FEBS Letters 588 (2014) 3770–3777

journal homepage: www.FEBSLetters.org

Chlorophyll *f* and chlorophyll *d* are produced in the cyanobacterium *Chlorogloeopsis fritschii* when cultured under natural light and near-infrared radiation

R.L. Airs [a,*], B. Temperton [a], C. Sambles [b], G. Farnham [a,1], S.C. Skill [a,2], C.A. Llewellyn [a]

[a] Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, Devon PL1 3DH, UK
[b] School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

ARTICLE INFO

Article history:
Received 21 July 2014
Revised 20 August 2014
Accepted 20 August 2014
Available online 28 August 2014

Edited by Richard Cogdell

Keywords:
Photosynthesis
Chlorophyll *f*
Chlorophyll *d*
Near-infra red
Cyanobacteria
Chlorogloeopsis fritschii

ABSTRACT

We report production of chlorophyll *f* and chlorophyll *d* in the cyanobacterium *Chlorogloeopsis fritschii* cultured under near-infrared and natural light conditions. *C. fritschii* produced chlorophyll *f* and chlorophyll *d* when cultured under natural light to a high culture density in a 20 L bubble column photobioreactor. In the laboratory, the ratio of chlorophyll *f* to chlorophyll *a* changed from 1:15 under near-infrared, to an undetectable level of chlorophyll *f* under artificial white light. The results provide support that chlorophylls *f* and *d* are both red-light inducible chlorophylls in *C. fritschii*.

1. Introduction

Chlorophyll *f*, the longest wavelength absorbing chlorophyll of oxygenic photosynthesis, was first discovered in samples taken from a stromatolite and enriched under near-infra red (near-IR) light [1]. A cyanobacterium was subsequently isolated and found to produce chlorophyll *f* when cultured under near-IR, but not white light [2,3]. A chlorophyll *f-producing* cyanobacterium has also been found in a Japanese Lake [4]. In organic solvent, the absorption spectrum of chlorophyll *f* shows a relatively large Q_Y transition at 706 nm, and a Soret band at 406 nm [1,5]. Stromatolite environments like that of the chlorophyll *f* producing filamentous cyanobacteria, *Halomicronema hongdechloris*, can be comparatively enriched in near-IR compared to UV/vis wavelengths [6], analogous to habitats of the chlorophyll *d*-producing cyanobacterium *Acaryochloris marina* [7–9]. Chorophyll *d* was first discovered in 1943 [10] but was thought to be an artefact of extraction for many years [11]. Chlorophyll *d* has been mistakenly associated with red algae in the past [10,12], whereas it is now known to be produced by epiphytic *Acaryochloris* sp. growing on red algae [13]. Chlorophyll *d* is the major chorophyll in *A. marina* [14,15]. Like chlorophyll *f*, chlorophyll *d* also has a red-shifted Q_Y transition (696 nm) compared to chlorophyll *a* [5,16], enabling light harvesting in the near-IR region [17,18].

Chlorogloeopsis fritschii is a subsection V cyanobacterium [19] which has a diverse morphology and diversity of function [20–22]. With a tolerance to a variety of growth conditions [22], and being amenable to large-scale culture, *C. fritschii* has potential for biotechnological applications [23,24]. We report production of chlorophyll *f* and chlorophyll *d* in the cyanobacterium *C. fritschii* cultured under near-IR and natural light conditions. We discuss these findings in context of the organism's natural habitat, morphotype and genome sequence.

Abbreviations: LC/MS^n, liquid chromatography/multistage mass spectrometry; PDA, photodiode array detector; HPLC, high performance liquid chromatography; APCI, atmospheric pressure chemical ionisation; CAO, chlorophyllide *a* oxygenase; HMM, Hidden Markov Model; t_R, retention time; *m/z*, mass to charge ratio

* Corresponding author. Fax: +44 (0) 1752 633101.
E-mail address: ruai@pml.ac.uk (R.L. Airs).
[1] Current address: Peninsula Medical School, Plymouth University, Drake Circus, Plymouth, Devon PL4 8AA, UK.
[2] Current address: Centre for Sustainable Aquatic Research, Swansea University, Singleton Park, Swansea SA2 8PP, Wales, UK.

http://dx.doi.org/10.1016/j.febslet.2014.08.026
0014-5793/

2. Materials and methods

2.1. Culture conditions

2.1.1. Large scale

C. fritschii was inoculated into BG11 media and grown on a large scale (50 L) in purpose built Perspex bubble columns, illuminated either by natural light (average light intensity 100 μmol m^{-2} s^{-1} (PAR), or by artificial white light (90 μmol m^{-2} s^{-1}; Grolux tubes). The bubble columns were inoculated with 500 mL culture of approximately 0.9 g L^{-1} density. The columns illuminated by natural light were set up on the roof of Plymouth Marine Laboratory, and the temperature of these bubble columns was maintained in the range 25–35 °C by use of a heat exchanger. The columns illuminated by artificial white light (Grolux tubes) were maintained at 35 °C in a temperature controlled room.

2.1.2. Small scale

Small scale cultures (100 mL) were grown in Pyrex culture dishes. *C. fritschii* (PCC6912) was inoculated into BG11 media at a concentration of 1 g L^{-1}, and grown under artificial white light at 60 μmol m^{-2} s^{-1} (Grolux fluorescent tubes) or near-IR light (720 nm) at 10 μmol m^{-2} s^{-1} provided by an LED light source (20 nm half width; 20° viewing angle; Epitex, Japan) in a light box that excluded external sources of light. The cultures were grown at 28 °C in continuous light for 7 days, after which time the cells were harvested by centrifugation and stored at −80 °C until analysis.

2.2. Pigment extraction

A known mass of frozen cell paste was transferred to an extraction tube containing 1 mL HPLC grade acetone and 1 mm glass beads. The sample was then lysed in a tissue lyser (Qiagen) at 30 Hz for 15 min. The sample was centrifuged (5 min at 20,000*g*, Microcentrifuge 5415, Eppendorf), and the supernatant removed, and transferred immediately to −20 °C. For exhaustive extraction, the pellet, which still appeared green, was subjected to 5 freeze-thaw cycles using dry ice. 1 mL of acetone was then added to the pellet and the tissue lyser program was repeated. The extraction process was repeated three times, after which the pellet appeared blue. Immediately after extraction, the samples were analysed by liquid chromatography/multistage mass spectrometry LC/MS^n.

2.3. LC/MS^n

The pigment extract was analysed using a high performance liquid chromatography (HPLC) method described previously (Method C in [25]). Pigment extracts (90 μL) were mixed with water (10 μL) in the autosampler and injected (100 μL) onto the HPLC column (2 Waters Spherisorb ODS2 cartridges coupled together, each 150 × 4.6 mm, particle size 3 μm, protected with a precolumn containing the same phase). Elution was carried out using a mobile phase comprising methanol, acetonitrile., ammonium acetate (0.01 M) and ethyl acetate (Method C in [25]) at a flow rate of 0.7 mL min^{-1}. HPLC was performed using an Agilent 1200 system comprising a degasser (G1379B), binary pump (G1312A), thermostated autosampler (G1367B and G1330B), thermostated column compartment (G1316A) and photodiode array (PDA) detector (G1315A). The PDA detector was set to monitor wavelengths at 406, 696 and 706 nm, in addition to the wavelengths routinely used in our laboratory for carotenoid and chlorophyll detection (440 and 660 nm). The HPLC was coupled to an Agilent 6330 ion trap mass spectrometer via an atmospheric pressure chemical ionisation (APCI) source. Ionisation conditions were as follows: ion polarity positive; drying temperature 350°C, vapouriser temperature 450°C; nebulizer pressure 60 psi; drying gas flow rate 5 L min^{-1}, high voltage capillary −4500 V. Mass spectra were collected over the range 400–1100 *m/z*. Post column addition of formic acid was used to improve mass spectrometric detection of the chlorophylls [26]. Quantification of long wavelength chlorophylls was calculated based on their HPLC peak areas, HPLC flow rate, path length of the flow cell and published extinction coefficients [27]. The extinction coefficients in 100% methanol were used [27], as this most closely matched the HPLC mobile phase at the elution point of chlorophylls *f* and *d* [25].

2.4. Phylogeny of genes putatively involved in the synthesis of chlorophyll *f* in *C. fritschii*

Bioinformatic analyses of the phylogeny of genes involved in the putative biosynthesis of chlorophyll *f* in *C. fritschii* are fully described in the Supplementary information (all analyses are available on GitHub at https://github.com/btemperton/airs). Briefly, chlorophyllide *a* oxygenase (CAOs) and CAO-like genes in *Prochlorococcus* and closely related taxa were aligned with MUSCLE (v. 3.8.31) and used to create a Hidden Markov Model (HMM). The CAO HMM was used to query the predicted proteins of *C. fritschii* to identify putative CAOs and CAO-like proteins, with a cutoff *e*-value of 1×10^{-5}, identifying 8 putative proteins. One of these proteins (WP_016873721) was excluded as it was much shorter than other candidates (115 amino acids vs. 4–500 amino acids). Sequences used to construct the HMM and putative targets in *C. fritschii* were used in an iterative process of identifying closely-related sequences with best-BLASTP hit analysis against the NCBI nr database, alignment, automatic gap removal with GBlocks (V. 0.91) and tree generation using FastTree (v. 2.1.7) [28]. Trees were left unrooted due to the lack of a suitable outgroup of known function in chlorophyll *f* or chlorophyll *d* biosynthesis.

3. Results

A pigment extract from *C. fritschii* cells grown to high cell density in a bubble column photobioreactor (50 L) under natural light was analysed using an HPLC method described previously (Method C in [25]). The resulting chromatogram (Fig. 1) showed four peaks with absorption at 706 nm. The peak eluting at t_R 42 min (peak 4; Fig. 1), gave retention time, UV/vis spectrum (Fig. 2A) and mass spectra characteristic of chlorophyll *a*. Although chlorophyll *a* has negligible absorbance at 706 nm, its high relative concentration in the extract (see absorbance scale in Fig. 2A) gave rise to absorbance at 706 nm on a scale comparable with peaks 1–3 (ie. <6 mAU). The predominant component absorbing at 706 nm and eluting at t_R 35 min (peak 2; Fig. 1), gave a UV/vis spectrum (Fig. 2B) characteristic of chlorophyll *f* [1,5,29]. Under LC/MS^n conditions using post-column addition of formic acid to aid ionisation of chlorophylls [26], peak 2 gave rise to ions in full MS at *m/z* 907 and *m/z* 885, corresponding to $[M+H]^+$ and $[M+H-Mg]^+$ of chlorophyll *f* [1,5,30]. Isolation and fragmentation of the ion at *m/z* 907 in the ion trap resulted in MS^2 spectra typical of a formyl substituted chlorophyll macrocycle [31], consistent with chlorophyll *f* [5], namely an ion at *m/z* 879 corresponding to loss of 28 daltons from $[M+H]^+$, and ions at *m/z* 629 and 569, corresponding to $[M+H-phytyl]^+$ and $[M+H-phytyl-CO_2Me]^+$, respectively (Fig. 3A). Isolation and fragmentation of the ion at *m/z* 885 in the ion trap resulted in MS^2 spectra dominated by ions at *m/z* 607 and 547 (Fig. 3B) corresponding to $[M+H-Mg-phytyl]^+$ and $[M+H-Mg-phytyl-CO_2Me]^+$, respectively [5]. Notably, the full mass spectra were dominated by ions of mass to charge ratio (*m/z*) corresponding to

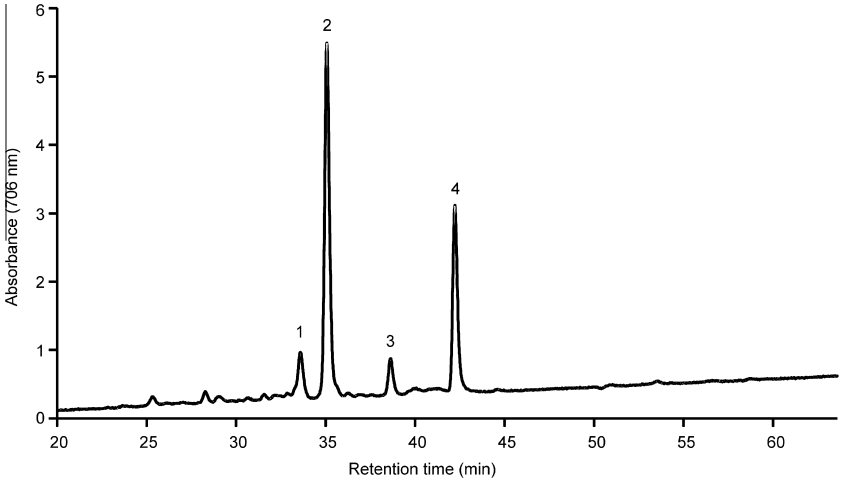

Fig. 1. Partial HPLC–PDA chromatogram (706 nm) of *Chlorogloeopsis fritschii* extract. Peaks are assigned as follows: (1) chlorophyll *d*; (2) chlorophyll *f*; (3) unassigned chlorin; (4) chlorophyll *a* (see text).

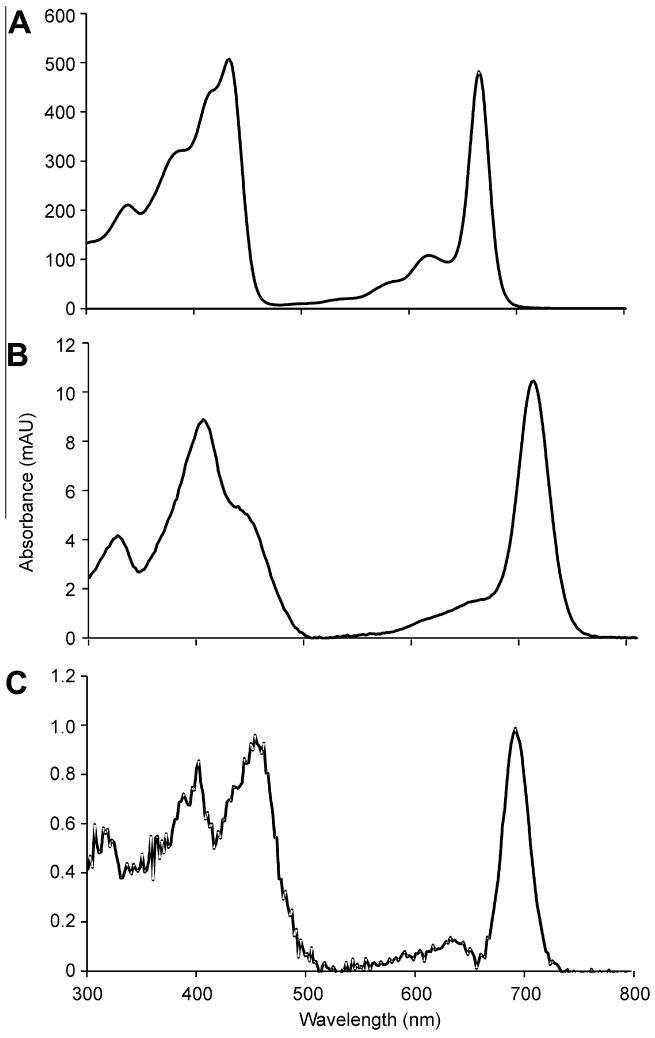

Fig. 2. On-line UV/vis spectra obtained during HPLC–PDA analysis of *C. fritschii* extract of (A) peak 4, (B) peak 2 and (C) peak 1.

the demetallated chlorins, due to the formic acid added to the LC flow in between the LC and MS detectors [26].

The peak eluting before chlorophyll *f* at t_R 33 min (peak 1; Fig. 1) gave rise to a UV/vis spectrum characteristic of chlorophyll *d* (Fig. 2C) [5,16]. Under LC/MS^n conditions, peak 1 gave rise to ions at *m*/*z* 873 and 895, corresponding to $[M+H-Mg]^+$ and $[M+H]^+$ for chlorophyll *d*. Isolation and fragmentation of the ion at *m*/*z* 873 in the ion trap gave rise to ions at *m*/*z* 595 and 535 in the MS^2 spectrum (Fig. 3C), consistent with a chlorophyll *d* structure [5]. The combination of expected relative retention time, on-line UV/vis absorption spectra, protonated molecule and fragmentation characteristics consistent with chlorophylls *f* and *d* confirm their presence in *C. fritschii*. Genome analysis revealed no evidence of contamination of *C. fritschii* with *Acaryochloris* strains (see Supplementary information).

To investigate the light conditions of chlorophyll *f* and chlorophyll *d* production in *C. fritschii*, the cyanobacterium was cultured in a bubble column photobioreactor under artificial, white light. When the culture had reached a density comparable with that of the bubble column under natural light, it was harvested and the pigments extracted and analysed by LC/MS^n. The long wavelength absorbing chlorophylls *f* and *d* were not detected. By comparison to the emission spectrum of sunlight, measured inside the bubble column, the light spectra of the fluorescent tubes used for artificial white light (also measured inside the bubble column) were deficient in the region above 650 nm, and particularly above 700 nm, where the long wavelength chlorophylls absorb (Fig. 4). To further investigate a possible wavelength dependence of chlorophyll *f* and chlorophyll *d* production, and following a report that chlorophyll *f* is inducible in other cyanobacteria under near-infrared radiation [2], *C. fritschii* was cultured on a small scale (100 mL) under either near-infrared or artificial white light. The chromatograms obtained (not shown) show the presence of chlorophylls *f* and *d* in the cells grown under near-IR, but not under artificial white light.

The highest ratio of chlorophyll *f*:chlorophyll *a* measured in our cultures was 0.064 (Table 1), which compares to 0.125 in a filamentous cyanobacterium isolated from stromatolites [2]. The highest ratio of chlorophyll *d*:chlorophyll *a* observed was 0.010. The ratio of chlorophyll *f*:chlorophyll *d* ranged from 5.8 under natural light to 6.5 under near-IR (Table 1). Under near-IR conditions, chlorophyll *f* reached 0.19 μg mg^{-1} dry weight (Table 1).

The effect of the different culture conditions (bubble column or small dish; artificial or natural light) on the carotenoid profiles was also examined. The total carotenoid to chlorophyll *a* ratio was highest for *C. fritschii* cultures in the bubble column under natural light (Table 1). This ratio was driven by a high relative content of xanthophyll carotenoids (myxoxanthophyll, nostoxanthin, caloxanthin and zeaxanthin), which was approximately ten times that

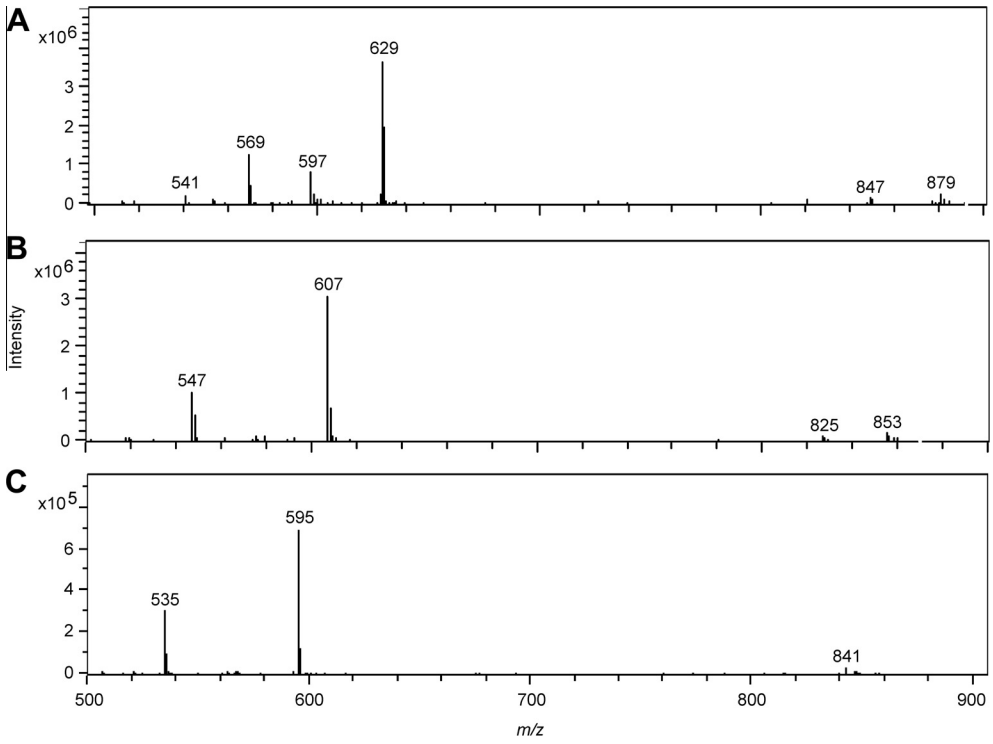

Fig. 3. On-line LC/MS^2 spectra generated from fragmentation of (A) *m/z* 907 (peak 2), (B) *m/z* 885 (peak 2) and (C) *m/z* 873 (peak 1).

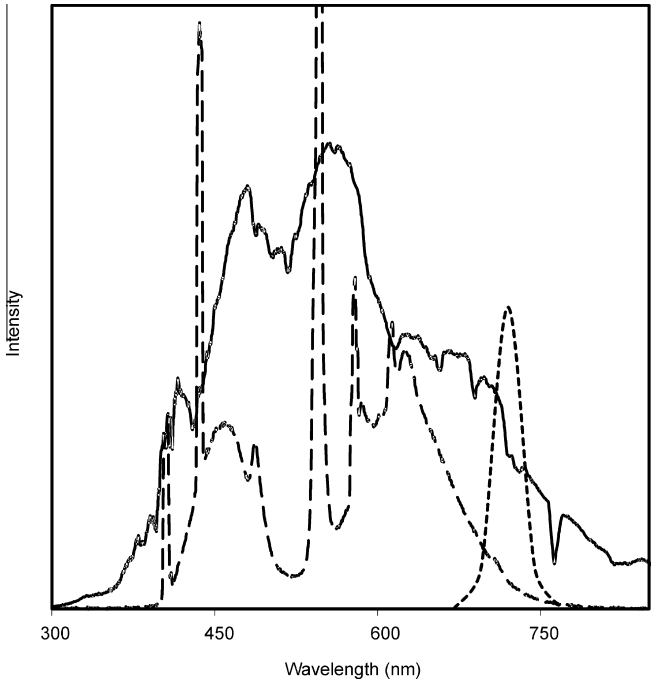

Fig. 4. Spectra of incident light inside the bubble columns under natural light (solid line) and artificial white light (dashed line), and emission spectrum of the near-IR LEDs (dotted line).

of cultures grown under artificial light. The cultures grown under artificial white or near-IR light showed low relative contents of xanthophyll carotenoids and high relative contents of echinenone and β,β-carotene (Table 1). For *C. fritschii* grown in small culture vessels, cells grown under white light showed a higher carotenoid content/dry weight for xanthophyll carotenoids than those grown under near-IR (Table 1), whereas the contents of echinenone and β,β-carotene were similar between the two light regimes.

4. Discussion

The long wavelength absorbing chlorophylls *f* and *d* have both been proposed to aid cyanobacteria habitat environments deplete in UV/vis, but rich in near-IR [1,13,32] and oxygenic photosynthesis has been shown to be energetically viable in long wavelength light environments [33]. *C. fritschii* was first isolated from the soils of paddy fields in India [34]. Such soil environments may well be seasonally deplete in UV/vis, when the foliage of rice plants is dense above them, but rich in near-IR wavelengths not typically utilised by higher plants. *Chlorogloeopsis* is also adapted to UV, as it induces mycosporine-like amino acid biosynthesis under UV radiation [35]. *C. fritshii* may therefore exhibit multiple light harvesting and photoprotective growth strategies based on its changing environment.

C. fritschii has been documented to take several morphological forms depending on growth conditions [22], including filaments, and aseriate (irregular clumps of cells) forms. Under the culture conditions used in this study, aseriate cells dominated. *C. fritschii* clumps may create a microenvironment deplete in UV/vis but rich in near-IR, due to self-shading. The production of long wavelength chlorophylls could therefore be proposed as a mechanism for cells within clumps to harvest the longer wavelength light energy that may be available. *C. fritschii* cells were in aseriate form under both bubble column photobioreactor and small vessel growth conditions, under natural, white and near-IR light regimes, but chlorophyll *f* and chlorophyll *d* were only detected when near-IR radiation was available. Production of the long wavelength chlorophylls in this organism is most likely therefore stimulated by the presence of near-IR, or possibly the ratio of near-IR to other

Table 1
Chlorophyll and carotenoid ratios and contents in *C. fritschii* cultured under different conditions. Tot.car = total carotenoid; nd = not detected; Echin = echinenone; ββ-Car = β,β-carotene.

Culture vessel	Light conditions	Chl *f* and *d* detected?	Chl *f*/ Chl *a*	Chl *d*/ Chl *a*	Chl *f*/ Chl *d*	μg/mg Dry weight			Tot.car/Chl *a*	Xanths[b]/Echin + ββ-Car	μg/mg Dry weight		
						Chl *a*	Chl *f*	Chl *d*			Xanths[b]	Echin	ββ-Car
Bubble column	White light	N	–	–	–	>2.91[a]	nd	nd	0.123	1.23	>0.193[a]	>0.084[a]	>0.073[a]
Bubble column	Natural light	Y	0.016	0.003	5.80	>0.30[a]	>0.005[a]	>0.001[a]	0.318	7.99	>0.082[a]	>0.005[a]	>0.005[a]
Pyrex culture dish	Near-IR only	Y	0.065	0.010	6.47	2.95	0.19	0.03	0.114	0.20	0.056	0.150	0.130
Pyrex culture dish	PAR only	N	–	–	–	5.03	nd	nd	0.069	0.28	0.075	0.145	0.125

[a] Culture extracts not exhaustively extracted.
[b] Xanths = myxoxanthophyll, nostoxanthin, caloxanthin and zeaxanthin.

wavelengths of photosynthetically active radiation, rather than just depletion of UV/vis, or a response to cell clumping. Notably, in a cyanobacterium isolated from a Japanese lake, chlorophyll *f* was induced under near-IR alone, but not under PAR and near-IR combined [4], which contrasts with the production of chlorophylls *f* and *d* in *C. fritschii* under natural light. The relative intensities of PAR and near-IR may therefore be important in chlorophyll *f* production.

The ratio of chlorophyll *f* to chlorophyll *a* increased from 0.016 under natural light to 0.065 (approximately 1:15) under near-IR. A chlorophyll *f*-containing filamentous cyanobacterium recently purified from stromatolites and inhabiting an environment deplete in UV/vis but with available near-IR, gave a chlorophyll *f* to chlorophyll *a* ratio of 1:8 after 2 weeks under near-IR in culture [2]. Notably, the same type of LEDs were used as a near-IR light source in both studies, but the length of time that the cells were exposed to the near-IR differed, which was 7 and 14 days, for our study and the study reported in [2], respectively. In the earlier study [2], the ratio of chlorophyll *a* to chlorophyll *f*, determined by the absorbance ratio of the Q_y bands, remained constant for *H. hongdechloris* cultured under near-IR for between 0 and 28 days, while under white light the ratio increased by 14 days. Notably the highest chlorophyll *f*/chlorophyll *a* index was observed under relatively low intensities (10–20 μE) of far-red light [3]. The chlorophyll *f* to chlorophyll *d* ratio in *C. fritschii* remained fairly constant between natural and near-IR light conditions (Table 1). *A. marina* uses chlorophyll *d* as the major antenna chlorophyll, and the special pair in PSI [36]. The structure of chlorophyll *f*, however, with the formyl group at C-2, shifts electron density to the periphery of the macrocycle, in a similar manner to chlorophyll *b* [18], resulting in a light harvesting pigment that is not likely to have redox activity, so having chlorophyll *a* or chlorophyll *d* in addition to chlorophyll *f* will be essential for photosynthesis to proceed. Chlorophyll *f* is likely therefore to act as an antenna pigment [18]. A previous study [4] also indicated that chlorophyll *f* is likely to act as an antenna constituent based on the detection of chlorophyll *a*′ and pheophytin *a* in their chlorophyll *f*-containing isolate which are common reaction centre components in cyanobacteria.

The genome of *C. fritschii* has been sequenced [37], which provides opportunity to search for genes which encode enzymes that may be responsible for chlorophyll *f* and chlorophyll *d* biosynthesis. Due to the structural similarity between chlorophylls *b* and *f*, which contain a single formyl group at positions C-7 and C-2, respectively, where chlorophyll *a* contains methyl groups, similar enzymes may be responsible for their biosynthesis. Chlorophyll *b* is produced via the action of chlorophyllide *a* oxygenase (CAO), a Rieske iron-containing protein, on chlorophyll *a* or chlorophyllide *a*, followed by esterification and reduction of the long chain alcohol at C-17[3] [38]. CAO oxidises the C-7 methyl of chlorophyllide *a* to a formyl group in vitro, thereby forming chlorophyllide *b* [39]. Seven CAO and CAO-like genes of suitable length were identified in the genome of *C. fritschii*, with many closely related to those of *A. marina* (Fig. 5). All CAO and CAO-like genes identified in this study contained a C-terminus Rieske iron-sulphur domain associated with redox processes. Four out of seven also contained a downstream Pheophorbide a oxygenase domain (PF08417) used in chlorophyll breakdown in *Arabidopsis thaliana* [40]. One gene (IMG accession 2509831456) instead contained a domain of unknown function (PB002321). This configuration was also found in CAO-like genes in *Cylindrospermum* sp. *PCC7417*, *Nostoc azollae* and *Anabaena cylindrica*. CAO and CAO-like genes in *Prochlorococcus* lacked either PB002321 or PF08417 and formed a distinct clade. The lack of a known biosynthetic pathway for chlorophyll *f* or chlorophyll *d* in cyanobacteria makes it difficult to infer which of the *C. fritschii* genes identified in this study as CAO or CAO-like would be the most suitable candidates for the formation of chlorophyll *f*. The

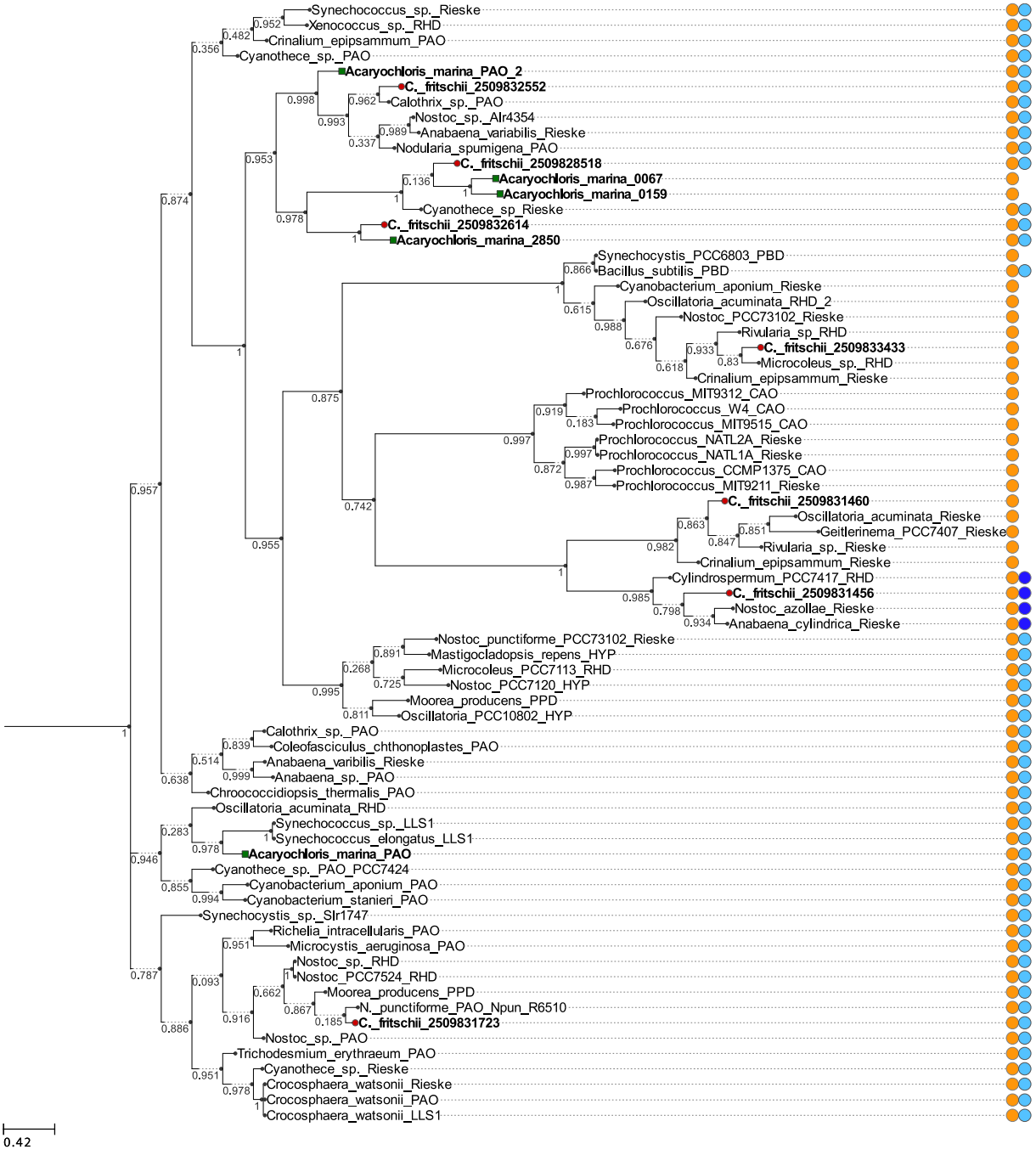

Fig. 5. Approximately maximum-likelihood unrooted phylogeny of putative chlorophyllide *a* oxygenase (CAO) and CAO-like genes identified in *C. fritschii* (bold), which may be involved in the conversion of chlorophyll *a* to chlorophyll *f*. Genes identified as putatively involved in the biosynthesis of chlorophyll *d* in *Acaryochloris marina* (bold) [7] are also included. Numbers represent Shimodaira–Hasegawa values for local branch support. Dashed branches represent shortening of branch lengths to reduce tree width (unaltered trees are available Supplementary Fig. 1). Circles on the right depict identified PFAM protein domains (orange: Rieske; light blue: Pheophorbide a oxygenase; dark blue: domain of unknown function PB002321).

formation of chlorophyll *d* must be catalysed by a different enzyme than CAO because it requires the breakage of a C=C double bond of the C-3 vinyl group [41]. Labelling studies have shown that the enzyme responsible for incorporation of the formyl group into chlorophyll *d* is an oxygenase, that the formyl group oxygen atom comes from molecular oxygen, and that in *Acaryochloris*, chlorophyll *a* is a biosynthetic precursor [41]. Whether the oxygenase acts on chlorophyll *a* or chlorophyllide *a* has yet to be determined. The formation of [7-formyl]-chlorophyll *d* in an *Acaryochloris* mutant transformed to contain a gene encoding CAO [42] lends support to the involvement of chlorophyllide *a* in chlorophyll *d* biosynthesis.

The carotenoid profiles of *C. fritschii* were dominated by xanthophyll carotenoids under natural light, but echinenone and β,β-carotene under artificial light (white or near-IR). This contrasts with previous findings in a second chlorophyll *f* producing cyanobacteria species [2], which showed increased β,β-carotene under white light, but a predominance of xanthophyll carotenoids under red

light. Nonetheless both studies reveal that chlorophyll *f*-containing cyanobacteria change their pigment profiles according to the spectrum of light available. *C. fritschii* has been shown to grow heterotrophically in the dark [43]. Under these conditions, formation of chlorophylls and carotenoids does not seem to be substantially impaired compared to light-grown cultures, but pigments are not incorporated into photosynthetic thylakoid membranes [43]. The content of long wavelength chlorophylls in dark grown cultures has not been established and is a topic for future study.

5. Conclusions

C. fritschii produces both chlorophyll *f* and chlorophyll *d* when cultured under both natural light and near-IR. The data support the hypothesis that long wavelength chlorophylls are produced in response to near-IR radiation.

Acknowledgements

This work received funding from the European Regional Development Funding through the INTERREG IVB EnAlgae project and from Plymouth Marine Laboratory.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.08.026.

References

[1] Chen, M., Schliep, M., Willows, R.D., Cai, Z.-L., Neilan, B.A. and Scheer, H. (2010) A red-shifted chlorophyll. Science 329, 1318–1319.
[2] Chen, M., Li, Y., Birch, D. and Willows, R.D. (2012) A cyanobacterium that contains chlorophyll *f* – a red-absorbing photopigment. FEBS Lett. 586, 3249–3254.
[3] Li, Y., Lin, Y., Loughlin, P.C. and Chen, M. (2014) Optimization and effects of different culture conditions on the growth of *Halomicronema hongdechloris* – a filamentous cyanobacterium containing chlorophyll *f*. Front. Plant Sci. 5, 1–12, http://dx.doi.org/10.3389/fpls.2014.00067.
[4] Akutsu, S., Fujinuma, D., Furukawa, H., Watanabe, T., Ohnishi-Kameyama, M., Ono, H., Ohkubo, S., Miyashita, H. and Kobayashi, M. (2011) Pigment analysis of a chlorophyll *f*-containing cyanobacterium strain KC1 isolated from Lake Biwa. Photomed. Photobiol. 33, 35–40.
[5] Kobayashi, M., Akutsu, S., Fujinuma, D., Furukawa, H., Komatsu, H., Hotota, Y., Kato, Y., Kuroiwa, Y., Watanabe, T., Ohnishi-Kameyama, M., Ono, H., Ohkubo, S. and Miyashita, H. () Physicochemical properties of chlorophylls in oxygenic photosynthesis – succession of co-factors from anoxygenic to oxygenic photosynthesis in: Photosynthesis (Dubinsky, Zvy, Ed.), http://dx.doi.org/10.5772/55460 (isbn: 978-953-51-1161-0, http://www.intechopen.com/books/photosynthesis).
[6] Farias, M.E., Rascovan, N., Toneatti, D.M., Albarracin, V.H., Flores, M.R., Poiré, D.G., Collavino, M.M., Aguillar, M., Vazquez, M.P. and Polerecky, L. (2013) The discovery of stromatolites developing at 3570 m above sea level in a high-altitude volcanic Lake Socompa, Argentinean Andes. PLoS One 8 (1), e53497.
[7] Swingley, W.D., Chen, M., Cheung, P.C., Conrad, A.L., Dejesa, L.C., Hao, J., Honchak, B.M., Karbach, L.E., Kurdoglu, A., Lahiri, S., Mastrian, S.D., Miyashita, H., Page, L., Ramakrishna, P., Satoh, S., Sattley, W.M., Shimada, Y., Taylor, H.L., Tomo, T., Tsuchiya, T., Wang, Z.T., Raymond, J., Mimuro, M., Blankenship, R.E. and Touchman, J.W. (2008) Niche adaptation and genome expansion in the chlorophyll *d*-producing Cyanobacterium *Acaryochloris marina*. Proc. Natl. Acad. Sci. USA 105 (6), 2005–2010.
[8] Duxbury, Z., Schliep, M., Ritchie, R.J., Larkum, A.W.D. and Chen, M. (2009) Chromatic photoacclimation extends utilisable photosynthetically active radiation in the chlorophyll *d*-containing cyanobacterium, *Acaryochloris marina*. Photosynth. Res. 101, 69–75.
[9] Loughlin, P., Lin, Y. and Chen, M. (2013) Chlorophyll *d* and *Acaryochloris marina*: current status. Photosynth. Res. 116, 277–293, http://dx.doi.org/10.1007/s11120-013-9829-y.
[10] Manning, W.M. and Strain, H.H. (1943) Chlorophyll *d*, a green pigment of red algae. J. Biol. Chem. 151, 1–19.
[11] Holt, A.S. and Morley, H.V. (1959) A proposed structure for chlorophyll *d*. Can. J. Chem. 37 (3), 507–514.
[12] Holt, A.S. (1961) Further evidence of the relation between 2-desvinyl-2-formyl-chlorophyll *a* and chlorophyll *d*. Can. J. Bot. 39 (2), 327–331.
[13] Murakami, A., Miyashita, H., Iseki, M., Adachi, K. and Mimuro, M. (2004) Chlorophyll *d* in an epiphytic cyanobacterium of red algae. Science 303, 1633.
[14] Miyashita, H., Ikemoto, H., Kurano, N., Adachi, K., Chihara, M. and Miyachi, S. (1996) Chlorophyll *d* as a major pigment. Nature 383 (6599), 402, http://dx.doi.org/10.1038/383402a0.
[15] Larkum, A.W.D., Chen, M., Li, Y., Schliep, M., Trampe, E. and West, J. (2012) A novel epiphytic chlorophyll *d*-containing cyanobacterium isolated from a mangrove-associated red alga. J. Phycol. 48, 1320–1327, http://dx.doi.org/10.1111/j.1529-8817.2012.01233.x.
[16] Chen, M. and Blankenship, R.E. (2011) Expanding the solar spectrum used by photosynthesis. Trends Plant Sci. 16 (8), 427–431.
[17] Larkum, A.W.D. and Kühl, M. (2005) Chlorophyll *d*: the puzzle resolved. Trends Plant Sci. 10 (8), 355–357.
[18] Schliep, M., Cavigliasso, G., Quinnell, R.G., Stranger, R. and Larkum, A.W.D. (2013) Formyl group modification of chlorophyll *a*: a major evolutionary mechanism in oxygenic photosynthesis. Plant Cell Environ. 36, 521–527.
[19] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1–61.
[20] Mitra, A.K. and Pandey, D.C. (1966) On a new genus of the blue-green alga *Chlorogloeopsis* with remarks on the production of heterocysts in the alga. Phykos 5, 106–114.
[21] Fay, P. (1965) Heterotrophy and nitrogen fixation in *Chlorogloea fritschii*. J. Gen. Microbiol. 39, 11–20.
[22] Evans, H.E., Foulds, I. and Carr, N.G. (1976) Environmental conditions and morphological variation in the blue-green alga *Chlorogloea fritschii*. J. Gen. Micorbiol. 92, 147–155.
[23] Balasundarum, B., Skill, S.C. and Llewellyn, C.A. (2012) A low energy process for the recovery of bioproducts from cyanobacteria using a ball mill. Biochem. Eng. J. 69, 48–56.
[24] Biller, P., Ross, A.B., Skill, S.C., Lea-Langton, A., Balasundaram, B., Hall, C., Riley, R. and Llewellyn, C.A. (2012) Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Res. 1 (1), 70–76.
[25] Airs, R.L., Atkinson, J.E. and Keely, B.J. (2001) Development and application of a high resolution liquid chromatographic method for the analysis of complex pigment distributions. J. Chromatogr. A 917, 169–179.
[26] Airs, R.L. and Keely, B.J. (2000) A novel approach for sensitivity enhancement in atmospheric pressure chemical ionisation liquid chromatography/mass spectrometry of chlorophylls. Rapid Commun. Mass Spectrom. 14, 125–128.
[27] Li, Y., Scales, N., Blankenship, R.E., Willows, R.D. and Chen, M. (2012) Extinction coefficient for red-shifted chlorophylls: chlorophyll d and chlorophyll f. Biochim. Biophys. Acta 1817 (8), 1292–1298.
[28] Price, M.N., Dehal, P.S. and Arkin, A.P. (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One, http://dx.doi.org/10.1371/journal.pone.0009490.
[29] Li, Y., Cai, Z.-L. and Chen, M. (2013) Spectroscopic properties of chlorophyll *f*. J. Phys. Chem. 117, 11309–11317, http://dx.doi.org/10.1021/jp402413d1.
[30] Willows, R.D., Li, Y., Scheer, S. and Chen, M. (2013) Structure of chlorophyll *f*. Org. Lett. 15 (7), 1588–1590, http://dx.doi.org/10.1021/ol400327j.
[31] Airs, R.L. and Keely, B.J. (2002) Atmospheric pressure chemical ionisation liquid chromatography mass spectrometry of bacteriochlorophylls from Chlorobiaceae: characteristic fragmentations. Rapid Commun. Mass Spectrom. 16, 453–461.
[32] Kühl, M., Chen, M., Ralph, P.J., Schreiber, U. and Larkum, A.W.D. (2005) A niche for cyanobacteria containing chlorophyll *d*. Nature 433, 820.
[33] Mielke, S.P., KInag, N.Y., Blankenship, R.E., Gunner, M.R. and Mauzerall, D. (2011) Efficiency of photosynthesis in a Chl *d*-utilizing cyanobacterium is comparable to or higher than that in Chl *a*-utilising species. Biochim. Biophys. Acta 1807, 1231–1236.
[34] Mitra, A.K. (1950) Two new algae from Indian soils. Ann. Bot. 14, 457–464.
[35] Portwich, A. and Garcia-Pichel, F. (1999) Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. Arch. Microbiol. 172 (4), 187–192.
[36] Hu, Q., Miyashita, H., Iwasaki, I., Kurano, N., Miyachi, S., Iwaki, M. and Itoh, S. (1998) A photosystem I reaction center driven by chlorophyll *d* in oxygenic photosynthesis. Proc. Natl. Acad. Sci. USA 95, 13319–13323.
[37] Dagan, T., Roettger, M., Stucken, K., Landan, G., Koch, R., Major, P., Gould, S.B., Goremykin, V.V., Rippka, R., Tandeau de Marsac, N., Gugger, M., Lockhart, P.J., Allen, J.F., Brune, I., Maus, I., Pühler, A. and Martin, W.F. (2013) Genomes of stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. Genome Biol. Evol. 5 (1), 31–44.
[38] Porra, R.J., Oster, U. and Scheer, H. (2011) Recent advances in chlorophyll and bacteriochlorophyll biosynthesis in: Phytoplankton Pigments, Characterization, Chemotaxonomy and Applications in Oceanography (Roy, C.A., Llewellyn, C.A., Egeland, E.S. and Johnsen, G., Eds.), Cambridge University Press.
[39] Oster, U., Tanaka, R., Tanaka, A. and Rüdiger, W. (2000) Cloning and functional expression of the gene encoding key enzyme for chlorophyll *b* biosynthesis (CAO) from *Arabidopsis thaliana*. Plant J. 21 (3), 305–310.
[40] Pruzinska, A., Tanner, G., Anders, I., Roca, M. and Hortensteiner, S. (2003) Chlorophyll breakdown: pheophorbide *a* oxygenase is a Rieske-type iron-sulfur protein, encoded by the accelerated cell death 1 gene. Proc. Natl. Acad. Sci. USA 100 (25), 15259–15264.

[41] Schliep, M., Crossett, B., Willows, R.D. and Chen, M. (2010) ^{18}O Labelling of chlorophyll *d* in *Acaryochloris marina* reveals that chlorophyll *a* and molecular oxygen are precursors. J. Biol. Chem. 285 (37), 28450–28456.

[42] Tsuchiya, T., Mizoguchi, T., Akimoto, S., Tomo, T., Tamiaki, H. and Mimuro, M. (2012) Metabolic engineering of the Chl *d*-dominated cyanobacterium *Acaryochloris marina*: production of a novel Chl species by the introduction of the chlorophyllide *a* oxygenase gene. Plant Cell Physiol. 53 (3), 518–527.

[43] Evans, E.H. and Britton, G. (1983) Relationship between growth conditions, cell morphology and carotenoid composition of the cyanobacterium *Chlorogloeopsis* (*Chlorogloea*) *fritschii*, and carotenoid compositions of photosystem 1 and photosystem 2 preparations. Arch. Microbiol. 135, 284–286.