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Original Research Article

Lipid composition of thermal isolate *Mastigocladus laminosus* at different temperatures

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A B S T R A C T

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^{o}C) and low temperature grown cells (26^{o}C). Total saturated fatty acid content was five times higher in low temperature grown cells (25^{o}C) compared to 45^{o}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^{o}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^{o}C.

K e y w o r d s

COD reduction; Electro-chemical; RSM; Ultra Violet

Introduction

Cyanobacteria are a common source of a wide range of fats, oils, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacologically and industrially important products. The application of cyanobacteria in the production of these latter compounds is only just being explored and their importance has yet to be determined. New developments in the chemical industries, particularly in the area of converting natural products to industrial feedstocks, will further enhance the range of commercially important products synthesized by cyanobacteria 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).In the present investigation quantitative changes in the fatty acid content at different growth temperature were investigated along with thin layer chromatography of lipids toward the functioning of photosynthesis process and growth of cyanobacterium which are ultimately responsible to get the industrial product of lipids of thermophilic cyanobacteria.

Materials and Methods

Extraction and separation of lipids

Mastigocladus laminosus was isolated from hot water spring Tattapani (HP).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.

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.

Thin layer Chromatography

Thin layer chromatography was carried out on the glass plates of size 20cm X 20cm size. Kieselgel Gnach Stahl silica gel of 5-25u 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.
Visualization lipids

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

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^{o}C

for 10min. Lipids appeared as charred black or pink spots depending upon their nature.

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.

Identification and quantification of fatty acids

Identification and quantification of fatty acid was done by gas chromatography (Miller and Berger, 1985). One gram of fresh weight of algal sample grown at various temperatures (25,35 and 45^{o}C) were extracted with sufficient volume of chloroform, methanol (2:1 v/v) reagent. The extracts were filtered through Whatman No.1 filter paper and extraction was repeated to ensure maximum removal of lipids from the cells of the organism. To the filtrates, equal volume of distilled water was added and vortexed thoroughly to remove water soluble impurities. The aqueous layer was removed by Pasteur pipette and moisture present in the liquid was removed by addition of sodium sulphate crystals in each sample. Each of the filtrate was transferred to pre-weighed bottle and dried by flushing nitrogen gas. The final weights were subtracted from the initial weights of the bottles to get weight of each lipid sample of particular temperature. 1ml of saponification regent (45g of NaOH in 300ml of ethanol water mixture, 1:1 v/v was added to 100ml each of lipid sample and tightly closed the tubes with teflon lined screw cap. The contents were vortexed and boiled for 30min. 2ml of methylation regent (325ml of 6N HCl in 275ml CH_3OH) was added and mixed thoroughly. The tubes were boiled for 20min in a water bath at 80^{o}C and cooled to room temperature and added 1.25ml of extraction solvent (200ml of hexane in 200ml of anhydrous dimethyl ether) in each of tubes. The aqueous lower phase in each tubes was discarded and added 3ml of base wash (10.8g of NaOH in 900ml distilled water). 2/3 of the organic extract of each of the tubes was transferred to a GC vial and run the GC in the following conditions:

Column DEGS
Column temp. 180^{o}C
Injection port temp. 200^{o}C
Detector temp. 230^{o}C
Carrier gas Nitrogen
Sample Volume 1.2ml
Detector FID
Integrator setting Zero
Chart speed
Peak width 0.04
Thrush 4
Chart speed 0.5 time
Peak width
P 0.64 time 5

Methyl esters of standard fatty acid (C8:0-C24:20) were run. The fatty acid at three temperatures (25,35 and 45^{o}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.

Results and 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. 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 8. 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 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^{o}C as compared to 45^{o}C. It is inter-esting 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^{o}C.

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^{o}C compared to 25^{o}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*

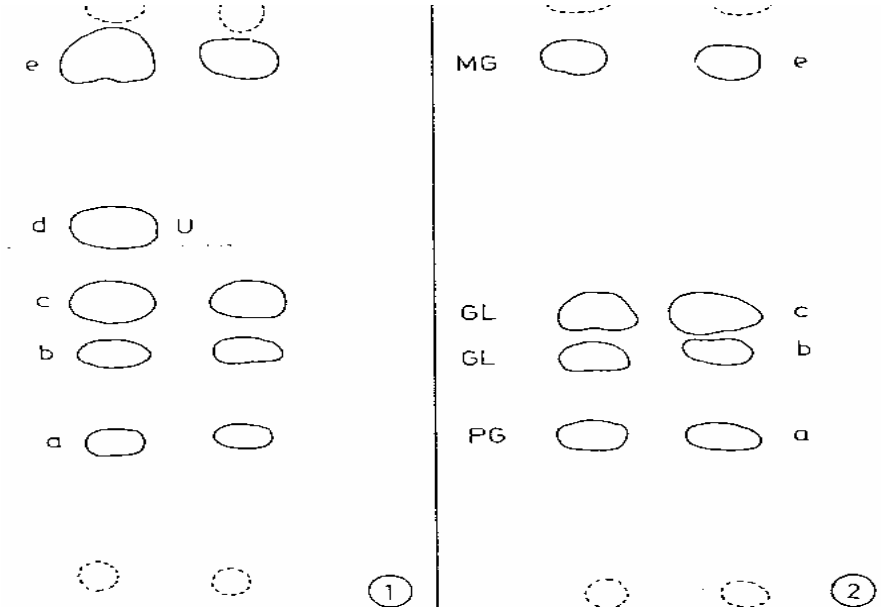
Table.1 Quantitative changes in the fatty acid content at different growth temperature

Fatty acid (Carbon Number : Doble Bond)	**Amont(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	-	-

- Not present

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

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



1. IODINE VAPOUR 2. SULPHURIC ACID

U Unidentified MG Monogalactosyl Diglyeride

GL glycolipid PG Phosphyatidyl Glycerol

Visualization 1 With iodine vapour

a,c&e -Deep yellow

b&d -Yellow

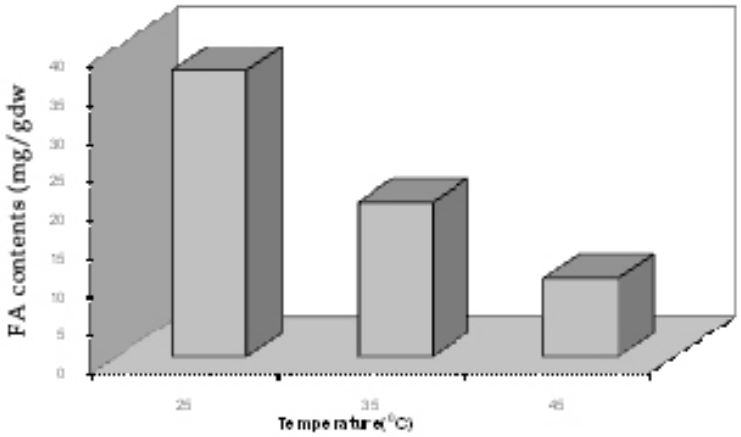
2 With sulphuric acid

a- Light Brown

d&e- Brown

d- Dark Brown

Fig.2 Variation in the total saturated fatty acids (fa) contents at different growth temperatures



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 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.

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.

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