| Title | Mutations in hik26 and slr1916 lead to high-light stress tolerance in Synechocystis sp. PCC6803 |
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Mutations in \textit{hik26} and \textit{slr1916} lead to high-light stress tolerance in \textit{Synechocystis} sp. PCC6803

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Increased tolerance to light stress in cyanobacteria is a desirable feature for their applications. Here, we obtained a high light tolerant (Tol) strain of \textit{Synechocystis} sp. PCC6803 through an adaptive laboratory evolution, in which the cells were repeatedly sub-cultured for 52 days under high light stress conditions (7000 to 9000 \(\mu\text{mol m}^{-2} \text{s}^{-1}\)). Although the growth of the parental strain almost stopped when exposed to 9000 \(\mu\text{mol m}^{-2} \text{s}^{-1}\), no growth inhibition was observed in the Tol strain. Excitation-energy flow was affected because of photosystem II damage in the parental strain under high light conditions, whereas the damage was alleviated and normal energy flow was maintained in the Tol strain. The transcriptome data indicated an increase in \textit{isiA} expression in the Tol strain under high light conditions. Whole genome sequence analysis and reverse engineering revealed two mutations in \textit{hik26} and \textit{slr1916} involved in high light stress tolerance in the Tol strain.
Cyanobacteria are promising host microorganisms for the production of industrial bio-molecules because these cells can directly fix carbon dioxide through the Calvin Benson cycle and converted it into target compounds using light energy. However, excess light, such as in summer, damages their photosystem and inhibits photosynthetic activity. Because high light (HL) stress is one of the major obstacles in the bio-production of cyanobacteria, it is highly desired to develop an HL tolerant strain for enhancing their growth and production rate of various bio-molecules for industrial applications.

Cyanobacteria can adapt their cellular system to survive under HL stress conditions by reducing their light-harvesting antenna size and decreasing contents of chlorophyll and photosystem I (PSI)1-6. Furthermore, they possess some mechanisms for preventing photoinhibition by allowing excess light energy to escape, such as state transition7, heat dissipation8, energy dissipation from phycobilisome and photosystem II (PSII)9,10, and synthesis and maintenance of protein complexes in thylakoid membrane by chaperone proteins11. However, because these mechanisms are insufficient for protecting the cellular system from excess light energy under further strong light intensity conditions (more than 2000 μmol m^{-2} s^{-1}), the cell growth is reduced due to inhibition of photosynthetic activity caused by damage to their photosystems. It has been reported that degradation of the PSII reaction center12 and inhibition of protein synthesis and repair of PSII protein13 by reactive oxygen species occur as by-products of photosynthesis. Although some mutants with reduced light-harvesting pigment content and minimized light-harvesting antenna size have been developed in cyanobacteria for improving the HL stress tolerance in previous studies, such strains have certain drawbacks that cause growth defects under low light conditions14,15. Furthermore, transcriptome analysis revealed a downregulation of genes related to light-harvesting systems, flagellum synthesis, and transhydrogenase, and upregulation of D1 protein turnover-related genes under excessive HL conditions16. However, effective mechanisms for protecting cells from excess light without decreasing the antenna size are still unknown.

Adaptive laboratory evolution (ALE) experiment is a method used to induce tolerance against stress conditions17. A serially passaged culture is performed under a stress environment with different phenotype or genotype strains, four single colonies of the Tol strain were isolated and named Tol(S1), Tol(S2), Tol(S3), and Tol(S4). All the four strains showed identical growth curves as that of the Tol strain under both low light and HL conditions (Supplementary Fig. 2). These results suggest that the population in the Tol strain was entirely composed of the strain possessing HL tolerance and high growth under low light conditions.

High light tolerant mechanism of Tol strain. The photosynthesis ability of the Tol strain obtained by the ALE experiment was investigated. Chlorophyll is an important pigment for light harvesting during photosynthesis. It has been reported that the amount of chlorophyll decreases to avoid absorption of excess light under HL conditions22. The chlorophyll contents of the PCC6803 and Tol strains under various light conditions are shown in Fig. 3. In the PCC6803 strain, chlorophyll content under light conditions of 7000 and 9000 μmol m^{-2} s^{-1} was 52% and 64% lower, respectively, than that under 4000 μmol m^{-2} s^{-1}, which was consistent with a previous report22. In the Tol strain, chlorophyll content was only decreased by 21% and 42% under 7000 and 9000 μmol m^{-2} s^{-1}, respectively. These results suggest that the Tol strain possesses a mechanism for HL tolerance other than decreasing the chlorophyll content.

In the cultures of PCC6803 and Tol strains under each light condition, absorption spectra of the cells were analyzed at 48 h (Fig. 4a). The phycobilisome and chlorophyll exhibited absorption bands around 620 and 675 nm, respectively. Although the relative content of chlorophyll to phycobilisome decreased as the light intensity increased in the PCC6803 strain, it was stable in the Tol strain. The comparison of absorption spectra suggests the relative carotenoid content in the Tol strain is decreased compared to PCC6803 strain. The relative intensities of PSII fluorescence (680–700 nm) under light conditions of 4000 and 7000 μmol m^{-2} s^{-1} were almost identical in the PCC6803 strain (Fig. 4b), whereas it increased under 9000 μmol m^{-2} s^{-1}, thereby suggesting a damage to PSII (Fig. 4c). By contrast, an increase in PSII fluorescence was not observed in response to a change from conditions of 4000 μmol m^{-2} s^{-1}, it was decreased under 7000 μmol m^{-2} s^{-1} and almost stopped under 9000 μmol m^{-2} s^{-1} (Fig. 1).
Fig. 1 Cell growth of PCC6803 and Tol strains under different light intensity conditions. Cultures under 40 μmol m$^{-2}$ s$^{-1}$ were performed using an LED plate, and cultures under other light intensities (4000, 7000, and 9000 μmol m$^{-2}$ s$^{-1}$) were performed using point source LED. All photos of cultures were taken at 72 h. No further growth was observed in the PCC6803 strain after 72 h under the 9000 μmol m$^{-2}$ s$^{-1}$ (Supplementary Fig. 1). The filled blue and red circles indicate average of OD$_{730}$ measurements of PCC6803 and Tol strains, respectively. Error bars indicate standard deviation from triplicate experiments. The open symbols represent individual data points in the triplicate experiments.

Fig. 2 ALE experiment under high light stress condition. a Experimental design of the ALE experiment. Cells were cultured in a test tube using a point source LED. Among four parallel cultures, the culture broth with the highest growth rate was inoculated into a fresh medium every 2-3 days. b Changes in specific growth rate during ALE experiment. Line plot indicates specific growth rate and bars indicate light intensity at each subculture experiment.

Fig. 3 Chlorophyll content of PCC6803 and Tol strains under different light intensities. The chlorophyll contents were measured at 48 h for all conditions. The bars and plots represent averages and individual data points in the biological triplicate experiments. Error bars indicate standard deviation from the triplicate experiments.
Fig. 4 Characterization of photosynthetic abilities in PCC6803 and Tol strains. 

a Absorption spectra normalized at the chlorophyll Qy band. 

b Fluorescence spectra normalized at the PSI band (<725 nm). 

c Fluorescence decay curves of PSII normalized to maximum intensities. 

d Fluorescence decay curves of PSI normalized to the maximum intensities. Graphs to the left and right represent PCC6803 and Tol strains, respectively. Blue, purple, and red lines represent 4000, 7000, and 9000 μmol m$^{-2}$ s$^{-1}$, respectively. The absorption spectra, fluorescence spectra, and fluorescence decay curves were measured at 48 h for all conditions.
7000 to 9000 μmol m$^{-2}$ s$^{-1}$ for the Tol strain. This result suggests that the ratio between PSII and PSI was maintained in the Tol strain under HL conditions.

As PSII in the Tol strain was observed to be stable under HL conditions (Fig. 4b), the excitation-energy flows in PSII and PSI were analyzed by time-resolved fluorescence spectroscopy (Fig. 4c, d). In the PCC6803 strain, a decrease in fluorescence decay rate in PSII was observed as the light intensity increased, suggesting an impairment of PSII. On the contrary, fluorescence decay curves were almost identical in the Tol strain regardless of the light intensity, suggesting the normal energy flow in PSII under HL conditions. However, a decrease in the fluorescence decay rate was not observed in PSI under HL conditions in both PCC6803 and Tol strains. These findings suggest that the growth inhibition of the PCC6803 strain under HL conditions was caused by a change in energy flow due to PSII damage. As the damage was alleviated and energy flow was maintained at normal state in the Tol strain, the cells could remain stable under HL condition without growth inhibition.

### Functional analysis of transcriptome data.

Transcriptome analysis was performed using a DNA microarray to understand the HL tolerance mechanisms in the Tol strain. Four sets of microarray data were obtained for the PCC6803 and Tol strains grown under 4000 and 7000 μmol m$^{-2}$ s$^{-1}$. The microarray data of the PCC6803 and Tol strains under 4000 and 7000 μmol m$^{-2}$ s$^{-1}$ were designated as W-4000 and W-7000, and T-4000 and T-7000, respectively. Gene expression data for 3527 genes spotted on the microarray were obtained in all four conditions (Supplementary Data 1).

Table 1 summarizes the number of genes showing significant difference in gene expression levels between the two conditions. Differences were compared with the two-tailed Student’s t test. To correct the p values for multiple comparisons, the q values were calculated with considering false discoveries by Storey’s method. When the expression data between 4000 and 7000 μmol m$^{-2}$ s$^{-1}$ were compared for each condition, only a small number of genes showed different expression levels (0.1% between W-4000 and W-7000; 0.1% between T-4000 and T-7000). This result suggested that the 4000 μmol m$^{-2}$ s$^{-1}$ condition for the Tol strain is also an HL condition for PCC6803. In the comparison between W-7000 and T-7000, the expression levels of 28% genes shows significant difference between PCC6803 and Tol strains under 7000 μmol m$^{-2}$ s$^{-1}$.

To identify genes whose expression was significantly altered in the Tol strain compared to those in PCC6803 strain, the ratios of numbers indicate the number of genes whose expression levels were significantly different between the two conditions among 2942 genes (q value < 0.05). The numbers in parenthesis indicate the percentage of the identified genes of the 2942 genes.

| Table 1 Number of genes showing significant difference in expression levels. |
|---|
| **W-4000** | **W-7000** | **T-4000** | **T-7000** |
| W-4000 | - | 2 (0.1%) | 13 (0.4%) | 455 (15.5%) |
| W-7000 | - | - | 147 (5.0%) | 824 (28.0%) |
| T-4000 | - | - | - | 2 (0.1%) |
| T-7000 | - | - | - | - |

### Effect of isiA overexpression on HL tolerance.

Since the isiA expression level in T-7000 was higher than that in T-4000 and W-7000, the enhanced expression of isiA might have been involved in HL tolerance in the Tol strain. To confirm this hypothesis, an isiA overexpressed strain of PCC6803, named PCC6803/isiA, was constructed by introduction of isiA, controlled under a strong promoter of psbA2, to the neutral site of PCC6803. The PCC6803/isiA showed increased growth under HL conditions (7000 and 9000 μmol m$^{-2}$ s$^{-1}$) compared with its parent strain PCC6803 (Fig. 5d). An increase in PSI fluorescence due to isiA overexpression has been reported, which is consistent with the results of chlorophyll fluorescence and fluorescence decay curve in the Tol strain under HL condition in our study.

However, the HL tolerance of the PCC6803/isiA was lower than that of the Tol strain (Fig. 5b), suggesting there are other mechanisms contributing toward HL tolerance of the Tol strain. isiA encodes CP43, which is required for the formation of PSI supercomplex and efficient state transition and is necessary for HL tolerance. Consequently, in our study, higher expression of isiA in the Tol strain may have enhanced non-photochemical quenching and efficient electron transfer under HL condition, thus increasing HL tolerance. Furthermore, because the isiA-isl0249 is an operon on the genome, the expressions of isiB and slr1916 as well as isiA were increased in the Tol strain (Table 2). Therefore, overexpression of these three genes may lead to stronger HL tolerance.

### Whole-genome sequence of the HL tolerant strain.

To identify mutations in the Tol strain, whole-genome sequences of PCC6803 and Tol strains were analyzed by a MiSeq Illumina sequencer using a 150 bp paired-end method. The mapping results that were compared to a reference genome sequence of Synechocystis sp. PCC6803 GT-I strain (NCBI Reference Sequence No.: NC_017038.1) showed around 100% coverage of the whole-genome sequence and average 500 read depth for each nucleotide sequence in both the strains (Supplementary Table 1). In the PCC6803 strain, seven mutations were identified compared with the reference genome sequence. In the Tol strain, additional two non-synonymous mutations were introduced in hik26 and slr1916 (Supplementary Table 2). slr1916 is annotated as a probable esterase, and its deletion was reported to cause increased PSI content under HL condition. hik26 encodes a two-component sensor histidine kinase, but its responsive stress conditions and response regulators have not been identified.

### Effects of the hik26 and slr1916 mutations on HL tolerance.

Effects of the identified mutations on HL tolerance were analyzed by deletion of hik26 or slr1916 in PCC6803 and Tol(S1) strains, and introduction of mutated hik26 or slr1916 into PCC6803. The slr1916 was deleted in both the PCC6803 and Tol(S1), generating 6803Δslr1916 and Tol(S1)Δslr1916, respectively. The 6803Δslr1916 strain could grow under 9000 μmol m$^{-2}$ s$^{-1}$ similar to the Tol strain and its culture broth showed higher viscosity (Fig. 5e). The Tol(S1)Δslr1916 strain maintained HL tolerance but its broth did not show high viscosity (Fig. 5f). These results indicate the possibility of the mutation of slr1916 in the Tol strain being a loss-of-function mutation, which may have increased HL tolerance. However, the 6803Δslr1916 and Tol strains showed different phenotypes, such as high viscosity and cell aggregation.
The different phenotypes between the Tol(S1)Δslr1916 and the 6803Δslr1916 strains may have been caused due to synergy effects of the slr1916 deletion and hik26 mutation.

The deletion strains of hik26 in PCC6803 and Tol(S1), designated as 6803Δhik26 and Tol(S1)Δhik26, showed severe growth inhibition under HL conditions and similar growth rate as that of with PCC6803 strain under low light condition (40 μmol m⁻² s⁻¹) (Fig. 5g, i). This indicates that hik26 is specifically needed for HL tolerance. To analyze the effect of the mutation of hik26 on HL tolerance, mutated hik26 (hereafter called as hik26m) with its own promoter region was amplified from the Tol(S1) strain and introduced into the genome of the 6803Δhik26, generating 6803Δhik26/hik26m. The 6803Δhik26/hik26m showed identical growth as that of the Tol strain under all conditions, even under 9000 μmol m⁻² s⁻¹ (Fig. 5); however, high viscosity and cell aggregation were not observed in the 6803Δhik26/hik26m. Both the hik26 mutation and slr1916 deletion also produced HL tolerance. Therefore, it seems that the mutations in either gene can cause HL tolerance, and the phenotype of the Tol strain could be attributed to either or both. hik26 encodes a histidine kinase, which is a component of two-component sensor-transducer system, but environmental conditions that activate Hik26 and its response regulator have not been identified. A deletion of hik26 caused severe growth inhibition under HL conditions, and introduction of hik26m improved HL tolerance, thus suggesting that Hik26 probably senses HL and regulates the genes required for HL tolerance through a response regulator that has not yet been identified. As well as hik26, mutated slr1916 (hereafter called as slr1916m) with its own promoter region was amplified from the Tol (S1) strain and introduced into the genome of the 6803Δslr1916, generating 6803Δslr1916/slr1916m. The 6803Δslr1916/slr1916m showed almost identical growth as that of the Tol and the 6803Δslr1916 strains under all conditions (Fig. 5g). Furthermore, the culture broth of PCC6803Δslr1916/slr1916m showed high viscosity and cell aggregation under the HL conditions, as well as PCC6803Δslr1916. These results suggest that the mutation of slr1916 in the Tol strain is loss-of-function mutation.

### Discussion

In the present study, an HL stress tolerant (Tol) strain of *Synechocystis* sp. PCC6803 was obtained by an ALE experiment under extreme HL stress condition (9000 μmol m⁻² s⁻¹). A previous study has reported that size reduction of the light-harvesting antenna increased HL tolerance but decreased the growth under low light condition. However, we observed that the growth rate of the newly obtained Tol strain was identical to that of the PCC6803 strain under low light condition (40 μmol m⁻² s⁻¹), suggesting that the Tol strain possesses a different mechanism to overcome HL stress. This is consistent with the findings that the decrease in chlorophyll content due to HL stress was partially alleviated in the Tol strain. Furthermore, evaluation of photosynthesis machineries revealed the HL tolerance mechanisms, which indicated that the PSII complex and the electron flow from PSI were maintained even under HL conditions in the Tol strain.

The HL tolerance mechanism in the Tol strain was also investigated based on transcriptome data of PCC6803 and Tol strains and known HL-responsive genes which are commonly induced or repressed under HL conditions. Although a comparison between W-4000 and T-4000 showed that the number of genes whose expression was significantly changed was small (0.4%); a comparison between W-7000 and T-7000 showed that the expression levels of many genes were changed (28%). Consequently, a change in expression of these genes would have contributed to the superior HL tolerance in the Tol strain. The transcriptome data revealed that isiA and isiB expressions were highly induced in the Tol strain under HL condition. The

### Table 2 Top 10 genes of increased or decreased expression levels with significant q values in Tol strain under HL condition (7000 μmol m⁻² s⁻¹).

| ORF ID   | Gene name | Function                                | Functional category            | Expression ratio | q value |
|----------|-----------|-----------------------------------------|--------------------------------|------------------|---------|
| sll0247  | isiA      | Iron-stress chlorophyll-binding protein | Photosystem II                 | 27               | 0.012   |
| sll0248  | isiB      | Flavodoxin                              | Soluble electron carriers      | 9.5              | 0.024   |
| sss2920  | ~         | Hypothetical protein                    |                                 | 7.7              | 0.014   |
| slr1417  | ycf57, isiA | Hypothetical protein                     |                                 | 7.5              | 0.010   |
| slr1054  | ~         | Hypothetical protein                    |                                 | 7.3              | 0.007   |
| sss2194  | ~         | Unknown protein                         |                                 | 6.9              | 0.044   |
| slr0249  | ~         | Hypothetical protein                    |                                 | 6.3              | 0.018   |
| slr5055  | ~         | Similar to UDP-α-acetyl-α-mannosaminuronic acid transferase | | 5.6              | 0.049   |
| slr1913  | ~         | Hypothetical protein                    |                                 | 5.3              | 0.023   |
| slr1282  | ISY508b   | Putative transposase                    | Transposon-related functions   | 5.2              | 0.013   |
| slr0226  | ~         | Photosystem I assembly related protein  | Photosystem I assembly related protein | 0.24 | 0.009   |

*Geometric mean of triplicate experiments under each condition was used for calculating the ratio of gene expression level in Tol strain to that in PCC6803 strain.*
**Fig. 5** Reverse engineering based on transcriptome and whole-genome re-sequencing analyses. 

- **a** PCC6803
- **b** Tol
- **c** Tol(S1)
- **d** 6803/OE-isiA
- **e** 6803Δslr1916
- **f** Tol(S1)Δslr1916
- **g** 6803Δslr1916/slr1916m
- **h** 6803Δhik26
- **i** Tol(S1)Δhik26
- **j** 6803Δhik26/hik26m

Culture under a light intensity of 40 μmol m⁻² s⁻¹ was performed using an LED plate, and cultures under other light intensities (4000, 7000, and 9000 μmol m⁻² s⁻¹) were performed using point source LED. The filled and open circles represent averages and individual data points in the biological triplicate experiments. Error bars indicate standard deviation from triplicate experiments. Note that the errors of the growth curve of the 6803Δhik26 strain under 4000 μmol m⁻² s⁻¹ were large because in the triplicate experiments similar growth as that of PCC6803 was observed in one culture experiment and severe-inhibited growth was displayed in remaining two culture experiments. In the 6803Δslr1916/slr1916m and 6803Δhik26/hik26m strains, the mutated genes were introduced at the neutral sites, not at their natural positions.
isiA was originally observed to be an iron deficiency stress-inducible gene, and it functioned as an antenna protein for PSI under iron-limiting condition. It has been reported that the isiA and isiB are induced by various stress conditions. For example, the gene expression is highly induced by hydrogen peroxide, which is generated under an extremely HL condition. The isiA deletion inhibits a redistribution of phycobilisome-absorbed energy between PSII and PSI and causes a growth defect under HL condition. Furthermore, the isiAB deletion resulted in a photosensitive phenotype under HL condition, with accumulation of reactive oxygen species and cell bleaching. Recently, it has been reported that IsiA is a member of a high light-inducible carotenoid-binding protein complex (HLCC) on a thylakoid membrane, and a lack of HLCC components causes impaired state transition and increased sensitivity to HL stress. The HLCC also plays a role in protecting the thylakoid membrane and D1 protein of PSI from oxidative stress. Therefore, the enhanced expression of IsiA in the Tol strain under HL condition would promote the formation of HLCC, which involves in the state transition for processing excess light energy, thus protecting the thylakoid membrane and D1 protein from oxidative stress.

Two mutations of slr1916 and hik26 in the Tol strain were identified by whole-genome sequencing, and the function was evaluated by reverse engineering. Especially, Hik26, a histidine kinase of a two-component system, can regulate many genes through a response regulator. Since the expression level of hik26 was not significantly altered in all the conditions, the mutation in hik26 might activate Hik26 at enzymatic level and affect its regulated gene expression level. The non-synonymous mutation in Hik26m (T29K) was located at the sensor domain predicted by NCBI database (ACCESSION No. YP_005652307.1). This suggests that the mutation might activate Hik26 and affect the expression levels of its regulatory genes. Considering functional analysis data and known HL-responsive genes, the known induced HL-responsive genes tended to be highly expressed and repressed HL-responsive genes were poorly expressed in the Tol strain than in PCC6803. The HL tolerance mechanisms related to these genes might have been more activated in the Tol strain, thereby increased HL tolerance. Moreover, these genes may be regulated through Hik26, and the mutation in hik26 might have caused these expression changes. Homology search using BLASTP (KEGG database) indicated that the homologous protein Hik26 was conserved in many cyanobacteria species except in PCC6803. BLASTP (KEGG database) indicated that the homologous protein Hik26 was conserved in many cyanobacteria species except in PCC6803. BLASTP (KEGG database) indicated that the homologous protein Hik26 was conserved in many cyanobacteria species except in PCC6803. The enhanced expression of Hik26 in the Tol strain may activate the histidine kinase activity. The Hik26 induces the expression of genes including isiA through a response regulator. The isiA contributes to protect the membrane proteins from the oxidative stress caused by HL conditions, and involves in light state transition from PSII to PSI.

Methods

Strains and culture conditions. The Synechocystis sp. PCC6803 glucose-tolerant, nonmotile, GT-1 strain was used as the parent strain. A slightly modified BG11 medium was used for the culture experiments. Pre-cultured cells were cultivated in 100 mL Erlenmeyer flasks with 20 mL modified BG11 medium at 34 °C with rotary agitation at 150 rpm (BR-40FL, TAITEC, Japan), under continuous light illumination using a light-emitting diode (LED) plate (about 40 µmol m$^{-2}$ s$^{-1}$; LC-LED 450 W, TAITEC). The pre-cultured cells (OD$\text{OD}_{730}=0.1$) were inoculated into fresh modified BG11 medium. The main culture was performed using a test tube (q$\phi$=20 cm, AGC Techno Glass, Shizuoka, Japan) containing 30 mL modified BG11 medium at 34 °C under continuous light illumination at 4000–9000 µmol m$^{-2}$ s$^{-1}$ using a point source LED (LA-HDF158KS, HAYASHI Non-electric Co. Ltd, Tokyo, Japan). The spectrum of the point source LED is shown in Supplementary Fig. 4. The cultures were aerated by mixing with sterile air. The light intensity (4000–9000 µmol m$^{-2}$ s$^{-1}$) used in the present study was significantly higher compared to that used in other studies. In our previous report, the PCC6803 strain exhibited photoinhibition when cultured under 1100–1300 µmol m$^{-2}$ s$^{-1}$. Although a flat LED panel with the photobioreactor was used in the previous study, we here culture the cells in a test tube with a point source LED that can irradiate more HL intensity. The light intensity was measured at the irradiate point on the surface of the test tube. Therefore, the normalized light intensity at the entire test tube becomes low. In the PCC6803 strain, although 70% growth reduction was observed at 1100 µmol m$^{-2}$ s$^{-1}$ with the flat LED device, that was observed at 7000 µmol m$^{-2}$ s$^{-1}$ with this point source LED device.

For the ALE experiment, cells were cultivated in a test tube with 30 mL of modified BG11 medium at 34 °C with aeration under continuous light illumination using a point source LED. Cells were inoculated into fresh medium every 2–3 days to maintain the growth phase. The specific growth rate was calculated from the values obtained at initial and final OD$\text{OD}_{730}$ for each cultivation. Light intensity was set to 7000 µmol m$^{-2}$ s$^{-1}$ at the initial stage of this experiment. After the growth rate increased under a light intensity of 7000 µmol m$^{-2}$ s$^{-1}$, the intensity was gradually increased up to 9000 µmol m$^{-2}$ s$^{-1}$. After the final cultivation, cells which were designated as the Tol strain, were stored at −80 °C with 15% glycerol solution. A frozen stock of the Tol strain obtained by ALE experiment was streaked on a plate containing modified BG11 medium and incubated under a light intensity of 40 µmol m$^{-2}$ s$^{-1}$ at 34 °C using an incubator MIR 154 (Sanyo Electric, Tokyo, Japan). Single colonies thus obtained were inoculated in modified BG11 medium and cultured at 34 °C with rotary agitation at 150 rpm (BR-40FL, TAITEC, Japan) under continuous light illumination by an LED plate (40 µmol m$^{-2}$ s$^{-1}$; LC-LED 450 W, TAITEC).

Construction of strains. All strains used in this study are listed in Supplementary Table 3. A slr1916-deleted strains of the PCC6803 and single-colony-isolated Tol(S1) strains, designated as 6803Δslr1916 and Tol(S1)Δslr1916, respectively, were constructed by replacing slr1916 with a chloramphenicol-resistance gene. For constructing the 6803Δslr1916 strain, mutated slr1916 (slr1916m) with its promoter region was amplified from genome DNA of the Tol(S1) strain and was introduced into a neutral site located in slr0168 of 6803Δslr1916 strain. The hik26-deleted strains of the PCC6803 and Tol(S1) strains, named PCC6803Δhik26 and Tol(S1)Δhik26, were constructed by replacing hik26 with a kanamycin-resistance gene. For constructing the 6803Δhik26 strain, mutated hik26 was inserted into the genome DNA of the Tol(S1) strain with its promoter region was amplified from genome DNA of the Tol(S1) strain.
and was introduced into a neutral site located at the downstream of the stop codon of mdtB in the 6803/hik26 strain. For constructing the isis overexpressed strain, named 6803hik26::isis, isis was amplified from genomic DNA of PCC6803. The isis gene with a strong phbA2 promoter was introduced into a neutral site located in strain 6803 of PCC6803 strain. The procedures for strain constructions are described in Supplementary Methods. The primers used in this study are listed in Supplementary Table 4.

**Analytical methods.** Cell concentration was measured as OD730 using a spectrophotometer (UVMmini-1240, Shimadzu, Kyoto, Japan). For measuring chlorophyll concentration, 1 ml of culture was centrifuged at 15,000 rpm for 10 min at 4 °C, and 1 ml of 100% methanol was added to the cell pellet. After 15 min, the sample was centrifuged at 15,000 rpm for 10 min at 4 °C, and absorbance of the supernatant thus obtained was measured at 660 nm (A660) by a spectrophotometer (UVMini-1240, Shimadzu, Kyoto, Japan). Chlorophyll concentration was calculated by the following formula

\[
\text{Chlorophyll concentration (μg ml}^{-1} \text{ OD}^{-1} \text{)} = (13.5 \times A_{660}^{-1} - 8.3 \times A_{680}^{-1}) / \text{OD}_{730}
\]

For measuring absorption spectra, steady-state fluorescence spectra, and time-resolved fluorescence spectra, cells were grown under light conditions of 4000, 7000, and 9000 μmol m^{-2} s^{-1}, and were subsequently collected at 48 h. Next, the samples were dark adapted for 10 min at 34 °C, and centrifuged. Pellets were suspended in 30% polyethylene glycol solution to final concentrations of OD730 = 2, and the light of the samples was stored in liquid nitrogen. The absorption spectra of whole cells were measured using a spectrophotometer (V-650/IVSC/747, JASCO, Tokyo, Japan) at 77 K. Steady-state fluorescence spectra were measured with a spectrophotometer (FP-6600/PMU-183, JASCO, Tokyo, Japan) at 77 K. The excitation wavelength was 440 nm. Fluorescence decay curves were measured using a time-correlated single-photon counting system at 77 K. Excitation wavelength was 440 nm.

**Whole-genome sequencing.** Total DNA of PCC6803 and tolerant strains were extracted using the Genomic DNA Buffer Set and QiAEN Genomic-tips 100/G (Qiagen, CA, USA). A DNA library was prepared using Truseq DNA PCR-Free LT sample prep kit (Illumina, CA, USA) and KAPA DNA library quantification kit (KK4804, KAPA Biosystems, MA, USA), and the sample was sequenced using MiSeq sequencer with MiSeq Reagent kit v2 (Illumina) generating 150 bp paired-end reads. The sequence data were mapped to the genome sequence of PCC6803 GT-1 strain (NCBI Reference Sequence: NC_017038.2) using bowtie 2 software version 2.2.3 with default parameters59. SNPs were identified using SAMtools ver 1.0 and BCfTools ver 1.052 with a threshold of a quality score of >150. The sequence data obtained from MiSeq was deposited in DDBJ Sequence Read Archive under accession number DRA010198. The mutations identified by whole-genome sequencing were confirmed by Sanger sequencing using DNA fragments, including the mutation site amplified by PCR using the primer pair listed in Supplementary Table 4.

**DNA microarray.** DNA microarray analysis was performed as described in our previous study with minor modifications.66 A custom design microarray of PCC6803 was designed using a web tool eArray (Agilent Technologies) and genomic sequences of open reading frame of PCC6803 genome and its cryptic plasmids, which were acquired using an Agilent G2565CA microarray scanner and Feature extraction software (Agilent Technologies). Detection was carried out using an Agilent Input Quick Amp WT Labeling Kit (Agilent Technologies), Cyanine 3-CTP peroxidase using Cy3; hybridization and washing were performed using a Low Ribopure yeast kit (Life Technologies Co., Carlsbad, CA). cDNA labeling was performed using Cy5 and cyanine peroxidase using Cy5; hybridization and washing were performed using a Low Ribopure yeast kit (Life Technologies Co., Carlsbad, CA). Microarray data were analyzed using Matlab 2014a (Mathworks Inc., Natick, MA). In this study, the median of the intensities of the ten probes representing each ORF was defined as the expression level of the corresponding gene.

**Statistics and reproducibility.** Expression data were normalized by quantile normalization to compare the data to each other. The geometric mean of the median fluorescence intensity of each gene obtained from triplicate experiments under each condition was used as the representative gene expression level. Gene expression data sets were deposited in Gene Expression Omnibus under accession number GSE149892. The significance of the difference of each gene expression level between the two conditions was analyzed by two-tailed t-test. To correct the p values for multiple comparisons, the q values were calculated by Storey’s method.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** The whole-genome sequence data were deposited in DDBJ Sequence Read Archive under accession number DRA010198. Gene expression data sets were deposited in Gene Expression Omnibus under accession number GSE149892. All source data for main figures as a Supplementary Data 2.

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