



# A seventh bacterial chlorophyll driving a large light-harvesting antenna

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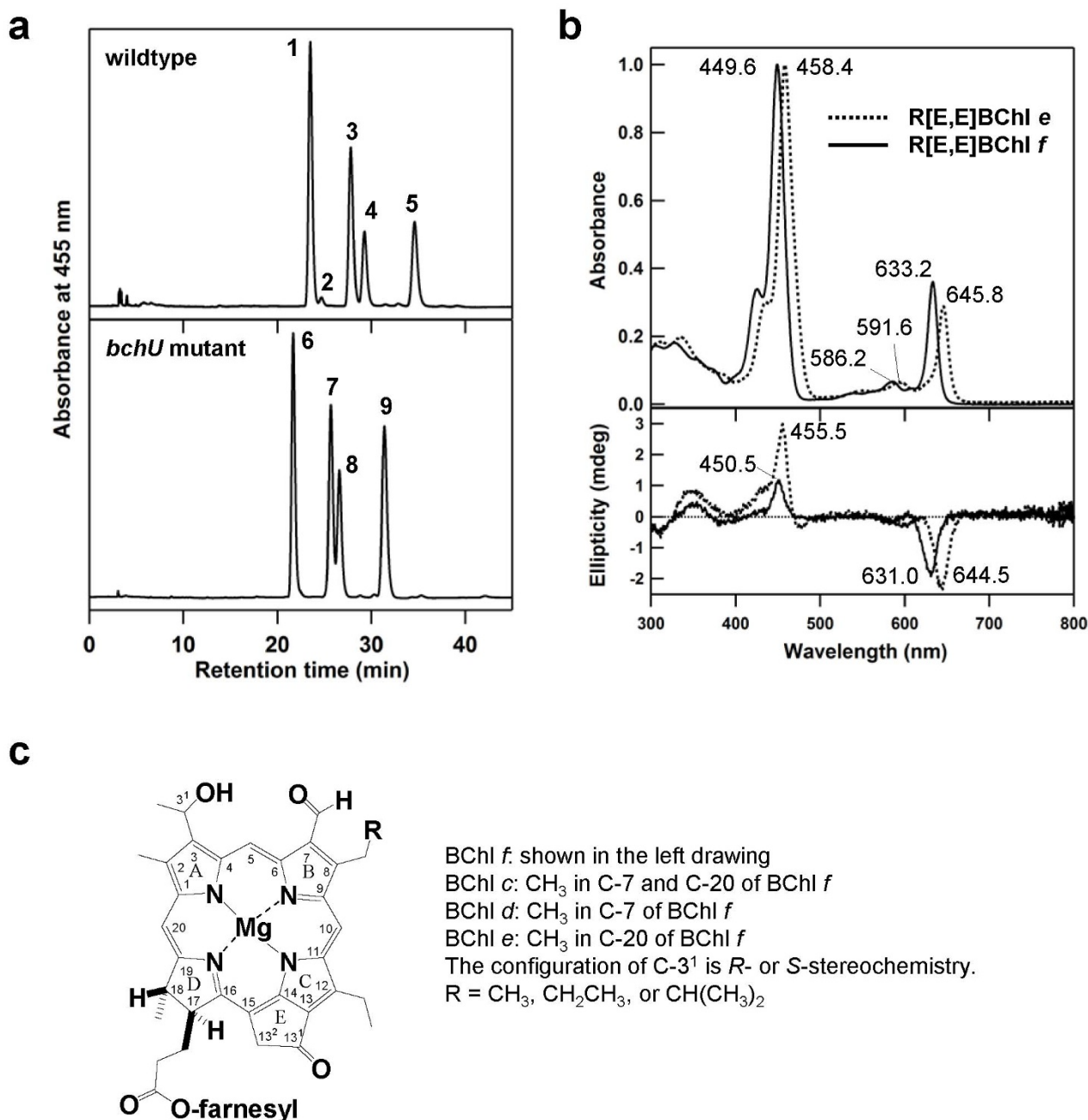
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The discovery of new chlorophyllous pigments would provide greater understanding of the mechanisms and evolution of photosynthesis. Bacteriochlorophyll *f* has never been observed in nature, although this name was proposed ~40 years ago based on structurally related compounds. We constructed a bacteriochlorophyll *f*-accumulating mutant of the green sulfur bacterium *Chlorobaculum limnaeum*, which originally produced bacteriochlorophyll *e*, by knocking out the *bchU* gene encoding C-20 methyltransferase based on natural transformation. This novel pigment self-aggregates in an *in vivo* light-harvesting antenna, the chlorosome, and exhibits a  $Q_y$  peak of 705 nm, more blue-shifted than any other chlorosome reported so far; the peak overlaps the maximum (~700 nm) of the solar photon flux spectrum. Bacteriochlorophyll *f* chlorosomes can transfer light energy from core aggregated pigments to another bacteriochlorophyll in the chlorosomal envelope across an energy gap of ~100 nm, and is thus a promising material for development of new bioenergy applications.

Chlorophyllous pigments are required by photosynthetic organisms in order to carry out primary production: harvesting sunlight energy, driving photosystems, and achieving charge separation. However, the specific pigments present in the cells of a photosynthetic organism influence its light-harvesting efficiency, because each pigment species has distinctive absorption characteristics. Most oxygenic phototrophs, such as plants and cyanobacteria, contain chlorophyll (Chl) *a*, whereas some plants have Chl *b*, and some cyanobacteria possess Chl *d*<sup>1</sup> and Chl *f*<sup>2</sup>. Differences among these Chls include oxidation of peripheral substituents of the chlorin ring structure. Chls *b* ( $Q_y$  and Soret maxima = 642.2 and 453.0 nm in diethyl ether), *d* ( $Q_y$ /Soret = 685.8/445.8 nm), and *f* ( $Q_y$ /Soret = 694.5/439.5 nm)<sup>3</sup> are differentiated from Chl *a* ( $Q_y$ /Soret = 660.8/429.6 nm) by the presence of a formyl group at the C-7, C-3, and C-2 positions, respectively. Changes in the peripheral substituents of the pigment ring alter visible light absorbance; the different Chls confer habitat- or niche-specific selective advantages on photosynthetic organisms that possess them.

Green sulfur bacteria (GSBs) are strict anaerobes that utilize reduced sulfur compounds (sulfide, polysulfide, or elemental sulfur) as electron donors in anoxygenic photosynthesis. They thrive in deep layers of the photic zone, where reduced sulfur compounds are abundant while light is limited. GSBs contain characteristic light-harvesting organelles, called chlorosomes, that contain large quantities ( $1.4\text{--}2.1 \times 10^5$  molecules per chlorosome) of bacteriochlorophylls (BChls) *c*, *d*, or *e* (see Fig. 1C)<sup>4,6</sup>. The  $Q_y$  and Soret absorption bands of BChls *c* ( $Q_y$ /Soret = 660.4/432.0 nm in diethyl ether), *d* ( $Q_y$ /Soret = 649.6/425.4 nm), and *e* ( $Q_y$ /Soret = 646.4/459.6 nm) in their monomeric states overlap those of Chls *a/b/d/f* in oxygenic phototrophs living in upper layers of the photic zone. However, BChls *c/d/e* can form self-aggregated structures in chlorosomes<sup>7,8</sup>, resulting in red-shifted absorption bands: typical chlorosomal  $Q_y$ /redmost Soret absorption maxima are 750–760/460 nm for BChl *c*, 730/450 for BChl *d*, and 710–720/520 for BChl *e* (Fig. 2a). GSBs, therefore, can utilize light energy at greater depths than other organisms possessing Chls. Fluorescence of free Chl *a* in a solution is completely quenched at 0.3 M, a phenomenon called concentration quenching<sup>9</sup>. Although the concentration of BChls in chlorosome is much larger [ $\sim 2$  M, based on data in ref 4], no concentration quenching is observed. Moreover, chlorosomal BChl concentrations are also larger than those in other photosynthetic pigment-protein antenna systems, e.g., 0.3 M for photosystem II light-harvesting complex of higher plants<sup>10</sup>, and 0.1 M for Fenna-Matthews-Olson (FMO) BChl *a* protein in GSB<sup>11</sup>. These observations suggest that the well-ordered supramolecular structures of chlorosomal self-aggregated pigments permit rapid and efficient migration of excitation energy within chlorosomes, allowing GSB to efficiently absorb light in ecological niches with illumination conditions as low as  $3\text{--}10 \text{ nE} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ <sup>12</sup>. Elucidation of the structural and photochemical mechanisms of chlorosomes would therefore be useful in developing bioenergy applications.



**Figure 1** | Determination of compositions of BChl *f* homologs in *C. limnaeum bchU* mutant. (a) HPLC elution profiles of extracted pigments from the *C. limnaeum* wildtype (upper panel) and *bchU* mutant cells (bottom panel). The elution of pigments was detected at 455 nm. Peak 1, R[E,E]BChl *e*; peak 2, S[E,E]BChl *e*; peak 3, R[P,E]BChl *e*; peak 4, S[P,E]BChl *e*; peak 5, S[I,E]BChl *e*; Peak 6, R[E,E]BChl *f*; peak 7, R[P,E]BChl *f*; peak 8, S[P,E]BChl *f*; peak 9, S[I,E]BChl *f*. (b) UV-Vis absorption (upper) and CD (bottom) spectra of R[E,E]BChl *f* in diethyl ether. (c) Chemical structure of BChl *f*, with comparison to other chlorosomal BChls.

The name BChl *f* was proposed ~40 years ago to denote the 20-unsubstituted form of BChl *e* (Fig. 1c)<sup>13</sup>. The structural relationship between BChl *e* and *f* is the same as that between BChl *c* and *d*, namely, a difference of a methyl or hydrogen group at C-20 position. Methylation at C-20 of BChl *c* is catalyzed by the gene product of the *bchU* gene<sup>14,15</sup>. The GSB *Chlorobaculum* (*C.*) *parvum* NCIB 8327 has a natural loss-of-function mutation in *bchU*<sup>14,16</sup>; therefore, this strain produces only BChl *d*, but it can be altered by a second mutation to produce BChl *c* under low-light conditions<sup>17,18</sup>. Restored production of BChl *c* in the strain under low light illumination was ascribable to that self-aggregated BChl *d* in chlorosomes was less efficient in energy transfer to the baseplate proteins in chlorosomal envelope<sup>19</sup>. Because such spontaneous mutation in *bchU* occurs frequently, we

hypothesized that a GSB that naturally produces BChl *e* could be easily altered to produce BChl *f*. However, although BChl *d* is common<sup>20,21</sup>, BChl *f* has never been detected in nature. A BChl *f* homolog was synthesized by modifying Chl *b*; it is stable *in vitro*, and its chemical and absorption properties have been characterized<sup>22</sup>. Using synthetic BChl *f* as a standard, detailed HPLC analyses were conducted on GSBs that produce BChl *e*; however, no natural BChl *f* molecule has previously been found. The absence of natural BChl *f* suggested that such a mutation in a BChl *e*-producing strain would dramatically reduce light harvesting and energy transfer, conferring a selective disadvantage on mutants producing BChl *f*. This prediction has heretofore not been tested, largely because BChl *e*-producing GSBs are difficult to culture and genetically manipulate.



We have serially cultured the BChl *e*-producing GSB strain *C. limnaeum* 1549 for about a decade, and have isolated a derivative strain that grows on plates (hereafter, called 'RK-j-1'). We performed insertional inactivation of *bchU* in RK-j-1 strain, yielding a mutant that produces BChl *f*. Here, we report the absorption properties of the resulting mutant, and characterize its chlorosomes, which are composed of self-aggregated BChl *f*. This work was initially submitted as a patent on February 13, 2012<sup>23</sup>. While the present paper was under review, Bryant's group reported similar results independently<sup>24</sup> (see also Supplementary information).

## Results

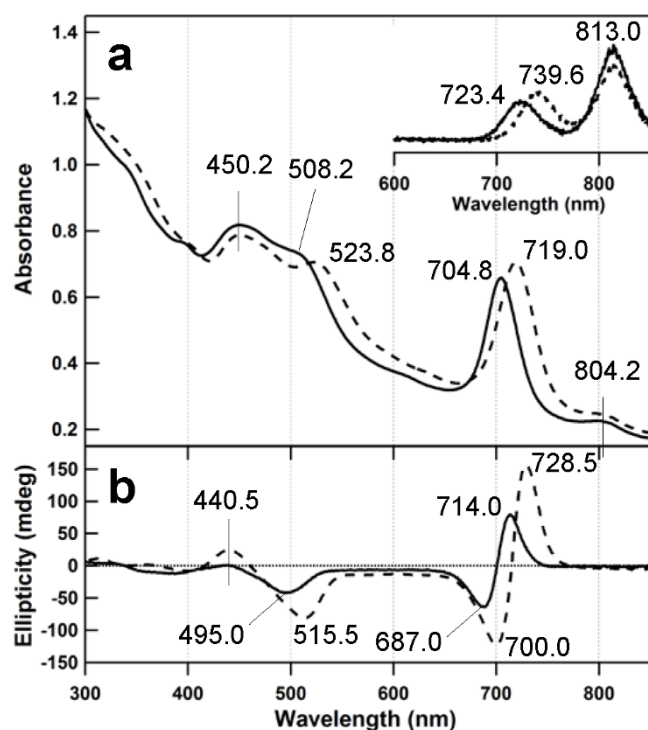
### Identification of BChl *f* homologs in *C. limnaeum bchU* mutant.

Mutation of *bchU* was achieved based on natural transformation and homologous recombination in the similar way previously described for the BChl *c*-producing bacterium *C. tepidum*<sup>25</sup> (Fig. S1). Pigments extracted from wildtype and mutant RK-j-1 cells were analyzed by HPLC (Fig. 1a). Elution profiles of pigment extracts from wildtype contained five BChl *e* homologs possessing an *R* or *S* stereo-configuration at the C-3' asymmetric position, and different degrees of methylation at C-8<sup>2</sup> (Figures 1a upper and 1c): (3<sup>1</sup>*R*)-epimeric 8,12-diethyl-(R[E,E])BChl *e* (peak 1), (3<sup>1</sup>*S*)-epimeric 8,12-diethyl-(S[E,E])BChl *e* (peak 2), (3<sup>1</sup>*R*)-epimeric 8-propyl-12-ethyl-(R[P,E])BChl *e* (peak 3), (3<sup>1</sup>*S*)-epimeric 8-propyl-12-ethyl-(S[P,E])BChl *e* (peak 4), and (3<sup>1</sup>*S*)-epimeric 8-isobutyl-12-ethyl-(S[I,E])BChl *e* (peak 5). The assignments for the *R* or *S* configuration at the C-3' position were based on the elution pattern described previously<sup>22</sup>. In HPLC profiles of mutant cells, four peaks were detected (Fig. 1a, lower), with the same online electronic absorption spectra:  $\lambda_{\max} = 639$  and 456 nm in 70:15:15 (v/v/v) acetonitrile:acetone:water. These spectra are consistent with that of the authentic BChl *f* standard<sup>22</sup>. The four bands were 14 Da less massive than the corresponding peaks in wildtype cells (Table S2). These observations established

that peaks 6, 7, 8, and 9 were assigned as R[E,E]-, R[P,E]-, S[P,E]-, and S[I,E]BChl *f*, respectively. The UV-Vis absorption spectrum of isolated R[E,E]BChl *f* (the main peak 6) in diethyl ether exhibits a 12.6-nm blue-shifted  $Q_y$  maximum at 633.2 nm and an 8.8-nm blue-shifted Soret maximum at 449.6 nm, in contrast to those of R[E,E]BChl *e* (Fig. 1b upper), which are identical to those of the R/S[E,E]BChl *f* standard<sup>22</sup>. The circular dichroism (CD) spectrum of R[E,E]BChl *f* exhibits blue-shifted peak positions relative to R[E,E]BChl *e* (Fig. 1b lower); intensities of the *e* peaks were higher than those of *f*. Furthermore, we determined the molecular structure of R[E,E]BChl *f* using <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy (Fig. S2). Based on these data, we conclude that the *bchU*-inactivated mutant exclusively accumulates BChl *f* homologs, and BChl *e* species are not present in mutant cells.

**Absorption properties of BChl *f* self-aggregation *in vivo*.** In the UV-Vis-NIR absorption spectrum of *bchU* mutant cells, we observed the  $Q_y$  peak at 704.8 nm and the Soret band at ~510 nm (Fig. 2a). These *in vivo* absorption bands were red-shifted by ~70 and 60 nm from those of monomeric BChl *f* (Fig. 1b upper), indicating that BChl *f* homologs formed self-aggregates in chlorosomes. The  $Q_y$  and red-most Soret absorption band in the mutant cells were 15-nm blue-shifted compared with wildtype, but the Soret bands on the blue side were almost identical ( $\lambda_{\max} = 450.2$  nm) (Fig. 2a). These spectroscopic relationships between BChl *e* and *f* were very similar to those between BChl *c* and *d*<sup>26</sup>. Notably, the BChl *f*-aggregates in chlorosomes exhibited the most blue-shifted  $Q_y$  peak reported so far among chlorosomes *in vivo*. Such self-aggregates in a BChl *f*-chlorosome have supramolecular structures similar to those in the corresponding BChl *e*-chlorosome, as revealed by similarities between the shapes of the CD spectra of *bchU* mutant and wildtype cells (Fig. 2b); however, the mutant peak positions were blue-shifted relative to the wildtype, except for the 440-nm positive peak.

**Photosynthetic properties of BChl *f*-chlorosomes.** The NIR bands at ~804 nm, observed in both wild and *bchU* mutant cells, were primarily derived from BChl *a* molecules in the chlorosomal baseplate and from FMO proteins as the first and second acceptors of excitation energy from chlorosomal core BChl aggregates, respectively (Fig. 2a). In the corresponding isolated chlorosomes lacking FMO proteins, 10-nm-blue-shifted bands at 793.0 nm (Fig. S3) were ascribed to BChl *a* molecules in baseplate proteins, while a small shift was observed in major chlorosomal  $Q_y$  peaks (704.8 to 703.0 nm). Thus, we estimated the energy gap between BChl *f*-aggregates and BChl *a* to be 90–100 nm (1600–1800  $\text{cm}^{-1}$ ), larger than that in wildtype chlorosomes. To clarify whether chlorosomal self-aggregates of BChl *f* in the *bchU* mutant are photosynthetically active over such a large energy gap, we measured fluorescence emission spectrum of the mutant cells (Fig. 2a inset). We observed singlet excited energy transfer from BChl *f*-aggregates ( $\lambda_{\text{em}} = 723$  nm) to the 810-nm emission species in mutant chlorosomes, similar to the transfer from BChl *e* to BChl *a* in the wildtype. The 810-nm emission species is ascribed to BChl *a* in baseplate and/or FMO proteins, and therefore, we could not discuss here about efficiency of net energy transfer from BChl *f*-aggregates to baseplate-BChl *a* which is next energy acceptor. Although the energy gap in the mutant was larger than in the wildtype, the energy transfer did indeed occur. Growth measurements of the *bchU* mutant cells at several light intensities supported this result; the more intense the illumination was, the faster the mutant grew (Table 1 and Fig. S4). Thus, we conclude that the *bchU* mutant could grow via phototrophic activity of BChl *f*, although the mutant grew 20–30% more slowly than wildtype under all conditions examined. The suppression of mutant growth rate could be due to Förster-type energy transfer in the chlorosomes<sup>19</sup>.



**Figure 2** | UV-Vis-NIR absorption (a) and CD (b) spectra of the cells of *C. limnaeum* wildtype (broken lines) and *bchU* mutant (solid lines). Panel (a) inset shows fluorescence emission spectra of the cells excited at the redmost Soret maxima.

Table 1 | Comparison of growth rates between *C. limnaeum* wildtype and *bchU* mutant under different light-illuminated conditions

Light intensity ( $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ )	Strain	Growth rate ( $\text{h}^{-1}$ ) [relative value]	Doubling time (h)
3	wildtype	$0.033 \pm 0.001$ [1]	21.1
	<i>bchU</i> mutant	$0.023 \pm 0.000$ [0.79]	30.7
30	wildtype	$0.102 \pm 0.007$ [1]	6.8
	<i>bchU</i> mutant	$0.070 \pm 0.003$ [0.69]	9.9
130	wildtype	$0.095 \pm 0.008$ [1]	7.3
	<i>bchU</i> mutant	$0.076 \pm 0.000$ [0.78]	9.2

## Discussion

Here we reported that the first time BChl *f* has been observed *in vivo*. If the *bchU* mutant can grow using photosynthetically active BChl *f* molecules, why has this pigment been never found in nature? Ecological studies revealed that BChl *e*-containing species predominate at the lowest depth in the photic zone, e.g., at 100 m in the Black Sea<sup>12</sup>. In such a region, almost all available light is green-yellow (~530 nm)<sup>21</sup>; BChl *e*-containing species, which possess a Soret band at ~520 nm, can utilize this light energy more efficiently than BChl *c*- or *d*-containing species. In contrast, BChl *f*-chlorosomes, with a Soret band at ~510 nm, capture this light less efficiently. Beyond this disadvantage in light-harvesting efficiency, self-aggregates of BChl *f* exhibit less effective energy transfer to BChl *a* due to their large energy gap. Therefore, BChl *f*-containing bacteria might find it very difficult to survive, especially in habitats where BChl *e*-producing bacteria are already predominant.

As in the study of *C. parvum* and *C. tepidum*<sup>14,16</sup> that demonstrated BChl *U*'s role in converting BChl *d* to BChl *c*, we have demonstrated that in *C. limnaeum* BChl *U* catalyzes conversion of BChl *e* from BChl *f* species. Among the chlorosomal pigments (BChls *c/d/e*), the biosynthetic pathway of BChl *e* is not well understood, especially regarding the formylation at C-7 (oxidation of a methyl group to formyl at C-7). Since the plating strain of *C. limnaeum*, RK-j-1, is now amenable to genetic manipulation, it will be possible to test genes potentially encoding enzyme(s) involved in this oxidation. After the pathway of BChl *e* has been characterized, it will be useful to create isogenic mutants that produce each of the BChls *c/d/e/f* individually or in different combinations. Because of the inherent compositional and morphological heterogeneity of chlorosomes, the structural arrangements of chlorosomal self-aggregates have not yet been determined; several distinct structural models have been proposed<sup>27–29</sup>. Therefore, structural studies on chlorosomes using mutants producing BChl *c*, *d*, *e*, and/or *f* will facilitate understanding of the bioenergetics of GSBs. Furthermore, elucidating the energetics of BChl *f*-chlorosomes, which exhibit the largest energy gap among all known phototroph chlorosomes, will be useful in developing artificial photosynthetic devices, capable of being driven by a wide range of sunlight energies.

## Methods

**Bacterial strains and culture conditions.** We continuously subcultured *Chlorobaculum limnaeum* 1549<sup>22</sup> for about ten years, and obtained a derivative strain, RK-j-1, used as wildtype in this study. This bacterium was anaerobically grown in screw-capped bottles filled with liquid CL media, or on solid CP plates<sup>6</sup>.

For molecular techniques, *E. coli* DH5 $\alpha$  was grown in LB medium containing 100  $\mu\text{g}/\text{mL}$  of ampicillin or 10  $\mu\text{g}/\text{mL}$  gentamycin, as appropriate.

**Determination of DNA sequences of the flanking region of *bchU* gene in *C. limnaeum*.** A partial DNA sequence of the *bchU* gene from *C. limnaeum* 1549 was determined by Maresca *et al.*<sup>14</sup> (Accession No. AY452767), and used for designing primers for inverse PCR amplified genes containing unknown regions of *bchU* and its flanking genes. The genomic DNA of *C. limnaeum* was extracted and digested by restriction enzyme *Hind*III. The resulting DNA fragments were self-ligated and used as the template for inverse PCR with a primer set of inverseU F and R (Table S1). An about 9-kbp DNA fragment containing *bchU* was amplified by the inverse PCR, and then the complete *bchU* gene and its flanking regions (1.9-kbp) were sequenced with an ABI PRISM@ 3100 Genetic Analyzer (Applied Biosystems, California, USA) (Accession No. AB721963).

**Plasmid constructions.** The PCR primers used in this study for the construction of plasmids are described in Table S1. To construct the *C. limnaeum* mutant lacking *bchU*, the plasmid pUCbchUGm (Fig. S1A) was produced as follows. A 1.53-kbp blunt-ended DNA fragment containing the *bchU* gene was amplified from the genomic DNA of *C. limnaeum* using KOD -plus- DNA polymerase (TOYOBO, Osaka, Japan), and primers Uaround F and Uaround R. The PCR product was digested at the *Eco*RI restriction enzyme site designed in Uaround R primer, and cloned into the *Sma*I and *Eco*RI sites of pUC118 (TAKARA, Shiga, Japan), yielding pUCbchU plasmid. To disrupt the unique *Eco*RI and *Bam*HI sites in pUCbchU for after cloning step, this plasmid was digested by *Eco*RI, blunted, and self-ligated, and the resulting plasmid was in turn treated with *Bam*HI, blunted, and self-ligated, producing pUCbchUEB. The *aacC1* gene, conferring resistance to gentamycin, was excised from pUCGM<sup>30</sup> using *Sac*I. After blunting, the gene fragment was sub-cloned into the *Sma*I site of pUC118. From the resulting plasmid, pUCGMF, the *aacC1* gene was excised by the treatment with *Bam*HI and *Eco*RI. To amplify the DNA fragment from pUCbchUEB without the inner portion of *bchU*, the plasmid was used as the template for PCR with primers (the directions of primers shown in Fig. S1A). The resulting PCR product was digested by *Bam*HI and *Eco*RI, (designed in the phaeU F and R primers, respectively), and ligated with the excised *aacC1* gene fragment, yielding pUCTbchUGm.

**Natural transformation of *C. limnaeum* RK-j-1.** The standard protocol for natural transformation was essentially according to the method described for *C. tepidum* by Frigaard and Bryant<sup>7</sup>. Cells from 3 mL of full-growth culture of *C. limnaeum* RK-j-1 were harvested in a microcentrifuge tube, and resuspended in 100  $\mu\text{L}$  of fresh CL medium. The cell suspension was mixed with  $\geq 0.1$  mg of *Hind*III-digested pUCbchUGm, and the mixture was spotted on a CP plate containing no antibiotics. To dry the spot, the plate was incubated in dark for 1 hour at room temperature. Next, the plate was placed upside-down in an anaerobic jar (#HP0031A, OXOID, Hampshire, UK) with catalysis (#BR0042A), an Anaerobic Gas Generating Kit (#BR0038B), and a small tube containing 100 mg of thioacetamide dissolved in 1 mL of aqueous 1 M HCl. The jar was kept in the dark at room temperature for 6–12 hours, and then incubated under illumination at 30°C for 5–7 days. Cells in each spot were scraped off and suspended in 1 mL of CL medium; the cell suspension was diluted and spread on CP plates containing 50  $\mu\text{g}/\text{mL}$  of gentamicin. The plates were placed in jar the same manner as described above, and incubated for 2–3 weeks to allow single colonies to appear.

PCR analysis was carried out to monitor segregation of wildtype and mutant alleles using primers limnI comf. F and R. A DNA molecular weight marker,  $\lambda$ /StyI digest (TOYOBO, Osaka, Japan), was used for molecular mass estimations of PCR products. Furthermore, DNA sequence analysis of the PCR fragments was performed using primers Uaround F, Uaround R, seq1, and seq2.

**Determination of compositions of BChl *f* homologs.** Pigments of the *C. limnaeum* mutant strain were extracted and analyzed as follows. A mixture of acetone and methanol (9:1, v/v) was added to the harvested cells and mixed using a vibrator. A mixture of diethyl ether and petroleum ether (1:1, v/v) and then distilled water were added to transfer the pigment components to the ether layer. The ether phase was collected and evaporated to dryness under a stream of N<sub>2</sub> gas, and the residues were dissolved in a small amount of HPLC eluent, described below. HPLC analysis was performed using a Shimadzu LCMS-2010EV system (Shimadzu, Kyoto, Japan) comprising a liquid chromatograph (SCL-10Avp system controller, LC-10ADvp pump, and SPD-M10Avp photodiode-array detector) and a quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) probe. Reverse-phase HPLC was performed under the following conditions: column, Cosmosil 5C<sub>18</sub>-AR-II (6.0  $\phi$  x 250 mm, Nacalai Tesque, Kyoto, Japan); eluent, acetonitrile:acetone:H<sub>2</sub>O (70:15:15, v/v/v); flow rate, 1.25 mL/min; detection wavelength, 455 nm. APCI-MS spectra were measured as follows: resolution,  $\pm 0.15$  Da; capillary temperature, 250°C; APCI vaporizer temperature, 400°C; ionization voltage, 4.5 kV; sheath gas flow, 2.5 L/min; drying gas pressure, 0.02 MPa.

**Isolation and structural determination of R[E,E]BChl *f*.** Pigment components were extracted from harvested *C. limnaeum* mutant cells as described above. R[E,E]BChl *f*, a major homolog of BChl *f* (corresponding to peak #6 in Fig. 1a lower), was purified by HPLC as follows: column, Cosmosil 5C<sub>18</sub>-AR-II (10  $\phi$  x 250 mm, Nacalai Tesque); eluent, methanol:H<sub>2</sub>O (95:5, v/v); flow rate, 1.5 mL/min. The 600 MHz <sup>1</sup>H-NMR spectra of the isolated R[E,E]BChl *f* were recorded in tetrahydrofuran (THF)-*d*<sub>8</sub> (Euriso-top, Saclay, France) using a JEOL ECA-600 NMR spectrometer (JEOL,



Akishima, Japan); the residual proton at the 3-position of THF- $d_7$  ( $\delta_H = 1.72$  ppm) was used as an internal standard. A set of assignments of  $^1H$  signals was obtained from correlation spectroscopy (COSY) and rotating frame Overhauser enhancement spectroscopy (ROESY) ( $\tau_m = 400$  msec).

**Spectroscopy measurements of bacterial cells, isolated chlorosomes and pigments.** UV-Vis-NIR absorption spectra were measured using a Hitachi U-3500 (Hitachi High-Tech, Tokyo, Japan). Steady-state fluorescence emission spectra were measured using a C9920-03G series fluorescence measurement system (Hamamatsu Photonics, Shizuoka, Japan). CD spectra were observed with a Jasco J-720W spectropolarimeter (Jasco, Tokyo, Japan). Cells of *C. limnaeum* strains grown in the early stationary phase under irradiation  $\sim 30 \mu E \cdot s^{-1} \cdot m^{-2}$  were collected, suspended in 50 mM Tris-HCl (pH 8.0), and used for UV-Vis-NIR absorption and CD spectra measurements. For fluorescence spectrum measurements, the cell suspension was mixed with 20 mM sodium dithionite and incubated for 30 min. Isolation of chlorosomes from 1 L full growth culture of the *C. limnaeum* wildtype and *bchU* mutant proceeded as previously described<sup>31,32</sup>.

**Growth rate measurements of *C. limnaeum bchU* mutant.** Pre-cultures of the wildtype and *bchU* mutant of *C. limnaeum* were grown to stationary phase under irradiation at  $\sim 30 \mu E \cdot s^{-1} \cdot m^{-2}$ . Three hundred  $\mu L$  aliquots of pre-cultures were inoculated into 30 mL screw-capped test tubes filled with freshly prepared CL media, and cultures were then grown under illumination of 3, 30, or 130  $\mu E \cdot s^{-1} \cdot m^{-2}$  at 30°C. Optical cell densities were measured at 660 nm using a Mini Photo 518R photometer (TAITEC, Saitama, Japan).

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## Author contributions

J.H., M.N., and H.T. conceived the project. J.H. performed the mutational analyses and bacterial growth measurements. J.H. and T.M. contributed spectroscopic analysis of the *bchU* mutant cells and its pigments. T.M. determined the compositions of BChl *f* homologs in the mutant, and structure of R[E,E]BChl *f*. Y.T. isolated chlorosomes from the mutant, and measured the UV-Vis-NIR absorption spectra. J.H., Y.T. and H.T. analyzed the data, interpreted the results and wrote the manuscript. All authors edited the manuscript.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

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