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*

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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

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/m^2/s at 25 $\pm$ 1 °C. BG-11 medium contains (g/L): $NaNO_3$ (1.5), K_2HPO_4 (0.04), $MgSO_4 \cdot 7H_2O$ (0.075), $CaCl_2 \cdot 2H_2O$ (0.036), citric acid (0.006), iron(III) ammonium citrate (0.006), Na_2-EDTA (0.001), and Na_2CO (0.02), and 1 mL of trace elements solution (mg/L): H_3BO_3 (61), $MnSO_4 \cdot H_2O$ (169), $ZnSO_4 \cdot 7H_2O$ (287), $CuSO_4 \cdot 5H_2O$ (2.5), and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (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-hydroxy-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/m^2/s) under a 12 h light/12 h dark cycle for 4 days, and then collected. Collected *M. aeruginosa* were centrifuged (6,000 $\times$ 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 5.49×10^9 ind/L and cultured at normal light intensity of 32 μmol/m^2/s for 6 days. All treatments had triplicate flasks. *M. aeruginosa* cultured at continuous light density of 32 μmol/m^2/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 (OD_{680}) using a spectrophotometer. A standard curve relating *M. aeruginosa* cell density to OD_{680} 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/m^2/s or darkness (0 µmol/m^2/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*psbA2*P-F and *SpkD*-*Eco*RI-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-*Sal*-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*-*Sac*II-F and *psbA2*-*Sac*I-R to create the downstream region of the homologous recombinant vector. The downstream fragment was cloned into the *Sac*II and *Sac*I sites of pBluescript SK + T1T2 to form p5ST1T2*psbA2*. A kanamycin resistance cassette carrying *npt* was then cloned into the single *Bam*HI site of p5ST1T2*psbA2* to form p5ST1T2*psbA2*npt. The fused *psbA2* promoter and *spkD* fragments were cloned into the *Sal*I and *Eco*RI sites of p5ST1T2*psbA2*npt to form p5S*spkD* (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 OD$_{730}$ 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 OD$_{730}$ = 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,
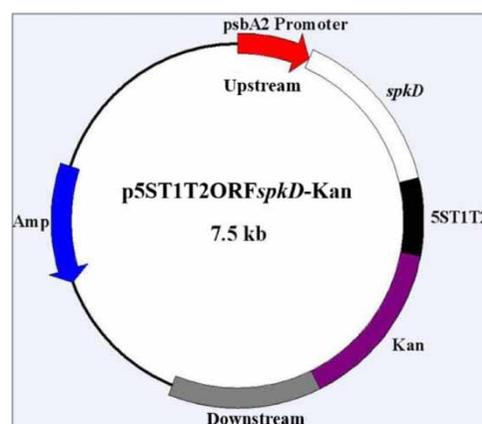

Figure 1 | Structure of homologous recombinant vector harboring *spkD* gene of *M. aeruginosa* PCC7806 in pBluescript II SK (+). The *psbA2* promoter (P*psbA2*), consisting of a 0.5-kb fragment upstream (UP) of *psbA2*, the kanamycin resistance cassette (Kan), a 1.0-kb fragment downstream of *psbA2* (*psbA2* ORF), and the *Escherichia coli* 5ST1T2 terminator were cloned into plasmids. Amp: ampicillin resistance cassette.

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/m^2/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 × 10^{10} ind/L and cultured at normal light intensity of 32 µmol/m^2/s for 6 days. All treatments had triplicate flasks, and *Synechocystis* cultured continuously under 32 µmol/m^2/s served as controls. In the light recovery stage, growth of *Synechocystis* was estimated each day from the OD$_{730}$ using a spectrophotometer. A standard curve relating *Synechocystis*

Table 1 | Primer sequences used in generation of STK heterologous recombination plasmids[a]

Primer	Sequence (5′—3′)
psbA2-Promoter-*Sal*I-F	AAT*GTCGAC*TGCCCAGATGCAGGCCTTC
psbA2-Promoter-R	TTGGTTATAATTCCTTATGT
spkD-f*psbA2*P-F	TAAGGAATTATAACCAAATGACGGATTTAATTCTAC
spkD-*Eco*RI-R	CGC*GAATTC*TCAAAATGAGAATTGCTG
*psbA2*ORF-*Sac*II-F	CTT*CCGCGG*ATGACAACGACTCTCCAAC
*psbA2*ORF-*Sac*I-R	AGT*GAGCTC*TTAACCGTTGACAGCAGG

[a]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: $\mu = (\ln N_t - \ln N_{t-1})/\Delta t$, 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 Δt 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 μL containing 2 × SYBR Green I PCR Master Mix (TaKaRa), 100 nM of each primer, and 1 μL 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

Gene name		Sequence (5′—3′)
MAE_RS26565 (putative *spkA*)	F	CCTCCTTTGGCAGTTGGT
	R	CCGTCGGCAGACTTGATA
MAE_RS23275 (putative *spkB*)	F	GCCACTTCCTCCGTTTCT
	R	GCTGCTGCGGGCTTTACT
MAE_RS08890 (putative *spkC*)	F	GCAGGAGTGGGATTGACGG
	R	AGGATCGAGTAAGGTGGC
AM778950 IPF_5717 (putative *spkD*)	F	AAGAAACTATGGGAACGG
	R	CTTGAGTAGGAGCGGGAG
MAE_RS06365 (putative *spkF*)	F	AGATGAGGGTGAGGGTAA
	R	AAACCTTCGCTAATGCTG
ropC1 (reference gene of *M. aeruginosa*)	F	CCTCAGCGAAGATCAATGGT
	R	CCGTTTTTGCCCCTTACTTT
sll1575 (*spkA*)	F	TGTAGCGGATGCTGGAC
	R	ACTCAACACGGATATGGAA
slr1697 (*spkB*)	F	CAAATTGATTCGGTCCTCT
	R	TTCCCAGTCCATCTCCC
slr0599 (*spkC*)	F	GCCACCAAGGTTTACACTC
	R	CCGCCAATCACTAGCAGTA
mpb (reference gene of *Synechocystis* sp. PCC6803)	F	GTGAGGACAGTGCCACAGAA
	R	GGCAGGAAAAAGACCAACCT

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 over-compensatory 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 *spkF* (GenBank: AM778936), *spkD* (GenBank: AM778938), *spkB* (GenBank: AM778950), and *spkC*

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 ($P < 0.05$).

(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
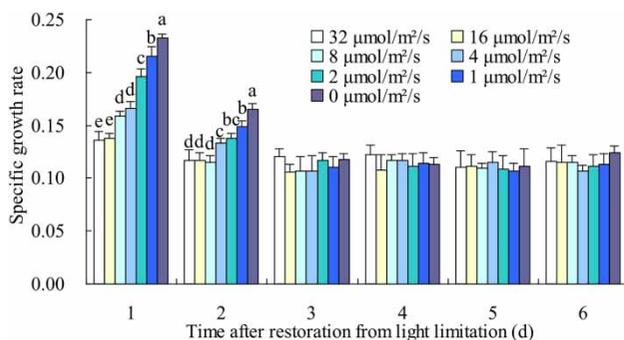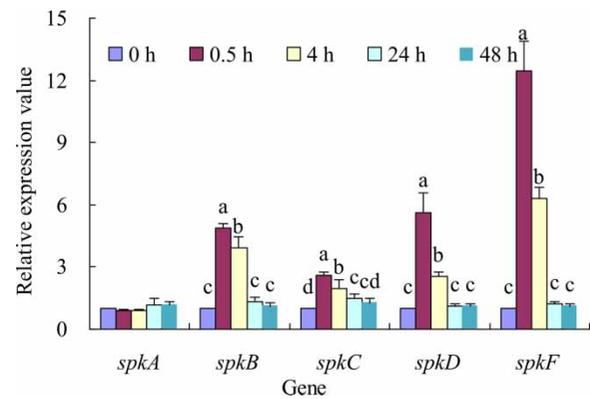
Figure 2 │ Effects of light limitation on overcompensatory growth of *M. aeruginosa*. Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).
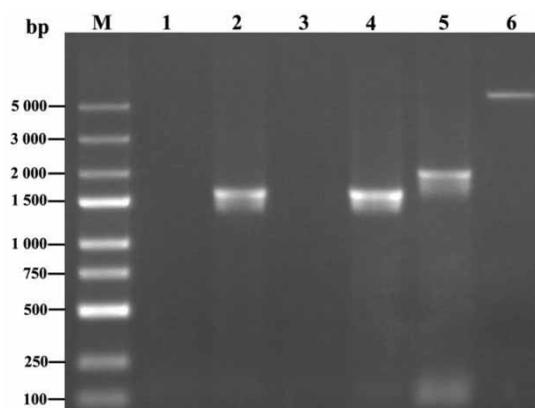
Figure 4 │ PCR analysis of wild-type *Synechocystis* and *spkD* transformant. M, Generay Normal Run 250 bp-II ladder; lane 1, PCR analysis of wild-type *Synechocystis* using primers Promoter-*Sal*I-F and *spkD-Eco*RI-R; lane 2, PCR analysis of *spkD* transformant using primers Promoter-*Sal*I-F and *spkD-Eco*RI-R; lane 3, PCR analysis of wild-type *Synechocystis* using primers *spkD*-f*psbA2*P-F and T1T2-R; lane 4, PCR analysis of the wild-type and *spkD* transformant using primers *spkD*-f*psbA2*P-F and T1T2-R; lane 5, PCR analysis of wild-type *Synechocystis* using primers Promoter-*Sal*I-F and T1T2-R; lane 6, PCR analysis of the wild-type and *spkD* transformant using primers Promoter-*Sal*I-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 2003). 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 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
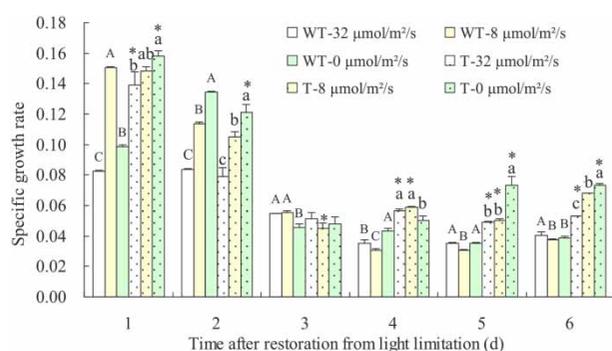

Figure 5 | Effects of light limitation on overcompensatory growth of wild-type and transgenic *Synechocystis*. The asterisk indicates significant difference between wild-type (WT) and transgenic (T) *Synechocystis* ($P < 0.05$). Values sharing the same letters in uppercase are not significantly different between wild-type *Synechocystis* under different light limitations ($P > 0.05$), whereas those with different letters in uppercase are significantly different between wild-type *Synechocystis* under different light limitations ($P < 0.05$). Values sharing the same letters or no letters in lowercase are not significantly different between transgenic *Synechocystis* under different light limitations ($P > 0.05$), whereas those with different letters in lowercase are significantly different between transgenic *Synechocystis* under different light limitations ($P < 0.05$).
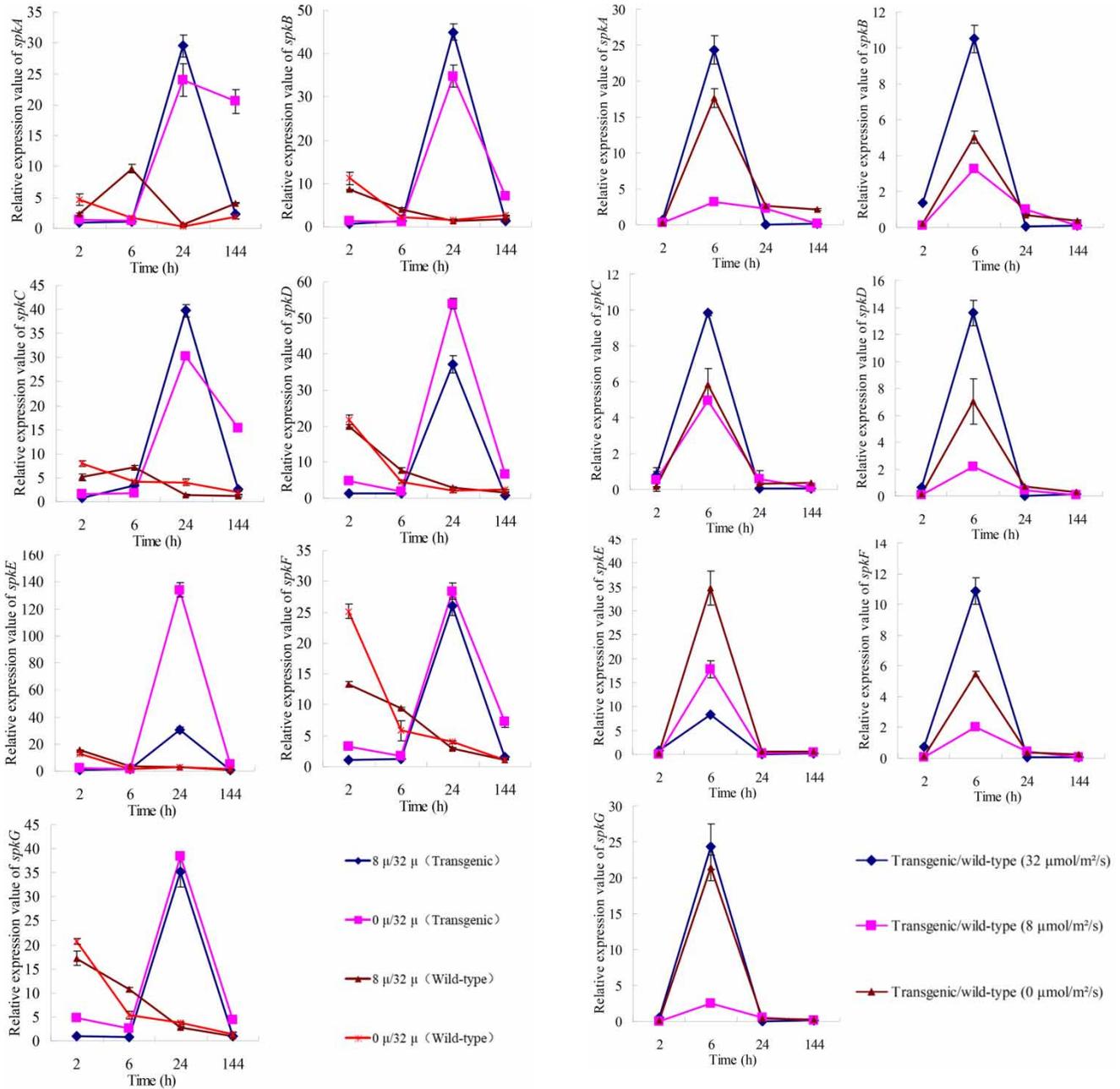
Figure 6 │ Changes in the relative expression of *STK* genes in wild-type and transgenic *Synechocystis* after light-limitation stress (0 and 8 μmol/m²/s). *STK* gene expression under normal light conditions (32 μmol/m²/s) was used as control. μ represents μmol/m²/s.

Figure 7 │ Changes in the relative expression of *STK* genes in transgenic *Synechocystis* after light-limitation stress (0, 8, and 32 μmol/m²/s). *STK* gene expression of wild-type *Synechocystis* was used as control.

dependent on the type and level of stress (Oesterheld & McNaughton 1991). 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). Giefing *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.* 2003), 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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