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Variation in Phospholipid Ester-Linked Fatty Acids and Carotenoids of Desiccated *Nostoc commune* (Cyanobacteria) from Different Geographic Locations

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Profiles of phospholipid fatty acids and carotenoids in desiccated *Nostoc commune* (cyanobacteria) collected from China, Federal Republic of Germany, and Antarctica and in axenic cultures of the desiccation-tolerant strains *N. commune* UTEX 584 and *Hydrocoleum* strain GOE1 were analyzed. The phospholipid fatty acid contents of the three samples of desiccated *Nostoc* species were all similar, and the dominant compounds were 16:1ω7c, 16:0, 18:2ω6, 18:3ω3, and 18:1ω7c. In comparison with the field materials, *N. commune* UTEX 584 had a much higher ratio of 18:2ω6 to 18:3ω3 (5.36) and a significantly lower ratio of 18:1ω7c to 18:1ω9c (1.86). Compound 18:3 was present in large amounts in the samples of desiccated *Nostoc* species which had been subject, in situ, to repeated cycles of drying and rewetting, but represented only a small fraction of the total fatty acids of the strains grown in liquid culture. This finding is in contrast to the data obtained from studies on the effects of drought and water stress on higher plants. Field materials of *Nostoc* species contained, in contrast to the axenic strains, significant amounts of apocarotenoids and a P384 pigment which, upon reduction with $NaBH_4$, yielded a mixture of a chlorophyll derivative and a compound with an absorption maximum of 451 nm. A clear distinction can be made between the carotenoid contents of the axenic cultures and the desiccated field materials. In the former, β-carotene and echinenone predominate; in the latter, canthaxanthin and the β-γ series of carotenoids are found.

Species of the genus *Nostoc* are among the most widespread of all nitrogen-fixing cyanobacteria. Communities of *Nostoc commune*, in particular, are prominent in those terrestrial limestone environments of tropical, polar, and temperate regions which are subject to extremes of water availability (25). An Aldabra Atoll (Indian Ocean), for example, macroscopic colonies persist in a desiccated state for approximately 6 months of the year (25). Throughout the monsoon season, however, the colonies resume and then cease cellular activities repeatedly as intermittent rains lead to cycles of rewetting and drying.

Both field and laboratory studies confirm that *N. commune* cells have a marked capacity to withstand long periods of desiccation and extremes of water stress (18, 22, 25). Upon rewetting, desiccated cells of field materials resume respiration first, then photosynthesis, and finally nitrogen fixation (22). A lag of 4 to 5 h was noted before the steady-state intracellular ATP pool was reached upon rewetting of field materials (21). With axenic laboratory-grown cultures of *N. commune* UTEX 584 this lag was of greater duration (19). Recent studies with *N. commune* UTEX 584 have shown that water stress induces marked changes in the protein index, the amounts of polysomes, and levels of chloramphenicol-sensitive protein synthesis (1, 17; M. Potts, Arch. Microbiol., in press), although there is, at present, no evidence for a water-stress regulon.

These preliminary studies suggest that the drying and rewetting of *Nostoc* cells can be used effectively to study the effects of water stress, an important environmental variable, on gene expression. However, if the molecular basis for desiccation tolerance is to be understood fully, it must be determined how *Nostoc* cells maintain the key components of their transcriptional and translational apparatus in a functionally intact state during prolonged periods of desiccation.

A previous investigation showed that the purified cytoplasmic membrane of *N. commune* UTEX 584 was enriched in carotenoids and contained significant amounts of a single fatty acid, compound 20:3ω3 (13). The latter was present at a level of 56.8% of the total membrane fatty acids; a unique feature which sets the membrane apart from all other cyanobacterial membranes which have been characterized to date (9, 12, 14, 15).

To assess further those features of *Nostoc* cells which may play a role in desiccation tolerance, we analyzed the phospholipid fatty acids (PLFA) and carotenoids of desiccated colonies from diverse geographic locations. The data were compared with those we obtained after the analysis of laboratory-grown cells of *N. commune* UTEX 584 and a desiccation-tolerant marine cyanobacterium.

MATERIALS AND METHODS

Microorganisms. Desiccated colonies of *N. commune* were collected from field sites in Hunan Province, China, and Reichenau (Konstanz), Federal Republic of Germany, and were provided kindly by T.-W. Chen and S. Scherer, respectively. The third sample was collected from an area of coastal lowland adjacent to the Ross Ice Shelf (Antarctica). For discussion purposes these three samples are referred to as *Nostoc* strain HUN, *Nostoc* strain REICH, and *Nostoc* strain ANT, respectively. At the time these samples were analyzed (see below), they had been stored in a desiccated state in the dark for either 18 months (*Nostoc* strain HUN, *Nostoc* strain REICH) or 7 years (*Nostoc* strain ANT).

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Laboratory cultures. For comparative purposes, analyses were performed on liquid cultures of *N. commune* UTEX 584 and a filamentous marine cyanobacterium which also expresses a marked tolerance to desiccation. Cultures of *N. commune* UTEX 584 were grown in liquid culture as described previously (18). The marine strain was isolated from stromatolitic crusts which were collected from an intertidal region of the Gulf of Elat, Israel, in 1978 (16). Cells were grown in an artificial seawater medium (ASN III) which lacked a source of combined nitrogen, at 32°C, under a photon flux density of 5 μmol of photons m^{-2} s^{-1}. This cyanobacterium has been assigned, provisionally, to the genus *Hydrocoleum* with the strain designation GOE1.

Lipid analysis. All glassware was rinsed with 6 N hydrochloric acid, distilled water, and chloroform-methanol (1:1, vol/vol). Between 100 and 200 mg of desiccated or lyophilized cells was subjected to a modified Bligh-Dyer extraction as described by Guckert et al. (8). The lipids were separated into three general classes by silicic acid chromatography (6, 8). The PLFA from the methanol fraction were methylated by milk alkaline methanolysis. The methods to quantify the fatty acid methyl esters (FAMES) are described in detail by Guckert et al. (8). The PLFA data are reported as the means of two analyses.

GC-MS. Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Hewlett-Packard 5996A system fitted with a direct capillary inlet. A cross-linked methyl silicone capillary column (8) was used. Samples were injected in the splitless mode at 100°C with a 30-s venting time, a 1-min isothermal interval followed by an increase in oven temperature of 10°C min^{-1} for 5 min, and then a rate of increase of 3°C min^{-1} until 280°C was reached. This was followed by an isothermal period of 10 min. This program was modified for the dimethyldisulfide (DMDS) adducts: the rate of temperature increase was changed from 3 to 1°C min^{-1} in the range of 250 to 280°C before the isothermal period for separation of *cis-trans* isomers. Helium was the carrier gas. The MS parameters were: electron multiplier voltage between 1,500 and 1,600 V, transfer line 280°C, source and analyzer 250°C, autotune file DFTPP normalized, optics tuned at *m/z* 502, electron impact energy equal to 70 eV. MS data were acquired and processed with a Hewlett-Packard RTE-6/VM data system.

After tentative identification by GC analysis, the PLFA were subjected to GC-MS analysis. Characteristic fragmentation patterns along with retention times were used to confirm the individual PLFA compounds.

Double-bond positions of compounds 16:1ω9c, 16:1ω7c, 16:1ω5c, 18:1ω9c, and 18:1ω7c were confirmed through the formation of their DMDS adducts as described by Dunkelblum et al. (4). Samples were dissolved in hexane, and then 100 μl of DMDS (gold label; Aldrich Chemical Co., Inc., Milwaukee, Wis.) and 1 to 2 drops of an iodine solution (6.0% [wt/vol] in diethyl ether) were added, and the samples were incubated at 50°C for 48 h. After cooling, additional hexane was added, and the iodine was removed by shaking with 5% (wt/vol) aqueous $Na_2S_2O_3$. The organic layer was removed, and the aqueous portion was reextracted with hexane-chloroform (4:1, vol/vol). The organic phases were evaporated under a stream of nitrogen in preparation for GC-MS analysis. The GC-MS analysis of the DMDS adducts indicated those major ions which arose from the fragmentation of the two CH_3S groups located at the site of unsaturation (Table 1).

Fatty acid nomenclature. Fatty acids are designated with respect to the total number of carbon atoms:number of double bonds followed by the position of the double bond from the omega (ω; aliphatic) end of the molecule. The suffixes c and t indicate *cis* and *trans* geometry, respectively. The prefixes i and a refer to iso and anteiso branching, respectively. Methyl branching is indicated by the prefix br. Cyclopropane fatty acids are designated with the prefix cy.

Extraction and analysis of carotenoids and chlorophylls. Before extraction, the desiccated or lyophilized sample was prewetted for 1 h on ice. After centrifugation (to remove excess water), the pellet was suspended in 10 ml of ice-cold methanol, and the solution was sonicated in five bursts of 30-s duration, with 30-s intermissions, on ice. Chloroform (10 ml) was added, and after 1 h of extraction the samples were centrifuged at 4°C. The supernatant was filtered through a Whatman 2V filter, and the pellet was reextracted with methanol and chloroform until it was colorless. A 0.5 volume of distilled water was added to the pooled supernatants, and the phases were allowed to partition at 4°C overnight. The chloroform fraction was filtered through a Whatman 2V filter, and then it was evaporated to dryness under a stream of nitrogen. The residue was dissolved in chloroform before its passage through a C18-SepPak cartridge and filtration in a centrifugal filter system (13). The pigments were analyzed by reverse-phase high-pressure liquid chromatography (HPLC).

Extracts were transferred to Whatman K6 silica gel thin-layer chromatography (TLC) plates, and chromatography was performed in a solvent mixture of petroleum ether-acetone-chloroform (3:1:1, vol/vol/vol). Individual bands were identified tentatively by their R_f values and their wavelength spectra which were recorded with a Hewlett-Packard 8450A diode array spectrophotometer. The area of gel within individual bands was scraped from plates and suspended in chloroform in the centrifugal filter system. After centrifugation, the wavelength spectrum was recorded in acetone, chloroform, ethyl acetate, hexane, and petroleum ether. The presence of allylic hydroxy groups was confirmed by treatment with HCl-chloroform; conjugated keto groups were detected after treatment with $NaBH_4$-ethanol.

RESULTS

The PLFA profiles of the three samples of desiccated *N. commune* were similar (Table 2). The dominant fatty acids present were compounds 16:1ω7c (10.12 to 18.29%), 16:0 (23.08 to 32.03%), 18:2ω6 (4.48 to 15.60%), 18:3ω3 (11.95 to 28.22%), and 18:1ω7c (10.38 to 20.63%). Iso-, anteiso-, methyl-, and cyclopropane-branched fatty acids were either not detected or represented a small percentage of the total FAMEs. The ratios of the amounts of 18:1ω7c to 18:1ω9c were similar for *Nostoc* strain HUN and *Nostoc* strain REICH (2.63 and 2.46, respectively) and significantly higher

TABLE 1. Characteristic ion fragments of derivatized products formed by reaction of monounsaturated FAMES with DMDS

FAME	M^+	Delta[a]	Delta-32	Omega[b]
il5:1ω11	ND[c]	ND	ND	215
16:1ω9c	ND	ND	157	173
16:1ω7c	362	217	185	145
16:1ω5c	ND	245	213	117
18:1ω9c	390	217	185	173
18:1ω7c	390	245	213	145

[a] Delta indicates double-bond position from carboxylic end of molecule.
[b] Omega indicates double-bond position from aliphatic end of molecule.
[c] ND, Not detected.

TABLE 2. PLFA profiles of field and laboratory cultures of cyanobacteria

Fatty acid[a]	% Composition[b]				
	Nostoc strain HUN	*Nostoc* strain REICH	*Nostoc* strain ANT	*N. commune* UTEX 584	*Hydrocoleum* strain GOE1
il5:1ω11	0.57	0.34	0.35	Tr[c]	Tr
il5:0	0.97	0.40	0.53	Tr	1.28
al5:0	0.47	0.42	0.97	0.75	Tr
il6:0	0.55	0.56	1.53	0.65	0.82
16:1ω9c	0.69	0.06	0.40	Tr	2.11
16:1ω7c	10.12	13.02	18.29	13.52	4.18
16:1ω5c	1.16	0.73	1.18	Tr	Tr
16:0	23.08	30.35	32.03	33.21	23.24
al7:0/17:1	1.28	0.56	0.80	2.65	0.76
cy17:0	Tr	Tr	Tr	Tr	0.37
17:0	0.44	0.14	0.16	0.43	0.35
br18:1	1.11	0.56	1.12	Tr	Tr
18:2ω6	15.60	9.34	4.48	17.80	13.59
18:3ω3	11.95	28.22	16.00	3.32	3.50
18:1ω9c	7.83	4.21	4.45	4.13	12.76
18:1ω7c	20.63	10.38	16.22	7.68	16.74
18:0	3.61	0.73	1.53	8.52	13.00
br19:1	Tr	Tr	Tr	Tr	7.43
cy19:0	Tr	Tr	Tr	7.33	7.68
Total 18:2ω6 + 3ω3	27.55	37.56	20.48	21.12	17.09
Ratio 18:2ω6/3ω3	1.31	0.33	0.28	5.36	3.88
Total FAME[d]	682.5	879.6	309.6	1,366.0	110.0

[a] Fatty acid identification based on GC retention data, GC-MS confirmation, and analysis of DMDS adducts of the monounsaturated components (Table 1) unless specified otherwise.
[b] Mean of two replicates expressed as a percentage of the total fatty acids.
[c] Tr, Trace amounts of fatty acids below selected cutoff of 0.05%.
[d] Total amount of FAME in picomoles per milligram (dry weight); mean of two replicates.

for *Nostoc* strain ANT (3.64). The total amounts of compounds 18:2ω6 plus 18:3ω3 were much less in Nostoc strain ANT than in the other two field *Nostoc* samples.

The PLFA profile of the laboratory-grown culture of *N. commune* UTEX 584 differed significantly from those of the field materials. Obvious differences, in comparison to the field samples, were the high ratio of compounds 18:2ω6 to 18:3ω3 (5.36), high concentrations of compounds cy19:0, 18:0, 17:0, and a17:1, and low concentrations of compounds 18:3ω3 and 18:1ω7c. The ratio of 18:1ω7c to 18:1ω9c was

TABLE 3. Characteristic wavelength spectra for total pigments of cyanobacteria (isolated by HPLC and TLC)[a]

EO index	Wavelength (nm) maxima				
	Nostoc strain HUN	*Nostoc* strain REICH	*Nostoc* strain ANT	*N. commune* UTEX 584	*Hydrocoleum* strain GOE1
0.24–0.29	385, 297, 414	384, 295	384, 262, 274, 297, 580		
0.47–0.51		475, 505, 446, 295 (67)			
0.53–0.55	478, 504, 446, 290 (66)	476, 505, 447, 295 (69)			504, 474, 444, 274 (88)
0.57–0.61					505, 477, 444, 275 (64)
0.58–0.63				449, 476, 425, 278 (42)	
0.63–0.67	477	469, 292, 370	479, 262		
0.70–0.73	482				
0.73–0.75					452, 476, 424, 276 (60)
0.75–0.76		426, 411, 658, 504 (1.0)		424, 378, 660, 616 (1.6)	556, 484, 661, 451 (1.1)
0.76–0.78				424, 660, 376 (1.3)	
0.77–0.80	427, 662, 614, 410 (1.2)	427, 661, 617, 410 (1.2)		276, 451, 478, 422 (58)	426, 660, 614, 557 (1.3)
0.78–0.80			427, 662, 611, 577 (1.3)	425, 377, 663, 614 (1.4)	429, 663, 613, 485 (1.2)
0.80–0.81		428, 493, 660 (1.0)		426, 394, 660, 612 (1.6)	
0.82–0.84		462, 296		454, 281, 354	428, 661, 484, 614 (1.2)
0.82–0.85				426, 408, 660, 613 (1.7)	457
0.83–0.84	461, 293				
0.94–0.96	405, 501, 662, 533 (3.7)	406, 664, 502, 532 (2.4)	406, 665, 501, 606 (2.3)		406, 664, 502, 533 (2.2)
0.95–0.97		407, 502, 532, 664 (2.4)	405, 533, 665, 505 (2.5)		410, 665, 534, 603 (2.2)
1.00	480, 451, 423	408, 505, 535, 665		450, 476, 422, 276 (13)	452, 478, 424, 276 (29)
1.00–1.01		449, 479, 417 (40)			

[a] Values in parentheses indicate the peak III/II ratio for carotenoids and soret/alpha peak ratio for chlorophylls. Elution order (EO) index is the retention time of the compound under the HPLC conditions employed divided by the retention time for β-carotene. Wavelength maxima are listed (left to right) for the highest to lowest peaks in the spectrum.

TABLE 4. Identity of pigments from cyanobacteria[a]

Order of elution	Compound
1	Tentative identity
2	Apo-beta-carotenal or -one
3	Myxol or 2-OH plectaniaxanthin
4	Plectaniaxanthin or saproxanthin
5	Saproxanthin or plectaniaxanthin
6	Zeaxanthin
7	Canthaxanthin/hydroxyechinenone
8	NI[b]
9	Crocoxanthin
10	Chlorophyll *a*
11	Chlorophyll *a*
12	Beta-cryptoxanthin
13	Chlorophyll *a*
14	Chlorophyll *a*
15	Echinenone
16	Chlorophyll *a*
17	NI[b]
18	Pheophytin *a*
19	Pheophytin *a*
20	Beta-carotene
21	Beta-carotene

[a] Pigments are listed in order with increasing time of retention on HPLC columns.
[b] NI, Not identified.

1.86, significantly lower than the range of values found for the field samples (2.46 to 3.64).

The phospholipid profile of laboratory-grown *Hydrocoleum* strain GOE1 most resembled that of *N. commune* UTEX 584, with a high ratio of 18:2ω6 to 18:3ω3 (3.88) and a low ratio of 18:1ω7c to 18:1ω9c (1.31). Of all the materials analyzed, this strain contained the lowest amounts of compounds 16:1ω7c and the highest amounts of compounds 18:1ω9c and 18:0. The concentrations of methyl-branched and cyclopropane fatty acids in *Hydrocoleum* strain GOE1 were high (7.43 and 8.05%, respectively).

The methods of HPLC and TLC described here made it possible to discriminate between, and identify, some 21 different pigments from the cyanobacterial samples (Tables 3 and 4). Apo-beta-carotenal (or -one), myxol- or 2-OH plectaniaxanthin, and saproxanthin (Table 4) were associated exclusively with the desiccated field materials and were present in high concentrations (Table 5). Pheophytin was also present in these field materials in high concentrations. The apocarotenoids were composed of a chromatophore of five conjugated double bonds, with at least one of the double bonds in a ring, and with either a conjugated aldehyde or a conjugated keto group. *Nostoc* strain HUN, *Nostoc* strain REICH, and *Hydrocoleum* strain GOE1 contained myxoxanthophyll-like pigments as deduced from the wavelength spectra, although the retention time on the HPLC columns did not correspond to that of pure myxoxanthophyll. *Nostoc* strain REICH was the only strain which contained a pigment which is identified either as myxoxanthophyll (lacking sugar) or 2-hydroxyplectaniaxanthin. *Nostoc* strain HUN, *Nostoc* strain REICH, and *Hydrocoleum* strain GOE1 contained a myxoxanthophyll-type carotenoid with two O atoms in the molecule identified as either plectaniaxanthin or saproxanthin. Plectaniaxanthin and saproxanthin were both present in *Hydrocoleum* strain GOE1. Ketocarotenoids were detected as canthaxanthin (*Nostoc* strain HUN, *Nostoc* strain ANT), hydroxyechinenone (*Nostoc* strain REICH), and echinenone (*Nostoc* strain HUN, *Nostoc* strain REICH, *N. commune* UTEX 584, and *Hydrocoleum* strain GOE1). Hydroxycarotenoids were present as zeaxanthin (*N. commune* UTEX 584), crocoxanthin (*Hydrocoleum* strain GOE1), and β-cryptoxanthin (*Nostoc* strain REICH, *N. commune* UTEX 584, *Hydrocoleum* strain GOE1).

DISCUSSION

The fatty acid profiles of cyanobacteria show great diversity (7). Based on a survey of laboratory-grown cultures of unicellular and filamentous strains, four metabolic groups were recognized by the degree of saturation of different major fatty acids (10). However, there does not appear to be a strict correlation between the presence of polyunsaturated fatty acids and the cellular organization in filamentous cyanobacteria (7). Like the higher-plant chloroplast, many cyanobacteria, particularly the filamentous forms, have fatty acid profiles with a high proportion of polyunsaturated C_{18} compounds, especially linolenic acid (18:3). Data suggest that the mechanism for the desaturation of C_{18} acids is different between cyanobacteria and the photosynthetic eucaryotes but that the mechanism for the desaturation of C_{16} acids seems to be similar between them (20).

Changes in the conditions used to grow laboratory cultures can lead to changes in the composition of lipid molecular species present in cells. In *Anabaena variabilis*, the composition of fatty acids is dependent upon temperature (20). The 18:1/16:1 and 18:2/16:1 species of monogalactosyldiacylglycerol are formed at 38°C, whereas the 18:2/16:0, 18:3/16:0, 18:3/16:1, and 18:3/16:2 species of monogalactosyldiacylglycerol are formed at 22°C. By limiting catalyst-mediated hydrogenation of fatty acids to cell-surface membranes, Vigh et al. (24) obtained direct evidence to support the hypothesis that the thermotrophic properties of lipids within cytoplasmic membranes, and not thylakoids, control chilling susceptibility of the unicellular cyanobacterium *Anacystis nidulans*. In contrast to these rather clear effects of temperature, the fatty acid compositions of *Anabaena* strain BCC 6310 and *Anabaenopsis* strain BCC 6720 were similar in cells grown photoautotrophically and in cells grown heterotrophically in the dark (10).

The phospholipid profiles of the desiccated *N. commune* colonies from different geographic locations are very similar, and the profile appears to change little upon prolonged storage of the cells in the desiccated state (7 years). Given the diverse environments from which the field materials were collected and the different times of collection, the similarities in the fatty acid profiles are striking. The profiles of desiccated *N. commune* are significantly different from those of the laboratory-grown cultures of *N. commune* UTEX 584 and desiccation-tolerant *Hydrocoleum* strain GOE1. In comparison with laboratory-grown cultures, the field materials, which have undergone multiple cycles of drying and rewetting, contain lower amounts of 18:0, equivalent amounts of 16:0, and elevated levels of 18:3ω3 with low ratios of 18:2ω6/3ω3. This trend is the opposite of that found when chloroplasts of *Gossypium hirsutum* L. cv. Reba were subjected to water stress (5). In this case, the galactolipid content, particularly digalactosyldiglyceride, decreased with decreasing water potential, and the percentage of linolenic acid (18:3), the major fatty acid of thylakoids, decreased, whereas that of linoleic (18:2) and oleic (18:1) acids increased. An accumulation of fatty acids having less than 16 carbon atoms was also observed (5). Rather different data were obtained in studies with the desiccation-tolerant and -intolerant mosses *Tortula ruralis* (Hedw.) Gaertn, Meyer

TABLE 5. Quantitative pigment composition of desiccated *N. commune* and laboratory-grown cultures of *Hydrocoleum* strain GOE1[a]

EO index[b]	Compound	Peak area/mg (dry wt)			
		Nostoc strain HUN	*Nostoc* strain REICH	*Nostoc* strain ANT	*Hydrocoleum* strain GOE1
0.26–0.29	Keto-		50,870	127,886	
0.49–0.51	Myxo-like		2,162		
0.57–0.58	Myxo-like				846
0.63–0.65	Canthaxanthin	3,931	12,145	3,897	
0.70–0.73	Chlorophyll *a*			503	
0.73–0.75	Chlorophyll *a*			4,645	
0.76–0.78	Chlorophyll *a*		1,956	9,277	2,185
0.77–0.80	Chlorophyll *a*	12,152	16,069	1,300	15,547
0.78–0.80	β-Cryptoxanthin	597			
0.80–0.81	Chlorophyll *a*		2,483		2,272
0.82–0.85	Echinenone	2,794	9,428	3,694	6,187
0.92–0.94	Pheophytin			1,279	
0.94–0.96	Pheophytin	8,743	18,459	22,225	10,228
1.00	β-Carotene	1,833	2,222	1,562	8,646

[a] Data for *N. commune* UTEX 584 are given in J. J. Olie and M. Potts, Appl. Environ. Microbiol., in press.
[b] EO, Elution order.

and Scherb and *Cratoneuron filicinium*, respectively (23). No changes in phospholipid composition occurred in either moss as a consequence of rapid drying, but after slow drying, there was a decline in some unsaturated fatty acids. Original levels were recovered upon rehydration of *T. ruralis* but not upon rewetting of *C. filicinum*. In addition, there was poor correlation between lipid peroxidation of fatty acids owing to desiccation and changes in the phospholipid fraction.

The total lipid composition of cyanobacterial cells may be representative of the thylakoid membrane fraction in view of the high mass of the thylakoids in comparison with the cytoplasmic and outer membranes (7). The similarities in the phospholipid profiles of the three desiccated materials suggest that thylakoid membranes undergo no gross changes upon prolonged desiccation. Furthermore, the differences noted in fatty acid compositions of field and laboratory cultures may reflect differences in composition of their thylakoids. As such, desiccation and water stress in field populations of *Nostoc* species may result in the differentiation of thylakoids with quite different properties from those of axenic cultures. We have shown already that the cytoplasmic membrane of *N. commune* UTEX 584 possesses features which may account, in part, for the resistance of the cells to the stresses of de- and rehydration (13) and that the membranes of desiccated *Nostoc* cells remain intact during prolonged periods of desiccation.

Studies have demonstrated relatively large amounts of carotenoids in the cytoplasmic and outer membranes of unicellular and filamentous cyanobacteria, and recently, a carotenoid-binding protein has been described (3, 9, 12, 14, 15). Apart from an intrinsic role in photosynthesis, carotenoids are thought to protect cyanobacteria and other bacteria (2) against photoxidative radiation (11). Radiation damage is likely to be of some consequence in field populations of nitrogen-fixing cyanobacteria, such as the desiccated colonies of *Nostoc* species. The similarity in the carotenoid contents of the field materials may therefore be of ecological significance. There are clear differences in the carotenoid compositions of laboratory-grown cultures and the desiccated field *Nostoc* samples. While beta-carotene is present in all samples, there is an evident shift from beta-carotene and echinenone, which predominate in the laboratory cultures, to canthaxanthin and the beta-gamma-carotene series in the desiccated cells. A major feature of the carotenoid content of desiccated *Nostoc* cells is the presence of high levels of apocarotenoids. The identity of these apocarotenoids proved difficult to establish. The P384 pigment which appeared through TLC analysis to be a chlorophyll derivative yielded, upon reduction with $NaBH_4$, a mixture of a chlorophyll derivative plus a compound with one absorption maximum at 451 nm. The absorption properties of this pigment(s) and its high concentration in desiccated cells suggest the potential for a role in protection against UV radiation.

The role of carotenoids in cyanobacterial membranes remains poorly understood. It is of interest that zeaxanthin in the cytoplasmic membrane of *Anacystis nidulans* undergoes a chilling-induced absorption increase around 390 nm, a sign of the phase change in the membrane, although it is not known whether this represents an alteration in the conformation or an aggregation of the pigment when the membrane lipids enter a phase-separated state (24). The absorption properties of carotenoids may be of some consequence in *Nostoc* membranes which have become "dehydrated."

Field populations of *N. commune* are pigmented, and the filaments are embedded in a thick dense mucilage. When dry the colonies appear black and are friable and brittle. When wet the colonies have the consistency of soft cartilage. These are quite different properties from those shown by dry immobilized cells of *N. commune* UTEX 584 and cells of this strain when grown in liquid culture. Such differences between the field populations and laboratory strains, as well as the differences in biochemical composition discussed above, suggest that a greater emphasis should be placed upon field materials with respect to the molecular analysis of desiccation tolerance.

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