Lipid and fatty acid composition of freshwater cyanobacteria

A. K. SALLAL, N. A. NIMER and S. S. RADWAN*

Department of Botany and Microbiology, Faculty of Science, Kuwait University, PO Box 5969, Safat 13060, Kuwait

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Four species of freshwater cyanobacteria (*Anabaena cylindrica*, *Anacystis nidulans*, *Nostoc canina* and *Nostoc muscorum*) contained as major lipid classes monogalactosyldiacylglycerols, digalactosyldiacylglycerols, sulphoquinovosyldiacylglycerols and phosphatidylglycerols. Unlike photosynthetic eukaryotes, cyanobacteria incubated for 7 d in the dark suffered no decrease in the concentrations of these classes, except for *N. muscorum*. Growth, photosynthesis and nitrogen fixation were 30–40% lower after dark incubation. The nitrogen-fixing cyanobacteria, *Anabaena cylindrica*, *N. canina* and *N. muscorum*, contained alcohol glycosides and a highly-polar unknown glycolipid at high concentrations. The proportion of these two lipid classes decreased in the dark in *N. muscorum* alone. Extracts from *Anacystis nidulans* and *N. canina*, and to a lesser extent *N. muscorum*, contained sterols, whose concentration increased after dark incubation. *Anabaena cylindrica* contained considerable concentrations of linolenic acid in its total lipid, which did not decrease on dark incubation, and was not present mainly in monogalactosyldiacylglycerols as in photosynthetic eukaryotes. Palmitoleic acid, which is primarily confined to phosphatidylglycerols in photosynthetic eukaryotes, was distributed among the major lipid classes of *N. canina*.

Introduction

Although the major lipid classes in cyanobacteria and photosynthetic eukaryotes are quite similar (Quinn & Williams, 1983) the fatty acid compositions of their lipids are not. Lipids from photosynthetic eukaryotes are rich in polyenoic fatty acids (for reviews see Kates, 1970; Radwan & Mangold, 1976, 1980), but with a few exceptions (Nichols & Wood, 1968a; Sato & Morata, 1980, 1982), they are absent from cyanobacterial lipids (Holton *et al.*, 1964; Parker *et al.*, 1967). Moreover, cyanobacteria, in contrast to eukaryotes, usually lack sterols (Quinn & Williams, 1983).

Dark incubation of photosynthetic eukaryotes is usually associated with decreases in the concentration of the major lipid classes, and of the constituent linolenic acid (Kates, 1970; Radwan *et al.*, 1979). An earlier study in our laboratory indicated that different marine cyanobacteria do not exhibit similar changes in their lipids in response to dark incubation (Al-Hasan *et al.*, 1989). The present paper contains data on the lipid and fatty acid composition of four freshwater cyanobacteria, three of which differ from the marine cyanobacteria we studied earlier, in that they can fix nitrogen. Preliminary analyses indicate considerable differences in lipid composition between these freshwater cyanobacteria and the marine species studied earlier.

Methods

Organisms and growth conditions. *Anabaena cylindrica* (CU 1403/2a) and *Nostoc muscorum* (1453/9) were obtained from the Culture Centre of Algae and Protozoa, Cambridge, UK. *Anacystis nidulans* and *Nostoc canina* were a gift from Professor G. A. Codd, University of Dundee, UK. The cyanobacteria were grown axenically at 28 °C in the basic BG-11 medium (Stainer *et al.*, 1971) in aspirators (10 litre) equipped with magnetic stirrers and sparged with sterile air. Light of 3500 lx at the surfaces of the vessels was provided by cool-white fluorescent tubes. The ferricyanide Hill reaction (PS II), Mehler reaction (PS I), NADP-photoreduction and oxygen evolution were measured as described by Sallal & Nimer (1988a, b). Nitrogen-fixing cyanobacteria were grown in nitrogen-free medium, and nitrogenase was assayed by the method of Haystead *et al.* (1970) using a Porapak-T column. For lipid analysis, cultures were either grown under white light for 7 d and then incubated for another 7 d in the dark, or else maintained under continuous illumination throughout the 14 d period. At the end of the culture period, the biomass was allowed to settle and the supernatant was decanted, reducing the 10 l volume to about 500 ml. The biomass was then harvested by centrifugation and the pellet was suspended in about 20 vols of 2-propanol/chloroform (1:2, v/v), ready for lipid analysis.

Lipid extractions and analysis. All solvents were freshly redistilled, and all steps were done under oxygen-free nitrogen, as far as feasible. The total lipid was extracted from cyanobacteria with 2-propanol/chloroform (1:2, v/v) (Nichols, 1964), and purified according to Folch *et al.* (1957). Boiling solvents were used in the first extraction to deactivate lipases (Kates, 1972; Radwan, 1984). The lipid extracts were analysed by thin-layer chromatography (TLC) on plates of silica gel. Nonpolar lipid classes were fractionated using hexane/diethyl ether/acetic acid (90:10:1, by vol.) (Mangold & Malins, 1960). To improve the resolution of sterols, we used hexane/diethyl ether/acetic acid

(50:50:1, by vol.). Ionic and other polar lipids were resolved by two-dimensional TLC using chloroform/methanol/7 M-ammonium hydroxide (65:25:4, by vol.) in the first direction and chloroform/methanol/acetic acid/water (170:25:25:4, by vol.) in the second (Nichols, 1964). The lipid fractions were detected with iodine vapour or by charring after spraying the chromatograms with 50% (v/v) H_2SO_4 . Individual lipid classes were identified by their chromatographic behaviour using standards and specific spray reagents (Dittmer & Lester, 1964; Siakotos & Rouser, 1965; Stahl, 1967; Vioque, 1984). Sterols were identified by the characteristic deep pink colour with 50% methanolic H_2SO_4 at 100 °C and characteristic fluorescence under UV with antimony trichloride/acetic acid, chlorosulphonic acid/acetic acid, perchloric acid, bismuth trichloride and methanolic ZnCl_2 (Stahl, 1967). Sterol fractions were eluted from thin-layer chromatograms with chloroform/diethyl ether/ethanol (1:1:1, by vol.) (Kates, 1972) and analysed by UV-spectrophotometry (Pye-Unicam SP 800) and gas chromatography (Pye-Unicam 204) as their trimethylsilyl ether derivatives using a column packed with OV-17 on Chromosorb W-HP. To confirm the identity of a glycolipid tentatively identified as alcohol glycoside, and that of a very polar unknown glycolipid, samples of the pure compounds were separated by preparative TLC, eluted (Kates, 1972), and subjected to acid hydrolysis (Gaver & Sweeley, 1965). Hydrolysis products soluble in hexane were analysed by TLC, and sugar moieties remaining in the aqueous phases were identified by paper chromatography (Menzies & Seakins, 1976). Samples of total lipids and individual lipid classes, separated by preparative TLC (Radwan, 1978), were subjected to methanolysis with 2% H_2SO_4 in absolute methanol at 90 °C for 90 min (Chalvardjian, 1964). The resulting methyl esters of fatty acids were recovered with hexane, purified by preparative TLC and analysed by gas chromatography using a glass column (1.83 m $\times$ 4 mm i.d.) packed with 15% (w/w) DEGS on Anakrom D (100:120 mesh) and operated isothermally at 170 °C with nitrogen as the carrier gas. Individual fatty acids were identified by comparing their retention times with those of standard samples, and by the 'equivalent chain length' method of Miwa *et al.* (1960).

Results

Growth and physiological activity

N. canina and *N. muscorum* grew for about 2 doublings under continuous illumination during the first 12 d, after which their growth slowed. Incubating 7 d light-grown cultures in the dark for another 7 d did not increase the biomass. The dark-incubated cells resumed slow growth after reillumination. When 7 d light-grown cultures of *N. canina* and *N. muscorum* were incubated for 7 d in the dark, there was a 30–40% decrease in their light-dependent photosynthetic and nitrogen fixation activities (data not shown).

Lipid composition

The total lipid content of the four cyanobacterial species ranged between 10.7 and 12.3% of dry weight. There was no obvious difference in the lipid content between light- and dark-incubated cultures. Thin-layer chromatograms indicated the presence of hydrocarbons, diacylglycerols,

Fig. 1. Typical TLC plate showing individual sterols in the lipids of light- and dark-incubated cells of *Anacystis nidulans* and *Nostoc canina*. TLC was done using silica gel G plates and hexane/diethyl ether/acetic acid (50:50:1, by vol.). Sterols were visualized by charring after spraying with 50% H_2SO_4 . Lanes 1 and 2, *N. canina* in the light (lane 1) and in the dark (lane 2); lanes 3 and 4, *Anacystis nidulans* in the light (lane 3) and in the dark (lane 4). Ls, lanosterol; C, cholesterol.

monoacylglycerols and pigments in the nonpolar lipid fraction. All extracts, except those from *Anacystis nidulans*, also contained free fatty acids. Lipids from *Anacystis nidulans* contained appreciable concentrations of cholesterol, and those from *N. canina* considerable amounts of cholesterol and lanosterol (Fig. 1).

The predominant polar lipid classes in all extracts were monogalactosyldiacylglycerols, digalactosyldiacylglycerols, sulphoquinovosyldiacylglycerols and phosphatidylglycerols (Fig. 2). In addition, all extracts except those from *Anacystis nidulans* contained high concentrations of alcohol glycosides and a highly-polar unknown glycolipid. The identity of the alcohol glycoside fraction, isolated from a light-incubated culture of *Anabaena cylindrica* by preparative TLC, was confirmed by identifying its acid-hydrolysis products. The aqueous phase contained glucose and the hexane phase contained long-chain alcohols, identified by their TLC behaviour and IR absorption band at 3500 cm^{-1} . The unknown glycolipid gave a positive reaction with α -naphthol

Fig. 2. Typical two-dimensional thin-layer chromatogram showing the composition of lipid classes of *N. canina*. TLC was done using silica gel G plates and the following solvent systems: chloroform/methanol/7 M-ammonium hydroxide (65:25:4, by vol.) in the first direction, and chloroform/methanol/acetic acid/water (170:25:25:2, by vol.) in the second direction. The lipid fractions were visualized by charring after spraying with 50% H_2SO_4 . NL, neutral lipids and pigments; MGDG, monogalactosyldiacylglycerols; P, pigments; AG, alcohol glycosides; PG, phosphatidylglycerols; DGDG, digalactosyldiacylglycerols; SQDG, sulphoquinovosyldiacylglycerols; X, unknown glycolipid.

(i.e. sugar positive), a weak reaction with ninhydrin, and contained no phosphorus. Acid hydrolysis of this fraction, isolated from a light-incubated culture of *N. muscorum*, showed that it was an acylglycolipid (the fatty acid composition is given in Table 2) containing approximately equal proportions of glucose and galactose (as indicated by their spot densities on paper chromatograms). Several other unidentified polar lipids occurred in various extracts in trace amounts.

The composition of major glycolipids in extracts from light-incubated cyanobacteria was similar to that from dark-incubated cultures (Fig. 3). The only exception was *N. muscorum*, in which dark-incubated cultures contained lower concentrations of the major glycolipids than did light-incubated cultures.

Fatty acid compositions

The fatty acid compositions of lipids from the test cyanobacteria are shown in Tables 1 and 2. The predominant fatty acids in lipids from *Anacystis nidulans* (Table 1) were palmitic (16:0) and palmitoleic (16:1) acids. In lipids of *Anabaena cylindrica* (Table 1) palmitic, linoleic (18:2) and linolenic (18:3) acids predominated. *N. canina* lipids contained palmitic, palmitoleic, oleic (18:1) and linoleic acids as major acyl moieties, whereas

Table 1. Fatty acid composition of total lipid from non-nitrogen-fixing (*Anacystis nidulans*) and nitrogen-fixing (*Anabaena cylindrica*) cyanobacteria

Light cultures were maintained for 14 d under continuous illumination. Dark cultures were grown for the first 7 d in the light and were then maintained for another 7 d in the dark. Values are expressed as wt%; each value is the mean of three determinations, $\pm$ SD. tr, trace.

Fatty acid	<i>Anacystis nidulans</i>		<i>Anabaena cylindrica</i>	
	Light	Dark	Light	Dark
16:0	41.8 $\pm$ 2.0	56.2 $\pm$ 2.0	47.6 $\pm$ 1.9	44.9 $\pm$ 2.1
16:1	30.1 $\pm$ 0.6	31.7 $\pm$ 1.5	1.7 $\pm$ 0.1	2.0 $\pm$ 0.1
16:2	7.6 $\pm$ 0.3	tr	10.1 $\pm$ 0.6	11.5 $\pm$ 0.9
18:0	6.4 $\pm$ 0.2	5.8 $\pm$ 0.2	tr	tr
18:1	11.2 $\pm$ 0.6	5.8 $\pm$ 0.3	8.4 $\pm$ 0.5	12.6 $\pm$ 0.7
18:2	2.3 $\pm$ 0.1	0.5 $\pm$ 0.0	17.5 $\pm$ 1.2	15.4 $\pm$ 0.8
18:3	tr	tr	13.7 $\pm$ 0.5	13.5 $\pm$ 0.7

in *N. muscorum* palmitic, palmitoleic, hexadecadienoic (16:2), oleic and linoleic acids were predominant (Table 2). In the total lipid from *Anacystis nidulans* and *N. canina*, the dark incubation was associated with increased concentration of palmitic acid and decreased concentration of oleic acid. The converse was true for the

Fig. 3. Typical TLC plate showing the effect of illumination on glycolipids of the four cyanobacteria. TLC was done using silica gel G and chloroform/methanol/acetic acid/water (85:12:12:1, by vol.). The glycolipid fractions were visualized by spraying with α -naphthol and H_2SO_4 and heating at $100^\circ C$. MGDG, monogalactosyldiacylglycerols; P, pigments; AG, alcohol glycosides; DGDG, digalactosyldiacylglycerols; SQDG, sulphoquinovosyldiacylglycerols; X, unknown glycolipid. Lanes 1 and 2, *Anacystis nidulans* in the dark (lane 1) and in the light (lane 2); lanes 3 and 4, *Anabaena cylindrica* in the dark (lane 3) and in the light (lane 4); lanes 5 and 6, *N. canina* in the dark (lane 5) and in the light (lane 6); lanes 7 and 8, *N. muscorum* in the dark (lane 7) and in the light (lane 8).

total lipid from *Anabaena cylindrica* and *N. muscorum*. In the lipids from *N. muscorum*, but not in lipids from the other cyanobacteria, the concentration of linoleic acid decreased dramatically in response to dark incubation.

The fatty acid composition of the major lipid classes of *Anabaena cylindrica* and *N. canina*, and the effects of light and dark incubation, are shown in Table 3. *Anabaena cylindrica* had the highest concentration of linolenic acid (18:3) in its total lipid, present mainly in digalactosyldiacylglycerols and, to a lesser extent, in sulphoquinovosyldiacylglycerols and phosphatidylglycerols, but not in monogalactosyldiacylglycerols. The presence or absence of illumination had no consistent effect on the relative concentrations of any fatty acid in the various lipid classes of *Anabaena cylindrica*. *N. canina*, unlike *Anabaena cylindrica*, contained high concentrations of palmitoleic acid (16:1) in its total lipid, distributed among all major lipid classes, with little difference between light and dark incubation except for phosphatidylglycerols.

Discussion

Our results show that freshwater cyanobacteria do not respond uniformly to the presence or absence of illumination with respect to changes in their lipid composition. This constitutes additional evidence for the heterogeneity of these photosynthetic prokaryotes as a systematic group and echoes our earlier results on marine

Table 2. Effect of dark-incubation on the constituent fatty acids of total lipid from two *Nostoc* spp. at different growth stages

Values are expressed as wt%, each value is the mean of three determinations, $\pm$ SD. tr, trace.

Fatty acid	<i>Nostoc canina</i>								<i>Nostoc muscorum</i>							
	7 d		9 d		12 d		14 d		7 d		9 d		12 d		14 d	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
16:0	23.0 ± 1.0	23.1 ± 1.0	30.3 ± 1.1	27.0 ± 1.5	38.8 ± 2.0	26.9 ± 1.7	39.1 ± 1.6	21.9 ± 1.5	21.9 ± 1.3	22.0 ± 0.6	22.0 ± 1.3	17.5 ± 1.0	21.6 ± 0.8	17.0 ± 0.8		
16:1	24.0 ± 1.4	24.0 ± 0.7	22.4 ± 1.2	22.9 ± 0.9	20.0 ± 1.0	23.2 ± 0.9	20.2 ± 1.2	19.5 ± 1.1	19.7 ± 0.7	20.0 ± 1.3	19.3 ± 0.6	20.2 ± 1.2	19.0 ± 1.1	20.0 ± 0.9		
16:2	2.8 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.2 ± 0.2	15.9 ± 0.4	16.0 ± 0.8	17.0 ± 1.0	16.0 ± 0.9	22.0 ± 0.8	16.0 ± 1.0	21.6 ± 1.7		
18:0	1.3 ± 0.0	0.9 ± 0.0	0.4 ± 0.0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1.6 ± 0.1	tr
18:1	30.2 ± 1.2	29.8 ± 1.1	15.8 ± 0.9	29.0 ± 1.3	11.0 ± 0.7	29.2 ± 1.8	10.9 ± 0.5	14.7 ± 0.8	14.5 ± 0.4	19.2 ± 0.5	15.3 ± 0.6	28.9 ± 1.4	14.2 ± 0.4	29.7 ± 1.7		
18:2	18.0 ± 1.0	17.8 ± 0.5	26.8 ± 1.3	16.0 ± 0.8	26.0 ± 1.0	16.0 ± 0.9	25.6 ± 1.0	26.4 ± 0.8	26.7 ± 1.0	21.5 ± 1.2	26.8 ± 1.4	11.4 ± 0.7	27.1 ± 0.8	11.7 ± 0.4		
18:3	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.9 ± 0.0	tr	0.9 ± 0.1	tr	0.4 ± 0.1	0.5 ± 0.1	tr	0.4 ± 0.0	tr	0.3 ± 0.0	tr		

Table 3. Constituent fatty acids of individual lipid classes from two cyanobacteria

Light cultures were maintained for 14 d under continuous illumination. Dark cultures were grown for the first 7 d in the light and were then maintained for another 7 d in the dark. Values are expressed as wt%; each value is the mean of two determinations, $\pm$ SD. tr, trace.

Fatty acid	<i>Anabaena cylindrica</i>		<i>Nostoc canina</i>	
	Light	Dark	Light	Dark
Monogalactosyldiacylglycerols:				
16:0	56.0 $\pm$ 1.0	24.4 $\pm$ 1.1	11.5 $\pm$ 0.4	12.1 $\pm$ 0.5
16:1	1.1 $\pm$ 0.0	tr	21.6 $\pm$ 0.9	26.1 $\pm$ 1.2
16:2	12.1 $\pm$ 0.5	24.2 $\pm$ 0.9	25.6 $\pm$ 1.1	24.9 $\pm$ 1.2
18:0	12.1 $\pm$ 0.4	tr	tr	tr
18:1	25.2 $\pm$ 0.8	29.1 $\pm$ 1.2	21.6 $\pm$ 0.4	24.7 $\pm$ 1.3
18:2	1.6 $\pm$ 0.1	20.5 $\pm$ 1.2	19.7 $\pm$ 1.0	8.7 $\pm$ 0.1
18:3	2.8 $\pm$ 0.1	1.8 $\pm$ 0.0	tr	3.4 $\pm$ 0.1
Digalactosyldiacylglycerols:				
16:0	20.0 $\pm$ 1.0	20.7 $\pm$ 1.0	1.7 $\pm$ 0.1	12.5 $\pm$ 0.7
16:1	tr	tr	28.7 $\pm$ 1.4	23.6 $\pm$ 1.1
16:2	26.5 $\pm$ 1.1	27.6 $\pm$ 1.6	28.8 $\pm$ 0.8	24.5 $\pm$ 1.2
18:0	0.3 $\pm$ 0.0	tr	tr	tr
18:1	24.3 $\pm$ 1.7	28.2 $\pm$ 0.8	27.0 $\pm$ 1.6	30.3 $\pm$ 1.8
18:2	12.3 $\pm$ 0.6	15.1 $\pm$ 0.4	5.6 $\pm$ 0.1	9.0 $\pm$ 0.2
18:3	16.5 $\pm$ 0.5	8.3 $\pm$ 0.1	tr	tr
Sulphoquinovosyldiacylglycerols:				
16:0	17.8 $\pm$ 0.8	24.6 $\pm$ 0.7	9.1 $\pm$ 0.5	8.0 $\pm$ 0.6
16:1	tr	tr	28.5 $\pm$ 1.7	29.2 $\pm$ 1.2
16:2	35.1 $\pm$ 1.1	23.1 $\pm$ 1.2	30.6 $\pm$ 1.2	30.3 $\pm$ 1.8
18:0	0.1 $\pm$ 0.0	tr	0.8 $\pm$ 0.0	tr
18:1	30.8 $\pm$ 1.2	25.1 $\pm$ 1.5	19.6 $\pm$ 1.0	32.5 $\pm$ 1.3
18:2	22.6 $\pm$ 0.7	18.7 $\pm$ 0.9	7.0 $\pm$ 0.3	tr
18:3	4.0 $\pm$ 0.2	8.5 $\pm$ 0.2	4.3 $\pm$ 0.1	tr
Phosphatidylglycerols:				
16:0	21.7 $\pm$ 1.7	33.4 $\pm$ 1.5	22.8 $\pm$ 1.3	11.4 $\pm$ 0.2
16:1	tr	tr	9.8 $\pm$ 0.6	20.0 $\pm$ 1.2
16:2	24.0 $\pm$ 1.1	29.7 $\pm$ 1.3	17.8 $\pm$ 1.0	5.6 $\pm$ 0.3
18:0	tr	tr	7.3 $\pm$ 0.5	tr
18:1	24.3 $\pm$ 0.7	19.9 $\pm$ 0.8	29.4 $\pm$ 1.6	50.0 $\pm$ 1.8
18:2	20.8 $\pm$ 0.5	10.6 $\pm$ 0.6	12.9 $\pm$ 0.7	tr
18:3	9.2 $\pm$ 0.4	6.4 $\pm$ 0.4	tr	13.6 $\pm$ 0.6
Unidentified glycolipid:				
16:0	1.4 $\pm$ 0.1	25.9 $\pm$ 1.0	11.0 $\pm$ 0.5	0.5 $\pm$ 0.0
16:1	tr	tr	32.1 $\pm$ 1.6	30.4 $\pm$ 1.1
16:2	35.7 $\pm$ 2.0	24.8 $\pm$ 1.2	32.1 $\pm$ 1.3	38.0 $\pm$ 1.2
18:0	10.0 $\pm$ 0.5	tr	1.9 $\pm$ 0.1	0.2 $\pm$ 0.0
18:1	23.9 $\pm$ 0.9	25.7 $\pm$ 0.8	15.2 $\pm$ 0.8	30.4 $\pm$ 1.3
18:2	12.9 $\pm$ 0.4	15.7 $\pm$ 0.4	7.8 $\pm$ 0.4	0.6 $\pm$ 0.0
18:3	5.7 $\pm$ 0.2	7.8 $\pm$ 0.3	tr	tr

cyanobacteria. All four freshwater cyanobacteria examined contain lipid classes known to predominate in photosynthetic prokaryotes and eukaryotes, specifically monogalactosyldiacylglycerols, digalactosyldiacylglycerols, sulphoquinovosyldiacylglycerols and phosphatidylglycerols (Quinn & Williams, 1983). However, several basic differences are apparent between these cyanobacteria and photosynthetic eukaryotes. The nitrogen-fixing cyanobacteria *Anabaena cylindrica*, *N. canina* and *N. muscorum* contain alcohol glycosides; this observation is

consistent with earlier results on other organisms (Nichols & Wood, 1968b; Walsby & Nichols, 1969; Winklenbach *et al.*, 1972). However, we have also found this lipid class in marine cyanobacteria which were apparently incapable of nitrogen fixation (Al-Hasan *et al.*, 1989). Alcohol glycosides have never been recorded in lipids of photosynthetic eukaryotes (for reviews see Kates, 1970; Quinn & Williams, 1983). The three nitrogen-fixing freshwater cyanobacteria contain, in addition, a highly-polar unknown glycolipid not previously recorded. This compound was not found in the non-nitrogen-fixing *Anacystis nidulans*, or in any of the marine cyanobacteria we studied earlier (Al-Hasan *et al.*, 1989), and has not been recorded in photosynthetic eukaryotes.

In *N. muscorum* (but not the other three cyanobacteria), the concentrations of the major lipid classes decrease in the dark (Fig. 3), although growth of all cyanobacteria slowed and photosynthetic activity and nitrogen fixation decreased 30–40%. This response of *N. muscorum* is similar to that of many photosynthetic eukaryotes (Radwan *et al.*, 1979).

Anabaena cylindrica contains a considerable concentration of linolenic acid (18:3) in its total lipid, showing similarity to photosynthetic eukaryotes (Kates, 1972; Quinn & Williams, 1983). However, in contrast to eukaryotes, this cyanobacterium does not show any decrease in linolenic acid in its total lipid after incubation in the dark, nor is this fatty acid mainly concentrated in monogalactosyldiacylglycerols. This result contrasts with that of Zepke *et al.* (1978), who reported that in *Nostoc calcicola*, the major lipid classes, including monogalactosyldiacylglycerols, contain linolenic acid as a major constituent fatty acid. Palmitoleic acid (16:1), which is known to be confined to phosphatidylglycerols in photosynthetic eukaryotes (Hitchcock & Nichols, 1971), is distributed among various major lipid classes of *N. canina*. *N. canina* and *Anacystis nidulans* also contain sterols, a lipid class also present in a few other cyanobacteria (De Souza & Nes, 1968; Kohlase & Pohl, 1986; Sallal *et al.*, 1987).

In conclusion, as with marine cyanobacteria (Al-Hasan *et al.*, 1989), light-associated variations in the lipid and fatty acid composition of freshwater cyanobacteria are distinctly different from those reported for photosynthetic eukaryotes.

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