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

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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.

Key words | compensatory growth, cyanobacteria, light limitation, serine/threonine kinase, *spkD*

HIGHLIGHTS

- Light limitation induced overcompensatory growth of *Microcystis*.
- Light limitation affected expression of most STK genes of *Microcystis*.
- Heterologous expression of *Microcystis spkD* enhanced and prolonged light-limitation-induced overcompensatory growth of transgenic *Synechocystis*.
- 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...

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rarely kept in check via herbivory/predation. Overcompensatory growth is a phase of accelerated growth that occurs upon availability of adequate resources following a period of resource deprivation (Ali et al. 2003). Microcystis shows marked overcompensatory growth after exposure to environmental stress, such as high temperature (Han et al. 2015), and high concentration of lead(II) (Bi et al. 2013). 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 under environmental stress (Bi et al. 2013; Han et al. 2015) and the molecular mechanism underlying 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 to occur 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 Na₂CO₃ (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)₂MoO₄·2H₂O (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-[hydroxyethyl]-1,1-bis[hydroxymethyl] ethyl] amino) 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-FpsbA2P-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-SalI-R to create the downstream region of the homologous recombinant vector. The downstream fragment was cloned into the SalII and SacI sites of pBluescript SK+T1T2 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 OD₇₃₀ 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 OD₇₃₀ = 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 OD₇₃₀ using a spectrophotometer. A standard curve relating *Synechocystis*
cell density to OD_{730} was established using serial dilutions of culture. Total RNA for subsequent RT-qPCR analysis was isolated from \textit{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. Primers used for RT-qPCR are listed in Table 2. Each PCR was repeated four times in a total volume of 20 \( \mu \)L containing 2 \( \times \) SYBR Green I 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 \(^\circ\)C, followed by 42 cycles of 10 s at 95 \(^\circ\)C, 30 s at 60 \(^\circ\)C, and 30 s at 72 \(^\circ\)C; an additional cycle of 10 s at 95 \(^\circ\)C, 30 s at 58 \(^\circ\)C, and 5 min at 72 \(^\circ\)C; and 10 s at 95 \(^\circ\)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 \( \pm \) 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 \textit{Synechocystis}.

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

| Gene name | Sequence (5’—3’) |
|-----------|------------------|
| MAE_RS26565 (putative spkA) | F CCTCTTTGGCAGTTGGT R CCGTCGGCAAGACTTGATA |
| MAE_RS23275(putative spkB) | F GCCACTTCCTCCGGTTTCT R GCTGCTGCGGGCTTTACT |
| MAE_RS08890 (putative spkC) | F GCAGGAGTGGATTGGACCG R AGGATCGAGTAAGGTGGC |
| AM778950 IPF_5717(putative spkD) | F AAGAAACTATGGGAACGG R CTTGAGTAGGAGCGGGAG |
| MAE_RS06365 (putative spkF) | F AGATGAGGGTGAGGGTAA R AAACCTTCGCTAATGCTG |
| ropC1 (reference gene of \textit{M. aeruginosa}) | F CCTCAGCGAAGATCAATGGT R CCGTTTTTGCCCCTTACTTT |
| slr1575 (spkA) | F TGTAGCGGATGCTGGAC R ACTCAACACGGATATGGAA |
| s1r1697 (spkB) | F CAAATTGATTCGGTCCTCT R TTCCCAGTCCATCTCCC |
| slr0599 (spkC) | F GCCACCAAGTTTACACTC R CCGCAATCAGTACAGTA |
| mlp (reference gene of \textit{Synechocystis} sp. PCC6803) | F GTGAGGAGCACTGCCAAGAA R GGCAGGAAAGAGCAACCT |
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 $\mu$mol/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 $\mu$mol/m$^2$/s. However, compared to the control group, specific growth rates in light-limited groups exposed to 4, 2, 1, and 0 $\mu$mol/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 $\mu$mol/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 $\mu$mol/m$^2$/s, putative *spkF* (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$_{730}$ ($y = 3.420x + 0.493$, $r^2 = 0.996$, $n = 6$), and transgenic *Synechocystis* cell density to OD$_{730}$ ($y = 3.380x + 0.538$, $r^2 = 0.993$, $n = 6$), respectively. Compared to the control group

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**Figure 2** | Effects of light limitation on overcompensatory growth of *M. aeruginosa*. Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

**Figure 3** | Changes in the relative expression of STK genes in *M. aeruginosa* after light-limitation stress. Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

**Figure 4** | PCR analysis of wild-type *Synechocystis* and *spkD* transformant. M, Generay Normal Run 250 bp-II ladder; lane 1, PCR analysis of wild-type *Synechocystis* using primers Promoter-SalI-F and *spkD*-EcoRI-R; lane 2, PCR analysis of *spkD* transformant using primers Promoter-SalI-F and *spkD*-EcoRI-R; lane 3, PCR analysis of wild-type *Synechocystis* using primers *spkD*-fpsbA2P-F and T1T2-R; lane 4, PCR analysis of the wild-type and *spkD* transformant using primers *spkD*-fpsbA2P-F and T1T2-R; lane 5, PCR analysis of wild-type *Synechocystis* using primers Promoter-SalI-F and T1T2-R; lane 6, PCR analysis of the wild-type and *spkD* transformant using primers Promoter-SalI-F and *psbA2*ORF-R.
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 spkA 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 microagal growth and proliferation. The photoautotroph *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). *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. 2015). 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.

REFERENCES

Agarwal, S., Pancholi, P. & Pancholi, V. 2011 Role of serine/threonine phosphatase (SP-STP) in Streptococcus pyogenes physiology and virulence. Journal of Biological Chemistry 286 (48), 41368–41380.

Ali, M. A., Nicieza, A. G. & Wootton, R. J. 2005 Compensatory growth in fishes, a response to growth depression. Fish and Fisheries 4 (2), 147–190.

Bi, X. D., Zhang, S. L., Dai, W., Xing, K. Z. & Yang, F. 2013 Effects of lead(II) on the extracellular polysaccharide (EPS)
production and colony formation of cultured Microcystis aeruginosa. Water Science and Technology 67 (4), 803–809.
Cai, Z. P., Duan, S. S. & Wei, W. 2009 Darkness and UV radiation provoked compensatory growth in marine phytoplankton Phaeodactylum tricornutum (Bacillariophyceae). Aquaculture Research 40 (13), 1559–1562.
Chen, G., Peng, Z. Y., Shan, L., Xuan, N., Tang, G. Y., Zhang, Y., Li, L., He, Q. F. & Bi, Y. P. 2012 Cloning of Acyl-ACP Thioesterase FatA from Arachis hypogaea L. and its Expression in Escherichia coli. Biomed Research International 2012, 652579.
Chen, G., Qu, S. J., Wang, Q., Bian, F., Peng, Z. Y., Zhang, Y., Ge, H. T., Yu, J. H., Xuan, N., Bi, Y. P. & He, Q. F. 2014 Transgenic expression of delta-6 and delta-15 fatty acid desaturases enhances omega-3 polyunsaturated fatty acid accumulation in Synechocystis sp. PCC6803. Biotechnology for Biofuels 7 (1), 1–10.
Chen, G., Chen, J., He, Q. F., Zhang, Y., Peng, Z. Y., Fan, Z. X., Bian, F., Yu, J. H. & Qin, S. 2017 Functional expression of the Arachis hypogaea L. Acyl-ACP thioesterases AhFatA and AhFatB enhances fatty acid production in Synechocystis sp. PCC6803. Energies 10 (12), 2093.
DeMott, W. R., Gullati, R. D. & van Donk, E. 2001 Daphnia food limitation in three hypereutrophic Dutch lakes: evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnology and Oceanography 46, 2054–2060.
Galkin, A. N., Mikheeva, L. E. & Shestakov, S. V. 2003 Insertional inactivation of genes encoding eukaryotic-type serine/threonine protein kinases in cyanobacterium Synechocystis sp. PCC 6803. Microbiology 72 (1), 64–69.
Giefing, C., Jelenccsics, K. E., Gelbmann, D., Senn, B. M. & Nagy, E. 2010 The pneumococcal eukaryotic-type serine/threonine protein kinase StkP co-localizes with the cell division apparatus and interacts with FtsZ in vitro. Microbiology 156 (6), 1697–1707.
Haande, S., Ballot, A., Rohrillac, T., Fastner, J., Wiedner, C. & Edvardsen, B. 2007 Diversity of Microcystis aeruginosa isolates (Chroococcales, Cyanobacteria) from East African water bodies. Archives of Microbiology 188 (1), 15–25.
Han, W., Jing, Y. & Li, T. 2005 Compensatory growth in Microcystis aeruginosa after moderate high-temperature exposure. Journal of Limnology 74 (3), 549–558.
Huisman, J., Codding, G. A., Paelinck, H. W., Wiltings, B. W., Verspanden, J. M. H. & Visser, P. M. 2018 Cyanobacterial blooms. Nature Reviews Microbiology 16, 471–483.
Kamei, A., Yuasa, T., Orikawa, K., Geng, X. X. & Ikeuchi, M. 2001 A eukaryotic-type protein kinase, SpkA, is required for normal motility of the unicellular cyanobacterium Synechocystis sp. strain PCC 6803. Journal of Bacteriology 183 (5), 1505–1510.
Kamei, A., Yuasa, T., Geng, X. & Ikeuchi, M. 2002 Biochemical examination of the potential eukaryotic-type protein kinase genes in the complete genome of the unicellular cyanobacterium Synechocystis sp. PCC 6803. DNA Research 9 (3), 71–78.
agalactiae reversibly phosphorylate an inorganic pyrophosphatase and affect growth, cell segregation, and virulence. *Journal of Biological Chemistry* 278 (16), 14429–14441.

Reichwaldt, E. S., Song, H. & Ghadouani, A. 2013 Effects of the distribution of a toxic *Microcystis* bloom on the small scale patchiness of zooplankton. *PLoS One* 8 (6), e66674.

Rippka, R. J., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. 1979 Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111 (1), 1–61.

Shen, H. & Song, L. R. 2007 Comparative studies on physiological responses to phosphorus in two phenotypes of bloom forming *Microcystis*. *Hydrobiologia* 592 (1), 475–486.

Thakur, M. & Chakraborti, P. K. 2006 GTPase activity of mycobacterial FtsZ is impaired due to its transphosphorylation by the eukaryotic-type Ser/Thr kinase, PknA. *Journal of Biological Chemistry* 281 (52), 40107–40113.

Tian, X. L. & Qin, J. G. 2003 A single phase of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. *Aquaculture* 224 (1–4), 169–179.

Turano, M. J., Borski, R. J. & Daniels, H. V. 2007 Compensatory growth of pond-reared hybrid striped bass, *Moronechrysops × Moronesaxatilis*, fingerlings. *Journal of the World Aquaculture Society* 38 (2), 250–261.

Wang, X., Qin, B., Gao, G. & Paerl, H. W. 2010 Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *Journal of Plankton Research* 32, 457–470.

Williams, J. G. K. 1988 Construction of specific mutations in the photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. *Methods in Enzymology* 167, 766–778.

Zhou, W. G., Li, Y., Min, M., Hu, B., Chen, P. & Ruan, R. 2011 Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production. *Bioresource Technology* 102 (13), 6909–6019.

Zorina, A. A., Bedbenov, V. S., Novikova, G. V., Panichkin, V. B. & Los, D. A. 2014 Involvement of serine/threonine protein kinases in the cold stress response in the cyanobacterium *Synechocystis* sp. PCC 6803: functional characterization of SpkE protein kinase. *Molecular Biology* 48 (3), 390–398.