Do Cyanobacterial Lipids Contain Fatty Acids Longer Than 18 Carbon Atoms?

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Fatty acids of twelve species of cyanobacteria grown under different photoautotrophic conditions were studied and their composition was compared with literature data of many other species. We have come to the conclusion that the lipids of cyanobacteria do not contain fatty acids with a chain longer than 18 carbon atoms. In our opinion, omission of an analytical procedure, *i.e.* purification of fatty acid methyl esters before gas chromatography, leads to incorrect interpretation of the results. Absence or presence of fatty acids was suggested as a useful taxonomic marker and a proper diagnostic indicator in the commercial application of cyanobacterial biomass.

Key words: Chemotaxonomy, Cyanobacteria, Fatty Acids

Introduction

Historically, the cyanobacteria were the first photoautotrophic, oxygen-evolving prokaryotic organisms. Their membranes have a simple lipid composition compared to eukaryotic algal taxa. Indeed, their glycolipids and sulfolipids do not differ from those of eukaryotic algae. The difference is in the phospholipids, which are represented with a lower number of substances. Phosphatidyl glycerol is ubiquitous and predominant, and it is the only phospholipid of cyanobacteria (Nichols and Wood, 1968; Petkov and Furnadzieva, 1988; Domonkos *et al*., 2004; Iliev *et al*., 2006; Okazaki *et al*., 2006). Lipids of cyanobacteria fulfil mostly a membrane function. Triacylglycerols, being storage substances, are normally present in small amounts and are not a part of the functional membranes. Also, small amounts of naturally occurring fatty acid methyl esters have been found (Petkov and Furnadzieva, 1993).

As a rule, the proportion of fatty acids in cyanobacteria follows temperature fluctuations (Wada and Murata, 1990; Varkonyi *et al*., 2000). This relationship is very strongly expressed by some cyanobacteria, for example, the cold- and heat-resistant *Arthronema africanum* (Iliev *et al*., 2006). This cyanobacterium maintains relatively constant fluidity of its membranes, adjusting the proportion of its fatty acids to the temperature. In other cases, the growth conditions have a smaller effect on fatty acid proportion (Piorreck *et al*., 1984; Ronda and Lele, 2008).

Many previous studies on fatty acids of cyanobacteria used samples collected from a natural ecosystem and grown in the laboratory as a mixture, which was highly dominated by a single species. Consequently, the found fatty acid composition was not that of a single taxon. Similarly, when cyanobacteria are grown in open ponds, it is not a monoculture. Such studies do not allow chemotaxonomic conclusion and they are not mentioned here.

Chain length and number and position of double bonds of fatty acids are genetically determined. It is reasonable to suggest that a taxon has a specific maximal length of the fatty acid chain and maximal number of double bonds. Here obviously arises a question: which is the taxon in the hierarchy where all organisms have similar qualitative composition of fatty acids?

Based on the analysis of a large number of literature data on fatty acids of cyanobacteria and on our own experiments, we conclude that

Table I. Fatty acids of cyanobacteria grown under different photoautotrophic conditions (% of total fatty acids).

Genus	Conditions	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3
Aphanizomenon klebahnii	180 μmol m^{-2} s^{-1}, (32 ± 1) °C	0.5	tr.	29.0	32.8	0.3	10.4	18.7	8.2
Arthronema africanum	180 μmol m^{-2} s^{-1}, 20 – 46 °C	0.1–0.9	-	26–40	18–36	0.3–1.0	3–20	4–30	0.5–33
Arthrospira maxima	180 μmol m^{-2} s^{-1}, (32 ± 1) °C	tr.	tr.	52	4	1.5	3	17.4	21.4[a]
Spirulina platensis	up to 1,300 μmol m^{-2} s^{-1}, 15–35 °C	1–3	1–2	41–55	5–18	0.1–0.9	4–9	5–15	17–48[a]
Plectonema boryanum	180 μmol m^{-2} s^{-1}, 15–44 °C	tr.	-	44 ± 3	25 ± 5	4 ± 1	12 ± 6	14 ± 4	5 ± 4
Lyngbya arboricum	180 μmol m^{-2} s^{-1}, 22 °C	1.8	1.1	26.8	24.4	5.5	9.7	26.0	4.8[a]
Microcystis aeruginosa	180 μmol m^{-2} s^{-1}, 32 °C	-	-	48 ± 2	9 ± 1	0.4 ± 0.1	3.6 ± 0.7	19.5 ± 0.4	20 ± 1
Nostoc calcicola	50 μmol m^{-2} s^{-1}, 22 °C	3.3	-	27.5	10.5	3.5	32.5	18.4	4.3
Scytonema ocellatum[b]	50 μmol m^{-2} s^{-1}, 22 °C	1.8	-	29.1	15.8	6.5	16.6	23.2	6.3[c]
Synechococcus elongatus	180 μmol m^{-2} s^{-1}, 20–32 °C	0.4 ± 0.1	2.6 ± 0.2	42 ± 4	46 ± 4	tr.	8 ± 0.6	-	-
Synechococcus leopoliensis	180 μmol m^{-2} s^{-1}, 20–32 °C	0.3 ± 0.1	2.6 ± 0.2	38 ± 3	49 ± 4	-	8 ± 0.5	-	-
Anabaena variabilis[d]	180 μmol m^{-2} s^{-1}, 28–32 °C	1–3	1–2	45–55	5–10	0.3–0.9	4–9	5–15	15–20

[a] gamma – 18:3. [b] 12:0 – 0.6%. [c] gamma – 3.3%, alpha – 3%. [d] 16:2 up to 2%. tr., trace amount.

the longest fatty acid chain consists of 18 carbon atoms. We have revised cases where higher numbers had been implied.

Material and Methods

Cultivation of the cyanobacteria

Twelve strains of cyanobacteria used in this study originated from the CCALA collection, Trebon, the Czech Republic. *Aphanizomenon klebahnii* (Elenkin) Pechar et Kalina, strain Jaworski 1970/FBA-218; *Plectonema boryanum* Gom. [*Leptolingbya boryana* (Gomont), Anagn. et Kom.], strain 594; and *Microcystis aeruginosa* Kutz, strain Zapomelova 2006/2, were cultivated in nutrient medium described by Allen and Arnon (1955). *Arthronema africanum* (Schwabe et Simons) Kom. et Luk., strain Lukavsky 1981/1 was grown in the medium described by Iliev *et al*. (2006).

Spirulina platensis (= *Arthrospira fusiformis*) (Voronich.) Anagn. et Kom., strain Hindak 1985/1, and *Arthrospira maxima* Setchell et Gardner in Gardner, strain Compere 1968/3768, were grown in the medium described by Zarrouck (1966). *Lyngbya arboricum* (Bruhl et Biswas), strain Adhikary BBSR 2003/225; *Nostoc calcicola* Born. et Flah, strain Badour 1963/23; *Scytonema ocellatum* Lyngbye ex Bornet et Flahault, strain Adhikary 231; *Synechococcus elongatus* Skuja, strain Kovrov 1972/8; and *Synechococcus leopoliensis* (Pringsheim) Kom. in Bourrelly, strain Kratz-Allen/UTEX 625 (syn. *Anacystis nidulans*), were cultivated on the medium of Zehnder (Staub, 1961). *Anabaena variabilis* (syn. *Trichormus variabilis*, Kom. et Anagn.) Kütz. ex Born et Flah., strain Greifswald/92, was cultivated according to Vonshak (1986).

The cyanobacteria *Nostoc* and *Scytonema* were cultivated in the laboratory at 22 °C, using uninterrupted light of 50 μmol m^{-2} s^{-1}. The other cyanobacteria were grown at 180 μmol m^{-2} s^{-1}, uninterrupted (24 h) light from 5 x 40 W luminescent lamps. *Aphanizomenon*, *Arthrospira*, *Plectonema*, *Microcystis*, and *Anabaena* were grown at (32 ± 1) °C; *Arthronema* was grown at 16, 20, 28, 32, 35, 40, 46 °C (± 1 °C); *Synechococcus* at 20 °C and 32 °C. Bubbling with 3 cm^3 s^{-1} air enriched with 0.5% CO_2 and pH 8 ± 0.5 was maintained. Cultivation of *Spirulina* (*Arthrospira*) in covered ponds was carried out in Nigrita, Greece at a temperature range of 18–38 °C, in sunlight, mechani-

cal stirring by paddle wheel, and a CO_2 supply to maintain a pH value of 8–8.5. The cyanobacteria were cultivated to a density of about 3–4 g dm^{-3} dry weight, which was achieved in 7–10 d depending on the volume and strain. Biomass was separated from the medium by centrifugation at 3,000 x *g*.

Chemical analyses

The fresh biomass was extracted with chloroform/methanol (2:1 v/v), three times for 0.5 h each under reflux. The solvent was evaporated *in vacuo*, and the residue was re-extracted with chloroform. Parts of the lipid samples were converted to fatty acid methyl esters (FAMEs), by heating in methanol containing 6% (m/m) anhydrous HCl at 60 °C for 1.5 h. The FAMEs were extracted with hexane. FAMEs of all samples were purified by thin layer chromatography (TLC) on silica gel with hexane/diethyl ether (10:1 v/v) right before gas chromatography (GC). GC of FAMEs was carried out on a Shimadzu GC-15A instrument (Kyoto, Japan), using a 30-m Supelcowax-10 capillary column, and on a Perkin-Elmer instrument, using two columns: 10% PEGA and 2.5% SE-52, at 195 °C and with flame ionization detection. A kit of reference substances, solvents, and TLC plates from Merck (Darmstadt, Germany) was used.

Results and Discussion

The fatty acid composition of twelve species of cyanobacteria cultures grown in the laboratory and at large scale is summarized in Table I. They were grown under different environmental conditions. Contrary to some claims in the literature (refer to Table I), linolenic acid, α-18:3 or γ-18:3, was the final substance of the cyanobacterial metabolic pathway of fatty acids, and there was no further elongation of the chain. So the question arose: are there fatty acids with 20 or more carbon atoms in cyanobacteria? We revised the available data as shown in Table II.

The individual fatty acid percentage follows the change of environmental conditions. The percentage of a fatty acid, which is the end product of its metabolic pathway, could be gradually reduced to almost zero when the cyanobacterium was grown under extreme conditions, for example at very high temperature, but no new fatty acid could suddenly appear (Iliev *et al.*, 2006). Therefore, the qualitative composition could be accepted as a chemotaxonomic marker.

According to Singh *et al.* (2002) the fatty acid 20:1 constitutes up to 10% of the total fatty acids of cyanobacteria. An extremely high content of fatty acid 20:3 was found within the membrane fatty acids of *Nostoc commune* (Table II) by Olie and Potts (1986). Later, Potts *et al.* (1987) reported on the fatty acid composition of the same strain, *Nostoc commune* (UTEX) 584, and three other strains, but they did not mention 20:3 and C_{20} fatty acids, nor did they comment on the reasons for their absence. The 20:3 fatty acid was not found in *Nostoc* studied by Schneider *et al.* (1970), Liu *et al.* (2003, 2005), and Miura and Yokota (2006), as shown in Table III, nor in our samples of *Nostoc calcicola* (Table I). As a whole, Table I includes data obtained under much more variable conditions compared to Table II and repeats almost all of them.

Using a desiccated biomass of *Tolypothrix*, Rajendran *et al.* (2007) found about 30.3% of 20:1 fatty acid, which was not present in the fresh biomass. Such a phenomenon is beyond the biochemistry of cyanobacteria and algae. We would assume that the GC peak of the questionable substance had the same RT value as the fatty acid 20:1, but the substance is not identical with this fatty acid. The same holds true for 22:0 and 23:0 fatty acids presented in the same paper. Moreover, the fatty acids were quantified, and about 0.46% were from lipids of fresh cells and 0.82% from lipids of desiccated cells. Thus one wonders about the composition of the *Tolypothrix* membranes? According to our experience, fatty acids normally constitute 30–32% of total lipids in all cyanobacteria and algae as well.

Micheli *et al.* (2007), reported that Antarctic *Leptolyngbya*, *Plectonema*, and *Nostoc* and a Mediterranean *Nostoc* strain do not synthesize fatty acids longer than 18 carbon atoms (Table III). According to the analytical certificate *Aphanizomenon*, a cyanobacterium widely used as food supplement, possesses C_{18} as longest fatty acid chain (OSC, 2009).

Considering the above mentioned facts, the conclusion can be drawn that the case where a fatty acid with 20 carbon atoms is reported in cyanobacteria probably ought to be classified as an error. All of the data in Table II were obtained without TLC purification of the FAMEs before

Table II. Previously reported fatty acids with more than 18 carbon atoms (% of total fatty acids).

Cyanobacterium	20:0	20:1	20:2	20:3	20:4	20:5	22:1	22:2	22:6	23:0	24:0	24:1	Reference
Anabaena	-	-	-	-	0.1	-	-	-	-	-	-	-	Caudales *et al.* (1992)
Anabaena	0.14	-	0.22	-	0.25	-	-	-	-	-	-	-	Caudales and Wells (1992)
Anacystis nidulans	0.7	-	-	-	-	-	-	-	-	-	-	-	Piorreck *et al.* (1984)
Aphanizomenon flos-aquae	-	2.17[a]	-	-	-	-	-	-	-	-	-	-	Parshikov and Kostlan (1976)
Chroococcus sp.	0.53[*]	-	0.27[*]	-	0.27[*]	-	0.27[*]	-	-	-	-	-	Patil *et al.* (2007)
Microcystis aeruginosa	-	-	-	0.15[b]	0.46[b]	-	-	-	-	-	-	-	Hayakawa *et al.* (2002)
Microcystis sp.	-	-	-	0.17[b]	0.20[b]	0.30[b]	-	-	-	-	-	-	Hayakawa *et al.* (2002)
Nostoc commune UTEX 584	3.0	-	-	56.8	-	-	-	-	-	-	-	-	Olie and Potts (1986)
Nostoc sp.	0.04	-	0.13	-	0.16	-	-	-	-	-	-	-	Caudales and Wells (1992)
Nostoc sp.	-	-	-	-	-	1.4	-	-	-	-	-	-	Vargas *et al.* (1998)
Nostoc pruniforme	1.2	-	-	-	-	-	-	-	-	-	-	-	Temina *et al.* (2007)
Oscillatoria hamelii	-	-	-	-	1.0	-	-	-	-	-	-	-	Shanab (2007)
Oscillatoria platensis	-	-	-	-	1.4	-	-	-	-	-	-	-	Shanab (2007)
Oscillatoria rubescens	-	-	-	-	-	3.2	-	-	-	-	-	-	Shanab (2007)
Phormidium sp. 64S01	-	-	-	-	32.4	3.8	-	-	-	-	-	-	Pushparaj *et al.* (2008)
Phormidium sp. 79S11	-	-	-	-	23.9	13.1	-	-	-	-	-	-	Pushparaj *et al.* (2008)
Spirulina platensis	-	6.3	5.6	-	-	-	-	-	-	-	-	-	Antonyan *et al.* (1986)
Spirulina pacifica	-	-	1.01	1.39	-	-	1.39	-	-	-	-	-	Ötleş and Pire (2001)
Spirulina platensis	-	-	0.16	-	-	0.19	-	-	-	-	0.15		Ötleş and Pire (2001)
Spirulina maxima	-	-	0.59	0.66	-	-	-	-	-	-	0.58	0.62	Ötleş and Pire (2001)
Spirulina platensis	-	-	-	0.2	-	-	-	-	-	-	-	-	Andrich *et al.* (2006)
Spirulina platensis	0.73	0.44	8.29	1.93	9.92	7.70	-	-	2.88	-	0.13	-	Diraman *et al.* (2009)
Spirulina sp.	12.6	0.14	0.08	0.36	0.49	-	-	-	-	-	-	-	Radmann and Costa (2008)
Synechococcus nidulans	0.13	-	7.64	0.19	0.12	0.10	-	0.11	0.13	1.15	0.09	2.85	Radmann and Costa (2008)
Tolypothrix scytonemoides	-	30.3[*]	-	-	-	-	-	-	-	tr.	-	-	Rajendran *et al.* (2007)

[*] After recalculation as % of total fatty acids. [a] In non-polar lipids only. [b] Highest concentration. tr., trace amount.

Table III. Studies in which fatty acids longer than 18 carbon atoms were not found.

Cyanobacterium	Conditions	Reference
Chroococcales		
Aphanothece	P, 30 μmol m^{-2} s^{-1}, NaCl stress	Laloknam *et al.* (2010)
Gloeocapsa, *Chlorogloea*	P, 25–48 °C	Kenyon (1972)
Gloeobacter	P, 17/26 °C, 10 μmol m^{-2} s^{-1}	Maslova *et al.* (2004)
Microcystis	P, 30 °C, 0.5% CO_2	Kenyon (1972)
	P, 3,000 lx	Parshikov and Kostlan (1976)
	P, 800 lx, 22 °C, [N]	Piorreck *et al.* (1984)
	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
Anacystis	M (Na-acetate), 30 °C	Nichols (1968)
Synechococcus	P, 35 °C, 1% CO_2	Schneider *et al.* (1970)
	P, 30 °C, 0.5% CO_2	Kenyon (1972)
	P, 25 °C	Bishop *et al.* (1986)
	P, 800 lx, 22 °C, [N]	Piorreck *et al.* (1984)
	P, 25 °C	Caudales *et al.* (2000)
	P, 50 W m^{-2}, 25–32 °C	Maslova *et al.* (2004)
	P, 143 μmol m^{-2} s^{-1}, 28 °C	Pratoomyot *et al.* (2005)
Synechocystis	P, 85 W m^{-2}, 26/36 °C, 1.7% CO_2	Klyachko-Gurvich *et al.* (1988)
	M (glucose), 1,000 lx, 28 °C	Kim *et al.* (1999)
	P, cold shock	Los and Murata (1999)
	P, 143 μmol m^{-2} s^{-1}, 28 °C	Pratoomyot *et al.* (2005)
	P, 350–3,500 lx, 10–30 °C, 1% CO_2	Chintalapati *et al.* (2006)
	P, 30 °C	Okazaki *et al.* (2006)
Pleurocapsales		
Myxosarcinia	P, 5% CO_2	Nichols and Wood (1968)
Chroococcidiopsis	P, 25 °C	Caudales *et al.* (2000)
Pleurocapsa, Xenococcus	P, 25 °C	Caudales *et al.* (2000)
Oscillatoriales		
Leptolyngbya	P, 5 μmol m^{-2} s^{-1}, 20 °C	Micheli *et al.* (2007)
Lyngbya	P, 35 °C, 1% CO_2	Schneider *et al.* (1970)
Oscillatoria	P, 30 m^2, natural light	Materassi *et al.* (1980)
	P, 800 lx, 22 °C, [N]	Piorreck *et al.* (1984)
	P, ND	Loura *et al.* (1987)
	P, 5,000 lx, 23 °C	Son *et al.* (2000)
Phormidium	P, 50 W m^{-2}, 47 °C	Maslova *et al.* (2004)
Planktothrix	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
Plectonema	P, 25 °C, 0.5% CO_2	Kenyon *et al.* (1972)
	P, 8,000 lx, 12.5–44.5 °C	Chaneva and Furnadzieva (1997)
	P, 5 μmol m^{-2} s^{-1}, 20 °C	Micheli *et al.* (2007)
Pseudoanabaena	P, 50 μmol m^{-2} s^{-1}, 20 °C, ND	Loura *et al.* (1987)
Spirulina (*Arthrospira*)	P, 3,000 lx	Parshikov and Kostlan (1976)
	P, 800 lx, 22 °C, [N]	Piorreck *et al.* (1984)
	P, 8,000 lx, 30 °C	Petkov and Furnadzieva (1988)
	P, 70 μmol m^{-2} s^{-1}, 22–30 °C, 5% CO_2	Cohen *et al.* (1995)
	P, different extraction methods	Reis *et al.* (1998)
	P, 1,900 lx, 30/35 °C	Colla *et al.* (2004)
	P, 30 W m^{-2}, 35 °C	Maslova *et al.* (2004)
	P, 30 μmol m^{-2} s^{-1}, 35 °C	Mühling *et al.* (2005)
	P, different extraction methods	Mendes *et al.* (2006)
	P, different extraction methods	Chaiklahan *et al.* (2008)
	P, H, M, 30 °C, 120 μmol m^{-2} s^{-1}	Choi *et al.* (2008)
Nostocales		
Anabaena	M (Na-acetate), 30 °C	Nichols (1968)
	P, 3,000 lx	Parshikov and Kostlan (1976)
	P, 3,500 lx, 28 °C	Sallal *et al.* (1990)
	P, 8,000 lx, 30 °C	Petkov and Furnadzieva (1993)
	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
	P, 40 μmol m^{-2} s^{-1}, 20 °C	Li and Watanabe (2001, 2004)
Aphanizomenon	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)

Table III continued.

Calothrix marchica	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
Cylindrospermum	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
Nostoc	P, 35 °C, 1% CO_2	Schneider *et al.* (1970)
	P, 3,500 lx, 28 °C	Sallal *et al.* (1990)
	P, 100 μmol m^{-2} s^{-1}, 26 °C	Caudales *et al.* (1992)
	P, 12 μmol m^{-2} s^{-1}, 20 °C, [N]	Gugger *et al.* (2002)
	P, 60 μmol m^{-2} s^{-1}, 30 °C	Liu *et al.* (2003, 2005)
	P, 100 μmol m^{-2} s^{-1}, 25 °C	Wang *et al.* (2000)
	P, 30 μmol m^{-2} s^{-1}, 20 °C	Miura and Yokota (2006)
	P, 350–3,500 lx, 10–30 °C, 1% CO_2	Chintalapati *et al.* (2006)
	P, 5 μmol m^{-2} s^{-1}, 20 °C	Micheli *et al.* (2007)
Tolypothrix	P, 30 W m^{-2}, 35 °C	Maslova *et al.* (2004)
	P, 90 μmol m^{-2} s^{-1}, 28/15 °C	Abulnaja *et al.* (2008)
Stigonematales		
Mastigocladus	P, 5% CO_2	Nichols and Wood (1968)
	P, 2,000 lx, 40–55 °C	Hirayama and Kishida (1990)

P, photoautotrophic cultivation; H, heterotrophic cultivation; M, mixotrophic cultivation; [N], different nitrogen concentrations/sources; ND, nitrogen deficiency.

GC. Plenty of impurities, such as free fatty acids, hydrocarbons, phytol, and other substances, for example ubiquitous phthalates, emerge from the GC column with the same RT value as some of the above mentioned fatty acids. The RT value is the only identification characteristic of GC, and TLC purification is therefore highly important. Besides, we have used a second chromatographic column, SE-52, which separates fatty acids according to the number of their carbon atoms. We never detected a C_{20} fatty acid in any of our samples.

Separation of fatty acids by TLC was done by Wang *et al.* (2000), Okazaki *et al.* (2006), Loura *et al.* (1987), and Maslova *et al.* (2004). As a result they analysed pure samples by GC revealing the presence of fatty acids with 18 carbon atoms as a maximum (Table III).

Son *et al.* (2000), using the methods of GC/MS and ^{13}C NMR spectroscopy, showed that there are no fatty acids with more than 18 carbon atoms in galactolipids of *Oscillatoria* sp. Studying *Synechocystis* sp. PCC 6803, Kim *et al.* (1999) provided GC/MS evidences that there is no fatty acid longer than 18 carbon atoms in the species of MGDG, DGDG, SQDG and PG. By gene expression and mutant analyses, respectively, Los and Murata (1999) and Okazaki *et al.* (2006) have concluded that the fatty acids contain up to 18 carbon atoms in the same strain. Wada and Murata (1990), Gombos *et al.* (1992), and Kis *et al.* (1998) found fatty acid 18:4 and a specific enzyme that catalyzes the desaturation of 18:3 to 18:4 in *Synechocystis* sp. PCC 6803, but there were no enzymes which would elongate the chain beyond 18 carbon atoms.

Production of arachidonic (20:4) and eicosapentaenoic (20:5) acids by a recombinant marine cyanobacterium, *Synechococcus* sp., was described by Yu *et al.* (2000). Of course, genetic manipulations are beyond the topic of this report. Our results show that *Synechococcus* had the most simple fatty acid composition (Table I). Moreover, the proportion of fatty acids does not vary significantly over a wide temperature range. The end product of fatty acid biosynthesis in *Synechococcus elongatus* and *Synechococcus leopoliensis* is oleic acid (18:1). The results of von Elert and Wolffrom (2001), who investigated *Synechococcus elongatus*, and of Kiseleva *et al.* (1999), who studied *Synechococcus vulcanus*, agree with our results. The same cyanobacteria have been studied under the historical name *Anacystis nidulans* by Piorreck *et al.* (1984) and Bishop *et al.* (1986). These studies confirmd the lack of fatty acids longer than 18 carbon atoms in the total and individual lipids of *Anacystis nidulans* and *Synechococcus* sp. strains. Patil *et al.* (2007) found fatty acid 16:1 as the final fatty acid in *Synechococcus*, which was not confirmed by other published studies.

Studying 24 strains of *Anabaena*, Li and Watanabe (2001) reported fatty acids of no more than 18 carbon atoms. A small amount of C_{20} fatty acids was detected by Caudales and Wells (1992) in *Anabaena* and *Nostoc* (Table II). In the same cy-

anobacteria they found about 1–2% of the fatty acids 16:3 and 16:4. This is a sure proof that the studied cyanobacterial cultures had been contaminated with eukaryotic algae.

Shanab (2007) found that caprylic acid (8:0) predominates in strains of *Oscillatoria* reaching 60–64%. Linoleic acid (18:2) was totally absent in two species, being merely 0.3% in *Oscillatoria rubescens*. There is no cyanobacterium or alga with such a fatty acid composition. Samples of algal fatty acids, being polyunsaturated, are very sensitive and easily affected by oxidation. That is why we purified FAMEs by TLC immediately before GC.

A high content of 14:0 and 14:1 and a low content or lack of 18:2 and γ-linolenic acid ($18:3^{6,9,12}$) in *Spirulina* was reported by Kenyon *et al.* (1972). The finding of low content of $18:3^{6,9,12}$ and high content of 14:0 fatty acids could have been due to the oxidation of samples. The percentages are rather different when fresh samples are analysed (Petkov and Furnadzieva, 1988; Maslova *et al.*, 2004). Also, the very small percentage of fatty acid 18:3 in *Plectonema*, described by Kenyon *et al.* (1972), seemed to be more abundant as reported by Chaneva and Furnadzieva (1997). Our results confirm that fatty acid 18:3 is about 6–8% of total fatty acids in *Plectonema*.

Petkov and Furnadzieva (1988, 1993), Maslova *et al.* (2004), Colla *et al.* (2004), Chaiklahan *et al.* (2008), and Choi *et al.* (2008) analysed total and individual lipids in axenic cultures of *Spirulina* (*Arthrospira*) and showed that there was neither α-linolenic acid ($18:3^{9,12,15}$) nor any other fatty acid with a longer carbon chain. Some *Spirulina*-like species, as described by Cohen and Vonshak (1991) and Cohen *et al.* (1995), did not contain $18:3^{6,9,12}$ but only $18:3^{9,12,15}$ fatty acids. Such a culture could easily be mistaken as *Spirulina*, if the fatty acid composition had not been analysed. *Spirulina* grown in open ponds often contain fatty acid $18:3^{9,12,15}$, which is valid evidence for contamination by other algae, sometimes difficult to distinguish microscopically. Taking into consideration the commercial importance of *Spirulina*, it is practical to suggest using the lack of fatty acid $18:3^{9,12,15}$ as an important indicator of purity when the biomass is standardized. Radmann and Costa (2008) found 7.6% $18:3^{9,12,15}$ fatty acid in *Spirulina*; so they must have grown a mixed culture. Besides, they displayed fatty acids that have never been found in *Spirulina* (Table II) and should not be expected.

Years ago, trace amounts of fatty acids 20:2 and 20:3 were found in *Spirulina platensis* by Nichols and Wood (1968). They found about 5% $18:3^{9,12,15}$ fatty acid in the neutral lipids and MGDG which again indicates a mixed culture, as shown above. What is more, the presence of two unidentified fatty acids, namely X (up to 2%) and Y (up to 4%), in the individual lipids confirms that the studied *Spirulina* was not a monoculture. Today we can state that there are no fatty acids with the same RT values of these X and Y substances previously reported. *Spirulina* grown in pig waste by Olguin *et al.* (2001), and after careful washing of the produced biomass, was found to have the usual fatty acid composition similar to those given in Table I.

Studying *Spirulina*, Antonyan *et al.* (1986) found 5.6% 20:2 fatty acid at a nitrogen supply of 70% of the normal requirement. There was no 20:2 fatty acid at 100% supply. It seems unlikely that a genetically determined substance, such as a fatty acid, can so easily disappear and reappear when exposed to small variations in nitrogen concentrations. Piorreck *et al.* (1984) found that the nitrogen concentration did not substantially alter the fatty acid proportion of *Microcystis*, *Oscillatoria*, and *Spirulina*.

Reis *et al.* (1998), Mendes *et al.* (2006), and Chaiklahan *et al.* (2008) employed different methods to extract lipids from *Arthrospira* (*Spirulina*) *maxima* biomass and reported that C_{18} fatty acids were the ones with the longest chain. Studying 35 strains of *Arthrospira*, Mühling *et al.* (2005) found fatty acids with chains not longer than 18 carbon atoms. We have kept large scale cultures in covered ponds for 18 years in Bulgaria and Greece, and we have never found a fatty acid with chains of 20 or more carbon atoms. That is why we suggest the lack of fatty acids with more than 18 carbon atoms as one of parameters in the standardization of *Arthrospira* (*Spirulina*) biomass.

We can conclude that the lipids of cyanobacteria do not contain fatty acids with a chain longer than 18 carbon atoms. A purification of FAMEs before GC is of importance for a correct interpretation of the results. Cyanobacterial fatty acids could be a valuable diagnostic index in the commercial use of cyanobacteria.

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