Cellular fatty acid composition of cyanobacteria assigned to subsection II, order *Pleurocapsales*

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The cellular fatty acid composition of five of the six genera of unicellular cyanobacteria in subsection II, *Pleurocapsales* (*Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina* and the *Pleurocapsa* assemblage) contained high proportions of saturated straight-chain fatty acids (26–41% of the total) and unsaturated straight chains (40–67%). Isomers of 16:1 were the main monounsaturated acid component (11–59%). Polyunsaturated acids were present at trace levels (0.1% or less) in *Xenococcus* and *Myxosarcina*, at concentrations of less than 7% in *Dermocarpa*, *Dermocarpella*, *Pleurocapsa* and CCMP 1489, and at high concentrations (35% or more) in *Chroococcidiopsis*. *Chroococcidiopsis* was also different in terms of the percentage of 16:1 isomers (10–12%) compared to other genera (30–59%), and in terms of total 16-carbon and 18-carbon fatty acids. In general, the composition and heterogeneity of fatty acids in the order *Pleurocapsales* was similar to that reported for the unicellular cyanobacteria of subsection I, order *Chroococcales*.

Keywords: cellular fatty acids, cyanobacteria, *Pleurocapsales*

INTRODUCTION

The oxygenic photosynthetic bacteria, or cyanobacteria, belonging to the taxonomic grouping of subsection II, order *Pleurocapsales* were classified, according to the system of Rippka *et al.* (1979), by morphology on the basis that they were unicellular and reproduced by forming small, spherical baeocytes through internal multiple fission. These characteristics separate them from unicellular cyanobacteria of subsection I, order *Chroococcales*, which reproduce by binary fission, and from filamentous forms of subsection III, order *Oscillatoriales*, subsection IV, order *Nostocales* and subsection V, order *Gleocapsales* (Castenholz & Waterbury, 1989).

Within the order *Pleurocapsales* there are six genera, each having members from marine, terrestrial and freshwater habitats: *Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina*, *Chroococcidiopsis* and the provisional *Pleurocapsa* assemblage (Waterbury, 1989). Giovannoni *et al.* (1988) analysed the 16S rRNA sequences of one strain each of *Dermocarpa*, *Pleurocapsa* and *Myxosarcina* and found that they were phylogenetically related. The other genera, however, have not yet been genetically analysed for coherence, particularly the frequently revised *Chroococcidiopsis*

(Compère, 1998) and the provisionally assigned *Pleurocapsa* assemblage. Since physiochemical data provide valuable support for classification systems based on morphology, the purpose of the present study is to describe the cellular fatty acid composition of representative strains of each of the six genera recognized in subsection II and to evaluate their relatedness based on that criterion.

General features of the fatty acid composition of many genera representing subsections I, III, IV and V have been described. Holton *et al.* (1964, 1968) and Parker *et al.* (1967) analysed a strain of *Anacystis*, a unicellular cyanobacteria of subsection I, and found an absence of polyunsaturated fatty acids; this observation was confirmed and extended by Kenyon (1972) and others (Kenyon & Stanier, 1970; Kenyon *et al.*, 1972; Scheuerbrandt & Bloch, 1962; Stanier *et al.*, 1971). The composition of filamentous strains of most genera of subsection III have been studied (Ahlgren *et al.*, 1992; Cohen *et al.*, 1993; Hudson & Karis, 1974; Tedesco & Duerr, 1989) as well as some from subsection IV (Ahlgren *et al.*, 1992; Caudales & Wells, 1992; Caudales *et al.*, 1995; Hudson & Karis, 1974; Sallal *et al.*, 1990; Sandmann & Boger, 1992; Sato & Murata, 1981) and subsection V (Kenyon & Stanier, 1970; Kenyon, 1972; Kenyon *et al.*, 1972).

Table 1. Cellular fatty acids (as percentages of the total) of cyanobacteria assigned to genera under subsection II, order *Pleurocapsales*

tr, Trace amounts.

Fatty acids by chemical class	ECL*	Strains of cyanobacteria low in polyunsaturated acids							Strains high in polyunsaturated acids			
		<i>Dermocarpa</i>		<i>Xenococcus</i>	<i>Dermocarpella</i>	<i>Myxosarcina</i>	<i>Unchar'd</i>	<i>Pleurocapsa</i>	<i>Chroococcidiopsis</i>		<i>Chroococcidiopsis</i>	
		PCC		PCC 7305	PCC 7326	PCC 7312	CCMP	PCC 7319*	PCC 7439		PCC 7203†	
		7301	7437				1489	Mean	SD		Mean	SD
Saturated straight chains												
12:0	12:00	1.0	0.5	2.7	1.0	tr	0.1	0.2	±0.1	tr	0.1	±0.1
14:0	14:00	2.8	2.4	3.0	1.6	1.1	2.0	2.8	±0.8	0.2	0.4	±0.1
16:0	16:00	23.1	20.4	33.8	26.8	23.6	25.9	25.9	±3.6	31.2	35.1	±4.0
18:0	18:00	0.2	1.0	0.7	2.2	1.3	0.3	0.6	±0.6	1.8	1.9	±0.6
Class total†		27.5	25.8	41.3	33.7	26.2	28.5	29.9	±4.1	34.5	38.9	±6.0
Unsaturated straight chains												
12:1	11:79	10.2	0.1	4.2	0	0	0	0.2	±0.2	0	0	
14:1 (<i>cis</i> 7)	13:83	0.5	6.5	1.2	1.5	9.3	0.2	1.1	±0.1	3.2	1.9	±1.0
14:1 (<i>cis</i> 9)	13:88	0	0.3	0	2.9	0.1	0.2	1.1	±0.4	6.1	0.2	±0.4
16:4 (<i>cis</i> 4)	15:53	0.4	0.9	0	1.3	0	0.3	0.9	±1.0	1.1	0.8	±1.5
16:3 (<i>cis</i> 6)	15:58	2.4	0.1	0	0	0	0.3	0.7	±1.0	0.4	1.1	±1.4
16:1 (<i>cis</i> 5)	15:71	1.8	0	0.6	0.7	0	0.6	0.5	±0.3	0.1	1.6	±0.4
16:1 (<i>cis</i> 7)	15:76	44.3	46.7	29.0	32.0	46.4	51.0	0		0.6	8.3	±1.5
16:1 (<i>cis</i> 9)	15:82	0.9	0	0	0.5	0	0	0		9.7	0	
16:1 (<i>cis</i> 11)	15:90	0.3	5.4	0	0.9	0	7.1	34.4	±0.7	0.9	1.4	±1.9
16:1 (<i>cis</i> 13)	15:94	0.2	0.4	0.5	0.9	3.6	0.3	9.9	±4.1	0	0	
18:3 (<i>cis</i> 6)	17:60	0	0.7	0	2.2	0	0.7	2.4	±1.4	17.8	0	
18:2 (<i>cis</i> 9)	17:72	0	1.8	0	4.2	0	0	0		13.5	25.0	±3.0
18:3 (<i>cis</i> 9)	17:73	0	0	0	0	0	1.5	0		1.7	6.7	±8.9
18:1 (<i>cis</i> 11)	17:75	0.4	1.5	1.1	3.7	4.5	0.8	2.9	±1.9	2.5	3.7	±3.9
18:1 (<i>cis</i> 9)	17:77	1.4	2.1	3.7	4.6	0	1.5	3.6	±1.7	0	0	
Class total‡		63.9	67.4	40.4	56.3	64.5	65.7	58.7	±3.0	59.2	52.4	±3.2
Subtotals												
Mono-acids		60.9	64.6	40.3	49.8	64.4	62.4	56.1	±2.1	23.7	17.5	±3.2
Poly-acids		2.9	2.8	0.1	6.4	0.1	3.4	3.7	±2.1	35.4	35.0	±3.8
Total 16:1		47.5	52.6	30.1	35.0	50.0	59.0	44.4	±6.2	11.3	11.2	±1.0
16 carbons		50.7	54.0	30.1	37.1	50.0	60.2	47.0	±5.2	13.4	13.5	±3.6
18 carbons		1.8	6.1	4.8	14.7	4.5	4.5	8.5	±6.4	35.6	35.5	±4.3
Hydroxy acids												
iso-3-OH-17:0	18:15	0.2	0	0	1.0	0	0.2	0.2	±0.3	0	0.4	±0.7
Class total§		1.6	0.5	0.4	1.2	0	0.2	1.0	±0.6	0.4	0.8	±0.5
Saturated branched-chains												
iso-13:0	12:61	tr	0	0	0.1	0	1.0	0		0	0.1	±0.1
anteiso-14:0	13:71	0	0	tr	0	0	2.1	3.5	±0.3	0	0.1	±0.2
iso-15:0	14:62	1.0	0.2	4.2	0.4	0	0.4	tr	±0.1	tr	0.4	±0.5
anteiso-15:0	14:71	1.1	0.2	4.0	1.0	tr	tr	0.2	±0.3	tr	0.8	±0.8
iso-16:0	15:63	0.1	0	1.3	0.2	1.1	0	0		0	0.9	±1.0
iso-17:0	16:63	0.7	0.7	1.1	1.2	0.3	0.3	0.4	±0.1	0.8	0.6	±0.1
Class total		3.3	1.4	11.4	3.2	1.7	3.1	4.8	±0.6	1.4	3.7	±1.9
Cyclic acids: cyclo-17:0	16:89	tr	0.6	0.1	1.2	tr	0.1	tr	±0.1	0.6	0.2	±0.1
Unsaturated branched chains												
iso-16:1	15:44	0	0.1	1.0	0	1.0	0	1.6	±0.2	0	0	
iso-18:1	17:45	0.7	0	0.7	0	1.9	0	0		0.4	0	
Class total¶		2.0	0.4	2.9	0.5	3.5	0.6	2.2	±0.2	1.0	0.3	±0.1
Unidentified		1.7	3.9	3.5	3.9	4.1	1.8	3.0	±0.9	2.9	3.8	±0.6
Ratios												
Unsaturated/saturated		2.1	2.5	0.8	1.5	2.4	2.1	1.8	±0.2	1.7	1.2	±0.2
Mono-/poly-unsaturated		20.6	23.4	671.8	7.8	538.7	18.5	11.8	±2.9	0.7	0.5	±0.2
16:0/16:1		0.5	0.4	1.0	0.8	0.5	0.4	0.6	±0.1	2.8	3.1	±0.4
16:0/14:0		8.2	8.5	11.1	16.3	20.7	12.9	9.2	±2.8	164.4	81.7	±40.0

* Mean of three analyses each performed about 6 months apart.

† Including trace and minor levels (less than 1 % of total) of 8:0, 10:0, 13:0, 15:0, 17:0, 19:0 and 20:0 (data not shown).

‡ Including trace and minor levels of 15:1, 17:3 (*cis*5), 20:4 (*cis*5) and 20:5 (*cis*3).

§ Including trace and minor levels of 3-OH-10:0, iso-3-OH-11:0, iso-3-OH-14:0, iso-3-OH-15:0 and iso-3-OH-17:0.

|| Including trace and minor levels of anteiso-13:0, iso-14:0, anteiso-17:0, iso-19:0 and anteiso-19:0.

¶ Including iso-17:1 and anteiso-17:1.

Unlike those of most strains of subsection I, the fatty acid profiles of strains from subsections III, IV and V generally include major concentrations of polyunsaturated acids. The fatty acid composition of the unicellular cyanobacteria comprising subsection II, order *Pleurocapsales*, however, has not been studied to the authors' knowledge.

METHODS

Strains of cyanobacteria and their cultivation. At least one representative strain of each genus in subsection II was selected and cultured for this study: *Dermocarpa* PCC 7301 and PCC 7439, *Xenococcus* CCMP 7305, *Dermocarpella* PCC 7437, *Myxosarsina* PCC 7312, *Chroococcidiopsis* PCC 7203 and PCC 7439, *Pleurocapsa* group PCC 7319 and an uncharacterized isolate CCMP 1489 having morphological characteristics typical of the subsection.

Cyanobacteria were obtained from the Pasteur Institute Culture Collection (PCC), Institut Pasteur, Paris, France, the American Type Culture Collection (ATCC) Rockville, MD, USA, or from the Provasoli-Guillard National Centre for Culture of Marine Phytoplankton (CCMP), West Boothbay Harbour, ME, USA. Cultures were acclimatized to the study conditions for an average of three generations before inoculation into growth media. Marine isolates were grown axenically on AS-III medium (Sigma), and the terrestrial or freshwater isolates on BG-11 medium (Rippka *et al.*, 1979), for 14 d with gentle agitation under white fluorescent light at $25 \pm 1^\circ\text{C}$. Cells were harvested by centrifugation at late-exponential phase, as described previously, and stored at -20°C for up to 2 months prior to analysis (Caudales & Wells, 1992).

Fatty acid analysis. Cells were saponified and esterified by adding approximately 400–500 mg (wet wt) to 1 ml of 1.2 M NaOH in 50% aqueous methanol in a tightly sealed screw-cap test tube and heating for 30 min in a boiling water bath. When cool, the mixture was combined with 0.5 ml N HCl and 1 ml 12% (v/v) boron trichloride/methanol, resealed, then heated for 5 min at 85°C . The methylated acids were then extracted with 1 ml hexane/diethyl ether (1:1) and washed with 3 ml 0.3 N NaOH; the lower (hexane/ether) fraction was transferred to a 1.5 ml vial by pipette and then concentrated with a stream of purified dry nitrogen gas to approximately 10 μl . A 2 μl portion of the concentrated sample was injected into a gas chromatograph (model 3700; Varian) equipped with a flame-ionization detector and a 15 m $\times$ 0.25 mm capillary glass column coated with SPB-1 (Supelco) as a non-polar stationary phase. Operating conditions were as follows: a 20 ml min⁻¹ flow rate of helium carrier gas, a 230°C injector temperature, a 130°C column temperature, a 230°C final temperature and a 4°C min^{-1} temperature programme rate.

Fatty acids that were between 8 and 20 carbons long were identified by co-chromatography with reference standards for bacterial fatty acids (Supelco). Major peaks in selected runs were confirmed with a mass spectrometer (model 8230HR; Finnegan). Unsaturated fatty acids were also confirmed chemically by hydrogenating methyl esters by the method described by Moss (1979) and then detecting the presence of the saturated straight-chain analogues. Hydroxy-substituted fatty acids were confirmed by trifluoroacetylation, also by the method of Moss (1979). Additional confirmation of peak identities was derived from comparisons of carbon equivalent chain-lengths for each fatty

acid with those in other published reports on fatty acid composition of cyanobacteria (Gilliam & Hogg, 1984; Schulte, 1993). The components of the fatty acid profile of each strain were organized according to chemical class, from which class totals and selected ratios were derived.

Two cyanobacteria, *Pleurocapsa* PCC 7319 and *Chroococcidiopsis* PCC 7203, were cultivated and analysed on three different dates to provide a measure of standard deviations. Other strains were analysed once.

RESULTS

Major features of the fatty acid profile of cyanobacteria of subgroup II, order *Pleurocapsales* were high proportions of saturated straight-chain fatty acids (26–41% of the total), the 16:0 being the main component (20–35%), and unsaturated straight chain acids (40–67%) with isomers of 16:1 being the major components (11–59%). Polyunsaturated acids were present at trace levels (0.1% or less) in *Xenococcus* and *Myxosarcina*, at concentrations of less than 7% in *Dermocarpa*, *Dermocarpella*, *Pleurocapsa* and CCMP 1498, and at high concentrations (35% or more) in *Chroococcidiopsis*. Saturated branched chains comprised 1–11% of the total in all groups and unsaturated branched chains 0.4–3.5%. Minor acids, generally less than 1% of the total, were the saturated straight odd-carbon, hydroxy-substituted and cyclic fatty acids. The ratio of unsaturated to saturated fatty acids ranged from 0.8 in *Xenococcus* to 2.5 in *Dermocarpa* PCC 7437 (Table 1).

Chroococcidiopsis was different from other genera in *Pleurocapsales* primarily in terms of the ratio of mono- to polyunsaturated acids. The principal mono-unsaturated acids, isomers of 16:1, ranged from 10 to 12% in *Chroococcidiopsis* and from 30 to 59% in the other genera. Polyunsaturated acids, principally 18:2 (*cis*9) and isomers of 18:3, averaged 35% in *Chroococcidiopsis* and from trace amounts up to 6% in the other genera. These differences were reflected in a ratio of mono- to polyunsaturated acids for the two strains of *Chroococcidiopsis* of less than 1 (compared to more than 7 in other genera). Total 16- and 18-carbon fatty acids were also quantitatively different in *Chroococcidiopsis*: the percentages of 16- and 18-carbon acids were less than half and more than double those of the other genera, respectively. These differences were also apparent in the ratios for 16:0/16:1 and 16:0/14:0 fatty acids (Table 1).

Minor, but distinguishing, differences were evident among genera with low levels of polyunsaturated acids. The composition of the two *Dermocarpa* strains (PCC 7301 and 7437) was similar but with differences in distribution of the shorter-chain unsaturated fatty acids 12:1 and 14:1. *Xenococcus* PCC 7305 was noticeably different in the ratio of unsaturated to saturated acids (0.8, the only strain with a ratio of less than 1) and in the ratio of mono- to polyunsaturated acids (672, the highest in the *Pleurocapsales*).

DISCUSSION

The fatty acid composition of unicellular strains of the order *Pleurocapsales* followed a pattern similar to that of subsection I, order *Chroococcales*. Kenyon (1972) analysed 35 unicellular strains of *Chroococcales* and found them clustered into heterogeneous groups with respect to fatty acids. Twenty strains contained mostly monounsaturated fatty acids, with a maximum of 4% polyunsaturated acids, and the remainder contained major quantities of polyunsaturated acids. In our analysis of nine strains of *Pleurocapsales*, six contained less than 4% polyunsaturated acids, one contained 6% and one, the genus *Chroococcidiopsis*, had noticeably high levels of polyunsaturated acids (Table 2).

By contrast, nearly all strains of the orders *Oscillatoriales* and *Nostocales* (subsections III and IV) that have been analysed contain high concentrations of polyunsaturated acids and ratios of mono- to polyunsaturated acids of 3 or less. Of 32 strains from these orders analysed by Kenyon *et al.* (1972), 30 contained 25% or more polyunsaturated acids and ratios of less than 1.5. Free-living and symbiotic strains of *Nostoc* and *Anabaena* (*Oscillatoriales*) are also high in polyunsaturated acids, having ratios of mono- to poly-

unsaturated acids averaging less than 1 (Caudales & Wells, 1992; Caudales *et al.*, 1995). Sallal *et al.* (1990) reported ratios of less than 1 for *Nostoc muscorum* and 2.5 for *Nostoc canina*. Of the few strains analysed from subsection V, order *Gleocapsales*, all were also high in polyunsaturated acids (Kenyon & Stanier, 1970; Kenyon, 1972; Kenyon *et al.*, 1972).

The unicellular strains of subsection II, order *Pleurocapsales* (excepting *Chroococcidiopsis*) were also similar to those of subsection I in having relatively high concentrations of 16:1 (30–59%) compared to filamentous cells of subsections III, IV and V. The percentages of 16:1 in the two strains of subsection I analysed by Parker *et al.* (1967) were 34 and 36%. Strains containing polyunsaturated acids in the study by Kenyon *et al.* (1972) averaged 42% for 16:1 but less than 16% in strains low in polyunsaturated acids. Genera in subsections III, IV and V (low in polyunsaturated acids) have concentrations of 16:1 generally below 30% (Caudales & Wells, 1992; Caudales *et al.*, 1995; Kenyon *et al.*, 1972).

The fatty acid profiles of the *Pleurocapsales* confirm the general observation that unicellular cyanobacteria are divided into different compositional groups, the

Table 2. Comparison of major classes of fatty acids of the unicellular cyanobacteria of subsection I, order *Chroococcales* and subsection II, order *Pleurocapsales*

tr, Trace amounts; —, the acid was not detected under our experimental conditions.

Cyanobacteria (no. strains)	Saturated			Monounsaturated			Polyunsaturated		
	14:0	16:0	18:0	14:1	16:1	18:1	16:2*	18:2	18:3
Order <i>Chroococcales</i>†									
<i>Synechococcus</i>									
Clusters 1–5 (12)	9	28	2	1	42	12	—	tr	—
Clusters 6–7 (6)	8	24	2	tr	9	15	—	22	6
Cluster 8 (2)	3	25	1	tr	17	4	14	24	4
<i>Anpanocapsa</i> ‡									
Cluster 1 (6)	1	33	2	1	8	5	—	18	24
Cluster 2–5 (6)	28	7	2	9	38	2	—	2	—
<i>Gloeocapsa</i> § (1)	11	19	3	3	46	4	—	3	—
<i>Chlorogloea</i> (1)	26	9	7	3	27	3	—	2	—
Order <i>Pleurocapsales</i>									
<i>Dermocarpa</i> (2)	3	27	1	4	50	2	2	1	tr
<i>Xenococcus</i> (1)	3	34	1	1	30	5	—	—	—
<i>Dermocapella</i> (1)	2	27	2	4	35	8	1	4	1
<i>Myxosarcina</i> (1)	1	24	1	9	50	5	—	—	—
CCMP 1489 (1)	2	26	tr	tr	51	2	1	—	2
<i>Pleurocapsa</i> (1)	1	26	1	1	44	6	2	—	2
<i>Chroococcidiopsis</i> (2)	tr	33	2	3	11	3	2	19	13

* Including 16:3 and 16:4 polyunsaturated acids.

† Data summarized from Kenyon (1972).

‡ All listed strains of *Aphanocapsa* reclassified into the provisional *Synechocystis* group by Waterbury & Rippka (1989).

§ *Gloeocapsa* strain PCC 6501 reclassified into the *Synechococcus* group by Waterbury & Rippka (1989).

|| *Chlorogloea* strain PCC 6712 reclassified as *Chroococcidiopsis* in subgroup II, order *Pleurocapsales* by Waterbury & Rippka (1989).

majority having low or undetectable levels of polyenoic fatty acids. Profiles with saturated straight and branched chains and monounsaturated acids are typical of photosynthetic and non-photosynthetic prokaryotes. Profiles with high concentrations of polyunsaturated acids, however, are more typical of the filamentous cyanobacteria as well as of algae and higher plants. The unicellular cyanobacteria of the order *Pleurocapsales*, therefore, as well as those of the order *Chroococcales*, appear to occupy a transitional position (with respect to fatty acid composition) between prokaryotes and higher plants.

Regarding the relatedness of the *Pleurocapsales*, all genera except *Chroococcidiopsis* share the same basic fatty acid profile; this also applies to *Pleurocapsa*, the provisional assemblage distinguishable from other members of *Pleurocapsales* only by the relative proportions of 16:1 isomers.

Despite the apparent morphological coherence of the *Pleurocapsales*, *Chroococcidiopsis* was distinct from other genera in its elevated percentages of polyunsaturated acids. Similar disparities occur in the unicellular *Chroococcales* (subsection I) in which, as documented by Kenyon (1972), half of the 40 strains analysed contained elevated levels of unsaturated acids. The division of the unicellular cyanobacteria between subsections I and II, based on morphology, is undoubtedly compromised by uncertainties regarding strain identification. For example, Waterbury & Rippka (1989) reclassified strain PCC 6712, formerly *Chlorogoea* as reported by Kenyon (1972), as *Chroococcidiopsis* while recognizing its difference from other strains. On the basis of the fatty acid profile of PCC 6712 (i.e. low levels of polyunsaturated acids), it is also different from strains of *Chroococcidiopsis* analysed in this report (i.e. high polyunsaturated acids). At present, however, the principal taxonomic criterion for cyanobacteria is morphology and development. Thus, the significance, if any, of these discrepancies must await additional lines of evidence such as a complete genetic analysis of the unicellular cyanobacteria.

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