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# EFFECT OF IRRADIANCE ON FATTY ACID, CAROTENOID, TOTAL PROTEIN COMPOSITION AND GROWTH OF *MICROCYSTIS AERUGINOSA*

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**Key Word Index**—*Microcystis aeruginosa*; cyanobacterium; irradiance; carotenoids; fatty acids; protein; growth.

**Abstract**—The cyanobacterium, *Microcystis aeruginosa*, was grown under low, standard and high irradiance intensities (20, 40 and 70 $\mu$mol m$^{-1}$ s$^{-1}$) to determine whether irradiance affected pigment, lipid and protein composition, growth yields and the total dry weights of the cell cultures. The components detected in the saponified lipid extracts included $C_{14}$ to $C_{20}$ fatty acids with various levels of saturation, odd chain-length fatty acids and long-chain alcohols. 16:0 was the main fatty acid detected in all samples and its percentage abundance was significantly higher in cells from the lower and standard irradiance intensities compared with the high irradiance cells. Conversely, the proportions of the 14:0, 15:0, 17:0, 18:0 and 20:0 fatty acids were higher in the high irradiance cells compared with the low and standard irradiance cells. Polyunsaturated fatty acid concentrations were reduced and those of monounsaturated fatty acids were increased in the high irradiance cells compared with the low and standard irradiance cells. Chlorophyll *a*, zeaxanthin and $\beta$-carotene were the most abundant pigments detected. Cells exposed to the standard irradiance treatment had substantially higher amounts of carotenoid, chlorophyll *a* and total protein after 15 days of growth compared with cells exposed to either the low or high irradiance intensities. The ratios of the zeaxanthin, echinenone and $\beta$-carotene with respect to chlorophyll *a* from the high irradiance cells were approximately double those observed in the low and standard irradiance regimes. It was concluded that the changes in the fatty acid composition occurred as a cellular response to reduce the susceptibility of the cyanobacterial membranes to photo-oxidation. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

Freshwater cyanobacteria have a similar membrane composition to that of the chloroplasts of higher plants [1], where *ca* 50% of the components consist of proteins primarily associated with photosynthesis, 26% photosynthetic pigments and the remaining 24% structural lipids, carotenoids and other integrated lipophilic components [2]. Previous research into the nature of cyanobacterial membranes has determined that the fatty acid composition can vary with both species and environmental conditions [3, 4]. For example, some species, such as *Anacystis nidulans*, have been reported to lack polyunsaturated fatty acids (PUFA) [5], whereas others, such as *Anabaena*, *Nostoc*, *Oscillatoria*, *Spirulina* and *Microcystis* were reported to have a high content of PUFA [6-8]. Consequently, the diversity of fatty acids observed in different species of cyanobacteria has been utilized for taxonomic purposes [9, 10, 6].

Although most cyanobacterial growth occurs during the warmer summer months, it is well documented that these organisms (particularly laboratory strains) prefer low irradiance, such as 30 $\mu$mol m$^{-2}$ s$^{-1}$, for growth [11, 12] and are extremely susceptible to photoinhibition [13, 14]. Photoinhibition can be defined as the reduction in photosynthetic capacity after exposure to irradiance in excess of that required to saturate photosynthesis [15, 16]. This process is dependent on incident light intensity, exposure time and wavelength [14]. The membranes of photosynthetic organisms are known to alter in their composition to improve species survival under conditions of low temperature [17-19]. Cyanobacterial adaption to a changing light environment can occur by altering the

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In memory of Professor D. J. D. Nicholas for his contribution and service to science.

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Table 1. Concentration of total protein ($\mu g\ mg^{-1}$ dry weight ± standard error), total dry weight (mg ± standard error) and cell yields ($cell\ ml^{-1}$ ± standard error) from *Microcystis aeruginosa* from the low (L), standard (S) and high (H) irradiance conditions. Significant differences are represented by $^{*}P < 0.05$ and $^{***}P < 0.001$ (one-way ANOVA). Duncan's multiple range rankings are shown

| | Low | Standard | High | Duncan's ranking |
|---|---|---|---|---|
| Protein concentration* | $140.3 \pm 18.6$ | $204.3 \pm 18.6$ | $150.0 \pm 17.6$ | $S > L = H$ |
| Total dry weight*** | $51.2 \pm 4.6$ | $76.2 \pm 5.4$ | $74.1 \pm 4.1$ | $S = H > L$ |
| Cell yields ($\times 10^5$) | $4.4 \pm 0.4$ | $5.3 \pm 0.4$ | $5.8 \pm 0.4$ | $L = S = H$ |

surface area and the proportion of light-harvesting complexes within the photosynthetic membrane [20]. Alterations in the carotenoid composition of higher plants and photosynthetic bacteria may also occur in response to increasing irradiance [21–23] as a protection mechanism against photo-oxidation [24, 25]. The protein components of photosystems I and II in cyanobacteria are also extremely susceptible to oxidation during high irradiance [26, 16, 27].

The cellular responses of *Microcystis aeruginosa* to growth under a range of low irradiance conditions have not been previously investigated. Therefore, the aims of this study were to determine whether growth yields, fatty acid, chlorophyll and carotenoid composition, as well as the total protein, of *M. aeruginosa* measured under standard irradiance (40 $\mu mol\ m^{-2}\ s^{-1}$) were altered when cells were grown under either low (20 $\mu mol\ m^{-2}\ s^{-1}$) or high (70 $\mu mol\ m^{-2}\ s^{-1}$) conditions.

## RESULTS

### *Cell density, total dry weight and total protein concentration*

Cell yields, total protein concentrations and total dry weights were determined at day 15 from cultures of *M. aeruginosa* grown under the three lights regime (Table 1). The protein content in the standard light regime was significantly higher than those measured in the low and high light regime cultures, whereas the total dry weight grown under the low light regime was significantly lower than those from the standard and high light regimes. Cell yields, however, were not found to be significantly different at harvest on day 15.

### *Fatty acids and other lipophilic components*

The fatty acid components identified in the saponified lipid extracts from *M. aeruginosa* are summarized in Table 2 in relation to the growth of the cyanobacterium under the different irradiances. The numbers assigned to each compound correspond to the peak numbers on the chromatogram of a typical lipid profile for *M. aeruginosa* grown under high irradiance (Fig. 1). Most of the lipophilic components detected were fatty acids with chain lengths varying from $C_{14}$ to $C_{20}$; 16:0 was the most abundant fatty acid component comprising 61–75% of lipids in cells from all of the light regime treatments. $C_{18}$ fatty acids were the most diverse group observed in *M. aeruginosa*, consisting of 11 fatty acids with degrees of unsaturation ranging from zero to four.

Individual fatty acids were assigned to one of three classes based on their degree of saturation, namely, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The concentration of SFA, PUFA and total fatty acids appeared to decrease with increasing irradiance but these changes were not statistically significant (Table 2). However, by expressing the fatty acid abundance as percentage composition of the total fatty acid concentration (Table 3), the proportions of PUFA in the low and standard irradiance regimes were 8.5% and 8.8%, respectively, compared with 5.8% ($P < 0.05$) under the high irradiance. The proportions of total SFA decreased significantly with increasing irradiance from 81.2% in low light to 71.8% in standard light and 69.3% in high light ($P < 0.01$). In contrast, the proportions of MUFA were lowest in the low light at 10.2% compared with 19.4% in the standard light and 24.9% in the high irradiance regime ($P < 0.001$).

PUFA were affected most by growth under the different irradiance conditions. The concentration of 18:3 (isomeric composition uncertain), was significantly higher under the standard irradiance conditions than the high and low irradiance. The concentration of 18:4Δ3 from cells grown under the high irradiance was significantly lower than those in cells from the standard and low irradiance conditions. In contrast, 20:3Δ6 and 20:2Δ6 levels were significantly elevated in the high irradiance regime compared with low and standard irradiance. The concentration of 20:0 was also significantly greater under the high irradiance regime compared with low and standard irradiance. Cells that were grown under high irradiance had a total $C_{20}$ fatty acid concentration that was *ca* three to six times higher than those from the other light regimes (Table 2). It was also interesting to note that the percentage composition of 16:0 was smaller ($P < 0.01$) in the high irradiance cells compared with the low irradiance cells, whereas 14:0,

Table 2. Major fatty acid components of saponified lipid extracts from *Microcystis aerunginosa* (nmol g$^{-1}$ dry wt ± standard error); peak number corresponds to those shown in Fig. 1. Concentrations of each component are listed for the low (L), standard (S) and high (H) irradiance regimes. Significant differences between concentrations, one-way ANOVA, are represented by $^{*}P < 0.05$, $^{**}P < 0.01$. Significant Duncan's rankings are shown ($P < 0.05$). Trace $\leqslant$ pmol g$^{-1}$ dry wt

| Peak No. | Compound | Fatty acid concentration<br>Low ($n = 9$) | Standard ($n = 9$) | High ($n = 8$) | Duncan's ranking |
|---|---|---|---|---|---|
| 1 | 14:0 | 9.8 ± 1.5 | 21.4 ± 6.6 | 27.1 ± 5.6 | |
| 2 | 15:0 | 1.9 ± 0.6 | 1.8 ± 0.8 | 3.3 ± 0.6 | |
| 3 | 16:1Δ10 | 14.7 ± 2.8 | 17.8 ± 4.2 | 17.3 ± 3.1 | |
| 4 | 16:1Δ9 | 61.7 ± 8.6 | 97.1 ± 33.2 | 71.1 ± 11.8 | |
| 5 | 16:0 | 2174.2 ± 218.9 | 1789.0 ± 408.4 | 1373.6 ± 188.9 | |
| 6 | 17:0 | 1.7 ± 0.5 | 2.3 ± 0.9 | 4.6 ± 1.3 | |
| 7 | 18:3Δ6 | Trace | 0.1 ± 0.04 | 6.6 ± 3.0 | |
| 8 | 18:4Δ3* | 111.3 ± 28.2 | 101.2 ± 20.1 | 32.3 ± 8.2 | L = S > H |
| 9 | 18:2Δ6 | 56.3 ± 17.1 | 62.8 ± 17.1 | 55.2 ± 10.1 | |
| 10 | 18:3Δ9 | 0.33 ± 0.10 | 0.09 ± 0.06 | 0.07 ± 0.02 | |
| 11 | 18:1Δ9 | 80.4 ± 13.9 | 233.5 ± 75.3 | 196.2 ± 25.2 | |
| 12 | 18:1Δ11 | 136.0 ± 23.9 | 173.2 ± 53.0 | 196.2 ± 52.7 | |
| 13 | 18:3Δ3 | 75.8 ± 17.4 | 68.1 ± 17.5 | 28.8 ± 7.0 | L = S > H |
| 14 | 18:4† | Trace | Trace | Trace | |
| | 18:3†** | n.d. | 0.01 | n.d. | S > L = H |
| | 18:1Δ12 | Trace | Trace | trace | |
| 15 | 18:0 | 137.8 ± 30.2 | 117.0 ± 33.6 | 151.6 ± 20.5 | |
| 16 | 20:3Δ6** | 0.3 ± 0.2 | n.d. | 3.3 ± 1.0 | L = S > H |
| 17 | 20:2Δ6** | n.d. | 1.4 ± 0.7 | 3.2 ± 0.7 | L < H |
| 18 | 20:3† | 0.3 ± 0.3 | 0.3 ± 0.2 | 1.7 ± 0.8 | |
| 19 | 20:3Δ3 | n.d. | 3.4 ± 1.8 | 2.9 ± 1.0 | |
| 20 | 20:0* | 1.8 ± 1.4 | n.d. | 7.1 ± 3.2 | |
| | Total $C_{16}$ | 2250.7 ± 226.4 | 1903.9 ± 444.5 | 1461.9 ± 283.5 | |
| | Total $C_{18}$ | 597.8 ± 81.5 | 756.0 ± 207.8 | 750.1 ± 161.3 | |
| | Total $C_{20}$* | 2.4 ± 1.7 | 5.1 ± 2.6 | 18.2 ± 5.7 | L < S < H |
| | SFA | 2327.2 ± 233.9 | 1931.6 ± 449.1 | 1567.3 ± 298.7 | |
| | MUFA | 292.8 ± 46.1 | 521.6 ± 163.3 | 563.7 ± 125.6 | |
| | PUFA | 244.3 ± 50.5 | 236.0 ± 50.5 | 130.9 ± 33.0 | |
| | Total FA | 2864.3 ± 303.8 | 2689.2 ± 658.8 | 2262.0 ± 504.7 | |

† Isomer uncertain.
n.d.: not detected.

15:0, 17:0, 18:0 and 20:0 had larger percentage values ($P < 0.05$–$0.001$) in the high compared with the low irradiance cells (Table 3). The concentrations of specific MUFA did not alter significantly between the three light regimes.

Several components detected by GC-mass spectrometry in the saponified lipid extracts from *M. aeruginosa* were analysed by percentage composition (of non-fatty acid components) because either standards were not available or the identity of the components could not be confirmed from available mass spectral reference data. Glycerol (6, 11 and 9% in the low, standard and high irradiance cells, respectively), butanedioic acid (0.6, 1.9 and 1.1%, respectively), hexanedioic acid (1.2, 7.6 and 2.9%, respectively), 1-octadecanol (0.4, 0.6 and 1.5%, respectively), disaccharide 1 (identity unknown; 2, 1 and 5%, respectively) and disaccharide 2 (identity unknown: 57, 61 and 59%, respectively) were detected in all samples; the percentage composition values were not statistically significant. The abundance of 3,7,11,15-tetramethyl-2-hexadecenol (phytol) was significantly greater ($P < 0.01$) in the low and high light regimes (32% and 21%, respectively) compared with the standard irradiance regime (17%). Only very small amounts of glycerophosphates were detected (with no irradiance treatment differences) and there was no detectable phosphate in the saponified lipid extracts.

### *Carotenoids and chlorophylls*

Chlorophyll *a*, β-carotene and zeaxanthin were the most abundant components of the pigment fraction from *M. aeruginosa* from all irradiance treatments (Table 4). The cultures from the standard irradiance regime showed a two- to ten-fold increase in concentration for zeaxanthin, β-carotene, echinenone, chlorophyll *a* and the chlorophyll *a* epimer, compared with either the high or low irradiance cultures. Comparison between the concentrations of chlorophyll *a* from the high and low irradiance regime were not significantly different (Table 4, S > L = H). However, β-carotene, echinenone and zeaxanthin were detected in significantly lower concentrations in the low

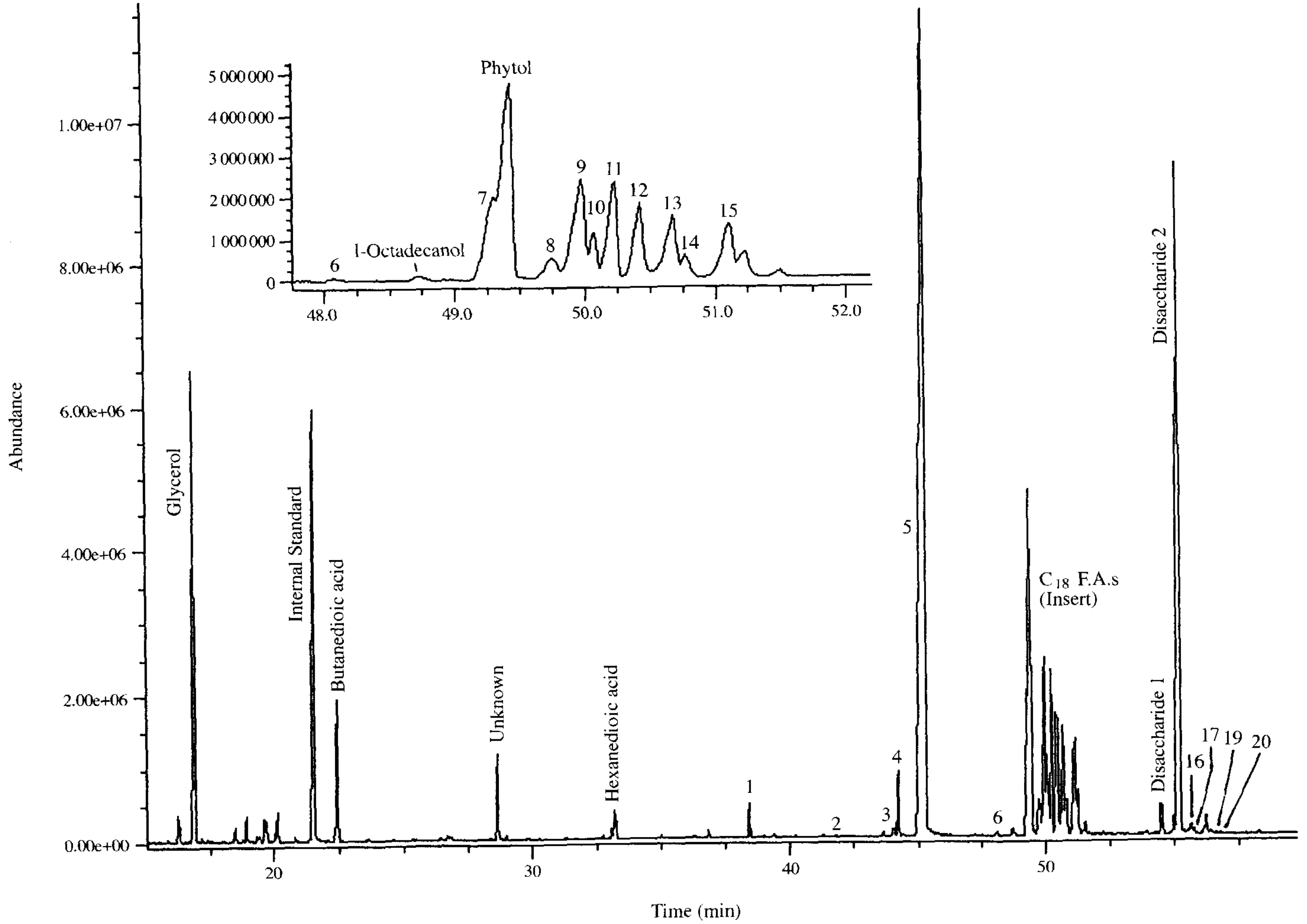

Fig. 1. GC-mass spectrometry ion chromatogram of the saponification products of lipid extracts from *Microcystis aeruginosa*. Numbered peaks correspond to the fatty acid components listed in Table 2; other saponification products are labelled.

Table 3. Proportional changes of individual and fatty acid classes in *Microcystis aeruginosa* from the low (L), standard (S) and high (H) irradiance regimes. Significant differences of arcsin transformed percentage composition data between irradiance regimes (one-way ANOVA) are represented by $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Significant Duncan's rankings are shown ($P < 0.05$)

| | Percentage composition of fatty acids | | | |
|---|---|---|---|---|
| | Low | Standard | High | Duncan's ranking |
| 16:0** | 75.9 | 66.5 | 60.7 | L > S = H |
| SFA-16:0*** | 5.3 | 5.3 | 8.6 | L = S < H |
| SFA** | 81.2 | 71.8 | 69.3 | L > S = H |
| MUFA*** | 10.2 | 19.4 | 24.9 | L < S < H |
| PUFA* | 8.5 | 8.8 | 5.8 | L = S > H |

Table 4. Concentrations of carotenoids and photosynthetic pigments ($\mu g\ g^{-1}$ dry wt ± standard error) from *Microcystis aeruginosa* grown under the low (L), standard (S) and high (H) irradiance conditions. Significant differences between concentrations (one-way ANOVA) are represented by $^*P < 0.05$ and $^{***}P < 0.001$. Significant Duncan's rankings are shown ($P < 0.05$)

| | Carotenoid concentration | | | |
|---|---|---|---|---|
| | Low ($n = 8$) | Standard ($n = 9$) | High ($n = 9$) | Duncan's ranking |
| Chlorophyll *a**** | 99.4±8.4 | 1004.7±73.9 | 184.1±40.9 | S > H = L |
| β-Carotene*** | 73.8±4.3 | 579.7±122.4 | 201.8±21.2 | S > H > L |
| Zeaxanthin*** | 37.6±2.2 | 431.2±29.0 | 181.6±14.2 | S > H > L |
| Echinenone*** | 15.6±0.7 | 143.3±7.9 | 47.7±10.0 | S > H > L |
| Chlorophyll *a* epimer* | 7.6±2.5 | 75.3±22.5 | 41.4±9.7 | S > H = L |
| Chlorophyll *a* allomer | 4.0±2.3 | 0 | 5.3±5.0 | — |
| Chlorophyllide *a* | 0.9±0.6 | 0 | 0 | S = H < L |
| Total pigments*** | 239.4±11.6 | 2234.2±236.6 | 646.7±80.7 | S > H = L |

irradiance, compared with the high irradiance regime (Table 4, S > H > L). The chlorophyll *a* concentration was significantly correlated with zeaxanthin ($r = 0.95$, $P < 0.001$), echinenone ($r = 0.96$, $P < 0.001$), the chlorophyll *a* epimer ($r = 0.57$, $P < 0.001$), β-carotene ($r = 0.87$, $P < 0.001$), total protein concentration ($r = 0.50$, $P < 0.02$) and phytol ($r = -0.53$, $P < 0.02$) where $n = 25$.

## DISCUSSION

The cyanobacterium, *M. aeruginosa*, contained a diverse range of lipophilic components including $C_{14}$–$C_{20}$ fatty acids with a higher proportion of unsaturated fatty acids, together with various antioxidant carotenoids. Most lipophilic components extracted from cyanobacteria are derived from the thylakoid membranes [3, 28, 29]. The presence of abundant glycolipids in the lipophilic fraction of *M. aeruginosa* was confirmed by the detection of the two disaccharide components found in the saponified lipid fractions. The absence of phosphate and glycerophosphates in the saponified lipid extracts was consistent with previous studies [3, 10, 30]. Previous investigations also reported the absence of triacylglycerols, but our detection of glycerol suggests either the presence of some triacylglycerols in this species or a high cellular content of free glycerol.

The range of fatty acids observed in the present study (Table 2) was in general agreement with the fatty acid composition previously reported by Ahlgren *et al.* [6] for *M. aeruginosa*. Piorreck *et al.* [4] also observed a similar distribution of fatty acids, although they did not detect $18{:}4\Delta 3$ or any of the $C_{20}$ fatty acids for *M. aeruginosa*. Both groups of workers observed 16:0, as the major fatty acid component for *M. aeruginosa*. The predominance of 16:0 is probably due to the specific fatty acid pairings present in the glycolipids of *Microcystis* species, where 16:0 is present in six out of seven fatty acid combinations with another MUFA or PUFA [31]. Only one recent study could be found that reported the detection of $C_{20}$ to $C_{22}$ fatty acids in cultured freshwater cyanobacteria, such as *Oscillatoria*, *Microcystis*, *Anabaena* and *Spirulina* species [6]. These fatty acids were previously thought to be limited to marine cyanobacteria and phytoplankton [1]. The concentrations of $C_{20}$ fatty acids in the higher irradiance cells of *M. aeruginosa* was substantially higher compared with the corresponding low and standard irradiance cells, suggesting that these fatty acids comprise an important cellular response to changing intensity of irradiance.

Since the cellular yields of *M. aeruginosa* were not found to be significantly different between irradiance regimes, the alterations observed in membrane components and dry weights were concluded to result from a cellular response to irradiance and not from differences in culture maturity at the time of harvest. The increases in dry weight in *M. aeruginosa* observed in the standard and high irradiance cells compared with the low irradiance cells may reflect an increased deposition of storage carbohydrate under the higher irradiance regime. The 3.0% reduction of total PUFA observed during growth under high irradiance compared with the low and standard irradiance conditions corresponded to a 3.3% combined increase in the 14:0, 15:0, 17:0, 18:0 and 20:0 fatty acids (SFA-16:0). This could be explained as a cellular response reflecting the substitution of SFA for PUFA (other than 16:0) in the membrane lipids in order to minimize lipid peroxidation under high irradiance (Table 3). Similarly, the 15.2% reduction in 16:0 observed during growth under the high compared with low irradiance conditions corresponds to a 14.7% increase in MUFA. This again may reflect a cellular response, where MUFA replace 16:0, presumably to maintain membrane fluidity and integrity when the PUFA, which are more susceptible to photo-oxidation, are reduced in response to high irradiance.

The branched-chain monounsaturated $C_{16}$ isoprenoid alcohol, 3,7,11,15-tetramethyl-2-hexadecenol is phytol, produced by saponification of chlorophyll *a*. Free intracellular phytol may also be present, since the addition of phytol to chlorophyllide *a* to form chlorophyll *a* occurs in the final stages of chlorophyll biosynthesis [2]. The negative correlation between phytol and chlorophyll *a* may reflect the utilization of an intracellular pool of phytol for chlorophyll *a* biosynthesis.

Photosynthetic units are comprised of a high proportion of proteins [32, 33] and a positive correlation was observed in *M. aeruginosa* between clorophyll *a* and the total protein concentrations, reflecting the high concentration of active photosynthetic units in the cells. The positive correlation between chlorophyll *a* concentration and zeaxanthin, echinenone and β-carotene indicates that changes in the photosynthetic capacity within the membrane are matched by changes in the antioxidant potential. The significantly greater concentrations of the carotenoids in the standard irradiance regime implies that these cells had the greatest antioxidant potential, presumably to match a higher photosynthetic capacity characterized by the corresponding higher chlorophyll *a* concentrations. However, it was interesting to note that the ratio of carotenoids to chlorophyll *a* was further increased in the high irradiance cells. That is, the ratio of the concentrations of zeaxanthin, echinenone and β-carotene with respect to chlorophyll *a* from the high irradiance regime (0.97, 0.25 and 1.1, respectively) was approximately double those from the low (0.38, 0.15 and 0.74, respectively) and standard irradiance regimes (0.43, 0.14 and 0.58, respectively). This increase in the ratio of antioxidant:chlorophyll *a* may reflect a cellular requirement for protection against saturation of the light harvesting complex under high irradiance and the subsequent production of free radical species.

## EXPERIMENTAL

*Culturing and irradiance treatment.* Stock cultures of *M. aeruginosa* were grown and maintained in 500 ml conical flasks containing 300 ml of ASM media with additional MBL trace metals [34, 35]. Ten cultures (300 ml) were grown under each experimental light regime. Two cultures were pooled to form each replicate to obtain sufficient material for analysis. Cultures were grown at 25° under 12:1 cyclic artificial illumination using cool white fluorescent lamps and an incident photon flux density on the culture flasks of 35–45 $\mu$mol m$^{-2}$ s$^{-1}$; *M. aeruginosa* is usually grown by many laboratories under reduced environmental irradiance (30–40 $\mu$mol m$^{-2}$ s$^{-1}$) [14, 36, 37]. An irradiance of 40 $\mu$mol m$^{-2}$ s$^{-1}$ was designated as the standard irradiance. Cells were grown under three different irradiance regimes representing low (20 $\mu$mol m$^{-2}$ s$^{-1}$) and high (70 $\mu$mol $^{-2}$ s$^{-1}$) irradiance relative to the designated standard.

*Harvesting.* Cultures were harvested after 15 days growth from each light regime during the exponential growth phase. For growth yield a 1 ml sample from each culture determination was heated for 10 min at 80° according to the method of ref. [38] and counted using a Neubauer haemocytometer after addition of Lugol's $I_2$ (0.05 ml). Care was taken to shield the cultures from exposure to direct light during subsequent processing. Cells were harvested by centrifugation (10 min, 3800 *g*), the supernatant discarded and the pellet transferred to a round-bottom flask. Samples were then snap-frozen for 5 min in liquid $N_2$, freeze-dried for 24 hr and total dry wt recorded.

*Extraction of fatty acids and other lipophilic components.* Freeze-dried cyanobacterial samples (30 mg) were extracted with 10 ml of $Et_2O$–hexane–MeOH (9:9:2) in a glass beaker. Each sample was sonicated for 3 min, filtered through a Whatman GF/C glass filter disc and the filtrate transferred to a separating funnel. The filtered residue was re-extracted twice more in 10 ml $Et_2O$:hexane (1:1). The filtered residue, along with the glass filters, were stored in McCartney bottles for total protein analysis. Dist. $H_2O$ (1 ml) was then added to the comb. $Et_2O$–hexane–MeOH filtrates and mixed thoroughly to remove any residual neutral sugar components from the lipid fr. The sepd lipid phase was transferred to a pear-shaped flask and taken just to dryness under red. pres. Fresh $Et_2O$–hexane (1 ml) was then added and the sample transferred to a derivatization tube were it was again taken just to dryness under $N_2$.

*Saponification and derivatization.* The dried cyanobacterial lipid extract was saponified by adding 100 $\mu$l fresh 10% KOH in 95% EtOH and heated for 2 hr at 100° [39]. The sample was then acidified by adding HCl (60 $\mu$l; 6 M) [40] and freeze-dried. The lipid material was redissolved in EtOH (200 $\mu$l) and transferred to a clean derivatization tube. Any remaining lipophilic material in the saponification tube was redissolved by washing twice more with EtOH (200 $\mu$l) and the combined EtOH fr. again freeze dried. TMSi derivatives were formed for GC-MS by adding 100 $\mu$l of bis(trimethylsilyl)trifluoroacetamide (BSTFA) in 1% (v/v) trimethylchlorosilane for 30 min at 100°. The sample was allowed to cool (10 min) and the int. standard, dimethyl benzoic acid (100 $\mu$l to give a final concn of 8 nmol $\mu l^{-1}$ in $Me_2CO$), was added to the sample; the mixt. was then heated for 30 min at 100°. Prior to analysis, the cooled sample was shaken just to dryness under $N_2$ and made up to a final vol. of 50 $\mu$l using fresh BSTFA. The sample was mixed, centrifuged (10 min, 500 $g$) and immediately injected (0.5 $\mu$l).

*GC-MS.* Derivatized sample components were analysed using a 25 m, 0.20 mm internal diameter HP1 fused silica capillary column (film, 0.33 $\mu$m) and a split-splitless injector. The GC was run with the following settings; injection temp. 300°, temp. program 80–300° at 3° $min^{-1}$, with a 2 min hold at 80° and a 10 min hold at 300°. The MS scan 45–650 mu $sec^{-1}$. Fatty acids and other lipophilic components were identified by comparison of their mass spectrum with a user-generated reference library combined with the retention index of standards. Fatty acid identification was confirmed using equivalent chain-length computations according to the method of ref. [41]. Co-eluting $C_{18}$ fatty acids were quantified by using ion-monitoring techniques [39] by monitoring the $[M-15]^+$ ions where 18:4, 18:3, 18:2 and 18:1 gave $m/z$ 333, 335, 337 and 339, respectively.

*Total protein analyses.* Dried, solvent-insoluble, cyanobacterial residues concd on Whatman GF/C glass filter discs during fatty acid extractions were dissolved in 8 ml of NaOH in McCartney bottles (20 min at room temp. followed by heating at 70° for 10 min). The soln was then centrifuged (10 min, 1000 $g$) to remove insol. cellular material and filter disc debris. Filter discs were not found to interfere with calculations of total protein conc. Protein concns were determined according to the method of ref. [42].

*Extraction of carotenoids.* Because carotenoids are extremely susceptible to UV light and oxidation, extractions were processed under minimal light conditions and all solvents and samples were maintained on ice where possible. Samples of freeze-dried cyanobacteria (20 mg) were extracted using a modified version of the fatty acid extraction protocol, where 2 ml of $Me_2CO$–MeOH (1:1) was used as the extraction solvent for all sonications. The extract was collected in a test tube, reduced in vol. under a stream of $N_2$ to *ca* 0.5 ml and then transferred to a 2 ml vial where the sample was taken just to dryness again using $N_2$ immediately prior to HPLC, the int. standard (retinol acetate, 10 $\mu g\ ml^{-1}$) was added and the sample diluted to 1 ml in $Me_2CO$–MeOH (1:1).

*HPLC.* Carotenoid and chlorophyll components were separated in a Spherisorb OSD-25 Micron $C_{18}$ 250 mm × 4.6 mm i.d. RP column. Carotenoids and chlorophylls were detected at 436 nm and retinol acetate (int. standard) at 375 nm; flow rate 1 ml $min^{-1}$. The solvent gradient system used was that described in ref. [43]. Carotenoids and chlorophylls were identified by comparison with $R_t$ values of available standards; their identity was confirmed by visible absorption spectra [43].

*Statistics.* Concentrations of fatty acids and carotenoids were obtained using relative molar response factors according to the method of ref. [44]. ANOVA and Duncan's multiple range test [45] were performed as indicated in the text.

*Glassware, standards and chemicals.* All glassware used during culturing and analyses were acid-washed (chromic or nitric acids) and oven-baked (180°) for at least 24 hr. HPLC grade solvents and double distilled $H_2O$ were used for all analyses. Because of the limitation of available standards for carotenoid analyses, $\beta$-carotene, lutein and chlorophyll $a$ were used to estimate the concentrations of individual carotene, xanthophyll and chlorophyll components.

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