JOURNAL OF ALGAL BIOMASS UTILIZATION
JABU - Copyright © 2009 PHYCOSPECTRUM

Total lipid and fatty acid composition in some freshwater cyanobacteria

Kodandoor Sharathchandra and Madaiah Rajashekhar

Department of Biosciences, Mangalore University, Mangalagangothri-574 199, Karnataka, India

E- mail: profmrajashekhar@yahoo.co.in; Fax: 0091 824 2287367

ABSTRACT

Fatty acid composition and total lipids in 13 species of freshwater cyanobacteria isolated from different aquatic habitats of Southern Karnataka were examined. The species grown in BG-11 medium with nitrate were harvested at exponential phase. The GC analysis showed variation in n-saturated, unsaturated and long chain branched fatty acids with respect to location and habitat. Of the 13 species investigated, two toxic bloom forming species such as *Microcystis aeruginosa* and *Nostoc linckia* were also involved. Among the fatty acids detected most of them belong to polyunsaturated fatty acids (more than 60%). The study also revealed that palmitic acid C16:0 was found in all the isolates followed by linoleic acid C18:2. In some, the long chain fatty acids (C20:1 and C24:0) were found in lower concentrations.

Keywords: Cyanobacteria, Lipids, Fatty acids, Gas chromatography.

INTRODUCTION

Cyanobacteria are a diverse group of photosynthetic organisms found in different habitats. In freshwaters they form an important component of primary producers and hence employed in aquaculture and mariculture practices. Their diversity and potential applications has been worked out by Tajuddin and Subramanian (2005). They store reserve food materials which are the source of pigments, lipids, vitamins and proteins (Rastogi and Sinha 2009). In the eutrophic lakes they form water blooms excreting secondary metabolites toxic to aquatic fauna (Bendarz, *et. al*., 2002; Carmichael, *et. al*., 2001). Bury et. al.

(1998) has shown that lipids of some cyanobacteria interfere with gill basolateral membrane ion-extrusion mechanisms and result in the fish deaths after a cyanobacterial bloom. There are some health benefits of polyunsaturated fatty acids (PUFA) for aquatic organisms which has spurred interest in their commercial production. The species of *Anabaena, Nostoc* and *Spirulina* are consumed as food due to their high protein and vitamin content (Anupama 2000 ; Ciferri *et. al.*, 1985).

Cyanobacteria contain significant quantities of lipids and some of them are also rich in essential fatty acids such as linoleic and gamma linolenic acids. Besides nutritional value, the fatty acids of cyanobacteria are generally used to clarify taxonomical problems (Li *et. al.*, 2001). According to Kenyon et. al. (1972) four types of fatty acids exist in cyanobacteria and are linked to morphological characteristics. The fatty acid composition of marine microalgae has been studied more extensively (Caudales *et. al.*, 2000; Renaud *et. al.*, 1999; Tran *et. al.*, 2009; Volkman *et. al.*, 1989) than the freshwater forms (Caudales and Wells, 1992; Kruger *et. al.*, 1995; Rezanka *et. al.*, 1982). The fatty acids of freshwater forms of tropical waters are little examined except the reports of Manoharan and Subramanian (1993); Mahajan and Kamat (1995); Renaud et. al. (1999). The present study reports on the lipids and fatty acid composition in thirteen species of cyanobacteria isolated from lakes and reservoirs of Southern Karnataka.

MATERIAL AND METHODS

Cyanobacterial isolates

The species namely, *Oscillatoria calcuttensis* and *Oscillatoria chlorina* were isolated from Mangalore dairy effluents and sewage drain of Mangalore, respectively; *Oscillatora acuminata* was from a water tank at Malavalli of Mandya District; *Nostoc linckia, Microcystis aeruginosa* and *Oscillatoria perornata* were isolated from Kukkarahalli tank of Mysore; *Lyngbya limnetica, Phormidium purpurescens, Calothrix fusca* and *Scytonema bohnerii* were from a sulfur spring in Dakshina Kannada District; *Lyngbya dendrobia* and *Phormidium anomala* were isolated from Shimsha reservoir of Mandya District; *Lyngbya spiralis* and *Phormidium ambiguum* were isolated from a fish tank at Lakkavalli and Bhadra reservoir of Chickmagalore District respectively and *Oscillatoria amoena* was isolated from Hemavathi reservoir of Hassan District.

Culture conditions

The species were cultured in the laboratory and grown aseptically on dry agar (Dor *et*. *al*., 1987) and then in BG-11 growth medium with nitrate (Stainer *et*. *al*., 1971) and were maintained at 26±2^{o}C under constant illumination (14 h light: 10 h dark at 2000 lux) before their harvest at exponential phase.

Extraction of total lipids

Extractions of total lipids were done by chloroform/methanol extraction method described by Rezanka et. al. (1982).

Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared from total lipid fractions using HCl-methanol as described elsewhere (Pauda- Resurreccion *et*. *al*., 1979 and Rezanka *et*. *al*., 1982).

Gas chromatography of FAME

Methyl esterified samples were diluted (40µl FAME sample+960 µl n-hexane HPLC quality) in the sample vial with micropipettes. The sample vials were put in auto-injector vial tray. The sample (1µl) was injected into the gas chromatograph (GC-2010, Shimadzu, Japan) by an autoinjector and capillary column (BPX 70, 30 m, 0.25 mm diam, 0.25 µm film thickness). The elutants were detected on flame ionization detector. The GC column temperature was set at 100^{0} C per 3 min and increased to 280^{0} C. The injector temperature was kept at 225^{0}C. The flow rate of carrier gas (nitrogen) was 1.29 ml per min. The amplified signals were transferred and recorded in a computer with GC-solutions software. The quantitative method was followed with external standard mixtures of fatty acids (C6-C24, Sigma, USA) and was run earlier under similar conditions.

The data of total lipids were statistically analyzed and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The total lipid content in 13 species of cyanobacteria is shown in **Table 1** and the same in a few species at different seasons is shown in **Table 2**. It was found that *Microcystis aeruginosa* showed high lipid content followed by *Phormidium purpurescens*, whereas in *Phormidium ambiguum* it was lowest. The total lipid content was high in summer season and it was least in monsoon.

Table 1- Total lipid content in some freshwater cyanobacteria.

Cyanobacteria	Total lipid content[a,b]
Oscillatoria calcuttensis	25.70±0.14
Oscillatoria acuminata	24.65±0.21
Nostoc linckia	18.45±0.07
Calothrix fusca	22.60±0.28
Lyngbya limnetica	18.10±0.14
Phormidium purpurescens	26.45±0.21
Microcystis aeruginosa	28.15±0.21
Lyngbya dendrobia	10.55±0.07
Oscillatoria perornata	14.10±0.14
Phormidium ambiguum	10.48±0.10
Oscillatoria amoena	18.63±0.18
Scytonema bohnerii	22.22±0.32
Oscillatoria chlorina	16.62±0.16

[a] **Mean ± standard deviation** [b] **percentage of total lipids**

Table 2- Total lipid content[a,b] of cyanobacteria at different seasons.

Cyanobacteria	Monsoon	Post monsoon	Pre monsoon
Oscillatoria culcutensis	19.18±0.18	22.68±0.13	28.10±0.10
Oscillatoria acuminata	15.60±0.20	19.28±0.02	26.23±0.23
Nostoc linckia	10.27±0.07	14.52±0.12	20.38±0.22
Microcystis aeruginosa	18.48±0.14	24.44±0.06	30.32±0.08
Oscillatoria perornata	07.82±0.13	09.37±0.37	16.62±0.08
Calothrix fusca	15.57±0.07	17.43±0.09	25.57±0.07
Lyngbya limnetica	11.53±0.37	16.62±0.12	20.33±0.16
Phormidium purpurescens	18.70±0.16	24.42±0.07	26.70±0.14

[a] Mean ± standard deviation **[b] percentage of total lipids.**

The total number and percentage of fatty acids of cyanobacteria isolated from tanks and reservoirs are listed in **Table 3** and **4**. In the present study most of the fatty acids were unsaturated (50-65 %) although n-saturated fatty acids were also found in most of the isolates (20-40%). In these, major fatty acids were palmitic acid (C16:0) ranging from 5 to 45% of the total fatty acids, oleic acid (C18:1) and lenoleic acid (C18:2) were also present as higher components (5 to 50%). The predominant unsaturated fatty acids obtained were oleic acid (C18:1) and lenoleic acid (C18:2) upto 50%. Other monounsaturated fatty acid observed was palmitoleic acid (C16:1) as lower component (upto 10%). There were n-saturated fatty acids such as lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0) heptadecanoic acid (C17:0) and stearic acid (C18:0) which were also determined in less amounts (upto 10%). Among these myristic acid (C14:0) was found only in *Oscillatora acuminata*. It was also found that n-saturated fatty acid namely, palmitic acid (C16:0) was present in all the 13 species.

Table 3- Fatty acids present in different species of cyanobacteria.

	Cyanobacteria												
Fatty acids	***O.c***	***O.a***	***N.l***	***C.f***	***L.l***	***P.p***	***M.a***	***L.d***	***O.p***	***P.a***	***O.a***	***S.b***	***O.c***
Lauric acid C12:0	+	+	-	-	-	-	-	-	-	+	-	-	-
Tridecanoic Acid C13:0	-	+	-	-	-	-	+	-	-	-	-	-	-
Myristic acid C14:0	-	+	-	-	-	-	-	-	-	-	-	-	-
Palmitic acid C16:0	+	+	+	+	+	+	+	+	+	+	+	+	+
Palmitoleic acid 16:1	+	+	-	-	-	+	-	+	+	-	-	-	-
Heptadecanoic acid C17:0	+	+	-	-	-	-	-	-	-	-	-	-	-
Stearic acid C18:0	+	+	-	-	-	+	+	-	-	-	-	-	+
Oleic acid C18:1	+	+	+	-	+	+	-	+	-	-	+	-	+
Linoleic acid C18:2	+	+	+	+	+	+	+	+	-	-	+	+	+
α-linolenic acid C18:3	-	-	-	-	-	-	+	-	-	-	-	-	-
γ-linolenic acid	-	-	-	-	-	-	+	-	-	-	-	-	-

C18:3													
Cis-11-eicosenoic acid C20:1	-	-	-	-	-	-	+	-	-	-	-	-	-
Lignoceric acid C24:0	-	-	-	-	-	-	-	-	-	+	+	-	+

+ Present, - Absent

Table 4- Fatty acid composition (%) of cyanobacteria[a] isolated from different habitats.

	Cyanobacteria												
Fatty Acid	*O.c*	*O.a*	*N .l*	*C.f*	*L.l*	*P.p*	*M.a*	*L.d*	*O.p*	*P.a*	*O.a*	*S.b*	*O.c*
n-Saturated													
Total	41.91	45.73	45.4	8.3	42.4	48.52	49.18	29.1	37.1	14.27	18.9	60.1	22.16
Dodecanoic (12:0)	3.67	2.82	-	-	-	-	-	-	-	4.87	-	-	-
Tridecanoic (13:0)	-	2.76	-	-	-	3.70	-	-	-	-	-	-	-
Tetradecanoic (14:0)	-	2.67	-	-	-	-	-	-	-	-	-	-	-
Hexadecanoic (16:0)	25.6	24.3	45.4	8.3	42.4	39.4	45.2	29.1	37.1	9.4	18.9	60.1	17.4
Heptadecanoic (17:0)	7.48	10.32	-	-	-	-	-	-	-	-	-	-	-
Octadecanoic	5.16	2.86	-	-	-	5.42	3..98	-	-	-	-	-	4.76

(18:0)													
Long chain saturated													
Tetracosanoic acid (24:0)	-	-	-	-	-	-	-	-	-	7.63	6.81	-	8.32
Unsaturated													
Total	47.9	44.59	54.57	6.4	65.77	27.54	27.7	52.37	-	-	26.92	40	89
9- Hexadecanoic (9-16:1)	9.72	7.03	-	-	8.18	-	18.3	16.9	-	-	-	-	-
9-Octadecanoic (9-18:1)	27.88	18.56	22.67	-	30.19	9.44	-	11.32	-	-	11.92	-	3.6
9,12-Octadecadienoic (9,12-18:2)	10.3	19	31.9	6.4	27.4	18.1	9.4	13.4	-	-	15	40	5.03
all- cis-9,12,15-Octadecatrienoic (9,12,15-18:3)	-	-	-	-	-	-	6.45	-	-	-	-	-	-
all-cis- 6,9,12-Octadecatrienoic (6,9,12-18:3)	-	-	-	-	-	-	10.75	-	-	-	-	-	-
Long chain unsaturated													
Cis-11-Eicosenoic acid (20:1)	-	-	-	-	-	-	3.88	-	-	-	-	-	-

[a]**O. c**- *Oscillatoria calcuttensis* **L. l**- *Lyngbya limnetica* **O. p**- *Oscillatoria perornata*
O. a- *Oscillatoria acuminata* **P. p**- *Phormidium purpurescens* **P. a**- *Phormidium ambiguum*
N. l- *Nostoc linckia* **M. a**- *Microcystis aeruginosa* **O .a**- *Oscillatoria amoena*
C. f- *Calothrix fusca* **L. d**- *Lyngbya dendrobia* **S. b**- *Scytonema bohnerii*
O. c- *Oscillatoria chlorina*

The polyunsaturated fatty acids such as α-linolenic acid (C18:3) (ALA) and γ- linolenic acid (C18:3) (GLA) were found only in *Microcystis aeruginosa* (upto 6-10 %). Similarly, Cis-11-eicosenoic acid (C20:1) was found only in *Microcystis aeruginosa* (upto 4%). In the study it was also observed that saturated long chain fatty acid namely, lignoceric acid (C24:0) was found in few isolates (upto 8%) as lower components.

Lipids have been recognized as essential components in human and animal nutrition and are used as feed additives in aquaculture. Microalgae are traditionally used for bivalves in mollusc hatcheries**.** They are primary producers in mariculture i.e. food for consumers such as rotifers, copepods, daphnia, brine shrimps etc. which are fed to late larval and juvenile fishes and crustaceans (Fraser *et. al*., 1989; Langdon *et. al*., 1981; Pernet *et. al*., 2003; Viso *et. al*. 1993). The fatty acids of cyanobacteria are either saturated or unsaturated. They can also tolerate environmental stresses such as heat, cold, desiccation, salinity etc. (Agarwal *et. al*., 2000, 2002 ; Benjamin *et. al*., 2008 ; Sinha, *et. al*., 1996 ; Tandeau *et. al*., 1993). Among the investigations (Holton *et. al*., 1964 ; Kenyon *et. al*., 1972 ; Lennarz *et. al*., 1966 ; Rezanka, *et. al*., 1982) the major types are hexadecanoic (16:0), 9-hexadecenoic (16:1), hexadecadienoic (16:2), octadecanoic (18:0) and 9-octadecenoic (18:1).

The fatty acids i.e. (PUFA) play an important role in human metabolic pathways, particularly as specific precursors for prostaglandin E1 (Mendes *et. al*., 2006). The PUFA including the essential fatty acids namely, linoleic acid, α-linolenic acid (ALA) and γ-linolenic acid (GLA) are important in pharmaceutical industry. The γ-linolenic acid is recognized as a promising therapeutic agent for numerous health disorders acting as a precursor for prostaglandin E1, an important compound necessary for reducing inflammation and in treatment of heart disease, Parkinson disease, multiple sclerosis, plasma cholesterol levels, dermatitis, diabetes, and pre-menstrual syndrome (Biagi *et. al*., 1991; Ghazala *et. al*., 2005; Tran *et. al*., 2009; Wainwright *et. al*., 1996). The cyanobacteria are capable of accumulating 1% of GLA in the dry cell mass. Under certain environmental conditions *viz*., high light intensity and low temperature, the GLA to total fatty acid ratio could be enhanced up to 31.7% (Cohen *et*. *al*., 1993). The PUFA play an important role in regulating cell membrane properties and serve as precursors for important animal hormones and are found to be critical in maintaining high growth, survival and reproductive rates and hence play an important role in the aquaculture studies (Brett *et*. *al*., 1997).

The previous studies have shown that the physical characteristics of thylakoid membrane of cyanobacteria are mainly determined by extant of PUFA in membrane lipids. These thylakoid membranes containing high level of PUFA tend to decrease the phase transition temperature and increase the fluidity of membrane lipids. Besides, it has been found that PUFA are important for the growth and the ability to tolerate photoinhibition of photosynthesis at low temperatures (Volkman *et*. *al*., 1989; Wada, *et*. *al*., 1990). Hence these molecules are essential to maintain the stability and fluidity of the membrane at low temperature.

CONCLUSION

The study documents the lipid and fatty acid content in cyanobacteria and indicates that the total lipid content and their constituent fatty acid composition vary with their groups, location and the habitat. Some of these freshwater cyanobacteria are a source of essential fatty acids that are of commercial interest, including linoleic, and α-and γ- linolenic acids, among others. Further, some cyanobacteria serve as an important source of essential fatty acids for aquatic animals; their survival and growth rates are related to fatty acid content of the feeds. The GC analysis has indicated the presence of palmitic acid in all the species followed by linoleic acid.

Acknowledgements The authors thank the U.G.C., New Delhi for financial assistance and the Director, C.P.C.R.I., Kasaragod for fatty acid analysis. The first author is grateful to the Sri Ramakrishna Mission, Mangalore for the academic encouragement.

REFERENCES

Agarwal, S.C. and Singh, V. 2000. Vegetative survival, akinete formation and germination in three blue green algae and one green algae in relation to light intensity, temperature, heat shock and UV exposure. *Folia*. *Microbiol*. **45**: 439-448

Agarwal, S.C. and Singh, V. 2002. Viability of dried filaments, survivability and reproduction under water stress, and survivability following heat and UV exposure in *Lyngbya maetensiana, Oscillatoria agardhii, Nostoc calcicola, Phormidium fluitans, Spirogyra* sp. and *Vaucheria geminata*. *Folia*. *Microbiol*. **47**: 61-68

Anupama, P.R. 2000. Value added food: single cell protein. *Biotech*. *Adv*. **18**: 459-479

Bednarz, T., Starzecka, G. and Mazurkiewicz, B. 2002. Microbiological processes accompanied by algal and blue-greens water blooms. *Wiad*. *Bot*. **46**: 45-55

Benjamin, P., Arianna, B., Raffaella, P., Raffaella, P., Alba, E., Pietro, C. and Claudio, S. 2008. Fatty acid composition of Antarctic cyanobacteria. *Phycologia*. **47**: 430-434

Biagi, P.L., Bordoni, A., Hrelia, S., Celadon, M. and Horrobin, D.F. 1991. γ-Linolenic acid dietary supplementation can reverse the aging influence on rat liver microsome Δ6- desaturase activity. *Biochim*. *Biophys*. *Acta*. **1083**: 187-192

Brett, M.T. and Muller-Navarra, D.C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwat*. *Biol*. **38**: 483-499

Bury, N.R., Codd, G.A., Bonga, S.E.W. and Flik, G. 1998. Fatty acids from the cyanobacterium *Microcystis aeruginosa* with potent inhibitory effects on fish gill Na+/K+

-ATPase activity. *J. Expt. Biol.* **201**: 81-89

Carmichael, W.W., Azevedo, S.M.F.O., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R. and Eaglesham, G.K. 2001. Human fatalities from cyanobacteria: chemical and biological evidences for cyanotoxins. *Environ. Heal. Persp.* **109**: 663-668

Caudales, R. and Wells, J.M. 1992. Differentiation of the free-living *Anabaena* and *Nostoc* cyanobacteria on the basis of fatty acid composition. *Int. J. Syst. Bacteriol.* **42**: 246-251

Caudales, R., Wells, J.M. and Butterfield, J.E. 2000. Cellular fatty acid composition of cyanobacteria assigned to subsection II, order *Pleurocapsales*. *Int. J .Syst. Evol. Microbiol.* **50**:1029-1034

Ciferri, O. and Tiboni, O. 1985. The biochemistry and industrial potentials of *Spirulina*. *Microbiol. Rev.* **39**: 503-526

Cohen, Z., Reungjitchachawali, M.W., Siangdung, W. and Tanticharoen, M. 1993. Production and partial purification of γ-linolenic acid and some pigments from *Spirulina platensis. J. Appl. Phycol.* **5**:109-115

Dor, I. 1987. Preservation and microscopy of blue-green algae (cyanobacteria) on dry agar. *Bot. Mar.* **30**: 507-510

Fraser, A.J., Sargent, J.R., Gamble, J.C. and Seaton, D. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus*) larvae. *Mar. Chem.* **27**: 1-18

Ghazala, B. and Shameel, M. 2005. Phytochemistry and bioactivity of some freshwater green algae from Pakistan. *Pharma. Biol.* **43**: 358-369

Holton, R.W., Blecker, H.H. and Onore, M. 1964. Effect of growth temperature on the fatty acid composition of blue - green algae. *Phytochemistry*. **3**: 595-602

Kenyon, C.N., Rippka, R. and Stanier, R.Y. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. *Archiv*. *Microbiol*. **83**: 216-236

Kruger, G.H.J., Wet, H.D., Kock, J.L.F. and Pieterse, A.J.H. 1995. Fatty acid composition as taxonomic characteristic for *Microcystis* and other coccoid cyanobacteria (blue- green alga) isolates. *Hydrobiologia*. **308**: 145-151

Langdon, C.J. and Waldock, M.J. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of Crassostrea *gigas* spat. *J. Mar*. *Biol*. *Assoc*. U. K. **61** : 431-448

Lennarz, W.J. 1966. Lipid metabolism in the bacteria. *Adv*. *Lipid*. *Res*. **4**: 175-225

Li, R. and Watanabe, M.M. 2001. Fatty acid profiles and their chemotaxonomy in planktonic species of *Anabaena* (cyanobacteria) with straight trichomes. *Photochemistry*. **57**: 727-731

Mahajan, G. and Kamat, M. 1995. Gamma linolenic acid production from *Spirulina platensis*. *Appl*. *Microbiol*. *Biotech*. **43**: 466-469

Manoharan, C. and Subramanian, G. 1993. Influence of effluents on fatty acid content of a cyanobacterium. *Curr*. *Sci*. **65**: 353-355

Mendes, R.L., Reis, A.D. and Palavra, A.F. 2006. Supercritical CO_2 extraction of γ-linolenic acid and other lipids from *Arthrospira (Spirulina) maxima*: comparison with organic solvent extraction. *Food*. *Chem*. **99**: 57-63

Pauda- Resurreccion, A.B. and Banzon, J.A. 1979. Fatty acid composition of the oil from progressively maturing bunches of coconut. *Philip. J. Coconut. Stud.* **4**: 1-15

Pernet, F., Tremblay, R., Demers, E. and Roussy, M. 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. Grown in a semicontinuous system. *Aquaculture*. **221**: 393-406

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

Renaud, S.M., Thinh, L.V. and Parry, D.L. 1999. The gross composition and fatty acids composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture*. **170**:147-159

Rezanka, T., Zahradnik, J. and Podojil, M. 1982. Hydrocarbons in green and blue green algae. *Folia*. *Microbiol*. **27**: 450-454

Sinha, R.P. and Hader, D.P. 1996. Response of a rice field cyanobacterium *Anabaena* sp. to physiological stressors. *Environ*. *Expt*. *Bot*. **36**: 147-155

Stainer, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. 1971. Purification and properties of unicellular blue –green algae (order *Chroococcales*). *Bacteriol*. *Rev*. **32**: 171-205

Tajuddin, N. and Subramanian, G. 2005. Cyanobacterial biodiversity and potential applications in biotechnology. *Curr*. *Sci*. **89**: 47-57

Tandeau De Marsac, N. and Houmard, J. 1993. Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS. *Microbiol*. *Rev*. **104**: 119-190

Tran, H., Hong, S. and Lee, C. 2009. Evaluation of extraction methods for recovery of fatty acids from *Botryococcus braunii* LB 572 and *Synechocystis* sp. PCC 6803. *Biotechnol*. *Bioeng*. **14**: 187-192

Viso, A.C. and Marty, J.C. 1993. Fatty acids from 28 marine microalgae. *Phytochemistry*. **34**:1521-1533

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I. and Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J .Expt .Mari*. *Biol*. *Ecol*. **128**: 219-240

Wada, H., Gombos, Z. and Murata, N. 1990. Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature*. **347**: 200-2003

Wainwright, P.E., Huang, Y.S., Levesque, S., Mutsaers, L., Mccutcheon, D., Balcaen, P. and Hammond, J. 1996. Effects of dietary γ- linolenic acid and prenatal ethanol on mouse brain and behaviour. *Pharmacol*. *Biochem*. *Beh*. **53**: 843-852