Light limitation inducing overcompensatory growth of cyanobacteria and function of serine/threonine kinase (STK) genes involved

Wei Dai, Gao Chen, Xiangdong Bi, Huairong Zhong, Xueying Wang, Shaojie Dong, Dong Lv, Shulin Zhang, Dajuan Zhang and Na Wang

ABSTRACT

The rapid overcompensatory growth that appears when cyanobacteria are supplied with adequate resources after a period of resource deprivation might contribute to the occurrence of cyanobacterial blooms. We investigated the changing characteristics of overcompensatory growth and serine/threonine kinase (STK) genes expression of cyanobacterium Microcystis aeruginosa in response to light limitation. The results showed M. aeruginosa exhibited overcompensatory growth for 2 days after light recovery, during which the increase in growth was inversely related to light intensity. Expression of STK genes, such as spkD, was upregulated significantly at 0.5–4 h after light recovery (P < 0.05).

To investigate the function of STK genes in the overcompensatory growth, M. aeruginosa spkD was heterologously expressed in Synechocystis. Transgenic Synechocystis exhibited greater and longer overcompensatory growth than wild-type Synechocystis after light recovery. Relative expression levels of STK genes in transgenic Synechocystis were significantly higher than those in wild-type Synechocystis at 24 h of light recovery (P < 0.05). Heterologous expression of Microcystis spkD might stimulate overcompensatory growth of Synechocystis by affecting its STK gene expression.

HIGHLIGHTS

- Light limitation induced overcompensatory growth of Microcystis.
- Light limitation affected expression of most STK genes of Microcystis.
- Heterologous expression of Microcystis spkD stimulated relative expression levels of STK genes in transgenic Synechocystis.

INTRODUCTION

Microcystis is a globally distributed bloom-forming genus of cyanobacterium (Haande et al. 2007; Shen & Song 2007). Frequent Microcystis blooms in natural waters are related to environmental factors, such as nutrients, light, and temperature (Huisman et al. 2018), and to the strong adaptability of this genus to adversity (Los et al. 2010; Zhou et al. 2011). Another cofactor in determining Microcystis blooms is that Microcystis is not very palatable to many zooplankters (DeMott et al. 2001; Wang et al. 2010; Reichwaldt et al. 2013), so that Microcystis blooms are
Accelerated proliferation during overcompensatory growth might be an endogenous factor responsible for transient bursts of cyanobacterial biomass when water blooms break out. Most studies of *Microcystis* overcompensatory growth are limited to a description of the phenomenon and changes in physiological and biochemical indexes during overcompensatory growth of *Microcystis* is unclear. Clarifying this mechanism can facilitate the early detection and control of cyanobacterial blooms.

Signal transduction systems enable prokaryotes to acclimate to changing environments by precisely regulating gene expression controlling division and differentiation (Los et al. 2010). Signal transduction in prokaryotes is perceived primarily via the two-component signaling system involving histidine kinases and cognate response regulators (Agarwal et al. 2011). As an alternative regulatory pathway, eukaryote-type serine/threonine kinases (STKs) have been found to be necessary for cellular functions of prokaryotes such as growth, division, and differentiation (Rajagopal et al. 2003; Pereira et al. 2011). STKs, a series of ATP-dependent protein kinases, could phosphorylate other proteins and catalyze their own phosphorylation by transferring gamma-phosphoric acid from ATP serine (Ser) and threonine (Thr) to residues of target proteins (Pereira et al. 2011). Previous studies of cyanobacterial STKs mainly focused on the functions of STKs in cell motility (Kamei et al. 2001; Kamei et al. 2003), stress tolerance (Liang et al. 2011; Zorina et al. 2014), and morphogenesis (Panichkin et al. 2006) in model cyanobacteria. However, the role of STKs in the overcompensatory growth of cyanobacteria has not been reported.

We characterized the overcompensatory growth of *Microcystis* after light-limitation stress and investigated the expression of key STK genes (e.g. *spkD*) during the subsequent overcompensatory growth. *Synechocystis* sp. PCC6803 is a cyanobacterial strain bearing the ability to be transformed naturally (Williams 1988), and its entire genome nucleotide sequence has been determined (Kaneko et al. 1996). We heterologously expressed *Microcystis* *spkD* in *Synechocystis* to analyze its role in light-limitation-induced overcompensatory growth of cyanobacteria.

### METHODS

#### Strains and growth conditions

The cyanobacteria *Microcystis aeruginosa* PCC7806 (hereafter *M. aeruginosa*) and *Synechocystis* sp. PCC6803 (hereafter *Synechocystis*) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. *M. aeruginosa* was grown in BG-11 medium (Rippka et al. 1979) under a 12 h light/12 h dark photoperiod with a light density of 32 μmol/m²/s at 25 ± 1 °C. BG-11 medium contains (g/L): NaNO₃ (1.5), K₂HPO₄ (0.04), MgSO₄·7H₂O (0.075), CaCl₂·2H₂O (0.036), citric acid (0.006), iron(III) ammonium citrate (0.006), Na₂-EDTA (0.001), and Na₂CO (0.02), and 1 mL of trace elements solution (mg/L): H₃BO₃ (61), MnSO₄·H₂O (169), ZnSO₄·7H₂O (287), CuSO₄·5H₂O (2.5), and (NH₄)₂MoO₄·4H₂O (12.5), pH 6.8 (Rippka et al. 1979). *Synechocystis* was cultivated in BG-11 medium (5 mM glucose) at 30 °C. For solid BG-11 medium, 1.5% (w/v) Difco Bacto-agar (Becton Dickinson, Sparks, MD, USA), 0.3% (w/v) sodium thiosulfate, and 10 mM TES (2-[2-hydroxyethyl] amino] ethyl] ethanesulfonic acid, pH 8.2) were added.

#### Analysis of overcompensatory growth performance of *M. aeruginosa* after light-limitation stress

*M. aeruginosa* grown to the exponential growth phase was reinoculated into 250-mL flask with 100 mL BG-11 medium and cultured for 24 h before the subsequent light-limitation experiment. *M. aeruginosa* were cultured at seven different light intensities (0, 1, 2, 4, 8, 16, and 32 μmol/m²/s) under a 12 h light/12 h dark cycle for 4 days, and then collected. Collected *M. aeruginosa* were centrifuged (6,000 × g for 10 min at room temperature) and then added to 250-mL flasks containing 100 mL BG-11 medium to a cell density of 5.49 × 10⁹ ind/L and cultured at normal light intensity of 32 μmol/m²/s for 6 days. All treatments had triplicate flasks. *M. aeruginosa* cultured at continuous light density of 32 μmol/m²/s served as controls. In the light recovery stage, the growth of *M. aeruginosa* was estimated each day from the optical density at 680 nm (OD680) using a spectrophotometer. A standard curve relating *M. aeruginosa* cell density to OD₆₈₀ was established using serial dilutions of culture. Total RNA for subsequent real-time quantitative PCR (RT-qPCR) analysis of *M. aeruginosa* was isolated from cells cultured at continuous
light density of 32 μmol/m²/s or darkness (0 μmol/m²/s) after light recovery for 0, 0.5, 4, 24, and 48 h.

**Generation of STK heterologous recombinant plasmids containing spkD from M. aeruginosa PCC7806**

For heterologous expression of spkD in *Synechocystis*, the 1.1-kb *spkD* gene (GenBank: AM778950) was amplified from *M. aeruginosa* PCC7806 using primers *SpkD-F* and *spkD-EcoRI-R* (Table 1). The upstream promoter region (0.5-kb fragment) of *Synechocystis* *psbA2* was amplified by PCR from genomic DNA. To fuse the *psbA2* promoter to *spkD*, the *psbA2* promoter was amplified using *psbA2-Promoter-SalI-F* and *psbA2-Promoter-R*, and the 1.0-kb fragment of *Synechocystis* genomic DNA encoding the *psbA2* open reading frame (ORF) was amplified by PCR using primers *psbA2-SalII-F* and *psbA2-SaclI-R* to create the downstream region of the homologous recombinant vector. The downstream fragment was cloned into the *SacII* and *Sacl* sites of pbBlueScript SK + TIT2 to form p5ST1T2psbA2. A kanamycin resistance cassette carrying npt was then cloned into the single *BamHI* site of p5ST1T2psbA2 to form p5ST1T2psbA2npt. The fused *psbA2* promoter and *spkD* fragments were cloned into the *SalI* and *EcoRI* sites of p5ST1T2psbA2npt to form p5SpkD (Figure 1) (Chen et al. 2017).

**Transformation of Synechocystis**

The *Synechocystis* strain was transformed as described by Chen et al. (2014). *Synechocystis* was grown in liquid BG-11 medium until the OD₇₅₀ reached 0.6. Cells were then harvested by centrifugation (6,000 × g for 10 min at room temperature) and resuspended in fresh BG-11 to a density of OD₇₅₀ = 4.8. Plasmid DNA was added to 500 μL of cell suspension and gently mixed; cells were incubated at 30 °C under low light for 6 h and then spread on BG-11 agar plates containing 50 μg/mL kanamycin (Dingguo Company, Beijing, China). Transformants were isolated after about 10 days of incubation and subcultured on BG-11 agar plates containing 100 μg/mL kanamycin. The transformants were then grown in liquid culture for further analysis.

**Analysis of overcompensatory growth performance of Synechocystis after light-limitation stress**

*Synechocystis* (wild-type and transgenic) was cultured at three different light intensities (0, 8, and 32 μmol/m²/s) under a 12 h light/12 h dark cycle for 4 days. After brief centrifugation (6,000 × g for 10 min at room temperature), *Synechocystis* was added to BG-11 medium to a cell density of 4.58 × 10¹⁰ ind/L and cultured at normal light intensity of 32 μmol/m²/s for 6 days. All treatments had triplicate flasks, and *Synechocystis* cultured continuously under 32 μmol/m²/s served as controls. In the light recovery stage, growth of *Synechocystis* was estimated each day from the OD₇₅₀ using a spectrophotometer. A standard curve relating *Synechocystis*

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**Table 1** | Primer sequences used in generation of STK heterologous recombination plasmids

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′→3′)</th>
</tr>
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<tbody>
<tr>
<td><em>psbA2</em>-Promoter-<em>SalI</em>-F</td>
<td>AATGTCGACTGCCAGATGCGAGCCCTTC</td>
</tr>
<tr>
<td><em>psbA2</em>-Promoter-R</td>
<td>TGGTTATAATTTCAATTGT</td>
</tr>
<tr>
<td><em>spkD</em>-fpsbA2P-F</td>
<td>TAAGGAATTATACCAATGATGGTTTATTCTAC</td>
</tr>
<tr>
<td><em>spkD</em>-EcoRI-R</td>
<td>CGCGAATTCTCAATTGAATAGATGGCTG</td>
</tr>
<tr>
<td><em>psbA2</em>ORF-<em>SalII</em>-F</td>
<td>CTTCCGGAGATGACACGACTCACCAC</td>
</tr>
<tr>
<td><em>psbA2</em>ORF-<em>SaclI</em>-R</td>
<td>AGTGAGCCTTACCGTGACACGAGG</td>
</tr>
</tbody>
</table>

*Italicized, underlined text indicates restriction enzyme sites.*
cell density to OD_{730} was established using serial dilutions of culture. Total RNA for subsequent RT-qPCR analysis was isolated from *Synechocystis* at 0, 2, 6, 24, and 144 h.

**Calculation of specific growth rate**

The specific growth rate was calculated using the following formula: \( \mu = \frac{(\ln N_t - \ln N_{t-1})}{\Delta t} \), where \( N_{t-1} \) is the cell density at the beginning of the time interval, \( N_t \) is the cell density at the end of the time interval, and \( \Delta t \) is the length of the time interval which equals 1 day.

**RNA isolation and cDNA synthesis**

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. First-strand cDNA was synthesized using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase and modified oligo (dT) following the manufacturer’s instructions (TaKaRa, Dalian, China).

**RT-qPCR analysis**

RT-qPCR examination of STK expression was carried out using a Bio-Rad iQ5 with reactions prepared following the manufacturer’s instructions. Each PCR was repeated four times in a total volume of 20 \( \mu \)L containing 2 \( \times \) SYBR Green 1 PCR Master Mix (TaKaRa), 100 nM of each primer, and 1 \( \mu \)L diluted (1:20) template cDNA. Reactions were carried out in 96-well optical-grade PCR plates with optical-grade membrane (TaKaRa). The amplification program was as follows: initial denaturing of 1 min at 95 °C, followed by 42 cycles of 10 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C; an additional cycle of 10 s at 95 °C, 30 s at 58 °C, and 5 min at 72 °C; and 10 s at 95 °C for melting curve analysis. Data were analyzed with Bio-Rad iQ5 software. Relative expression of STKs was calculated using the relative \( 2^{-\Delta\Delta Ct} \) method (Chen *et al.* 2012).

**Statistical analysis**

Data was expressed as means ± standard deviation (n = 3). Significant differences \( (P < 0.05) \) between treatments under different light limitations or at different times after restoration from light limitation were analyzed by one-way analysis of variance (ANOVA) followed by the least significant difference multiple-range test using SPSS 10.0. T-test was used to test significant difference between wild-type and transgenic *Synechocystis*.

### Table 2 | The primers of different STKs used in qRT-PCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sequence (5’—3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAE_RS26565 (putative spkA)</td>
<td>F CCTCCTTTGGCAGTGGT&lt;br&gt;R CCCTGGGAGACTTGATA</td>
</tr>
<tr>
<td>MAE_RS23275 (putative spkB)</td>
<td>F GCCACTTCCTCCGGTTTCT&lt;br&gt;R GCTGCTGCGGGCCTTTACT</td>
</tr>
<tr>
<td>MAE_RS08890 (putative spkC)</td>
<td>F GCAGGAGTGGATGAGCC&lt;br&gt;R AGGATCGAGTGATGGGCG</td>
</tr>
<tr>
<td>AM778950 IPF_5717 (putative spkD)</td>
<td>F AAGAAACTATGGGAACGG&lt;br&gt;R CTTTGAGTAGGAGCGGGAG</td>
</tr>
<tr>
<td>MAE_RS06565 (putative spkF)</td>
<td>F AGATGAGGGTGAGGGTAA&lt;br&gt;R AAACCTTCGCTAATGCTG</td>
</tr>
<tr>
<td>ropC1 (reference gene of <em>M. aeruginosa</em>)</td>
<td>F CCTCAGCGAAGATCAATGGT&lt;br&gt;R CCGTTTTTGCCCCTTACTTT</td>
</tr>
<tr>
<td>slr1575 (spkA)</td>
<td>F TGTAGCGGATGCTGGAC&lt;br&gt;R ACTCAACACGGATATGGAA</td>
</tr>
<tr>
<td>slr1697 (spkB)</td>
<td>F CAAATTGATTCGGTCCTCT&lt;br&gt;R TTCCCAGTCCATCTCCC</td>
</tr>
<tr>
<td>slr0599 (spkC)</td>
<td>F GCCACAAAGTTTACACTC&lt;br&gt;R CCGCAATCATGTCAGTA</td>
</tr>
<tr>
<td>mnpb (reference gene of <em>Synechocystis</em> sp. PCC6803)</td>
<td>F GTAGGAGAAATGCAAGG&lt;br&gt;R GGAGAAACGAGGACCAACCT</td>
</tr>
</tbody>
</table>
Characteristics of overcompensatory growth of *M. aeruginosa*

We established a standard curve relating *M. aeruginosa* cell density to OD$_{680}$ ($y = 24.67x + 0.108, r^2 = 0.993, n = 7$). After the end of light limitation, *Microcystis* exhibited overcompensatory growth for 2 days, during which the increase in growth was inversely related to light intensity (Figure 2). At day 1 of light recovery, specific growth rate of *M. aeruginosa* was higher for cells previously grown at lower light intensities. Compared to the control group, specific growth rates in light-limited groups (except 16 μmol/m$^2$/s) were significantly greater ($P < 0.05$). At day 2 of light recovery, no significant differences in specific growth rates were detected between the control group and groups exposed to limited light of 16 and 8 μmol/m$^2$/s. However, compared to the control group, specific growth rates in light-limited groups exposed to 4, 2, 1, and 0 μmol/m$^2$/s were significantly greater ($P < 0.05$). Insignificant differences were observed among the specific growth rates of *M. aeruginosa* exposed to the seven light intensities ($P > 0.05$) when light recovery exceeded 2 days.

**Gene expression of *M. aeruginosa* STKs after light-limitation stress**

No significant changes were observed between relative expression values (in darkness to in continuous light of 32 μmol/m$^2$/s) of *spkA* under light-limitation stress (Figure 3). However, relative expression values of the other four STK genes first increased significantly, peaked at 0.5 h, and then decreased. Compared to the control group cultured under continuous light of 32 μmol/m$^2$/s, putative *spkB* (GenBank: AM778936), *spkD* (GenBank: AM778938), *spkB* (GenBank: AM778950), and *spkC* (GenBank: AM778896) in darkness were upregulated 12.44, 5.62, 4.86, and 2.61 times, respectively, at 0.5 h.

**Characteristics of overcompensatory growth of transgenic *Synechocystis***

PCR analysis of wild-type and transformed *Synechocystis* is shown in Figure 4, and the transformant lines were the expected transgenic *Synechocystis*. We established standard curves relating wild-type *Synechocystis* cell density to OD$_{730}$ ($y = 5.420x + 0.493, r^2 = 0.996, n = 6$), and transgenic *Synechocystis* cell density to OD$_{730}$ ($y = 3.580x + 0.538, r^2 = 0.993, n = 6$), respectively. Compared to the control group...
cultured under continuous light of 32 μmol/m²/s (Figure 5), the specific growth rates of wild-type Synechocystis in light-limited groups increased during the first 2 days of light recovery, suggesting that overcompensatory growth in wild-type Synechocystis lasted for 2 days. However, compared to the control group, specific growth rates of transgenic Synechocystis in light-limited groups increased during the first 2 days and the last 2 days of light recovery. Overcompensatory growth lasted longer in transgenic Synechocystis than in wild-type Synechocystis. After 4 days of light recovery, specific growth rates of transgenic Synechocystis were all significantly higher than those of wild-type Synechocystis (P < 0.05). Transgenic Synechocystis exhibited greater overcompensatory growth than wild-type Synechocystis, and this growth lasted longer.

**Effects of heterologous spkD on gene expression of STks in Synechocystis after light-limitation stress**

During the light recovery stage, expression levels of most STK genes in both wild-type and transgenic Synechocystis previously exposed to limited light (0 and 8 μmol/m²/s) were higher than those in cells exposed to normal light (32 μmol/m²/s) (Figure 6). At 2 h and 6 h of light recovery, transgenic Synechocystis exhibited lower relative STK gene expression than wild-type Synechocystis. Relative STK gene expression levels were significantly higher in transgenic Synechocystis than in wild-type Synechocystis at 24 h of light recovery (P < 0.05). After 24 h of light recovery, relative expression of STK genes (except spkA) in transgenic Synechocystis decreased significantly (P < 0.05) and the difference in relative expression between wild-type and transgenic Synechocystis decreased. Except for spkB and spkF in wild-type Synechocystis, relative expression levels of all STK genes in both wild-type and transgenic Synechocystis exposed to 0 μmol/m²/s light were significantly higher than those in cells exposed to 8 μmol/m²/s light at 144 h of light recovery (P < 0.05).

At 2 h after light recovery, except for spkB after exposure to normal light (32 μmol/m²/s), expression levels of all STK genes in transgenic Synechocystis were lower than those in wild-type Synechocystis (Figure 7). At 6 h of light recovery, STK gene expression was triggered to a greater extent in transgenic Synechocystis than in wild-type Synechocystis, and expression levels of all STK genes in transgenic Synechocystis were much higher than those in wild-type. Except for spkE, relative STK gene expression levels decreased with light intensity in the following order: 32 μmol/m²/s > 0 μmol/m²/s > 8 μmol/m²/s. After 6 h of light recovery, except for spkA, expression levels of all STK genes in transgenic Synechocystis were lower than those in wild-type Synechocystis.

**DISCUSSION**

Compensatory growth has been studied extensively in a variety of animals and plants (Oesterheld & McNaughton 1991) and is considered an adaptive response of organisms to wide fluctuations in environmental factors (Turano et al. 2007). Compensatory growth in fish was classified into three types depending on the degree of recovery following relief from resource restriction: overcompensation, leading to fish growing larger than control fish; complete compensation, leading to fish achieving the same body mass as control fish; and partial compensation, leading to fish exhibiting accelerated growth, but not achieving the mass of control fish (Tian & Qin 2005). Overcompensatory growth was induced in microalgae by subjecting them to periods of resource restriction, such as high-temperature stress (Han et al. 2015), and darkness stress (Cai et al. 2009). Light is a key environmental factor influencing microalgal growth and proliferation. The phototrophic Microcystis relies heavily on light, which acts as the main energy source for its growth. In this study, overcompensatory growth occurred immediately upon light recovery. The amount and duration of compensatory growth are
dependent on the type and level of stress (Oesterheld & McNaughton 1994). Overcompensatory growth, might be one of the adaptation strategies used by Microcystis for light intensity variation. Based on overcompensatory growth in response to light limitation, Microcystis might proliferate exceptionally fast when subjected to continuous rainy days with low light intensities followed by normal clear weather. Mass growths of Microcystis leading to the production of blooms can threaten ecosystem functioning and degrade water quality for recreation, drinking water, and fisheries (Huisman et al. 2018).

Overcompensatory growth of microalgae depends on improved cell division during the recovery stage, and bacterial STKs are known to regulate bacterial cell division (Manuse et al. 2016). \textit{pknA} phosphorylates and regulates...
the functionality of FtsZ, a protein central to cell division throughout the bacterial lineage (Thakur & Chakraborti 2006). Interplay between PknA and PknB in mycobacteria allows phosphorylation of Wag31, an ortholog of the cell division protein DivIVA. PknA-mediated phosphorylation of both FtsZ and FipA stabilizes the FtsZ/FipA/FtsQ complex required for cell division under oxidative stress (Manuse et al. 2016). Gieffing et al. (2010) proposed that StkP played a currently undefined role in cell division of pneumococcus based on its cell-division-dependent localization and interaction with FtsZ. Expression of pknA and pknB in Mycobacterium tuberculosis was markedly higher during exponential growth than during the stationary phase, suggesting that the regulatory function of these essential kinases was required during active cell replication (Kang et al. 2005). Following darkness stress, we found that expression of four STK genes (spkB, spkC, spkD, and spkF) was upregulated significantly in the initial recovery stage (from 0.5 to 4 h) and then downregulated after 4 h. Translation of transcribed STK genes into proteins and their regulation of growth via signal transduction requires time. As a result, overcompensatory growth of *Microcystis* appeared later than upregulated STK gene expression. We found that overcompensatory growth of *Microcystis* occurred at day 1 of light recovery from darkness and lasted for only 2 days, suggesting that STKs might play a role in overcompensatory growth of *Microcystis* by stimulating cell division.

STKs in *Synechocystis* might be involved in autophosphorylation and phosphorylation of general substrate proteins (Kamei et al. 2001, 2002, 2003), regulation of nitrogen metabolism (Galkin et al. 2005), post-translational modification of pilin for pili assembly (Kim et al. 2004), and acclimation to abiotic changes (Liang et al. 2011; Liu et al. 2013). To confirm the roles of STKs during overcompensatory growth of *Microcystis* after light-limitation stress, we heterologously expressed *Microcystis* spkD, an STK gene essential for survival of the species (Kamei et al. 2002; Laurent et al. 2008), in the model cyanobacterium *Synechocystis*. Both wild-type and transgenic *Synechocystis* exhibited overcompensatory growth in the light recovery stage. However, transgenic *Synechocystis* exhibited greater overcompensatory growth than wild-type *Synechocystis*. Moreover, overcompensatory growth of transgenic *Synechocystis* lasted longer than wild-type *Synechocystis*. These results suggested that heterologous expression of *spkD* had stimulatory effects on overcompensatory growth in *Synechocystis*. After light recovery, marked changes in the expression of STK genes accompanied the overcompensatory growth in transgenic *Synechocystis*. How *Microcystis* spkD affects STK genes expression when heterologously expressed in *Synechocystis* merits further investigation, which will help us to further declare the molecular mechanism underlying overcompensatory growth of cyanobacteria.

CONCLUSIONS

*Microcystis* exhibited overcompensatory growth following light-limitation stress, and overcompensatory growth might be considered a factor contributing to transient bursts of cyanobacterial biomass when algal blooms break out. The overcompensatory growth was accompanied by the changes of STK gene expression in *Microcystis*. Furthermore, heterologous expression of *Microcystis* spkD in *Synechocystis* had stimulation effects on the overcompensatory growth caused by light limitation. STK genes might play an important role in the overcompensatory growth of cyanobacteria.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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