, Ethayuathulla AS, Kumar RP, Singh NK, Sinha M, Kaur P, Yadav S, Sharma S, Maddamwar D, Singh TP, "Structure of novel 14 kDa fragment of α-subunit of phycoerythrin from the starving cyanobacterium *Phormidium tenue"*. Journal of Structure Biology, 171, pp. 247- 255, 2010.
[18] Kim JJ, Jeon YM, Noh JH, Lee MY, "Isolation and characterization of a new phycoerythrin from the cyanobacterium *Synechocoocus* sp. ECS-18". J Appl Phycol 23:137-142, 2011.
[19] Ranjitha K and Kaushik BD, "Influence of environmental factors on accessory pigments of *Nostoc muscorum"*. Indian Journal of Microbiology, vol. 45, pp. 67-69, 2005a.
[20] Czeczuga B, "Light harvesting Phycobiliprotein pigment of red algae *Leptosomia simplex* from the Antarctic". Polar Biology, vol. 4, pp. 179-181, 1985.
[21] Vanitha A and Chandra S, "Studies on photosynthetic pigments of some red algae of Covelong, Chennai (India)". Int J. of Curr Sci, vol. 20, pp. 149-154, 2012.
[22] Kim JK, Raemer PK, Neefus CD, Chung IK, Yarism C, "Effects of temperature and ammonium on growth pigment production and nitrogen uptake by 4 species of *Porphyra* (Bangiales, Rhodophyta) native to the New England Coast" Sahoo DB and Kaushik BD In: Algal Biotechnology and Environment, IK international Publishing House Pvt Ltd, New Delhi and Bangalore, 2012.
[23] Poza-Carrion C, Fernadez-Valiente E, Fernadez-Pinas F, Leganes F, "Acclimation of photosynthetic pigments and photosynthesis of the cyanobacterium *Nostoc* sp strain UAM 206 to combined fluctuations of irradiance, pH and inorganic carbon availability". J Plant Physiol, vol. 158, pp. 1455-1461, 2001.
[24] Ashokkumar P and Anand N, "Studies on growth and phycobilins pigments of the cyanobacterium *Westiellopsis iyengari"*. Int J of Biotech and Biochem, vol. 6, pp. 315-323, 2010.
[25] Tomaseli L, Margheri MC, Sacchi A, "Effects of light on pigments and photosynthetic activity in a PE- rich strain of *Spirulina subsalsa*". Aquatic Microbial Ecology, vol. 9, pp. 27-31, 1995.
[26] Carnicas E, Jeminez C, Niell FX, "Effects of changes of irradiance on the pigment composition of *Gracilaria tenuispitata* var, *liui* Zhnget Xia". J of Photochem Photobiol B, vol. 50, pp. 149-158, 1999.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 3, March 2014

[27] Wang J, Chem B, Rao X, Huang JLi M, "Optimization of culturing conditions of *Porphyra cruentum* using uniform design". World J of Microbiol Biotechnol, vol. 23, pp. 1345-1350, 2007.
[28] Tandeu De Marsac N, "Occurrence and nature of chromatic adaptation in cyanobacteria". J Bacteriol, vol. 130, pp. 82-91, 1977.
[29] Mishra SK, Shrivastav A, Maurya RR, Patidar SK, Haldar S, Mishra S, " Effect of light quality on the C-phycoerythrin production in marine cyanobacteria *Pseudoanabaena* sp. Gujrat Coast, India. Protein Expression and Purification, vol. 81, pp. 5-10, 2012
[30] Bell PRF and Fu FX, "Effect of light on growth pigment and nitrogen fixation of cultured *Trichodesmium* sp. from Great Barrier Reef lagoon". Hydrobiologia vol. 543, pp. 25-35, 2005.
[31] Mihova SG, Georgiev DI, Minkova KM, Tchernov AA, "Phycobiliproteins in *Rhodellareticulata* and photoregulatory effects on their content". J Biotechnol, 48, pp. 251–257,1996.
[32] Schmitt-Goff CM and Federspeil NA, "In vivo and in vitro footprinting of a light- regulated promoter in the cyanobacterium *Fremyella diplosiphon"*. J Bacteriol, vol. 175, pp. 1806–1813, 1993.
[33] Prassana R, Pabby A, Saxena S, "Modulation of Pigment profile of *Calothrix elenkini* in response to environment changes". J. Plant Physiol., vol. 161, pp.125-132, 2004.
[34] Austin PA, Ross IS, Mills JD, "Regulation of pigment content and enzyme activity in the cyanobacterium *Nostoc* sp. Mac grown in continous light-dark photoperiod, or darkness". Biochimica et Biophysica Acta, vol. 1277, pp. 141-149, 1996.
[35] Yokoya NS, Neechi O, Martins AP, Gonzalez SF, Plastino EM, "Growth responses and photosynthetic characteristics of wild and phycoerythrin deficient strains of *Hypneamusciformis* (Rhodophyta)". J Appl Phycol, vol. 19, pp. 197-205, 2007.
[36] Kumar M, Kumari P, Gupta V, Reddy CRK, Jha B, " Biochemical responses of red alga *Gracilaria corticata* (Gracilariales, Rhodophyta) to salinity induced oxidative stress". J Exp Mar Biol Ecol, vol. 391, pp. 27–34, 2010.
[37] Jayasankar R, "Effect of salinity on physiology of *Gracilaria* spp. (Gigertinais, Rhodophyta)". Seaweed Res Utln, vol. 27, pp. 19-24, 2005.
[38] Nirmal JIK, Rita NK, Anubhati AB, Manmeet KA, " Differential sensitivity of nitrogen- fixing, filamentous cyanobacterial species to an organochlorine insecticide-6,7,8,9, 10, 10-hexachloro-1,5,5a,6,9,9a-Hexahydro-6,9-Methano-2,4,3-Benzodioxathiepine-3Oxide". World Academy Science Eng Technol, vol. 75, pp. 732-742, 2011.
[39] Prasad SM, Kumar D, Zeeshan M, "Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to endosulfan stress. J Gen Appl Microbiol, vol. 51, pp. 115–123, 2005.
[40] Mohapatra PK, Patra S, Samantaray PK, Mohanty, "Effect of the pyrethroid insecticide cypermethrin on photosynthetic pigment of cyanobacterium *Anabaena dolilolum* Bhar. Polish J of Envirinmental Studies, vol. 12, pp. 207-212, 2003.
[41] Xia J, "Response of growth photosynthesis and photo inhibition of the edible cyanobacterium *Nostoc sphaeroides* colonies to thiobencarb herbicide". Chemosphere, vol. 59, pp. 561-566, 2005.
[42] Mohapatra PK, Scheiwer U, "Dimethoate and quinalphos toxicity: pattern of photosynthetic pigment degradation and recovery in *Synechococcus* sp. PCC 6803". Algol Stud, vol. 99, pp. 79–94, 2000.
[43] Mostafa FI and Helling CS, "Impact of four pesticides on the growth and metabolic activities of two photosynthetic algae". J of Environ Sci Health, vol.37, pp. 417-444, 2002.
[44] Li YX, Zhou S, Zhao FJ, Liu Y, Fan PP, Wang GC, " Physiological responses of *Porphyridium haitanesis* to different copper and zinc concentrations". Brazilian J of Oceanography, vol. 58, pp. 261-267, 2010.
[45] Ahmad IZ and Aftab KU "Biochemical characterization of *Nostoc muscorum* under multiple stresses". Proceedings of the 7th International Confrence on Hands-on-Science 25-31 July, Rethymno-Crete pp 419-426, http://www.clab.edu.uoc.gr/HSci 2010
[46] Arunkumar KKIU and Zhang X, "Effect of Pb^{2+} on phycobiliprotein content of *Spirulina platensis*, an edible Cyanobacterium". Tropical Agricultural Research, vol. 19, pp. 150-159, 2007.
[47] Rangsayatorn N, Upatham ES, Kruatrachue M, Pokethityook P, Lanza GR, "Phytoremediation potential of *Spirulina (Arhtrospira) platensis*: Biosorption and toxicity of studies of cadmium. Environment Pollution, vol. 119, pp. 45-53, 2002.
[48] Zaccaro MC, Salazar C, Zulpa de Caire G, Storni de Cano M, Stella AM, "Lead toxicity in cyanobacterial porphyrin metabolism". Environ Toxicol, vol. 16, pp. 61–67, 2001.
[49] Suroz W and Palinska KA, "Effects of heavy metal stress on cyanobacterium *Anabaena-flos-aquae"*. Arch Environ ContamToxicol, vol. 48, pp. 40-48, 2004.
[50] Heng LY, Jusoh K, Ling CHM, Idris M, "Toxcity of single and combinations of lead and cadmium to the cyanobacteria *Anabaena-flos-aquae"*. Bull Environ Contam Toxicol vol. 72, pp. 373-379, 2004.
[51] Andrade L, Azevedo SMFO, Pfeiffer WC, "Effects of high zinc concentrations in phytoplankton species from Sepetiba Bay (Brazil)". Arq Biol Technol, vol 37, pp. 655–666, 1994.
[52] Krupa Z and Basznski T, "Some aspects of heavy metal toxicity towards photosynthesis apparatus directed and indirect effects on light and dark reactions". Acta Physiol Plant, vol. 17, pp. 177-190, 1995.