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A cyanobacterium that contains chlorophyll *f* – a red-absorbing photopigment

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ABSTRACT

A Chl *f*-containing filamentous cyanobacterium was purified from stromatolites and named as *Halomicronema hongdechloris* gen., sp. nov. after its phylogenetic classification and the morphological characteristics. *Hongdechloris* contains four main carotenoids and two chlorophylls, *a* and *f*. The ratio of Chl *f* to Chl *a* is reversibly changed from 1:8 under red light to an undetectable level of Chl *f* under white-light culture conditions. Phycobiliproteins were induced under white light growth conditions. A fluorescence emission peak of 748 nm was identified as due to Chl *f*. The results suggest that Chl *f* is a red-light inducible chlorophyll.

1. Introduction

Chlorophylls (Chls) are the essential molecules of oxygenic photosynthesis. There are five structurally characterized species of chlorophylls, Chl *a*, *b*, *c*, *d* and *f*. They exhibit different absorption maxima due to relatively minor modifications of their chemical structure [1]. Chl *a* is the essential molecule for nearly all oxygenic photosynthetic organisms, from cyanobacteria to higher plants, excluding the Chl *d*–containing cyanobacterium, *Acaryochloris marina* [2,3]. *A. marina* is the only cyanobacterium reported that uses Chl *d* as its major photosynthetic photopigment. It is found in filtered light environments in various ecological niches [4–9]. The advantage of using Chl *d* and long wavelength absorbing chlorophylls in oxygenic photosynthetic organisms is intriguing due to its unique absorption properties and its potential for increased photosynthetic efficiency [1,10].

Chlorophyll *f* has a maximum Q_Y absorption peak at about 706 nm and a maximum fluorescence emission at 722 nm at room temperature in methanol, making it the most red-shifted chlorophyll discovered to date [11]. The photophysical and photochemical functions of Chl *f* are unknown. We have isolated and cultured a cyanobacterium using red-light enrichment [11] that contains Chl *f* as an accessory photopigment. We report here the characterization of this newly isolated cyanobacterium based on its morphology, ultrastructure, 16S rDNA-based phylogeny and studies on the photosynthetic function of Chl *f* in this organism.

2. Materials and methods

2.1. Enrichment, isolation and cultivation

Red-light enrichments were plated on 1% agar of K + ES seawater medium [3] and grown under the same light-regime as described in Chen et al. 2010 [11]. Once cyanobacteria started to appear as a clone, individual tapering filaments were picked up and transferred to K + ES seawater media and grown under the same conditions for 4–6 weeks, then re-plated on 1% agar of K + ES seawater medium for further purification. This procedure was repeated until an axenic culture was obtained.

To test the influence of different light growth conditions, cells were cultured for two weeks, under continuous illumination of either white fluorescent light with an intensity of 20 μmol photons m^{-2} s^{-1}, or 720 nm LEDs (Cat. No. L720–04AU, Epitex, Japan) with an intensity of 10–15 μmol photons m^{-2} s^{-1} [11]. The 720 nm LEDs are 5 mm diameter LEDs with 20 nm spectral half width and a 20° viewing angle. Light intensities were measured with a Quantum Li-189 light meter (LiCor Corp, USA).

Abbreviations: Ca, carboxysome; Chl, chlorophyll; Cm, cytoplasmic membrane; fs, fibrous sheath-like layer; HPLC, high-performance liquid chromatography; LP, longpass optical filters; ML, Maximum-Likelihood; NJ, neighbour-joining; Om, outer membrane; Pg, peptidoglycan layer; PBS buffer, phosphate buffered saline buffer; PS, photosystem; Th, thylakoid membranes; TEM, Transmission electron microscopy

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2.2. PCR amplification and sequence analysis

Genomic DNA was obtained from the purified culture using standard methods and the gene coding for 16S rDNA was amplified using universal 16S rDNA primers: 16S_27F, 5′-AGAGTTTGATCCTGGCTCAG-3′ 16S_1494R, 5′-TACGGCTACCTTGTTACGAC-3′ with 94 °C for 3 min following by 30 cycles of 94 °C for 15 s, 54 °C for 25 s and 72 °C for 90 s. The PCR products were sequenced and confirmed from three individual repeat PCR reactions.

The 16S rDNA sequences were aligned using Clustal W in MEGA (version 5.0) [12] and the alignment was manually refined based on the published alignment [9,13]. The NJ tree was constructed based on the Kimura 2-parameter model with 10,000 repeats and the ML tree based on the GTR model with 1000 repeats using MEGA 5.0. Only bootstrap values $\geqslant$50% are illustrated at nodes. Accession numbers of sequences were listed in Supplementary Table 1. The length of sequences used to generate the phylogenetic tree was $\geqslant$1025 bp (without gap).

2.3. Pigment analysis

Pigments were extracted from cells in 100% pre-chilled methanol and injected onto a 4.6 × 150 mm Synergi 4 μm Max-RP column (Phenomenex, Australia) attached to a Shimadzu HPLC (model 10A series) equipped with a diode array absorption detector (SPD-M10Avp). The ratio of chlorophylls were calculated based on the peak area and their extinction coefficients at the assigned wavelengths, Chl *a* at 665 nm and Chl *f* at 707 nm [14]. Carotenoids were collected from HPLC peaks, dried under N_2 gas and analyzed by MALDI-TOF mass spectrometer using terthiophene as the matrix [15].

A homogenized culture suspension was obtained using a glass homogenizer and in vivo absorbance spectra were recorded using a Shimadzu UV–Vis spectrophotometer with a Taylor-sphere attachment (ISR-240A, Shimadzu, Japan). The spectral curves obtained were smoothed using the Savitzky-Golay method (Origin version 8.5).

2.4. Steady-state fluorescence spectral analysis

Steady-state fluorescence spectra were measured using a Varian Cary Eclipse Fluorescence spectrophotometer at low temperature (77 K) with aid of a Cryostat attachment (Oxford Instruments, UK). The cells were homogenized in filtered seawater with 65% glycerol and diluted to a maximum absorbance at the Q_y transition of 0.1. Spectra were recorded with a slit of 5 nm. To increase the signal to noise ratio, the fluorescence spectra were obtained as an average of 50 repeats recorded and smoothed using the Savitzky-Golay method (Origin version 8.5).

2.5. Confocal microscopy

The cells were mounted on slides under glass cover slips. The cells were visualized using emission fluorescence with a 60× objective (PlanApo oil, N.A 1.42) and recorded on a Fluoview FV300 confocal system, equipped with IX 70 inverted microscope (Olympus, Japan). The cells were excited with an Argon laser (488 nm) and the emission fluorescence was recorded in the presence of a 565 nm LP filter or a 666 nm LP filter.

2.6. Transmission electron microscopy

Cells were fixed in 3% glutaraldehyde, 6% sucrose in PBS buffer (pH 7.5) overnight at 4 °C, rinsed in PBS buffer and then embedded in 1% low melting agarose. The small blocks of agarose-containing cells were further fixed in 1% osmium tetroxide, 6% sucrose in PBS for 1 h and rinsed in distilled water; *en bloc* stained in 2% uranyl acetate (aqueous) for 20 min and then dehydrated in a graded series of ethanol from 50% to 100%, 10 min each step; infiltrated with LR White resin (London resin company) and embedded in gelatine capsules. The samples were polymerised at 65 °C for 48 h. Semithin sections (1 μm) were cut using glass knives on an ultramicrotome (Ultracut S, Leica, Austria), mounted and dried on glass slides and stained with 1% methylene blue.

Ultrathin sections (70 nm) were cut on the ultramictrotome, using a diamond knife (Drukker International), collected on 300 mesh, pioloform coated, thin bar copper grids (Proscitech) and stained with saturated aqueous uranyl acetate for 30 min and lead citrate (Reynold's) for 4 min. All sections were visualized using a transmission electron microscope (CM10, Phillips) at 100 kV. Images were captured using a MegaView III TEM CCD camera and iTEM, TEM imaging platform (Olympus, Japan).

3. Results

3.1. Isolated Chl *f*-containing cyanobacterium

An oxygenic photosynthetic filamentous prokaryote containing Chl *f* was isolated from red-light enrichment cultures obtained from a ground stromatolite sample as described in Chen et al., 2010 [11]. We tentatively name this newly isolated Chl *f*-containing organism as "*hongdechloris*", as "*hong-de*" means red in Chinese and thus "*hong-de-chloris*" represents "*red chlorophyll*". Cells are filamentous without branches (Fig. 1A and B) surrounded by a fibrous sheath-like layer (fs) with a thickness of 150–200 nm and helical fibrils are observed at the end of filaments (Fig. 1C). The cells are cylindroids of 0.6–0.8 μm diameter and 1.0–1.3 μm long depending on the extent of cell elongation since the last division (Fig. 1). The cell size of *hongdechloris* is rather small and is in the same size range of *Prochlorococcus marinus*, which is about 0.6–0.8 × 1.5–1.7 μm in size [16], except that it has filamentous structure. The cytoplasm is surrounded by the typical cell wall of cyanobacteria [17], which is comprised of three layers (Fig. 1E and F): cytoplasmic membrane (Cm), an electron-dense layer of peptidoglycan (Pg), and an outer membrane (Om). Asexual binary division by an invaginating septum occurs in the middle and along the entire lengths of filaments, but not in apical cells. The terminal apical cells are triangle shape with degraded cellular structure (Fig. 1D). No nodule structure or heterocyst cells are observed. Gas vacuoles are absent.

The polyhedral particles in the center of the cytoplasm (Fig. 1E and F) are consistent with the reported size and structure of carboxysomes [18,19]. Three to four layers of thylakoid membranes (Th) are arranged along cells peripherally and are separated by a gap of 25–35 nm width filled with an electron-dense substance (Fig. 1F, white arrows), which have a phycobilisome-like structure [20]. Each membrane pair that forms a thylakoid membrane sheet is separated by an electron-lucent lumen that is about 6–9 nm. Only 1–2 layers of thylakoid membrane are observed in the septa region (intersection between cells in filamentous cyanobacteria), which agrees with the ultrastructure of *Halomicronema* sp. reported [19,21]. The strong fluorescence observed symmetrically around cells under confocal microscopy with a 565 nm LP filter (Fig. 1A) is consistent with the emission from phycobilisomes and chlorophylls in these membranes [22]. Surprisingly, strong fluorescence is observed at cell septa by confocal microscopy with 666 nm LP filter, which are the main emission from chlorophylls (Fig. 1B).

3.2. SSU rDNA sequence and phylogenetic analysis

The partial small subunit (SSU) rDNA sequence (1378 bp) of *hongdechloris* was obtained from a number of independent PCR reactions and was submitted to Genbank with accession number

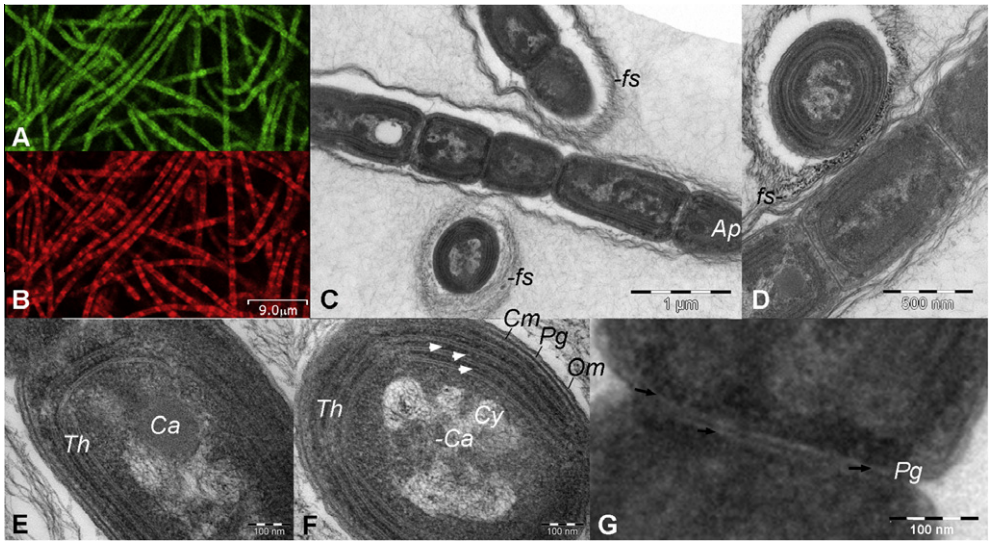

Fig. 1. Confocal light microscopic and transmission electron microscopic photography. A and B, confocal light microscopic images of *hongdechloris* cells by λ_{ex} = 488 nm, and λ_{em} < 565 nm LP filter (A) and λ_{em} < 660 nm LP filter (B); C–G, Transmission electron micrograph of ultrathin section of *hongdechloris* cells. The central cytoplasm (*Cy*) is surrounded by several concentrically arranged thylokoid membranes (*Th*). In the center of the cells a carboxysome (Ca) is visible. Outer membrane (*Om*), electron dense peptidoglycan layer (*Pg*) and cytoplasmic membrane (*Cm*) are observed (F). G, Detailed image of the intersection (septa region) between filamentous cells. White arrows (F) indicate phycobilisome-like structures filling the stroma side of the thylakoid membranes. The peptidoglycan layer (Pg) is shared between the cells with their own cytoplasmic membranes (Black arrows, G). *fs*, fibrils sheath; *Ap*, apical cells.

JX089399. By the NCBI nucleotide-nucleotide BLAST search, the sequence has similarity from 85% to 93% of those of known cyanobacteria.

The 16S rDNA sequence of *hongdechloris* was analyzed for its taxonomic position using 43 selected cyanbacterial sequences (Supplementary Table 1) and *Chlorobium tepidum* TLS as an outgroup. Both Neighbor Joining (NJ) and Maximum-likelihood (ML) trees indicate that *hongdechloris* is located in the subtree of the *Leptolyngbya* group within the cyanobacterial LPP-group and in the cluster together with *Halomicronema* sp. (Fig. 2). The cyanobacterial LPP-group corresponds to those filamentous non-heterocystous cyanobacteria resembling *Lyngbya* sp., *Plectonema* sp. or *Phormidium* sp. To refine the phylogenetic tree, 31 sequences for the cyanobacterial LPP-group were downloaded from the NCBI gene bank and *Prochlothrix hollandcia* used as an outgroup (Supplementary Table 1). NJ phylogenetic trees (Fig. S1) confirmed the phylogenetic position of *hongdechloris.* It is clustered with *Halomicronema Scyano39* [13], *Halomicronema sp* TFEP1 [21] and *Halomicronema sp*. Goniastrea-1 [23] together with high bootstrapping support (Fig. S1). Hence, the Chl *f*-containing *hongdechloris* belongs to the genus of *Halomicronema* nov. gen. The name for the newly isolated Chl *f*-containing cyanobacterium is formally proposed here as a new species, *Halomicronema hongdechloris* gen., nov. sp. *Halomicronema Scyano39* was reported from culture collections of Hamielin pool pustular mat and smooth mat [13], where *hongdechloris* was originally isolated, and showed 93% identity.

3.3. Culture spectral characteristics

Halomicronema hongdechloris gen., nov. sp. was maintained on solid media in K + ES seawater medium with 1% agar, but it grew in liquid culture more rapidly, although the analysis of the relative rates of growth is being carried out in another study. The *in vivo* absorbance spectrum of purified *hongdechloris* cultured under far-red light conditions (Fig. 3A) showed that Chl *a* is the main chlorophyll and a broader shoulder around 740 nm indicated the presence of Chl *f* (arrow in Fig. 3A). Interestingly, the *in vivo* absorbance spectrum of a white-light induced culture showed an increased absorbance of phycobiliproteins (star in Fig. 3A) and a decreased spectral absorbance at about 740 nm, compared to the far-red light-induced culture (Fig. 3A). The decreased absorbance around 740 nm demonstrated the loss of Chl *f* under white light growth conditions. The spectral analysis of methanolic pigment extracts (*in vitro*) agrees with the in vivo spectra, in that no Chl *f* is observed in the white-light induced culture (arrow in Fig. 3B). The ratio of $A_{665\ nm}$ to $A_{707\ nm}$ represents the changes of chlorophyll composition under different light condition, Chl *a* at 665 nm and Chl *f* at 707 nm in 100% methanol. Fig. 3C demonstrated that the ratio of $A_{665\ nm}/A_{707\ nm} \approx 10{:}1$ in the initial cells and the ratio keeps almost no changes during 4 weeks culture time under red-light condition, however, the ratio was dramatically increased to ~120:1 under white light culture condition for 4 weeks.

The HPLC analysis of methanolic pigment extracts were in agreement with absorbance spectral profiles (Fig. 3D). Chl *f* is about 12.5% of total chlorophylls under far-red light and is decreased to an undetectable level under white light illumination conditions. However, when the white-light grown cultures were returned to the red-light condition for 1–2 weeks, the pigment profile returned to that of the cultures grown exclusively under red-light. The results suggested that *hongdechloris* can acclimatize its pigment profiles to meet the requirement of the light environment: using Chl *f* to absorb red-light under red-light condition and using phycobiliproteins and Chl *a* to absorb the main light region of white light. The phenomena observed here suggest that Chl *f* is a red-light-induced chlorophyll, although the regulatory mechanism for pigment adaptation is unknown.

Pigment compositions of *hongdechloris* (two weeks culture) were determined by HPLC analysis (Fig. 3D). Six main pigments were resolved by HPLC in methanolic pigment extracts (Fig. 3D). Two chlorophyll derivatives: Chl *a* (peak 5) and Chl *f* (peak 4) were determined based on their retention time and online absorbance spectral properties [11]. Chl *f* was about 12.5% of total isolated chlorophylls when *hongdechloris* was cultured under red-light condition (720–730 nm). Surprisingly, the Chl *f* (peak 4) was undetectable in methanolic pigment extracts from white light cultured cells (Fig. 3D).

Four resolved carotenoids were identified based on their retention time, their visible spectral properties and their molecular mass

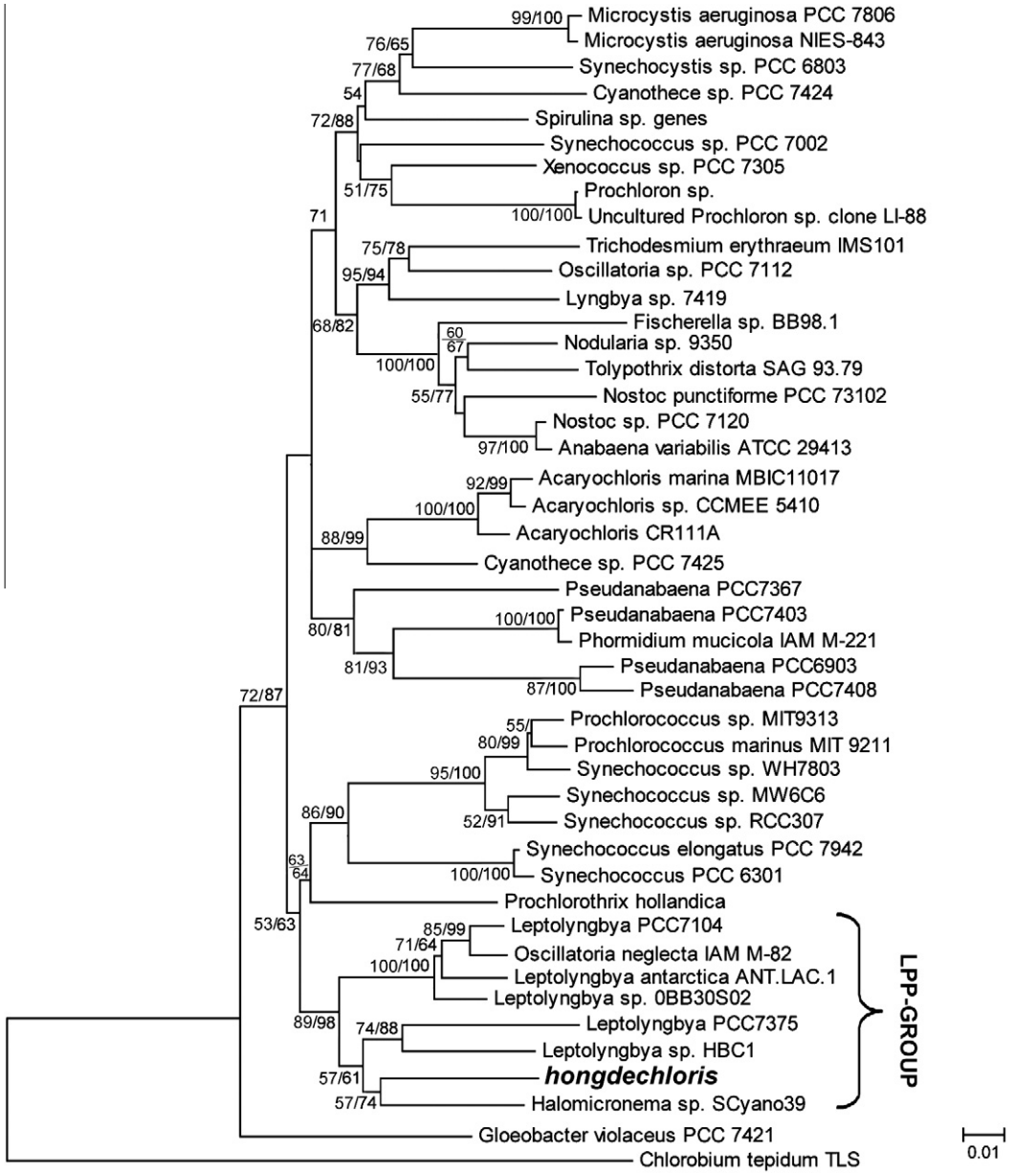

Fig. 2. Phylogenetic classification of *hongdechloris* using 16S rDNA sequences. The phylogenetic relationship was constructed using the Neighbor Joining (NJ) and the Maximum Likelihood (ML). The green sulfur bacterium *Chlorobium tepidum* TLS was used as an outgroup. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Scale bar represents 0.01 substitutions per nucleotide position. The numbers above the branches indicate bootstrap support based on 1000 replicates for ML tree and 10,000 replicates for NJ tree (ML/NJ). Only bootstrap values $\geqslant$50% are illustrated at nodes. Accession numbers of sequences are listed in Supplementary Table 1.

based on MALDI-TOF mass spectrometry. Peak 3 with $[M+H]^+$ of 568.4 is zeaxanthin. Peak 1 and Peak 2 with $[M+H]^+$ of 600.4 and 584.4, were products of epoxidation from zeaxanthin, violaxanthin and antheraxanthin, respectively (Fig. S2). Peak 6 is β-carotene according to its absorbance spectrum and retention time, which was confirmed with $[M+H]^+$ of 536.

The cells transferred from red- to white-light showed a much higher percentage of β-carotene as the main carotenoid (Fig. 3D), and this change was observed after 48 h under white light illumination. The relative ratios of violaxanthin, antheraxanthin and zeaxanthin to total pigment, were significantly decreased when cells were cultured under white light according to normalized HPLC chromatography (Fig. 3D). A further examination of optimal culture conditions and chromatic acclimation in *hongdechloris* cells will be carried out in the near future.

3.4. Low temperature fluorescence analysis

A major fluorescence emission peak of 748 nm was observed from *hongdechloris* cells grown under red-light conditions (Fig. 4A) using an excitation wavelength of 405 nm at low temperature (~77 K). There is a different fluorescence profile recorded from the cells grown under the white-light growth conditions, with fluorescence peaks of 640, 658, 682 and 730 nm (Fig. 4A). The fluorescence emission peaks of 640 and 658 nm were attributed to the presence of phycobiliproteins in the cells grown under white light, which were missing in the cells grown under red-light condition (Fig. 4 and S3). The emission peaks of 682 nm and ~720 nm were attributed to Chl *a* in photosystems (PS) II and PS I, respectively. Interestingly, the major fluorescence peak of 748 nm with two minor, but well resolved peaks centered at 682 and 720 nm, respec-

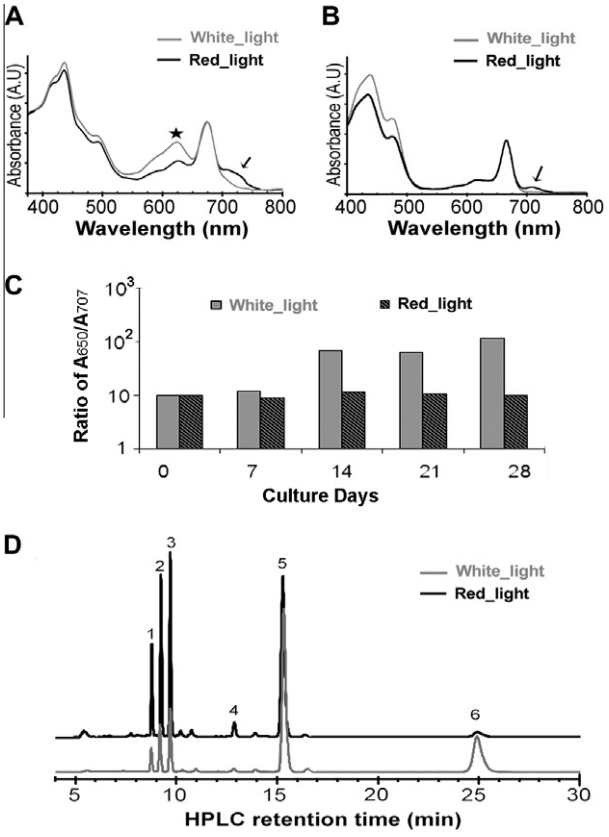

Fig. 3. Pigment analysis of *hongdechloris* at room temperature. (A) In vivo absorbance spectral comparison of cell suspensions in filtered seawater. Star indicates the presence of phycobiliproteins; (B) in vitro absorbance spectral comparison of total pigment extraction in 100% methanol; (C) The kinetic changes of chlorophyll composition in *hongdechloris* cells grown under different light condition. The absorbance ratio of $A_{665\ nm}/A_{707\ nm}$ represents the ratio of Chl *a* / Chl *f*; (D) The pigment composition analysis by HPLC. The HPLC chromatographs were normalized at Chl *a* peak. There are 6 main resolved photopigments: (1) violaxanthin; (2) antheraxanthin; (3) zeaxanthin; (4) chlorophyll *f*; (5) chlorophyll *a*; (6) β-carotene. Black lines are samples from *hongdechloris* cultured under red-light (720 nm) and grey lines represent the *hongdechloris* cells grown under white-light condition. Black arrows in (A and B) indicated the presence of Chl *f*.

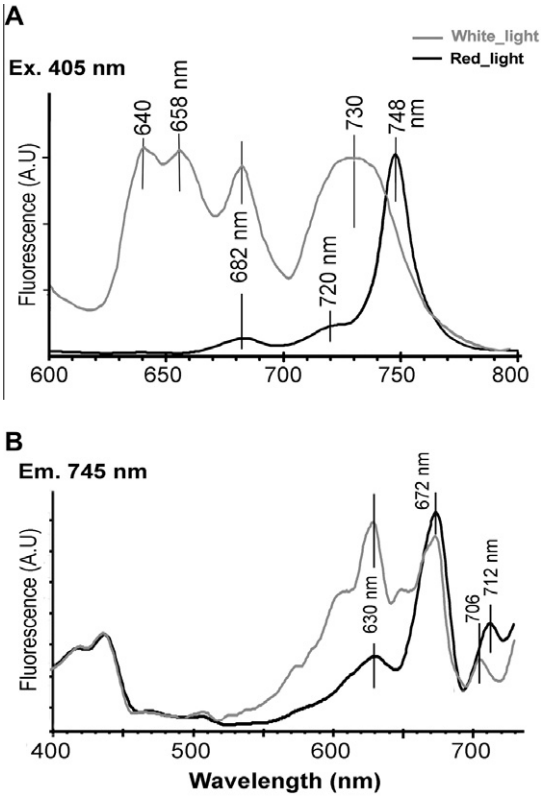

Fig. 4. Low-temperature (77 K) fluorescence spectral analysis of *hongdechloris* intact cells suspended in 65% glycerol. (A) Steady-state fluorescence emission spectral comparison using excitation wavelength of 405 nm. The spectra were normalized at the most-red fluorescence peaks, 730 nm for cells grown under white light and 748 nm for the cells grown under red-light condition; (B) Steady-state fluorescence excitation spectral comparison using emission wavelength of 745 nm. The spectra were normalized at the Soret bands (~440 nm). Black lines are *hongdechloris* cells grown under red-light (720 nm) and grey lines represent the *hongdechloris* cells grown under white-light condition.

tively, were only observed from the cells grown under red-light conditions, which contain ~12.5% Chl *f* (Fig. 3C). The emission fluorescence spectra showed similar profiles with excitation at 405 and 435 nm for cells cultured under red-light conditions (Fig. S3_A). Enriched phycobiliprotein emission is observed in the cells cultured under white-light conditions for 2 weeks, especially with excitation wavelength of 405 nm (Fig. S3_B).

The fluorescence excitation spectra were recorded using the emission wavelength of 745 nm (Fig. 4B). There are two main components of 672 nm and 712 nm from the cells grown under red-light with emission wavelength at 745 nm; but the main center excitation peaks were at 630 and 672 nm in the cells grown under white light. The excitation component of 712 nm was missing in the white-light grown cells (Fig. 4B). Regarding the different pigment composition of the two growth conditions, the fluorescence peak of 712 nm in the red-light cells was attributed to presence of Chl *f*, although its function and the protein environment of the pigment remained uncertain in this study. The nature of fluorescence profiles recorded from the red-light grown cells and white-light grown cells is consistent with phycobiliproteins as the major light-harvesting component for *hongdechloris* grown under white-light conditions (Fig. 4B and S3_B), which is similar to other cyanobacteria. However, the light-harvesting component binding Chl *f* in *hongdechloris* cells when they were cultured under red-light condition is unknown. A possible up-hill energy transfer mechanism may be considered for *hongdechloris* using the red-light for its oxygenic photosynthetic reactions. A further examination of Chl *f* and its pigment–protein complexes is underway.

4. Discussion

Non-heterocystous, very thin filamentous cyanobacteria are typically assigned to the former genus *Phormidium* and to the revised genus *Leptolyngbya* in the botanical revision [24,25]. In the bacteriological system, the LLP-group (*Lyngbya/Plectonema/Phormidium*-group) is synonymous with *Leptolyngbya* [26]. 16S rDNA analysis revealed this isolate containing Chl *f* is closest to *Halomicronema SCyano39*, with 93% identities. However, the morphology and other physiological characterization of *Halomicronema SCyano39* have not been reported [12]. The similarities in transmission electron microscopy ultrastructure of *hongdechloris* to *Halomicronema* TFEP strains [21] together with the 16S rDNA analysis support our proposal that *hongdechloris* is a new species in *Halomicronema* nov.*gen*. designated as: *Halomicronema hongdechloris* nov. sp. *Hongdechloris* cells are in the size of 0.6–0.7 μm × 1.0–1.3 μm, the thinnest filamentous cyanobacterium reported to date. The symmetrical arrangement of thylakoid membranes on both sides of cells with strong uniform fluorescence emission shown using a 565 nm LP filter suggests the even distribution of phycobiliproteins. The strong autofluorescence observed at septa region using the 666 nm LP filter, the main fluorescence emission from chlorophylls, may be attributed to the constricted distribution of phycobilipro-

teins in the region. The spatial difference of fluorescence (Fig. 1A and B) suggests that phycobiliproteins are mainly distributed along the filamentous direction. An electron microscopic image of the thin sections of cells revealed that the distance between thylakoid membranes was decreased in the region of septa (Fig. 1G), which agrees with the bulk fluorescence measurement in vivo (Fig. 1). In this study, we cannot distinguish fluorescence derived from Chl *a* and that of Chl *f* using an LP filter of 660 nm. Whether there is spatial separation of Chl *a* and Chl *f* binding protein complexes in the thylakoid membranes remains to be determined.

The red-light induced production of Chl *f* in the cells was confirmed by HPLC and the unique low temperature fluorescence profiles of these cells confirm that Chl *f* in the cells is responsible for the far-red fluorescence emission peak at 748 nm. These results suggest that cells take advantage of the extreme far-red absorbance of Chl *f* to capture red-light when it is the only available light source. The optimal culture condition and the function of Chl *f* in *hongdechloris* are still under investigation.

Halomicronema hongdechloris nov. sp. was purified from red-light enrichments of stromatolites collected from Shark Bay, Western Australia [11]. Allen et al. (2009) reported there were 19 isolated cyanobacteria from the mats [12], which were designated as *Halomicronema* sp. SCyano39 showed high 16S rDNA sequence similarity to *hongdechloris* with 93% identity based on the Pile-up sequences comparison. The microscopic morphological features of *Halomicronema* SCyano40 revealed the structural similarities to *hongdechloris* as reported here, except for the size of cells, *hongdechloris* filament has the thickness of 0.6–0.8 μm and *Halomicronema* SCyano40 of 1.8–2.2 μm [12].

The reported partial 16S rDNA sequence, extracted from an uncultured cyanobacterium (accession number: HM748584) related to Chl *f* isolation [11], from the same strometolite sample collection, showed a long branch, but 100% bootstrap support as a sister group of *hongdechloris* 16S rDNA (Fig. S1). Sequence comparison revealed the high similarity between two sequences at 5'-sequencing sites (between 30 and 800 bp), but more differences near 3′-sites (900–1200 bp), which may be the result from PCR or sequence errors (Fig. S4). The 16S rDNA sequences reported here are based on three individual repeat PCR reactions and sequencing from both ends (using forward and reverse primers), while the partial sequence (HM748584) was a PCR product and was sequenced using a forward primer only.

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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.2012.06.045.

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