

# Fatty Acid Composition of Unicellular Strains of Blue-Green Algae<sup>1</sup>

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The fatty acids of 34 strains of unicellular blue-green algae provisionally assigned to the genera *Synechococcus*, *Aphanocapsa*, *Gloeocapsa*, *Microcystis*, and *Chlorogloea* by Stanier et al. have been chemically characterized. The strains analyzed can be divided into a series of compositional groups based upon the highest degree of unsaturation of the major cellular fatty acids. Twenty strains fall into the group characterized by one trienoic fatty acid isomer ( $\alpha$ -linolenic acid), and seven strains fall into a group characterized by another trienoic acid isomer ( $\gamma$ -linolenic acid). These groups in many cases correlate well with groupings based upon other phenotypic characters of the strains, e.g., deoxyribonucleic acid base composition. The assignment of a strain to a compositional group is not altered when the strain is grown under a variety of different culture conditions. All strains contain glycolipids with the properties of mono- and digalactosyldiglycerides.

Blue-green algae have a prokaryotic cell structure, a property that they share with bacteria. However, their photosynthetic metabolism resembles that of eukaryotic algae and vascular plants with respect both to its mechanism and its machinery. The photosynthetic apparatus of blue-green algae contains the two pigments well-nigh universally associated with aerobic photosynthesis, chlorophyll *a* and  $\beta$ -carotene, as well as ferredoxin that is chemically of the plant type (2, 26). The major cellular lipids of the blue-green algae include the three glycolipids characteristic of the chloroplast: monogalactosyldiglyceride, digalactosyldiglyceride, and sulfoquinovosyldiglyceride (18). The other two groups of photosynthetic prokaryotes, purple bacteria and green bacteria, never contain chlorophyll *a*,  $\beta$ -carotene, or ferredoxins of the plant type. In most of them, the predominant cellular lipids are phospholipids, although green bacteria have been found recently to contain one of the glycolipids characteristic of the chloroplast, monogalactosyldiglyceride (4, 6).

The chloroplast fatty acids of eukaryotic algae and of vascular plants are largely polyenoic acids, of which  $\alpha$ -linolenic acid (18:3 $\alpha$ ), almost exclusively found in chloroplasts, is a major constituent in the leaf tissues of higher

plants and in certain groups of algae (18). The bacteria, on the other hand, including the purple and green bacteria, contain almost exclusively saturated fatty acids (including branched-chain acids) and monounsaturated fatty acids (or their cyclopropane group containing derivatives) in their cellular lipids. For this reason, the nature of the cellular fatty acids of blue-green algae is of particular comparative biochemical interest. The analyses so far conducted (9) have shown that the members of this group are uniquely diverse with respect to fatty acid composition: some have a fatty acid composition of the bacterial type, some of the chloroplast type, and some of types hitherto not described either in bacteria or in chloroplasts. As we have already briefly reported (13), the bacterial type of fatty acid composition is relatively common among unicellular blue-green algae, whereas the presence of large quantities of polyenoic fatty acids is characteristic of most filamentous blue-green algae. We shall describe in this paper the results of a comparative survey of fatty acid composition in a total of 34 strains of unicellular blue-green algae.

## MATERIALS AND METHODS

**Biological material.** The unicellular strains analyzed were derived from the collection of axenic blue-green algae currently maintained in this department. Strain histories and properties are fully described elsewhere (25).

<sup>1</sup> A preliminary report of this work (and that in the paper on filamentous strains) has been presented (Nature 227:1164-1166, 1970).

**Conditions of cultivation.** Except where specifically noted, all strains were grown in the light in mineral medium. The growth conditions employed are summarized in Table 1. The media were BG11 medium (25), modified Kratz and Meyers medium (20), and Van Baalen medium (25). More detailed information on the conditions for cultivation has appeared elsewhere (25).

The conditions employed in a representative experiment for each organism are noted in Tables 2 through 7.

**Harvesting of cultures.** Cells were harvested by centrifugation and washed twice with distilled water or with 0.1 M phosphate buffer (pH 7.0) before analysis.

**Fatty acid analysis.** The method described previously (13) was used. During the course of these studies, several minor modifications of the procedure for isolation and methylation of fatty acids were employed. These modifications included such changes as the use of  $\text{BF}_3$ -methanol rather than diazomethane for methylation. All determinations of the fatty acids of the same strain using any of the modifications were identical within experimental error.

**Proof of structure of fatty acids.** The first analysis of each strain was by gas-liquid chromatography on 10% diethylene glycol succinate (DEGS). Preliminary identification of the fatty acids was by co-chromatography with known standards, by comparison of relative retention times (palmitate = 1.0) with those of fatty acids previously identified chemically in other photosynthetic organisms, e.g., *Euglena gracilis* (12, 14), and by use of a plot of the log of the retention time versus chain length (16). A variety of other methods were used for further identification of the major fatty acids: (i) gas-liquid chromatography on Carbowax using known standards for reference; (ii) gas-liquid chromatography on Apiezon L using known standards for reference; (iii) hydrogenation by the mild procedure of Brian and Gardner (3); and (iv) bromination of the hydrogen-

ated sample by the procedure of Brian and Gardner (3).

Retention times and co-chromatography were used for identification of fatty acids on columns (i) and (ii).

The major unsaturated fatty acids were isolated by collection of their methyl esters from a 10% DEGS gas-liquid chromatograph column in cooled U-tubes. The column was maintained at about 150 C, and the injection port and detector were maintained at 120 to 130 C. These fatty acids were identified by hydrogenation (3) and by oxidation with permanganate-periodate (23).

**Lipid analysis.** Washed cells of unicellular strains were lyophilized and extracted with chloroform-methanol (2:1, v/v). The extract was filtered through extracted Whatman no. 1 paper, and the solvent was evaporated under vacuum. The lipids were redissolved in ethyl ether and transferred to a small tube. The ether was removed under nitrogen, and the lipids were stored in chloroform until analyzed.

The lipids of each strain were analyzed by thin-layer chromatography on silicic acid (Adsorbosil-1, Applied Science Laboratories, Inc.) in three solvent systems. For separation of neutral lipids, petroleum ether (bp 30 to 60 C)-ethyl ether-acetic acid (90:25:0.2, v/v/v) was used. The neutral lipids were revealed by staining with iodine and then with 10% phosphomolybdic acid in ethanol (heated 10 to 20 min at 110 C). For separation of glycolipids, the solvent chloroform-methanol-acetic acid (80:30:5, v/v/v) was used. Glycolipids were revealed with iodine and diphenylamine (8). Phospholipids were separated in chloroform-methanol-water (65:30:5, v/v/v). They were revealed with iodine, ninhydrin (stain 108, reference 24) and the phospholipid stain of Dittmer and Lester (7). The lipids were identified from their staining properties and by comparison of their  $R_f$  values with those of known standards. Neutral lipid and phospholipid standards were obtained from Applied Science Laboratories, Inc. The diphen-

TABLE 1. Conditions of cultivation

Condition	Growth medium	Temp (C)	Atmosphere	Light intensity <sup>a</sup>
I	BG11	30	Air	30
II	BG11	25	Air	30
III	BG11	30	0.5% $\text{CO}_2$ - $\text{N}_2$ <sup>b</sup>	100f
IV	Van Baalen	30	0.5% $\text{CO}_2$ - $\text{N}_2$	100f
V	Van Baalen	48	0.5% $\text{CO}_2$ - $\text{N}_2$	100f
VI	Kratz and Meyers	48	0.5% $\text{CO}_2$ - $\text{N}_2$	100f
VII	Kratz and Meyers	30	0.5% $\text{CO}_2$ - $\text{N}_2$	100f
VIII	BG11	37	0.5% $\text{CO}_2$ - $\text{N}_2$	100f
IX	BG11	25	350 ppm $\text{CO}_2$ - $\text{N}_2$ <sup>c</sup>	100f

<sup>a</sup> Illumination was by means of a tungsten bulb except where it is indicated that a fluorescent tube (f) was used. The values for light intensity are approximate and are given in foot candles.

<sup>b</sup> Composition: 0.5%  $\text{CO}_2$ -balance nitrogen.

<sup>c</sup> Composition: 350 ppm  $\text{CO}_2$ -balance nitrogen. The rate of flow of gas over the culture was adjusted so that the growth rate was the same as that for the cultures grown under condition VIII. The growth rate is very dependent upon rate of gas flow and far less dependent upon the concentration of  $\text{CO}_2$  in the gas mixture used.

ylamine-positive lipids of *Chlorella vulgaris* were used as standards for mono- and digalactosyl-diglycerides.

**Analysis of sugars in the glycolipids of strain 6301.** The total lipids of strain 6301 were fractionated on silicic acid (5). The purity of the mono- and digalactolipid fractions was verified by thin-layer chromatography in the system described above for glycolipids. Each of these fractions was hydrolyzed by refluxing in 1 ml of 2 N HCl plus 0.2 ml of methanol plus 0.1 ml of ethyl ether at 100 C for 3 hr. The HCl and water were evaporated under air at 50 C. The resulting sugars were redissolved in water and analyzed by thin-layer chromatography on silicic acid impregnated with boric acid in benzene-acetic acid-methanol (1:1:3, v/v/v) and in 2-butanone-acetic acid-methanol (3:1:1, v/v/v) (22). The sugars were revealed with diphenylamine and the naphthoresorcinol stain described by Patuska (22). A variety of known sugars were used as standards.

## RESULTS

**Fatty acid identification: total fatty acids.** The total fatty acids of almost all strains were first chromatographed on 10% DEGS and then either rechromatographed on Carbowax and Apiezon L or treated with hydrogen and, in some cases, bromine. A few strains were analyzed by all of the above methods. The results of rechromatography on Carbowax and Apiezon L were entirely consistent with the identifications reported below.

The sum of the postulated 16-carbon fatty acids and that of the 18-carbon fatty acids in the total cellular fatty acids was compared to the amounts of palmitic and stearic acids found in each strain after hydrogenation. In every case, the results were consistent with the proposed identifications of the fatty acids. Thus, for strain 6805, the sum of the relative areas of the peaks on the gas-chromatographic trace identified as methyl palmitate and methyl palmitoleate was 44% of the total fatty acids; the hydrogenated sample contained 41% methyl palmitate. The sum of the peaks identified as methyl stearate, methyl oleate, methyl linoleate, and methyl  $\gamma$ -linolenate was 47%; the hydrogenated sample contained 40% methyl stearate. In all cases, after hydrogenation, gas-chromatographic traces showed no peaks or very small peaks with the retention times of the postulated unsaturated acids.

No evidence for unsaturated fatty acids containing 20 or more carbon atoms was found. There was no change in the composition of any of the mildly hydrogenated samples upon bromination, indicating the absence of fatty acids containing cyclopropane groups.

The use of silicic acid in the preparation of the fatty acids for analysis eliminated hydroxy

acids from the samples. We therefore have no information on the presence or absence of hydroxy acids in the strains analyzed. None of the fatty acid methyl esters remaining after hydrogenation co-chromatographed with a variety of commonly occurring branched-chain fatty acid methyl esters.

**Fatty acid identification: isolated unsaturated fatty acids.** Certain fatty acids were isolated in preparative amounts from representative strains. A sample of each of the isolated fatty acids was hydrogenated to determine its chain length (Table 2). When the remainder of each fatty acid sample was oxidized and the resulting dicarboxylic acid analyzed by gas-liquid chromatography, the products were as described in Table 2. The length of the dicarboxylic acid defines the location of the first double bond in each acid. The identifications reported in Table 2 are therefore inferred from the dicarboxylic acid found, and from other information available as described above, in addition to the assumption of methylene interrupted double bonds. The unknown reported to be present in the isolated acid "18:3 $\gamma$ " may be an artifact. This possibility was not further explored, since it was not essential to the taxonomic conclusions drawn from the fatty acid compositions which are described below.

**Fatty acid composition.** Data on fatty acid composition of unicellular strains are summarized in Tables 3 through 6. In these tables, the strains have been arranged according to genus and, within each genus, grouped into numbered strain clusters, following the taxonomic treatment proposed by Stanier et al. (25).

Rod-shaped strains, dividing in a single plane at right angles to the long axis of the cell, were all assigned to the genus *Synechococcus* by these authors; they distinguished a total of nine distinct strain clusters. As shown in Tables 3 through 5, the strains of this genus are heterogeneous with respect to fatty acid composition; however, the members of each strain cluster (probably representatives of a single species) have a uniform fatty acid composition.

The 12 strains assigned to clusters 1, 3, 4, and 5 are all organisms with a low or undetectable (0 to 2%) content of polyunsaturated fatty acids. It should be noted that the strains of clusters 3, 4, and 5 are closely similar in most phenotypic respects and in deoxyribonucleic acid (DNA) base composition, which ranges from 50 to 56 moles per cent guanine plus cytosine (% GC) (25). The strains of cluster 1, although not readily distinguishable in gross structure from those of the remaining three

TABLE 2. Identification of purified, unsaturated fatty acids by hydrogenation and oxidation

Fatty acid	Source <sup>a</sup>	Product of hydrogenation	Major dicarboxylic acid product of oxidation	Identity
16:1	6715	16:0	Nonanedioate	Hexadeca-9-enoic acid
16:2	6910	16:0	Nonanedioate	Hexadeca-9,12-dienoic acid
18:1	6715	18:0	Nonanedioate	Octadeca-9-enoic acid
18:2	6714	18:0	Nonanedioate	Octadeca-9,12-dienoic acid
18:3 $\alpha$	6304 <sup>b</sup>	18:0	Nonanedioate	Octadeca-9,12,15-trienoic acid
18:3 $\gamma$	6714	18:0	Adipate + unknown	Octadeca-6,9,12-trienoic acid + unknown C <sub>18</sub> acid

<sup>a</sup> Strains 6714, 6715, and 6910 were grown in 1,400 ml of BG11 medium in a Fernbach flask with stirring at 33 C. Strain 6304 was grown in 75 ml of Kratz and Meyers medium at 30 C. The atmosphere was 0.5% CO<sub>2</sub>-balance nitrogen and the fluorescent light intensity was about 100 foot candles.

<sup>b</sup> Strain 6304 is a filamentous strain whose properties are described elsewhere (*manuscript in preparation*).

TABLE 3. Major fatty acids of rod-shaped unicellular blue-green algae: genus *Synechococcus* (strains containing monounsaturated fatty acids)

Strain cluster <sup>b</sup>	Strain no.	Growth conditions <sup>c</sup>	Fatty acid <sup>a</sup>									Poly unsaturated fatty acids <sup>d</sup> (%)
			14:0	16:0	18:0	14:1	16:1	18:1	18:2	18:3 $\alpha$	18:3 $\gamma$	
1	6307	I	17	22	1	1	50	4	1	— <sup>e</sup>	—	1
	6603	II	23	23	1	2	46	2	—	—	—	0
	6706	II	15	28	4	1	37	11	—	—	—	0
	6707	II	18	30	1	1	37	6	—	—	—	0
	6708	II	14	29	2	2	40	6	—	—	—	0
	6709	II	15	22	2	1	48	9	—	—	—	0
3	6715	V	tr <sup>f</sup>	31	2	tr	29	27	1	—	—	1
	6716	VI	tr	29	1	tr	37	27	1	—	—	1
	6717	V <sup>g</sup>	tr	26	2	tr	30	35	tr	—	—	0
4	6301	IV	1	32	1	6	46	4	2	—	—	2
	6311	II	2	35	2	1	47	8	—	—	—	0
5	6312	I	1	29	1	1	61	4	—	—	—	0

<sup>a</sup> Values in all tables given as percentage of total fatty acids as determined by gas-liquid chromatography.

<sup>b</sup> Defined in Stanier et al. (25).

<sup>c</sup> As described in Table 1.

<sup>d</sup> Content of 18-carbon polyunsaturated fatty acids as a percentage by weight of the total C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> acids identified in each strain.

<sup>e</sup> Not detected under conditions employed.

<sup>f</sup> Trace, less than 1% of total.

<sup>g</sup> Van Baalen's medium with 10 times the Ca<sup>2+</sup> content.

clusters 2, 6, and 9 (Table 4) are all character- higher GC content (66 to 71 moles %).

The six *Synechococcus* strains assigned to clusters 2, 6, and 9 (Table 4) are all characterized by a high content of polyunsaturated fatty acids, represented by linoleic (18:2) and  $\alpha$ -linolenic (18:3 $\alpha$ ) acids, with the exception of one strain in cluster 9, which contains a large amount of  $\gamma$ -linolenic (18:3 $\gamma$ ) acid. In phenotypic respects, these three clusters are readily differentiable from one another (25).

Two *Synechococcus* strains (Table 5), the unique representatives of clusters 7 and 8, are

also characterized by a high content of polyunsaturated fatty acids but are distinguishable from all other unicellular blue-green algae examined by virtue of the fact that they contain comparatively large amounts of a C<sub>16</sub>-polyunsaturated fatty acid, hexadecadienoic acid. This represents 7% of the total cellular fatty acids in strain 6605 (cluster 7) and 21% in strain 6910 (cluster 8).

Data on the fatty acid composition of unicellular blue-green algae with spherical cells dividing in two or three planes are shown in Table 6. Of the 14 strains examined, 11 were

assigned to the genus *Aphanocapsa* and fell into five strain clusters. The five strains of *Aphanocapsa* cluster 1 were very similar to one another phenotypically and differed markedly from the remaining *Aphanocapsa* strains in DNA base composition [46 to 48 moles %GC

for the strains of cluster 1, 35 to 37 moles %GC for the strains of the remaining clusters (25)]. As can be seen in Table 6, the strains of cluster 1 all have a high content of polyunsaturated fatty acids (42 to 55%), represented largely by linoleic acid (18:2) and  $\gamma$ -linolenic

TABLE 4. Major fatty acids of unicellular blue-green algae: genus *Synechococcus* (strains containing polyunsaturated fatty acids)

Strain cluster <sup>a</sup>	Strain no.	Growth conditions <sup>b</sup>	Fatty acid										Polyunsaturated fatty acids <sup>a</sup> (%)
			14:0	16:0	18:0	14:1	16:1	18:1	18:2	18:3 $\alpha$	18:3 $\gamma$		
2	7001	I	7	14	3	tr	16	16	17	—	—	23	
6	7002	I <sup>c</sup>	tr	38	2	—	13	18	17	6	—	24	
	7003	I <sup>c</sup>	1	29	2	—	11	18	18	10	—	31	
9	6801	III	—	41	1	—	2	9	21	3	16	43	
	6903	III	18	11	1	1	8	20	26	7	—	36	
	6901	I	22	12	1	1	6	12	36	8	—	45	

<sup>a</sup> See footnotes to Table 3 and Stanier et al. (25).

<sup>b</sup> As described in Table 1.

<sup>c</sup> BG 11 supplemented with vitamins (25).

TABLE 5. Major fatty acids of rod-shaped unicellular blue-green algae: genus *Synechococcus* (strains containing hexadecadienoic acid)

Strain cluster <sup>a</sup>	Strain no.	Growth conditions <sup>b</sup>	Fatty acid										Polyunsaturated fatty acid <sup>a</sup> (%)
			14:0	16:0	18:0	14:1	16:1	18:1	16:2	18:2	18:3 $\alpha$	18:3 $\gamma$	
7	6605	I	6	20	2	1	2	3	7	43	4	—	61
8	6910	III	1	29	1	tr	30	5	21	5	1	3	31

<sup>a</sup> See footnotes to Table 3 and Stanier et al. (25).

<sup>b</sup> As described in Table 1.

TABLE 6. Major fatty acids of coccoid unicellular blue-green algae: genera *Aphanocapsa*, *Gloeocapsa*, *Microcystis*, *Chlorogloea*

Genus <sup>a</sup>	Strain cluster <sup>a</sup>	Strain no.	Growth conditions <sup>b</sup>	Fatty acid									Polyunsaturated fatty acids <sup>a</sup> (%)
				14:0	16:0	18:0	14:1	16:1	18:1	18:2	18:3 $\alpha$	18:3 $\gamma$	
<i>Microcystis</i>	1	7005	I	1	40	2	tr	3	6	15	—	27	45
		6702	I	1	30	2	—	13	6	21	—	25	47
		6714	IV	tr	28	1	1	4	5	17	—	31	55
		6803	I	tr	32	2	1	9	5	18	1	21	45
		6805	I	2	35	3	1	10	4	21	2	18	43
		6806	I	1	35	1	1	9	5	17	2	19	42
		6308	I	27	6	4	11	34	3	4	—	—	4
		6701	I	34	7	2	8	35	3	1	—	—	1
		6711	I	29	7	1	13	39	1	1	—	—	1
		6807	I	34	5	2	9	36	2	2	—	—	2
		6808	I	18	9	2	9	50	2	3	—	—	3
		6804	I	26	10	2	5	35	3	3	—	—	4
<i>Gloeocapsa</i>		6501	II	11	19	3	3	46	4	3	—	—	3
<i>Chlorogloea</i>		6712	I	26	9	7	3	27	3	2	—	—	3

<sup>a</sup> See footnotes to Table 3 and Stanier et al. (25).

<sup>b</sup> As described in Table 1.

acid (18:3 $\gamma$ ). The strains of *Aphanocapsa* clusters 2 to 5 are all of low polyunsaturated fatty acid content (1 to 4%). Polyunsaturated fatty acids are represented exclusively by small quantities of linoleic acid (18:2).

One other coccoid unicellular blue-green alga, *Microcystis aeruginosa*, resembles the strains of *Aphanocapsa* cluster 1 both qualitatively and quantitatively in polyunsaturated fatty acid content. This strain has DNA with a base composition similar to that of *Aphanocapsa* cluster 1 [45 moles %GC (25)]. It is separated generically from *Aphanocapsa* on the basis of a special structural character, the possession of gas vacuoles (25).

Single strains of the genera *Gloeocapsa* and *Chlorogloea* had a low content of polyunsaturated fatty acids, similar to the strains of *Aphanocapsa* clusters 2 to 5. However, these two organisms are readily distinguishable both in structure and in DNA base composition from *Aphanocapsa* clusters 2 to 5.

A few generalizations concerning the fatty acid composition of unicellular blue-green algae can be derived from the data in Tables 3 through 6. In strains of low polyunsaturated fatty acid content (0 to 4%), the only polyunsaturated fatty acid that is detectable is the dienoic acid, linoleic acid; trienoic acids are invariably undetectable (Tables 3 and 6). Among most strains of high polyunsaturated fatty acid content (23 to 61%), there is a very marked dichotomy with respect to the predominant trienoic acid, which is either the  $\alpha$  or the  $\gamma$  isomer of linolenic acid (Tables 4 and 6).

**Effect of medium on fatty acid composition.** The data reported in this paper were collected over the course of several years. During

this time, various modifications of the growth conditions (media, atmosphere, temperature) were tested in an effort to optimize growth. The results of parallel analyses of five strains using five sets of growth conditions are reported in Table 7. It is clear that the growth conditions do not significantly affect the qualitative nature of the major fatty acids of any of these strains. The only changes observed were quantitative, were relatively minor, and would not affect the assignment of strains to groups on the basis of the highest degree of unsaturation of the major fatty acids found in the cell. Furthermore, when the effect of temperature and other more minor modifications of growth conditions on the fatty acid composition of strains 6301 and 6714 was determined, it was found that only in the case of 6714 was the expected increase in polyunsaturated acid content found at a lower temperature. For this strain, the percentage of polyunsaturated acids was 40% at 37 C and 58% at 25 C. In strain 6301, the percentage of polyunsaturated acids decreased from 9% at 37 C to 3% at 25 C.

**Lipid composition.** The lipids of the following unicellular strains were examined: 6301, 6307, 6308, 6311, 6312, 6501, 6603, 6605, 6706, 6707, and 6708. The strains appeared to be similar in both neutral and phospholipid composition. Spots corresponding both to diglycerides and to free fatty acids were present in all the samples, as were ninhydrin-positive phospholipids. None of these lipids was further identified. All strains contained diphenylamine-positive lipids (glycolipids) with the  $R_f$  values of mono- and digalactosyldiglycerides. There were also trace amounts of other more polar glycolipids in these strains. The nature of the sugar components of the glycolipids was

TABLE 7. Effect of growth medium, light source, and temperature on the fatty acid composition of strains 6307, 6308, 6714, and 6501

Strain no.	Growth conditions <sup>a</sup>	Fatty acid									Polyunsaturated fatty acids <sup>a</sup> (%)
		14:0	16:0	18:0	14:1	16:1	18:1	18:2	18:3 $\alpha$	18:3 $\gamma$	
6301	VIII	1	32	5	1	30	11	7	1	—	9
	IX	1	34	1	2	44	6	2	2	—	3
6307	I	17	22	1	1	50	4	1	—	—	1
	IV	19	20	3	1	41	3	2	—	—	2
6308	I	27	8	2	11	42	4	4	—	—	4
	IV	27	6	4	11	34	3	4	—	—	4
6714	I	1	28	4	1	7	6	17	—	15	41
	IV	tr	28	1	1	4	5	17	—	31	55
	VIII	1	29	3	tr	5	11	22	2	8	40
6501	IX	1	17	2	tr	5	6	21	3	19	58
	I	11	19	3	3	46	4	3	—	—	3
	VII	13	16	1	4	44	5	6	—	—	7

<sup>a</sup> See footnotes to Table 3.

<sup>b</sup> As described in Table 1.

investigated only in strain 6301. The major sugar component of the two glycolipids having  $R_F$  values corresponding to those of mono- and digalactolipids was shown, by thin-layer chromatography after hydrolysis, to be galactose. In each case, however, significant quantities of other sugars could be detected, so that the chemical identification of these two glycolipids remains incomplete.

### DISCUSSION

In terms of their fatty acid composition, the 34 strains of unicellular blue-green algae that we have examined can be divided into a series of compositional groups (Table 8), most of which were recognized by Holton and Blecker (9). Three primary groups are distinguishable, in terms of the highest degree of unsaturation (mono-, di-, or tri-) of the major cellular fatty acids. A further subdivision can be made among strains that contain trienoic fatty acids, since some contain the  $\alpha$ -isomer of linolenic acid and others the  $\gamma$ -isomer; only one or two strains synthesize significant quantities of both isomers.

A few discrepancies with previously published data deserve brief discussion. Strain 6301 (*Synechococcus* cluster 4) had been previously analyzed under the name *Anacystis nidulans* by several workers (9) and was uniformly reported to contain no polyunsaturated

fatty acids. However, we have consistently found that this strain contains small quantities of linoleic acid (18:2) and sometimes of linolenic acid. The amount present is to some extent dependent on the conditions of growth (Tables 3 and 7). There are likewise conflicting reports about the presence or absence of linoleic acid in a filamentous blue-green alga, *Mastigocladus (Hapalosiphon) laminosus*. This acid could not be detected by Holton et al. (11), but it accounted for 2% of the total fatty acids in the analysis by Nichols and Wood (19). Such minor discrepancies are probably not significant, since the detection of a fatty acid that accounts for as little as 2% of the total may depend on the specific conditions of isolation and analysis employed.

The present chaotic state of the nomenclature and taxonomy of the blue-green algae may explain some seeming discrepancies between the data from different laboratories. For example, in strain 7001 (*Synechococcus* cluster 2), which was received in this laboratory under the designation of *Anacystis marina*, linoleic acid accounted for 17% of the total fatty acids. A different strain carrying the same taxonomic designation was analyzed by Parker et al. (21), who were unable to detect any polyunsaturated fatty acids. It is probable that these two strains, despite their identical taxonomic designations, are members of two different species.

Our data for strains 7002 and 7003 (*Synechococcus* groups 7 and 8, respectively) are similar to those published by Parker et al. (22) for the same two strains. They designated strain 7002 as *Agmenellum quadruplicatum* and strain 7003 as *Coccochloris elabens*.

An interesting observation concerning the nature of the fatty acids of the thermophilic members of this group of organisms can be made. The three thermophilic unicellular strains 6715, 6716, and 6717, all contain very large amounts of oleic acid (Table 3). In a variety of organisms (1, 10, 16), the ratio of saturated to unsaturated fatty acids increases as the temperature of growth increases.

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TABLE 8. Numbers of strains of unicellular blue-green algae containing fatty acids of different degrees of unsaturation

Strain	Highest degree of unsaturation			
	Mono-	Di-	Tri-	
			$\alpha$	$\gamma$
<i>Synechococcus</i> <sup>a</sup>				
Cluster 1	6			
2		1		
3	3			
4	2			
5	1			
6			2	
7		1		
8		1		
9			2	1
<i>Aphanocapsa</i>				
Cluster 1				5
2	1			
3	2			
4	2			
5	2			
<i>Gloeocapsa</i>	1			
<i>Microcystis</i>				1
<i>Chlorogloea</i>	1			

<sup>a</sup> See Stanier et al. (25).

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