ISSN 2348-6201
Volume 6 Number 3
JOURNAL OF ADVANCES IN BIOTECHNOLOGY

Alterations In Proteins And Amino Acids Of The Cyanobacterium *Anacystis Nidulans* In Response To Different Inorganic Formulations

*Gajendra Kumar Dautania and Gajendra Pal Singh
Algal Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan. Pin code-302004, INDIA.

ABSTRACT

Anacystis nidulans is a small, rod-shaped, unicellular, colonial, obligatory phototrophic microalga isolated from Sambhar Lake, Jaipur (Rajasthan). To find out the best inorganic composition cultures were grown in five different defined inorganic medium such as Modified BG-11 medium (pH 7.31), BG-11 medium (7.1), CHU-10 (pH 7.65), Zarrouk's medium (pH 10.2) and Kratz & Myer medium (pH 9.5) and kept at the temperature of 25 ± 2°C, illuminated with white fluorescent lamps at a light intensity of 2.5 Klux with 12:12 hours light/dark photoperiod in departmental laboratory. Protein content is determined by Bradford assay and qualitatively by SDS-PAGE. Protein expression levels were determined through densitometry. Highest protein and amino acid content were obtained in Modified BG-11 medium as compared to other medium. Two polypeptides of 54.3 and 56.2 kDa were uniquely observed, but the genotype of 35.8 kDa polypeptide was completely degraded under Modified BG-11 inorganic formulation. 35.8, 54.3, 56.2 and 61.8 kDa polypeptides were completely degraded in Zarrouk's as well as Kratz and Myer medium. The expression of some polypeptides of 14.0, 34.1, 42.3, 45.9, 49.5 and 75.0 kDa were greatly reduced and expressed only 1mm level in Zarrouk's and Kratz and Myer medium. Quantity of free amino acids maximum was in Modified BG-11 medium and minimum was in the Kratz and Myer Medium. Total 17amino acids were observed in the HPLC chromatogram. No detectable amount of asparagine, glutamine and tryptophan were found throughout the course of the algal life cycle

KEY WORDS: - *Anacystis,* Amino acid, Bradford assay, densitometry, HPLC, microalgae, Protein, SDS-PAGE.

INTRODUCTION

Algal biotechnology has shown a range of applications from the traditional extensive biomass production in human and animal nutrition, soil conditioning in agriculture, technologies for bioremediation, products for the cosmetics and pharmaceutical industry, research and medical diagnostic products. Algae contain high amounts of protein, β- carotene, and omega-3 and help to improve immune reaction and reproduction of animals, since they are a good source of vitamins, minerals and fatty acids (Varfolomeev and Wasserman, 2011). It can also help in stomach ulcers and wounds. Algae show great potential for diabetes, cancer and AIDS treatment (Holdt and Kraan, 2011).

Nutrient manipulation should be viewed as the best approach for turnover growth of algae as well as the massive accumulation of certain metabolites if needed by such approach (Lembi, 2000). It is well known that the culture medium not only affects cell productivity, but also affects cell composition and yield of specific products (Imamoglu et al. 2007). The inorganic constituents of media have been credited to be responsible for the pattern of growth and variation in metabolite biosynthesis of algal forms growing either in natural or laboratory conditions. Optimum growing conditions must be identified choosing a suitable nutrient medium and especially a nitrogen and phosphate source, which is a particularly important factor defining the yield and composition of algal biomass. Various suggested media have inflicted differential impacts on the biochemical composition of the algae, but there is presently good evidence that C, N, P, K, Mg, Ca, and S are required by one or more algal species (O'kelly, 1968).

The present study was conducted to determine the differential and deleterious effects of various media on the protein and amino acid content of *Anacystis nidulans.* Protein content was measured quantitatively by Bradford assay (Bradford, 1976) and qualitatively by SDS-PAGE (Sambrook et al. 1989). Variations in the protein expression due to different culture conditions were determined through densitometry. High performance liquid chromatography (Christian 1990, Shad et al. 2002) and paper chromatography method were used for amino acid profiling. Quantitative estimation of amino acids was carried out following the method suggested by Lee and Takahashi (1966).

MATERIAL AND METHODS

Organism- The experimental organism *Anacystis nidulans* was isolated from Sambhar Lake, Jaipur, Rajasthan. The cells were grown in BG 11 medium at 25 ± 2°C; pH was 7.1. To remove the inhibitory substances and curb any possible mineral deficiency, the entire growth medium was replaced with fresh medium from time to time.

Culture medium- In order to find out optimum culture medium, cultures were subjected to five different media of different chemical compositions and pH i.e. Modified BG 11 medium, pH; 7.4, BG-11 medium, pH;7.1, Chu-10, pH;7.65, Zarrouk's medium, pH;10.0, Kratz and Myer medium pH;7.31. Three sets of flasks for each medium were prepared. Each flask contained 70 ml of culture medium added with 30 ml healthy and profusely growing cultures and used for protein and amino acid estimation.

Protein profiling- The effect of various inorganic composition on polypeptide profile was determined by SDS-PAGE. Soluble proteins were extracted by homogenizing 0.5g of algal tissues with extraction buffer composing of 20 ml solution of 0.5 M Tris (pH 7.0) 2.5 ml., urea (4.0 g), SDS (0.5 g), glycerol (4.0 ml), B-mercaptoethanol (500 µl), dH_2O (10.0

ml); final pH 6.8) and centrifuged at 4°C for 20 min at 15,000 rpm. The supernatant was used as crude protein extract and protein quantity was measured following the method of Bradford (1976). The sample extracted from the algae (50 µl) was mixed with equal quantity of sample buffer [0.5 M Tris (pH 7.0) 2.5 ml, urea (9.6 g), SDS (1.0 g), glycerol (4.0 ml), B-mercaptoethanol (500 µl), dH_2O (5.0 ml)]. The mixture were heat denatured in water bath at 100°C for 8 minutes and after that put on ice till loading. Protein extracts from all the treatments were resolved on 12 % SDS-polyacrylamide gel (Sambrook *et.al*. 1989) and stained with 0.1 % Coomassie Brilliant Blue (R250) dye. In order to score and preserve the banding pattern, the gel was subjected to image scanning using BIO-RAD GS –700 Imaging Densitometer (USA) and the protein profiles were obtained for each variety. The presence of each band was scored as (+) plus and when absence as minus (-) [Table. 1].

AMINO ACIDS ANALYSIS

Amino acids extraction was carried out by taking fresh material 500 mg of each sample following the method suggested by Singh, 1992. Quantitative estimation of amino acids was carried out following the method suggested by Lee and Takahashi 1966. The amino acids were estimated qualitatively by paper chromatography, employing the methods of Block *et al*. (1951). Amino acids were extracted according to the method proposed by Shad et al. (2002), and separated by method recommended by Christian (1990) using HPLC system (HP1050) with a UV detector at 254 nm. Identification of each amino acid peak was confirmed by comparison with the retention time of individual amino acids.

OBSERVATIONS AND RESULTS

1.) Modified BG 11 medium

This was favourable medium for algal growth as well as protein biosynthesis. Maximum quantity of the protein was observed under this growth medium i.e. 4.48mg/ml at the end of experiment (Graph: 1). Total 15 polypeptides were observed that were highest in number as compared to other medium (Figure: 1). Two polypeptide of 22.7 and 39.1 kDa were not affected under different inorganic solutions. Two other polypeptides of 54.3 and 56.2 kDa were uniquely observed in this medium. The genotype of 35.8 kDa polypeptide was completely degraded under this condition (Table: 1). Expression level of some polypeptides such as 14.0, 22.7, 42.3, 45.9, 75 and 98.7 kDa were high under this medium as compared to other inorganic formulations. One polypeptide of 47.1 kDa was expressed only at 1mm level as compared to other solutions (Graph: 2A).

Quantity of extracted whole cell free amino acids were estimated i.e. 739 µg/gfw (Graph: 3). Total17amino acids were observed in the HPLC chromatogram of *Anacystis nidulans* cultures grown in Modified BG-11 medium. In this medium most abundant amino acids were aspartic acid, glutamic acid, glycine, alanine, arginine, isoleucine, leucine, glutamine and valine. Cysteine, methionine, serine and histidine concentration were low i.e. determined according to peak area and peak height of the chromatogram. No detectable amount of asparagine, glutamine and tryptophan were found throughout the course of the algal life cycle (Figure: 2).

In the chromatogram of paper chromatography total 17 amino acids were observed. Three amino acids i.e. asparagine, glutamine and tryptophan were not determined under all cultures of *Anacystis nidulans* (Table: 2). Histidine was only amino acids that observed in free state. Aspartic acid, arginine, cysteine, leucine, methionine, proline and threonine were recorded as protein bound state (Table: 2). The concentration of alanine, aspartic acid, glutamic acid, glycine and leucine were higher as compared to other inorganic solutions and favour the HPLC results. On the other hand, the concentration of cysteine, methionine, phenylalanine, serine, tyrosine and histidine were lower as compared to other amino acids (Graph: 4).

2. BG 11 medium

Extracted whole cell protein quantity was 4.22 mg/ml. Under this medium total 13 polypeptide species were observed, but their expression level were slightly lower as compare to Modified BG11 medium record (Figure: 1). The genotype of 35.8, 54.3 and 56.2 kDa were not observed under this medium, probably this medium cannot support the expression of the genotypes of these polypeptides (Table: 1). The BG 11 media also supported the genotypes of some polypeptides of 14.0, 22.7, 42.3, 45.9 and 75 kDa that were highly expressed under this inorganic medium as compared to other medium. Polypeptide of 45.9 kDa was expressed in low quantity upto 2mm level as compare to Modified BG11 medium (Graph: 2B).

Quantity of extracted whole cell free amino acids were estimated i.e. 692 µg/gfw (Graph: 3).Total 17 bands of amino acids were observed in the chromatogram of paper chromatography that was slightly less dense than modified BG-11 medium. Histidine and phenylalanine were observed only in the free form (Table: 2). Aspartic acid, arginine, cysteine, leucine, methionine, proline and threonine were recorded as protein bound state (Table: 2). The biosynthesis of aspartic acid, glutamic acid, valine and leucine were not affected, whereas arginine, alanine, glycine isoleucine and proline biosynthesis were slightly decreased in this medium (Graph: 15).

3. CHU 10 medium

This medium also enhanced the metabolic activity of the cell, but it was however lower as compare to Modified BG11 medium and BG11 medium. The quantity of total protein was 4.08 mg/ml in cultures grown this culture media (Graph: 1). In this medium total 13 polypeptide species were observed, but their expression level were slightly lower

(Figure: 1). Three polypeptides of 35.8, 54.3 and 56.2 kDa were not observed under this inorganic medium (Table: 1). The expression of some polypeptides such as 14.0, 22.7, 42.3, and 75 kDa decreased under this condition as compared to both to Modified BG11 medium and BG11 medium. Unlike Modified BG11 medium and BG11 medium, 47.1 kDa polypeptide was highly expressed in this media (Graph: 2C).

Extracted free amino acids quantity was 627 µg/gfw (Graph: 3). In this medium, total 15 amino acids were observed (Table: 2). Histidine, tyrosine and lysine were recorded as free amino acids. Methionine and phenylalanine were disappeared in the cultures grown in this medium. Some protein bound amino acids i.e. arginine, aspartic acid, cysteine, leucine, proline and threonine were fractionated and recorded free form as well (Table: 2). The biosynthesis of some amino acid i.e. aspartic acid, glutamic acid, glycine, isoleucine, threonine, valine and leucine were reduced under this culture condition (Graph: 4).

4. Zarrouk's medium

Protein biosynthesis was reduced in this culture medium. The total quantity of extracted protein, was 3.05 mg/ml (Graph: 1). Total 13 bands were observed in the SDS-PAGE profile of the extracted proteins (Figure: 1). The expression of some genotypes highly decreased in this medium. The expression of some polypeptides of 34.1, 42.3, 45.9, 49.5 and 98.7 kDa were greatly reduced and expressed only 1mm level in this inorganic composition. Polypeptide of 47.1 kDa was expressed upto 2mm in this composition (Graph: 2D). One novel polypeptide of 35.8 kDa expressed under this condition upto 1mm level that was not observed in above three media (Table: 1). Unlike the other media studied genotype of 61.8 kDa was completely degraded in this medium (Table: 1).

Total quantity of extracted whole cell free amino acids was 586 µg/gfw (Graph: 3).This culture medium was not so effective for biosynthesis of amino acids and only 10 amino acids were observed in chromatogram of paper chromatography (Table: 2). Alanine and valine were recorded only in the free form. The amino acids which were not observed in aminogram were cysteine, histidine, lysine, methionine, serine and phenylalanine. Arginine, aspartic acid, glycine, glutamic acid, isoleucine, leucine, proline and threonine were observed free as well as in protein bound form (Table: 2). The biosynthesis of alanine, aspartic acid, glutamic acid, glycine, valine and glycine were highly reduced under this nutrient limited culture medium (Graph: 4).

5. Kratz and Myer Medium

The algal physiology was highly affected in this inorganic composition due to deficiency and different composition of inorganic components. Lowest quantity of the protein i.e. 2.98mg/ml was estimated in the cultures grown in this medium (Graph: 1). The SDS-PAGE profile showed the differential and deleterious banding pattern of protein (Figure: 1). A genotype of 37.0 kDa polypeptide was degraded under this inorganic formulation as compared to other all four medium. Three polypeptide 61.8, 56.2 and 54.3 kDa were also inhibited in this medium. Similar to CHU-10 medium, one polypeptide of 35.8 kDa also expressed in this K M Solution (Table: 1). Expression level of some polypeptides highly decreased under this medium, but a polypeptide of 22.7 kDa was not affected. The expression of some polypeptides of 14.0, 34.1, 42.3, 45.9, 49.5 and 75.0 kDa were decreased (1mm) under this inorganic composition. Two polypeptides of 47.1 and 98.7 kDa were expressed upto 2mm level in this composition (Graph: 2E).

The amino acid biosynthesis in this medium was also inhibited. Quantity of extracted whole cell free amino acids were estimated to i.e.548 µg/gfw (Graph: 3). The banding pattern of paper chromatogram showed that under this culture medium only 9 amino acids were observed (Table: 2). Valine disappeared in this culture medium. As similar to CHU-10 media, biosynthesis of cysteine, histidine, lysine, methionine, serine and phenylalanine were also inhibited and not observed in the chromatogram of paper chromatography. Arginine, aspartic acid, glycine, glutamic acid, isoleucine, leucine, proline and threonine were observed free as well as protein bound form (Table: 2).Under this inorganic condition band density of alanine, aspartic acid, glutamic acid, leucine, isoleucine, valine and glycine were highly reduced under this culture condition (Graph: 4).

DISCUSSION

Some new ref @017

Variation in biochemical composition due to growth stage is frequently related to culture age and nutrient depletion, particularly if an organism is grown in batch culture (Morris et al. 1983). Typically, algal cultures become depleted in nutrients, as they enter stationary stages of growth, and total lipid and CHO increase while protein declines (Ogbonna and Tanaka 1996; Zhu *et al*. 1997; Lourenco *et al*. 1997). The availability of non-mineral nutrients, macronutrients, and micronutrients, greatly influence the biochemical composition of microalgae (Fabregas *et al*. 2002). General responses that accompanied nutrient deprivation have been studied in various laboratories (Grossman *et al.,* 1993; Flores and Herrero, 1994).

The C, N, and P have been shown to be much essential macronutrient for the growth and metabolism of algae, as much as these are required for the rest of the living organisms. Carbon is the principal nutrient required by cyanobacteria, and in alkaline lakes this organism is the dominant species because of the presence of high concentrations of sodium carbonate. $CO_2/H_2CO_3/HCO_3$, which is a very useful buffer system for maintained the alkaline pH which is important for the optimum growth of *Spirulina,* which helps to prevent carbon depletion (Richmond, 1990).

Nitrogen, an essential macronutrient is required for the synthesis of amino acids, proteins, nucleotides, nucleic acids, co-enzymes, chlorophyll-*a* and photosynthetic pigments. Nitrate, nitrite and ammonium ions are the main sources of nitrogen for the algae, used mainly for the synthesis of amino acids and proteins (Reynolds, 1984). Nitrogen is identified as a major limiting nutrient for biotic productivity (O'Donohue and Dennison, 1997; Udy and Dennison, 1997). Similarly Yeesang and Cheirsilp, (2011) have reported loss of biomass when green alga, *Botrycoccus* spp. was exposed to nitrogen deficient conditions. Magnesium is universally needed by all algal species, as it is known to carry out molecular phosphate transfer. Mg is also a part of photosynthetic Mg-dependent enzymes (Shaul, 2002) and a constituent of chlorophyll. Mg also can regulate the transport trough the Ca-channels of the cell membrane by improving their permeability (Pasternak *et al.,* 2010).

The significance of other macronutrient such as phosphorous, sodium, potassium and sulfur was beneficial in biomass production and physiology of the cyanobacteria. These elements were present in Modified BG-11 medium in the form of Na_2CO_3, K_2HPO_4 and K_2SO_4. Phosphorus may become a major limiting nutrient for cyanobacteria growth (Martin and Gordon, 1988) with blooms of cyanobacteria, often related to phosphorus loadings from the surrounding environment (Paerl, 2008; Seitzinger, 1991). Wanger *et.al,* 2002 investigated that under phosphate-limiting growth conditions some membrane proteins were induced in cyanobacterium *Anacystis nidulans*. Studies have found that there is a co-limitation of primary production by both nitrogen and phosphorus (Mosich *et.al,* 2001).

Sulphur is essential for the synthesis of amino acids i.e. cysteine and methionine, secondary metabolic production and the synthesis of co-enzymes such as glutathione, thioredoxin and co-enzyme A. The loss of phycocyanin in both nitrogen and sulphur deprived cells was a consequence of the rapid and nearly complete degradation of phycobilisome (Yamanaka and Galzer, 1980; Collier and Grossman, 1992). Grossman *et al;* (1993) has demonstrated that in nitrogen starved cells the phycobilisome was actively and rapidly degraded. Starvation for inorganic carbon, phosphorus (Collier & Grossman, 1992), or iron (Singh & Sherman, 2000) results in partial loss of pigment.

Many other trace elements or micronutrients such as Fe, Ca, Mn, Cl, etc. were present only in Modified BG-11medium that promotes the algal growth and protein biosynthesis. Allakhaverdiev *et al.* (2002) demonstrated that salt stress inhibits the repair of photo-damaged PSII by concealing the synthesis of D1 protein in *Synechocystis.* Similarly, under salt stress, there is also a loss in the chlorophyll protein (47-kDa) and a core membrane linker-protein (94-kDa) that can attach phycobilisomes to thylakoids (Garnier *et al*., 1994). The enhanced Na^+ ions also affected energy transfer within the PBS (Verma and Mohanty 2000).

Protein content is affected by variabilities in nutrient supply (Stengel *et al.,* 2011). The protein expression levels were highly induced in the nutrient rich Modified BG-11 medium, whereas, it highly decreased in the all other nutrient deficient medium. Many previous studies reported that protein content under nitrogen starvation may decrease (Kilham *et al*., 1997; Lynn *et al*., 2000; Heraud *et al*., 2005). In present study, some novel polypeptides of 54.3, 56.2 in *Anacystis nidulans* were uniquely expressed in only Modified BG-11 medium. Rosales *et al*. (2005) reported about the physiological competence of *Synechococcus* species in hypersaline medium and observed maximum protein biosynthesis in good nutrient conditions. Similarly, other studies showed that different environmental conditions are responsible for induction or inhibition of certain peptides expression and also increase or decrease level of the protein expression (Chan *et.al* 2004). Subhashini *et al.* (2003) observed significant variations in protein content among the four isolates of *Anabaena azollae.* Becker (2007) reported that protein content of different algae strains ranges from 6% (dry matter) for *Spirogyra* sp. to 71% for *Arthrospira maxima*. In addition, a novel polypeptide of the 35.8 kDa was appeared in the cultures of Kratz and Myer medium that hardly support the growth of *Anacystis nidulans*. Wanger et.al, (1994) investigated that under phosphate limiting growth conditions some membrane proteins were induced in cyanobacterium *Anacystis nidulans*. Appearance of this new protein band in the SDS-PAGE profile have been assigned to originate either from the degradation of larger molecular weight proteins or from de novo synthesis induced by nutrient starvation (Allen 1984). Weber and Jung (2002) demonstrated that changes in protein profiling and newly formed proteins might be helping cyanobacteria to tolerate adverse stress conditions.

Under adverse culture condition some polypeptides expression were inhibited. Nutrient deficient conditions also degrade or inhibit the genotype of some polypeptide such as 37.0 and 61.8 kDa in *Anacystis nidulans.* Dortch *et al*. (1984) found that reduced amount of protein in algal cells is generally a result of nitrogen starvation. Phycobiliprotein synthesis also depends on the supply of assimilable nitrogen in the environment (Liotenberg *et al*. 1996). The loss of antenna pigments due to nutrient starvation (Allen & Smith 1969; Yamanka and Glazer 1980) and the degradation of the PBS due to nitrogen, sulphur or phosphorus deprivation (Collier and Grossman 1994) showed the involvement of these elements in the assembly and functioning of the PBS. *Anacystis nidulans* phycobilisome contain colorless polypeptides which are believed to participate in the assembly of phycobilisomes (Glazer, 1994). At the level of gene expression and protein synthesis, an overall reduction in the rate of protein synthesis is observed upon starvation (Aldehni *et al.,* 2003; Sauer *et al*., 2001).

There are many factors (inorganic composition, pH, photoperiods, light intensity and temperatures) that might affect the amino acid profile. The amino acids contents observed in free forms as well as in the fractions of bulk protein and peptides. The processes of formation of free amino acids and peptides are on different culture conditions varying in different inorganic composition, photoperiods and temperatures. A number of other groups have shown a seasonal variation of protein patterns present in algal tissue (Fleurence *et al.,* 1999). The nutritional quality of food can be determined by the content, proportion and availability of its amino acids, particularly for evaluation of a new protein resource (Gressler *et al.,* 2009).

It is more common to be reported seventeen amino acids in miroalgae in which aspartic acid, glutamic acid, glycine, alanine, proline, arginine, threonine, lysine, isoleucine, leucine, glutamine, valine and tyrosine are most abundant in cyanobacterium *Anacystis nidulans*. Cysteine, methionine and histidine were found in low concentrations in all experimental blue-green-algae. Aspartic acid, glutamic acid, alanine, glycine, serine were major components of the total free amino acid in cyanobacterium *Anacystis nidulans.* These amino acids constituted more than 50% of the total free amino acid in cyanobacteria, and are major contributors to flavour and taste of microalgae. Glutamine, asparagine and tryptophan were not observed in the chromatogram of HPLC as well as paper chromatography. Similar work on amino acid profiling in *Spirulina platensis* was carried out by Aly and Amber (2010).

In conclusion, in the present work, the optimal inorganic medium and pH for protein and amino acids biosynthesis were demonstrated for *Anacystis nidulans.* Various inorganic formulations affect synthesis of metabolic end products synthesized by cyanobacteria are essential since they contribute to understanding the control of metabolic activities and optimising yields of metabolic end products of interest.

ACKNOWLEDGEMENT

We thank to CSIR (Council of Scientific and Industrial Research) New Delhi (INDIA) for financial assistance in support of the study.

REFERENCES

1. **Aldehni, M. F.; Sauer, J.; Spielhaupter, C.; Schmid, R. and Forchhammer, K. (2003).** Signal transduction protein PII is required for NtcA-regulated gene expression during nitrogen deprivation in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. *J Bacteriol* 185: 2582–2591.
2. **Allakhaverdiev, S. I.; Sakamoto, A.; Nishiyama, Y.; Inaba, M. and Murata, N. (2000).** Ionic and osmotic effects of NaCl-induced inactivation of photosystem I and II in *Synechococcus sp. Plant Physiol.* 123: 1047-1056.
3. **Allen, M. M. (1984).** Cyanobacterial cell inclusions. Annu Rev Microbiol 38, 1–25.
4. **Allen, M.M. and Smith A.J. (1969).** Nitrogen chlorosis in blue-green algae. *Arch Microbiol,* 69:114–120.
5. **Aly, M. S and Amber. S.,**. Chemical Composition and Potential Application of Spirulina platensis Biomass. Journal of American Science 2010;6(10):1283-1291
6. **Becker E.W (2007).** Microalgae: Biotechnology and Microbiology. Cambridge University Press, Camb ridge
7. **Block, R.J. (1951)** Quantitative paper chromatography, a simplified procedure. *Proc. Soc. Exp. Biol. Med.* 72(2):337–341.
8. **Bradford, M. M.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72 (1976)** 248–254.
9. **Chan L. L.; Hodgkiss I. J.; Wan J. M. F.; Lum J. H. K.; Mak A. S. C.; Sit W. H. and Lo S. C. L. (2004).** Proteomic study of a model causative agent of harmful algal blooms, *Prorocentrum triestinum* II: The use of differentially expressed protein profiles under different growth phases and growth conditions for bloom prediction. *Proteomics* 4*:* 3214–3226.
10. **Christian, G. (1990).** HPLC Tips and Tricks. Great Britain at the Iden Press, Oxford, 608.
11. **Collier, J. L. and Grossman A R. (1994).** A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. *EMBO J.*13: 1039–1047.
12. **Fabregas, J. Maseda, A. Domınguez, A. Ferreira, M. and Otero A. (2002).** Changes in the cell composition of the marine microalga, *Nannochloropsis gaditana*, during a light : dark cycle. *Biotechnol Lett*. 24: 1699–1703.
13. **Fleurence. J. (1999).** Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology,* 10: 25-28.
14. **Flores, E. and Herrero, A. (1994).** Assimilatory nitrogen metabolism and its regulation. In The *Mol Bio of Cyanobacteria,* 487–517.
15. **Garnier, F.; Dubacq, J. P. and Thomas J. C. (1994).** Evidence for transient association of new proteins with the *Spirulina maxima* phycobilisome in relation to light intensity. *Plant Physiol.*106: 747-754.

16. **Glazer, A.N. (1994).** Phycobiliproteins—a family of valuable, widely used fluorophores. *J Appl Phycol* 6:105–112.

17. **Gressler, V., Yokoya, N. S., Fujii, M. T., Colepicolo, P., Filho, J. M., Torres, R. P., & Pinto, E. (2009).**. Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chemistry, 120(2), 585-590.

18. **Grossman, A.; Schaefer, M.; Chiang, G. and Coller, J. (1993).** Environmental effects on the light-harvesting complex of cyanobacteria. *J. Bacteriol.* 175: 575–582.

19. **Heraud, P.; Wood, B.R.; Tobin, M.J.; Beardall, J. and McNaughton, D. (2005).** Mapping of nutrient-induced biochemical changes in living algal cells using synchrotron infrared microspectroscopy. *FEMS Microbiol. Lett*. 249: 219–225.

20. **Holdt, S. L. and S. Kraan (2011).** Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology* 23: 543–597.

21. **Imamoglu E, Sukan EFV, Dalay MC (2007).** Effect of different culture media and light intensities on growth of *Haematococcus pluvialis*. Int. J. Nat. Eng. Sci. 1: 5-9.

22. **Kilham, S.S.; Kreeger, D.A.; Goulden, C.E. and Lynn, S.G. (1997).** Effects of nutrient limitationon biochemical constituents of *Ankistrodes musfalcatus*. Freshw. Biol. 38: 591–596.

23. **Lee, Y. P. and T. Takahashi. (1966).** An improved colorimatric determination of amino acids with the use of inhydrin. *Anal. Biochem.* 14(1): 71-77.

24. **Lembi, C. A. 2000.** Relative tolerance of mat-forming algae to copper. Journal of Aquatic PlantManagement.38:68–70.

25. **Liotenberg, S. Campbell, D. Rippka, R. Houmard, J. and Tandeau de Marsac, N (1996).** Effect of the nitrogen source on phycobiliprotein synthesis and cell reserves in a chromatically adapting filamentous cyanobacterium. *Microbiology* 142: 611–622.

26. **Lourenco S.O., Marquez U.M.L., Mancini-Filho J., Barbarino E. and Aidar E. 1997.** Changes in biochemical profile of *Tetraselmis gracilis* I. Comparison of two culture media. Aquaculture 148: 153–168.

27. **Lynn, S.G.; Kilham, S.S.; Kreeger, D.A. and Interlandi, S.J. (2000).** Effect of nutrient availabilityon the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *J. Phycol*. 36: 510–522.

28. **Martin, J.H. and Gordon, R.M. (1988).** Northeast Pacific iron distributions in relation to phytoplankton productivity. *Deep-Sea Res.,* 35: 177-196.

29. **Morris R.J., McCartney M.J. and Robinson G.A. 1983.** Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. J. Exp. Mar. Biol. Ecol. 70: 249–262.

30. **Mosich, T. D.; Bunn, S. E. and Davies, P. M. (2001).** The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology, 46: 1269-1278.

31. **O' Kelley, J.C. (1968)**. Mineral nutrition of algae. *A. Rev. Pl. Physiol*. 19: 89-112.

32. **O'Donohue, M.J.H., Dennison, W.C. (1997).** Phytoplankton response to nutrient concentrations, light availability and temperature along an Australian estuarine gradient. Estuaries 20: 521–533.

33. **Ogbonna J.C. and Tanaka H. 1996.** Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of *Chlorella pyrenoidosa*. J. Ferm. Bioeng. 82: 558–564.

34. **Paerl, H. (2008).** Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum, in: Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, edited by: Hudnell, K., Springer, North Carolina, USA, 217–237.

35. **Pasternak O, Sochen N, Basser PJ. (2010).** The effect of metric selection on the analysis of diffusion tensor MRI data. Neuroimage.; 49;2190–2204.

36. Reynolds, C.S. (1984). The ecology of freshwater phytoplankton. Cambridge University Press**,** London.

37. **Richmond A. (1990).** Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL. *ISBN,* 8493-3240.

38. **Rosales, N.; Ortega, J.; Mora, R. and Morales, E. (2005).** Influence of salinity on the growth andbiochemical composition of the cyanobacterium *Synechococcus* sp. *Ciencias Marinas,* 31: 349 – 355.

39. **Sambrook, J. Fritsch E.F. and Maniatis T. (1989).** *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

40. **Sauer. J.; Schreiber, U.; Schmid R; Völker U; Forchhammer K. (2001).** Nitrogen starvation-induced chlorosis in Synechococcus PCC7942. Low-Level Photosynthesis as a mechanism of long-term survival. *Plant Physiol,* 126: 233–243.

41. **Seitzinger, S. P. (1991).** The effect of pH on the release of phosphorus from Potomac estuary sediments – implications for blue-green-algal blooms, Estuar. Coast. Shelf Sci., 33: 409–418.

42. **Shad, M.A.; Ansari, T. M.; Pervez, H.; Rubab, M. and Mahmood, T. (2002).** Changes in sugar, amino acid and mineral contents of leaves of two mango varieties affected by quick decline disease. J. Biol. Sci., 2: 694-696.

43. **Shaul, O. (2002).** Magnesium transport and function in plants: the tip of the iceberg. *Biometals*. 15: 309–323.

44. **Singh, A.K. & Sherman, L.A. (2000).** Identification of ironresponsive, differential gene expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803 with a customized amplification library. *J Bacteriol* 182: 3536–3543.

45. **Stengel, D. B., S. Connan and Z. A. Popper. 2011.** Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol. Adv. 29:483-501.

46. **Subhashini, R.; Kumar, K. and Kannaiyan, S. (2003).** Intrinsic antibiotic resistance and biochemical characteristics of *Anabaena azollae* isolated from *Azolla*– cultures. *Indian J. Microbiol.*, 43: 65 -169.

47. **Tandeau De Marsac NT, (2003).**Phycobiliproteins and phycobilisomes: the early observations. *Photosynth. Res*. 6:197-205.

48. **Udy, J.W. and Dennison, W.C. (1997).** Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *J. Exp. Mar. Biol. Ecol*.217: 253–277.

49. **Varfolomeeva S. D. and L. A. Wasserman. (2011).** Microalgae as Source of Biofuel, Food, Fodder, and Medicines. *Applied Biochemistry and Microbiology* 47(9): 789–807.

50. **Verma, K. and Mohanty, P. (2000).** Changes of the photosynthetic apparatus in *Spirulina* cyanobacterium by sodium stress. Zeitsch-rift fuÈrNaturforschung 55C, 16-22.

51. **Wagner, C., Saizieu Ad, A., Schonfeld, H.J., Kamber, M., Lange, R., Thompson, C.J., Page, M.G. (2002)**. Genetic analysis and functional characterization of the *Streptococcus pneumoniae vic* operon. *Infect Immun* **70**: 6121–6128

52. **Weber A. and Jung K. (2002).** "Profiling Early Osmostress-Dependent Gene Expression in *Escherichia coli* Using DNA Macroarrays." *J. Bacteriol.*, 184: 5502-5507.

53. **Yamanka, G. and Glazer, A.N. (1980).** Dynamic aspects of phycobilisome structure. Phycobilisome turnover during nitrogen starvation in *Synechoccous* sp. *Archives of Microbiology* 124: 39-47.

54. **Yeesang, C. and Cheirsilp, B. (2011).** "Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand". *Bioresource Technol*., 102: 3034-3040.

55. **Zhu C.J., Lee Y.K. and Chao T.M. 1997.** Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J. Appl. Phycol. 9: 451–457.