ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

Lipid Profile Of Thermophlic Cyanobacterium "Mastigocladus Laminosus" At Different Temperatures

*A.C.Mongra

Department of Biosciences, Himachal Pradesh University, Shimla

*Present address- Professor &Head Department of Biomedical Engineering &Biotechnology

Adesh Institute of Engineering &Technology (Punjab Technical University) Faridkot, Punjab- 151203

Abstract: Thin layer chromatography of total lipids of Mastigocladus laminosus and comparison with values of standard indicated the presence of lipid spots, monogalactosyl diglyceride (MGDG), glycolipid (GL) and phosphatidyl diglyceride (PG) common in both high (45°C) and low temperature grown cells (26°C). Total saturated fatty acid content was five times higher in low temperature grown cells (25°C) compared to 45°C. Among important fatty acids detected in cells were caprylic acid (C_8:0), nonanoic acid (C_9:0), capric acid (C_{10}:0), undecanoic acid (C_{11}:0), lauric acid (C_{12}:0), tridecanoic acid (C_{13}:0), myristic acid(C_{14}:0), pentadecanoic acid (C_{15}:0), palmitic acid (C_{16}:0), heptadecanoic acid (C_{17}:0), stearic acid (C_{18}:0), nondecanoic acid (C_{19}:0), arachidic acid (C_{20}:0), heneicosanoic acids (C21:0), behenic acid (C_{22}:0), trichosanoic acid (C_{23}:0), and lignoceric acid (C_{24}:0). Low molecular weight saturated fatty acids species e.g. caprylic acid (C_8:0) and nonanoic acid (C_9:0), were totally absent in low temperature grown cells, while large molecular weight saturated fatty acid from carbon chain length C_{11} to C_{24}. were abundant in low temperature grown cells. Higher amount of saturated fatty acid in cells grown at suboptimum temperature (25°C) indicated that membranes become highly rigid and as a result most of the membrane linked processes such as photosynthetic electron transport remains non-functional or less efficient at 25°C.

Keywords: TLC,HPLC,Mastigocladus laminosus,Lipid profile, thermophilic cyanobacteria,extreme environment

I.INTRODUCTION

Lipids are esters of fatty acids and alcohols that comprise a large group of structurally distinct organic compounds including fats, waxes, phospholipids, glycolipids etc. Cyanobacteria may contain significant quantities of lipids (fats and oil) with compositions similar to those of vegetable oils. The lipids of some cyanobacterial species are also rich in essential fatty acids such as the C18 linoleic and y-linolenic acids and their C20 derivatives, eicosapentaenoic acids and arachidonic acid). These fatty acids are essential components of the diet of humans and animals and are becoming important feed additives in aquaculture (Borowitzka 1988). The lipids of cyanobacteria are generally esters of glycerol and fatty acids They may be either saturated or unsaturated. Some of the filamentous cyanobacteria tend to have large quantities (25 to 60 % of the total) of polyunsaturated fatty acids (Parker et al. 1967, Holton and Blecker 1972, Kenyon et al. 1972). A few cyanobacterial strains, which show facultative anoxygenic CO_2 photoassimilation with sulphite as electron donor, lack polyunsaturated fatty acids in their lipids (Oren et al. 1985).

Cyanobacteria contain four major glycerolipids:monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol(SQDG), and phosphatidylglycerol (PG).MGDG represents 50-60% of the total glycerolipid content, and DGDG, SQDG, and PG each amount to 10-20%(Murata,1989).The cyanobacteria also contain minor amounts of monoglucosyl diacylglycerol (MGlDG), which is a precursor in MGDG synthesis. The fatty acids known to be present in cyanobacteria are palmitic acid (16:0), Palmitoleic acid can be abbreviated as $16:1^{\Delta 9}$, hexadecadienoic acid (16:2 with unknown double bonds positions), stearic acid (18:0), oleic acid ($18:1^{\Delta 9}$), linoleic acid ($18:2^{\Delta 9,12}$), α-linolenic acid ($18:3^{\Delta 9,12,15}$), γ-linolenic acid ($18:3^{\Delta 6,9,12}$), and octadecatetraenoic acid ($18:4^{\Delta 6,9,12,15}$) (Murata,

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

1989 and Murata, et al 1992). Some cyanobacterial strains contain in addition myristoleic acid ($14:1^{\Delta 9}$) and cisvaccenic acid ($18:1^{\Delta 11}$). (Larisa etal 1999)

The temperature-dependent changes in fatty acid composition were extensively studied in the mesophilic cyanobacteria Anabaena variabilis (Sato et al 1979 and Sato and Murata 1980), Synechocystis sp. PCC 6803 (Wada and Murata 1990) and Anacystis nidulans (Sato and Murata 1988) It was suggested that accelerated unsaturation of membrane lipids helps to maintain the membrane fuidity that is reduced under low temperature conditions(Murata and Wada 1995 ; Murata and Los 1997). Thus, fatty acid unsaturation is considered to be the key process in temperature acclimation in the mesophilic organisms.In present investigation quantitative changes in the fatty acid content at different temperatures fatty acid composition of the thermophilic cyanobacterium Mastigocladus laminosus were investigated in shifting of culture from optimum growth temperature to sub optimum temperatures .Induced changes in the the fatty acids in relation to fluidity of member has been studied

II.MATERIAL & METHODS

II.1 Culture conditions

The culture of Mastigocladus laminosus was isolated from hot water spring Tattapani(HP)48 KM from Shimla.The cells were cultured photoautotrophically at 45^{o}C with continuous illumination at 100 µmol quanta m^{-2} s^{-1} and aeration with air that contained 2% CO_2, in BG-11 medium (Stanier et al 1971) supplemented with 25 mM HEPES-NaOH, pH 7.5.. The cells were grown at 45^{0}C until the OD at 790 nm (OD790) reached 0.4 (about 3×10^{7} cells ml^{-1}).Then they were transferred to 45^{0}C,35^{0}C and 25^{0}C . Lipid extraction and fatty acid analysis were done from all the samples after 7 days of shifting of temperatures from initial growth temperature of 45^{0}C . All experiments were performed in duplicate

II.2 Extraction and seperation of lipids

The methods of extraction and sepration of lipids by thin layer chromatography used were of Nichols and Wood (1968) and Walsby and Nichols (1969), with a slight modification.

II.3 Extraction

Algal material was washed by centrifugation and extracted with chloroform: methanol, 2:3(v/v), clear extract was separated after centrifugation. The process was repeated until blue coloured residue remained. The extracts were pooled and concentrated by evaporating at 37^{o}C.

II.4Thin layer Chromatography

Thin layer chromatography was carried out on the glass plates of size 20cm X 20cm size. Kieselgel Gnach Stahl silica gel of 5-25µ grain size containing 13% was mixed with distilled water in the ratio of 1.2(w/v) and layered on glass plates having 0.25cm thickness. Plates were activated at 120^{o}C for 2 h After the application of extracts, the plates were developed in the chromatographic tank saturated with the solvent. The solvent system used was chloroform : methanol : glacial acetic acid : water, 85 : 15 : 10 : 3 (v/v). After development, the plates were removed and dried at room temperature.

II.5 Visualization lipids

II.5.1 Iodine method (Randerath,1964)

Plates were exposed to iodine vapours in an air tight tank. All unsaturated and saturated lipids appeared as brownish yellow to yellow spots

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

II.5.2 Sulphuric acid method (Randerath, 1964)
The plates were wetted with 25% sulphuric acid by dipping the lower end of the plates in the acid and dried at 150°C for 10min. Lipids appeared as charred black or pink spots depending upon their nature.

II.6 Determination of Rf valves

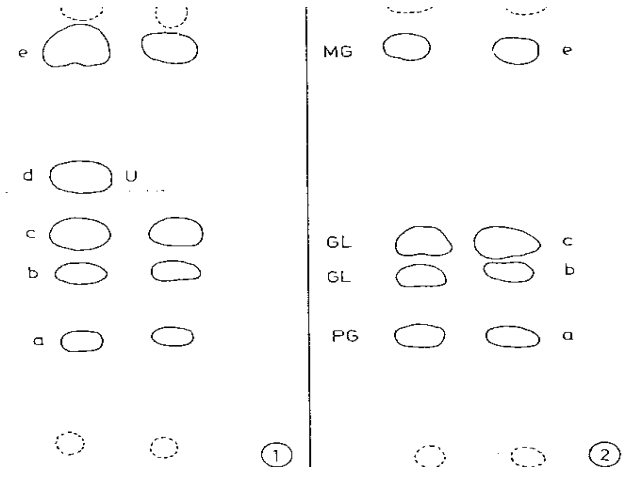
The plates were always run upto a known distance. The distance of each band migrated was measured and the Rf value was calculating in relation to the solvent front.

II.7 Identification and quantification of fatty acids

Identification and quantification of fatty acid was done by gas chromatography (Miller and Berger, 1985). using a Hewlett-Packard HP 5890 gas chromatograph equipped with a SP2330 capillary column. The fatty acid at three temperatures (25,35 and 45°C) was found out from the retention time (RT) and quantified them by using peak area and expressed as mg/g dry weight of sample.

III. RESULTS & DISCUSSION

Lipid and fatty acid composition in diverse cyanobacteria have been studied by several workers (Wahal et al., 1973; Yadav, 1975; Rao and Talpsayai, 1982). Since cyanobacteria differ in their morphology and growth characteristics at different growth temperature, relative changes in the lipid spectrum are also expected. Results, from thin layer chromatography of total lipids in the present study and comparison with values of standards, indicated the presence of lipid spot, monogalactosyl diglyceride (MGDG) glycolipid (GL) and phosphatidyl glycerol (PG) common in both highand low temperature grown cells, when TLC plates were developed with H_2SO_4 acid treatment as shown in Fig. 1.



LTG HTG LTG HTG
1. IODINE VAPOUR 2. SULPHURIC ACID
U Unidentified MG Monogalactosyl Diglyeride
GL glycolipid PG Phosphyatidyl Glycerol

Fig.1 Thin layer chromatogram (Diagrammatical) of lipids of the alga Mastigocladus laminosus showing effect of temperature on lipid components

Visualization 1 With iodine vapour
a,c&e -Deep yellow
b&d -Yellow

2 With sulphuric acid

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

a- Light Brown
d&e- Brown
d- Dark Brown

One unidentified lipid spot was observed in low temperature adapted cells when TLC plate was developed with iodine vapours. This additional spot was absent in high temperature grown cells Fig 1. Nicholas (1973) reported the presence of glycolipid in cyanobacteria which includes MGDG, DGDG and SQDG. Except for some minor differences in the lipid spots found in LTG and HTG cells, no quantitative changes were observed. However amount of individual lipid species has not been quantified. Changes in both fatty acid species and their quantity were observed when the cyanobacterium was preadapted to different growth temperature for one week as shown in Table 1.

Table 1
Quantitative changes in the fatty acids content at different growth temperatures

Fatty acid (Carbon Number: Double Bond)	**Amount (mg/g dry weight) growth temperature (^{0}C)**		
	25	**35**	**45**
C_7:0	-	-	-
C_8:0	-	5.580	0.115
C_9:0	-	-	1.335
C_{10}:0	-	-	-
C_{11}:0	0.991	0.884	0.652
C_{12}:0	0.898	0.533	0.626
C_{13}:0	0.652	0.393	0.628
C_{14}:0	0.441	0.282	0.304
C_{15}:0	0:074	0.042	0.069
C_{16}:0	9.438	6.879	3.309
C_{17}:0	6.823	1.049	0.920
C_{18}:0	14.427	4.390	2.094
C_{19}:0	0.000	0.000	0.108
C_{21}:0	3.205	-	-
C_{24}:0	0.036	-	-

Fatty acids Not present

Carbon No.	Fatty acids	Carbon No.	Fatty acids
C_7:0	Heptonic acid	C_{18}:0	Stearic acid
C_8:0	Caprylis acid	C_{18}:1	Oleic acid
C_9:0	Nonanoic acid	C_{18}:2	Cis Linoleic acid
C_{10}:0	Capric acid	C_{18}:3	Linolenic acid
C_{11}:0	Undecanoic acid	C_{19}:0	Nondecanoic acid
C_{12}:0	Lauric acid	C_{20}:0	Arachidic acid
C_{13}:0	Tridecanoic acid	C_{20}:4	Arachidonic acid
C_{14}:0	Myristic acid	C_{21}:0	Heneicosanoic acid
C_{15}:0	Pentadecnoicacid	C_{22}:0	Behenic acid
C_{16}:0	Palmitic acid	C_{23}:0	Trichosanoic acid
C_{16}:1	Palmitoleic acid	C_{24}:0	Lignoceric acid
C_{17}:0	Heptadecanoic acid		

Low molecular weight saturated fatty acid species e.g. caprylic acid (C_8:0) and nonanoic acid were totally absent in low temperature grown cells, while large molecular weight saturated fatty acid from carbon chain length C_{11} to C_{14} were abundant in low temperature grown cells compared totheir high temperature grown counterpart. Heneicosanoic acid (C21:0) and lignoceric acid (C_{24}:0) were totally absent at high temperature. Among predominant forms of saturated fatty acids, palmitic acid (C16:0,) heptadecanoic acid (C17:0) and stearic acid (C18:0) were significantly higher in 25^{o}C grown cells and the trend of the above three species of saturated fatty acids progressively decreased with increase of growth temperature. Total amount of saturated fatty acids calculated

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

from Table 1 at different growth temperatures have been depicted in the form of a histogram in Fig 2. A significantly higher saturated fatty acid content was in cells grown at 25°C as compared to 45°C. It is interesting to note that these variations in fatty acid profiles at different growth temperatures was a result of shifting of the culture originally grown at 45°C.

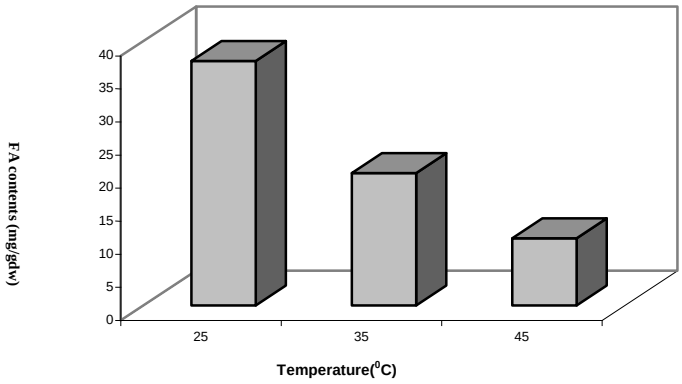


Fig2. VARIATION IN THE TOTAL SATURATED FATTY ACIDS (FA) CONTENTS AT DIFFERENT GROWTH TEMPERATURES

In mesophilic cyanobacteria, the temperature-dependent changes in fatty acid composition were mainly assigned to MGDG, the neutral major galactolipid of the membranes (Sato,1979; Sato and Murata 1980; Sato, and Murata1988). It was also reported that in A. nidulans, which is able to produce only saturated and monounsaturated fatty acids, the decrease in temperature caused conversion of 16:0 to 16:1 in all lipid classes (Murata,1989). Analogous results were reported for lipid molecular species of the filamentous thermophilic cyanobacterium Mastigocladus laminosus (Hirayama and Kishida, 1991).In S. vulcanus, neutral galactolipids were not significantly affected by the temperature dependent desaturation. The changes in unsaturation pattern in S. vulcanus were caused mainly by desaturation of the charged lipids PG and SQDG (Kiseleva etal .1999)

The composition of fatty acids depends on the growth temperature.A change in faty acids and lipid after a shift of the growth temperature has been reported in Anabaena variabilis (Sato and Murata, 1980). A decrease in the saturated fatty acids at high temperature 45°C compared to 25°C does not reflect a conversion of saturated fatty acids to unsaturated ones as the later were not quantified. A reduction in the level of unsaturated fatty acids in S. lividus grown at high temperature as compared to low temperature grown cells has also been reported. Brock (1967a) reported that in the thermophilic M. laminosus, there is a total lack of polyunsaturated fatty acids. This low content of unsaturated fatty acids in the membrane of this thermophilic cyanobacterium may be a crucial factor for thermal adaptation. Higher saturation of fatty acids provides a stability to the membrane at higher temperature (David et.al., 1979). Due to the unique nature of M. laminosus i,e. a complete lack of polyunsaturated fatty acids, it is cosmopolitan and wide spread in almost all hot water springs in the world. The significance of higher saturated fatty acid content at lower temperature compared to higher growth temperature can not be explained. Miller et al. (1988) reported that the growth of a strain of Synechococcus species at 38°C caused an increase in the proportion of unsaturated fatty acids compared to its growth at 58°C. Unsaturated fatty acids have a lower melting point compared to their saturated analog and an increase in the degree of unsaturation causes greater fluidity of

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

the membrane. The fatty acid compositions of cyanobacterial lipids show a considerable variation both when comparing different strains of the same genus and when comparing different growth conditions for a particular strain (Kenyon, 1972; Olsen and Ingram, 1975). A thermophilic strain of Synechococcus species contais no unsaturated fatty acid, while another species contains both mono and diunsaturated fatty acids (Kenyon, 1972). The proportion of mono unsaturated fatty acids in various thermophilic strains of Synechcococcus sp. when grown around 50°C varies from 25% (ForK et al., 1979) to 65% (Kenyon, 1972). Thus a general tendency towards less unsaturation and also a shorten carbon chain length is evident in thermophilic strains. The overall fatty acid composition is in general, a rather crude measure of the functionally important aspects of membrane composition.

IV.CONCLUSION

In cyanobacterial cells, lipids are typically found only in the membranes Higher amount of saturated fatty acid in cells grown at suboptimum temperature (25°C) indicated that membranes become highly rigid and as a result most of the membrane linked processes such as photosynthetic electron transport remains non-functional or less efficient at 25°C. This type of study is useful to understand the function of plasma membrane at extreme temperature in shifting of temperatures .

REFERENCES

[1] Borowitzka M. A,. Fats,oils and hydrocarbons. In: Micro-Algal Biotechnology, (Eds. M. A. Borowitzka, L. J. Borowitzka).Cambridge University Press, Cambridge, pp. 257-287, 1988
[2] Brock, T.D.,and Brock, M.L., The measurement of chlorophyll, primary productivity, photophosphorylation and macromolecules in benthic algae mats, Limnol. Oceanogr,vol.12, pp. 600-605,1967a
[3] David, C.E., Murata, N., and Sato, N., Effect of growth temperature on the lipid and fatty acid composition and the dependence on temperature of light energy redistribution in the thermophilic blue-green alga Synechococcus lividus, Plant Physiol.,vol 63, pp. 524-530, 1979.
[4] Fork, D.C., Murata, N., and Sato, N. , Effect of growth temperature on the lipid and fatty acid compo- sition and the dependence on temperature of lightinduced redox reactions of cytochrome-f and light energy redistribution in the thermophilic blue-green alga Synechococcus lividus,Plant physiol., vol.63, pp. 524-530, 1979
[5] Hirayama, O., and Kishida, T., Temperature-induced changes in the lipid molecular species of a thermophilic cyanobacterium, Mastigocladus laminosus, Agric. Biol. Chem, vol. 55, pp. 781-785, 1991
[6] Holton R. W., and Blecker H. H., Fatty acids in blue-green algae. In: Properties and Products of Algae, (Ed. J. E. Zaick). Plenum,New York, pp. 115-127,1972
[7] Kenyons, C.N. ,Fatty acid and compositioin of unicellular strains of blue green algae, J. Bacteriol., 109: pp.827-834, 1972
[8] Kenyon ,C. N., Rippka R., and Stanier ,R. Y., Fatty acid composition and physiological properties of some filamentous bluegreen algae, Arch. Mikrobiol. Vol.83, pp. 216-236 ,1972
[9] Larisa L.,Kiseleva, Ibolya Horvaéth., Laészlo ,Vigh., and Dmitry ,A. Los., Temperature-induced specific lipid desaturation in the thermophilic cyanobacterium Synechococcus vulcanus, FEMS Microbiology Letters ,vol.175, pp. 179-183, 1999
[10] Miller, L.,and Berger, T., Bacteria identification by Gas Chromotography of whole cell fatty acids. Gas Chromotography Application, Note, pp. 228-241, 1985
[11] Murata, N., Low-temperature e¡ects on cyanobacterial membranes,J. Bioenerg. Biomembr, vol.21, pp. 61-75, 1989
[12] Murata, N., Wada, H., and Gombos , Z. ,Modes of fatty-acid desaturation in cyanobacteria, Plant Cell Physiol, vol.33, pp.933-941, 199
[13] Murata, N. and Wada, H., Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria,Biochem, J. vol. 308, pp.1-8, 1995
[14] Murata, N. and Los, D.A. Membrane fuidity and temperature perception, Plant Physiol,Vol.115, pp. 875-879 , 1997
[15] Nichols, V.W., and Wood, B.J.B., New glycolipid specific to nitrogen fixing blue-green alga., Nature (London),vol. 127, pp.767-775, 1968
[16] Olsen, G.J., and Ingram, I.O.,Effects of temperature and mutational changes on the fatty acids of Anmenellum quadroplicatum, J. Bot., vol.124, pp. 373-379, 1975
[17] Oren A., Fattom A., Padan E., and Tietz A., Unsaturated fatty acid composition and biosynthesis in Oscillatoria limnetica and other cyanobacteria,Arch. Microbiol. Vol.141, pp. 138-142,1985
[18] Parker P. L., Van Baalen C., and Maurer L., Fatty acids in eleven species of blue-green algae: geochemical significance, Science ,vol. 155,pp. 707-708, 1967
[19] Rao, P.N. S., and Talpasayi, E.R.S. Changes in soluble amino acids, cellular carbohydrates and lipids in brine alga Anabaena fertilissima under salt stress, Indian J. Exp.Biol., vol.20, pp. 765-767, 1982
[20] Randerath, K. Thin layer chromatography (Academic Press Inc., New York) vol.124, 1964
[21] Sato, N. and Murata , Temperature shift-induced responses in lipids in the blue-green alga, Anabaena variabilis, the central role of diacylmonogalactosyl glycerol in thermo- adaptation, Biochem. Biphys, Acta, vol. 619 ,pp. 353-356, 1980
[22] Sato, N., Murata, N., Miura, Y. ,and Ueta, N., Effect of growth temperature on lipid and fatty acid compositions in the blue-green algae, Anabaena variabilis and Anacystis nidulans, Biochim. Biophys. Acta, vol.572, pp. 19-28, 1979

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

[23] Sato, N., and Murata, N.,Temperature shift-induced responses in lipids in the blue-green alga, Anabaena variabilis:the central role of diacylmonogalactosylglycerol in thermoadaptation, Biochim. Biophys. Acta vol,619, pp. 353-366 , 1980
[24] Sato, N. , and Murata, N., Membrane lipids, Methods Enzymol,Vol.167, pp.251-259,1988
[25] Stanier, R.Y., Kunisawa, R., Mandel, M., and Cohen-Bazire,G. , Purification and properties of unicellular blue green algae (order Chroococcales), Bacteriol. Rev,vol. 35, pp.171-205, 1971
[26] Yadava, D.N, Effect of chemicals on the heterocyst pro-duction, function and effect of stress on Anabaena ambigua Rao, Ph.D. Thesis, Banaras Hindu University, India. , 1975
[27] Wada, H., and Murata, N., Temperature-induced changes in the fatty acid composition of the cyanobacterium,Synechocystis PCC6803.,Plant Physiol.vol. 92, pp.1062-1069, 1990
[28] Wahal, C.K., Reddy, P.M., and Talpasayi, E.R.S., Lipid composition of some blue-green algae, Ind. J. Expt. Biol., vol.11,pp.588-589,1973
[29] Walsby, A.E. and Nichols, B.W., Lipid components ofheterocyst. Nature, London, vol.221 pp. 673-674, 1969