Biosynthesis of 2-methylisoborneol is regulated by chromatic acclimation of *Pseudanabaena*

Ming Su[a,b,g,*], Jiao Fang[a,h], Zeyu Jia[a,c], Yuliang Su[d], Yiping Zhu[e], Bin Wu[d], John C. Little[f], Jianwei Yu[a,b,g], Min Yang[a,b,g]

[a] *Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing, 100085,*

[b] *National Engineering Research Center of Industrial Wastewater Detoxication and Resource Recovery, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing, 100085,*

[c] *Yangtze Eco-Environment Engineering Research Center, China Three Gorges Corporation, Beijing, 100038,*

[d] *Zhuhai Water Environment Holdings Group Ltd., Zhuhai, 519020,*

[e] *Shanghai Chengtou Raw Water Co. Ltd., Beiai Rd. 1540, Shanghai, 200125,*

[f] *Department of Civil and Environmental Engineering, Virginia Tech., Blacksburg, VA, 24061-0246,*

[g] *University of Chinese Academy of Sciences, Beijing, 100049,*

[h] *School of Civil Engineering, Chang'an University, Xi'an, 710054,*

Abstract

Cyanobacteria can sense different light color by adjusting the components of photosynthetic pigments including chlorophyll *a* (Chl *a*) , phycoerythrin (PE), and phycocyanin (PC), etc. Filamentous cyanobacteria are the main producer of 2-methylisoborneol (MIB) and many can increase their PE levels so that they are more competitive in subsurface layer where green light is more abundant, and have caused extensive odor problems in drinking water reservoirs. Here, we identified the potential correlation between MIB biosynthesis and ambient light color induced chromatic acclimation (CA) of a MIB-producing *Pseudanabaena* strain. The results suggest *Pseudanabaena* regulates the pigment proportion through Type III CA (CA3), by increasing PE abundance and decreasing PC in green light. The biosynthesis of MIB and Chl *a* share the common precursor, and are positively correlated with statistical significance regardless of light color ($R^2 = 0.68, p < 0.001$). Besides, the PE abundance is also positively correlated with Chl *a* in green light ($R^2 = 0.57, p = 0.019$) since PE is the antenna that can only transfer the

energy to PC and Chl *a*. In addition, significantly higher MIB production was observed in green light since more Chl *a* was synthesized.
Keywords: *Pseudanabaena*, 2-methylisoborneol, phycoerythrin, chlorophyll *a*, chromatic acclimation

1. Introduction

As an ancient group of photosynthetic prokaryotes, cyanobacteria have evolved a diverse set of phytochromes and cyanobacteriochromes that enable them to sense and respond to ambient light conditions in the range from 300 nm to 750 nm to optimize their photosynthetic activity (Ikeuchi and Ishizuka, 2008; Gutu and Kehoe, 2012; Ho et al., 2017; Wiltbank and Kehoe, 2019). In addition to the ubiquitous pigment Chl *a* that absorbs both blue and red light for principle light harvest within photosystem II (PSII) and photosystem I (PSI) (Kirk, 2011; Luimstra et al., 2020), cyanobacteria use phycobilisomes (PBS) as antenna of the photosynthetic pigment apparatus to broaden their absorbable light color to red, orange, yellow and green within PSII (Stadnichuk et al., 2015; Zheng et al., 2021). Therefore, the regulation of components of photosynthetic pigments is essential to maximally absorb the ambient light color spectrum as it changes, namely chromatic acclimation (CA) (Kehoe and Gutu, 2006; Gutu and Kehoe, 2012; Grébert et al., 2018; Wiltbank and Kehoe, 2019). As a result, light color becomes an important driver of competition between phytoplankton species (Stomp et al., 2007; Luimstra et al., 2020; Holtrop et al., 2021).

In view of light wavelength affecting the penetration distance in water (Kirk, 2011; Wiltbank and Kehoe, 2019), organisms growing in shallow waters tend to contain phycobilins (phycocyanin, PC) that can capture yellow/red light, while those at greater depth often contain more of the phycobilins (phycoerythrin, PE) that can capture green light (O'Carra et al., 1980). Filamentous cyanobacteria tend to grow in subsurface and/or deep water layers according to field observations (Halstvedt et al., 2007; Su et al., 2015) and modeling studies (Su et al., 2014, 2019), and they also proved to be the most important producer of an earthy-musty odorant - MIB. PE is

* Corresponding author
Email addresses: mingsu@rcees.ac.cn (Ming Su), jia_zeyu@ctg.com.cn (Zeyu Jia), jwyu@rcees.ac.cn (Jianwei Yu), yangmin@rcees.ac.cn (Min Yang)

one of the major photosynthetic accessory pigments of red algae (Ficner and Huber, 1993), cryptophytes (van der Weij-De Wit et al., 2006), and marine cyanobacteria (Bryant, 1982), e.g. *Synechococcus* (Grébert et al., 2018) and *Pseudanabaena* (Mishra et al., 2011). *Pseudanabaena* has been widely observed as the MIB producer in lakes and reservoirs (Izaguirre and Taylor, 1998; Zhang et al., 2016; Izaguirre et al., 1999). Note that, MIB is the secondary metabolite synthesized through the isoprenoid pathway (Bentley and Meganathan, 1981), that is also used for biosynthesis of photosynthetic pigments (Zimba et al., 1999). Therefore, MIB production might be affected by the biosynthesis of photosynthetic pigments, which may be regulated by CA in *Pseudanabaena* in response to the ambient light conditions. The relationship between these two physiological process remains unclear, more specially, whether the PE-dominated photosynthesis promotes MIB synthesis is critical to understanding the mechanism of MIB episodes in reservoirs/lakes.

To date, six types of CA (naming CA1 through CA6) that have been identified for cyanobacteria in sensing ambient light color via regulation of phycobilisome composition (Sanfilippo et al., 2019). CA1, CA2 and CA3 belong to green/red acclimation type, CA1 regulates the production of PBS with different linkers , CA3 changes the levels of both PE and PC abundances, and CA2 changes PE abundance (de Marsac, 1977; Sanfilippo et al., 2019). CA4 is blue/green acclimation type that changes the relative amounts of the bilin chromophores but not PBS proteins (Sanfilippo et al., 2019). CA5 and Ca6 belong to red/far-red acclimation type. CA5 leads to a loss of PC-containing phycobilins in far-red light, which are replaced by chlorophyll d–based light-harvesting antennae in the membrane, and CA6 (also called FaRLip) has been recently identified in filamentous cyanobacteria *Leptolyngbya* as a new CA form that extends the wavelengths range to far-red light (Gan et al., 2014). The strains with different light-harvesting strategies can enhance their competition on ambient light according to field investigation (Tan et al., 2020) and model study (Luimstra et al., 2020), which is probably driven by CA. On the other hand, the spectrum of underwater light in lakes and reservoirs is changing due to the increasing eutrophication and/or enhanced influx of orangic matter (Leech et al., 2018; Solomon et al., 2015), suggesting an unintended influence on the PE-containing MIB-producers.

On the basis of the studies described above and our previous MIB-related studies in freshwater systems, we put forward the hypothesis that MIB biosynthesis is regulated by chromatic acclimation of PE-containing cyanobacteria. Here, we investigate the influence of light color on the levels of various photosynthetic pigments to examine whether CA occurs in a PE-containing *Pseudanabaena*. We also evaluate the growth and MIB production under different light conditions via culturing experiments, revealing the causal mechanism of MIB biosynthesis in response to CA process. Finally, we evaluate the potential applicability of this study for controlling the unpleasant MIB-deduced odor in drinking water reservoirs by adjusting underwater light color spectrum based upon turbidity regulation.

2. Methods and Materials

2.1. Cyanobacterial culture and experimental conditions

The MIB-producing cyanobacterium *Pseudanabaena* (FACHB-1277) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-Collection, China, (Zhang et al., 2016)) and used in this study. It was originally isolated from Xionghe Reservoir, and was identified according to 16S rDNA sequence. A *Pseudanabaena* cell exhibits an average length of about 4 µm. The filaments of *Pseudanabaena* consist of 2-40 cells, and usually form compact brown mats and attach to the flask surface, which would turn into pink in the post-exponential growth stage (Fig. S1).

The pre-cultured strain was grown under 30 µmol m^{-2} s^{-1} (cool white tubes, Philips, Netherland) on a 12/12h light/dark cycle in BG11 medium (Nichols, 1973). Cells in exponential stage were collected by centrifugation (17 g-force, 2 min), then the supernatant was removed, and the fresh medium was added. This operation was repeated twice to remove the extracellular odor compounds. Cultures with an initial cell density of approximately 2×10^6 cell L^{-1} were grown in plastic tissue culture flasks (NEST® T25) with PTFE cap (0.22 µm) containing 30 mL medium within a custom-made incubator where both temperature and light were controlled. All the treatments were performed in triplicate except white light condition as the control and the position of each flask was changed randomly every day to reduce the inhomogeneity of light acceptance. The

temperature was controled at 25 °C in the experiments. Cultures were sampled every 3 or 4 d during a 35 d culture period for analyzing cell growth and MIB concentration. The samples collected during the logarithmic phase (approx. day 15-day 30) were used to evaluate the MIB cell quota (the mean cellular MIB production) and fluroscense intensities of Chl *a* and PE.

LED lamps with a wavelength of 455 nm (blue), 520 nm (green), 620 nm (red) (half-wavelength < 5 nm) and white fluorescent lamps (cool white tubes, Philips, Netherland) were used as light sources. The light intensity was set at 30 µmol $m^{-2}s^{-1}$ for all color conditions. An spherical quantum sensor (LI-193, LI-COR Inc., Lincoln, Nebraska, USA) was used to measure the light intensities.

2.2. Sample analysis

Because *Pseudanabaena* tends to form mats on the flask surface, samples were vigorously shaken, then 200 µL samples were collected to quantify the pigments, and 15 mL samples were filtered using GF/F (Whatman, USA) membrane to obtain the extracellular MIB. After that, the samples were dispersed using a SONIC Sonifier (Model: VC 105PB) with tapered microtip for 10 s at 300 W. Five mL samples were preserved with 1% Lugol's iodine for cell counting later according to the method described by Li et al. (2012). The residual samples were used to determine total MIB concentration. The total and extracellular MIB concentration were measured using the solid phase micro-extraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) described by Su et al. (2015). All the standards and reagents were purchased from Supelco (Sigma-Aldrich Co., USA).

Pigment abundances were determined by the *in-vivo* fluoroscopy (IVF), which has been adopted by oceanographers and limnologists for over 50 years (Lorenzen, 1966). This method is based on the fluorescence emission from photopigments in living cyanobacterial cells and can provide a rapid estimation of the concentration of photopigments (Bertone et al., 2018). Samples were diluted to ensure proportional relationships between fluorescence intensity and pigments abundance, and the pigments abundances were evaluated according to the integration of instant fluorescence intensity over a period of 40 µs, which is longer than their

fluoresence lifetime (BEDDARD et al., 1975; Holzwarth, 1986). The pigment compositions were identified by their excitation spectrum and emission spectrum, and the relative abundances were determined by the corresponding emission fluorescence intensity. The 427/680 nm (excitation/emission fluroscense wavelengths) were used to identify and quantify Chl *a* (Chang et al., 2012), 488/588 nm were used to identify and quantify phycoerythrin (PE) (Teale and Dale, 1970), 600/660 nm for phycocyanin (PC) (Chang et al., 2012), 633/660 nm for allophycocyanin (APC) (Glazer and Stryer, 1983), and 513/556 nm for Carotenoids (Gillbro and Cogdell, 1989). In addition, the absorption spectrum of samples were used to calibrate the proportion of detected pigments based on the standard absorption spectrum of pure pigments (Fig. S3), as described in `Supplementary Material`. Absorption spectrum and fluorescence emission intensities were measured using a multimode microplate reader platform (Spark, Tecan). Corning 96-well clear plates were filled with 200 µL samples and shaken for 5 s before measurement of fluorescence intensity.

2.3. Data analysis

Maximum growth rate at low cell densities μ_{log} (d^{-1}) was obtained for each light intensity using a solution for the classic logistic growth model (Oberhaus et al., 2007):

$$N_t = \frac{KN_0}{N_0 + (K - N_0)e^{-\mu_{log}t}} \tag{1}$$

where N_0 and N_t are the cell density on day 0 and t, respectively; and K is the carrying capacity of the culture environment, namely maximum attainable cell density in the given culture environment. The fitting was carried out using least-squares method in R language (Team, 2017) over the duration of each experiment.

The one-way analysis of variance (ANOVA) with wilcox test was applied to compare the difference in cell density, odor production and pigment production between different light intensities and colors using R language. Values of $p < 0.05$ were regarded as significant.

3. Results

3.1. *Regulation of photosynthesis pigments of* Pseudanabaena *in response to different light color*

The composition of main photosynthetic pigments within *Pseudanabaena* cell changes along with culturing light color (Fig. 1). Mainly 3 peaks of absorption spectrum were observed for *Pseudanabaena* cells cultured under white light. The peaks are 440 nm, 570 nm and 680 nm, corresponding to the characteristic absorption peaks of Chl *a* (blue peak), PE and Chl *a* (red peak). Four pigments were detected according to the absorption spectrum including Chl *a* (41.9%), Carotenoids (24.6%), PE (21.9%) and PC (11.6%) under white culturing light, as shown in Fig. 1b. This strain showed a different light-harvesting strategy after the culturing light shift to red or green colors. The most significant difference between the two culturing conditions is the abundance of PE (Fig. 1a). It was not detected under red light, while the proportion showed a significant high value of 28.9% under green light. Meanwhile, the Chl *a* also showed a higher proportion under green light (42.9%) than red light (39.8%) and white (41.9%). The proportion of Carotenoids of 13.5% under green color, is significant lower than that under red (36%) and white (24.6%) color. Besides, the proportion of PC is much higher under red (24.2%) than under green (14.7%) and white (11.6%) color, respectively.

3.2. *Cell growth and MIB yield of* Pseudanabaena *in response to light color*

The growth characteristics of *Pseudanabaena* varied under different culturing light colors with same light intensity of 30 µmol m^{-2} s^{-1} (Fig. 2a). The samples cultured within red light reached a maximum cell density of (30 ± 2) × 10^9 cell L^{-1} on day 30, with the growth rate of 0.787 ± 0.181 d^{-1} during the logarithmic phase. In comparison, the samples cultured under green light showed much lower maximum cell density ((8.1 ± 0.6) × 10^9 cells L^{-1}) on day 25, with sightly lower growth rate of 0.756 d^{-1}. The logarithmic phase of white light cultured samples started earlier than others, and the maximum cell density (19 × 10^9 cells L^{-1}) was observed at day 19, with the growth rate of 0.562 d^{-1}. Noted that, the *Pseudanabaena* could not survive under solely blue light.

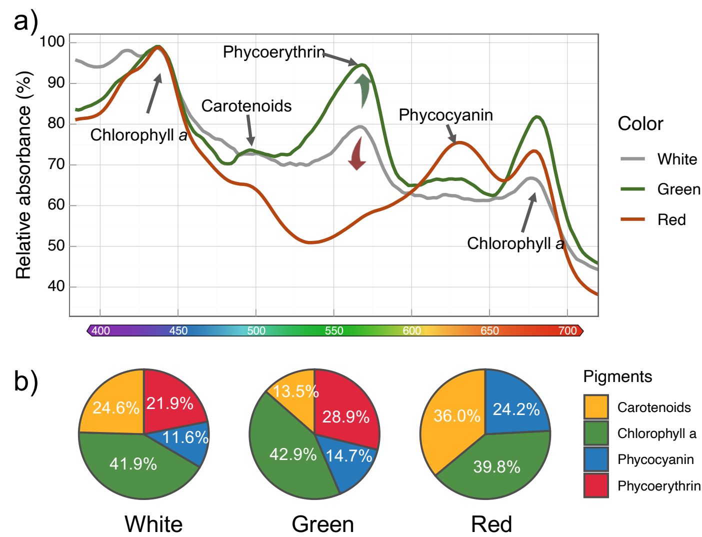

Fig. 1Light absorption spectra (a) and the photosynthetic pigments' composition (b) of *Pseudanabaena* cultured under white, green and red light color

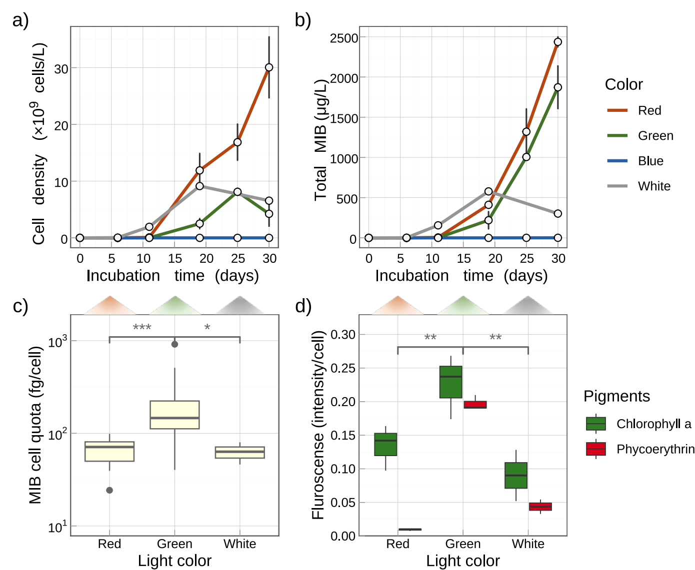

Fig. 2Effect of light color on the cell growth (a) and MIB production (b) of *Pseudanabaena* (25°C, n = 3 for red, green and blue light, n = 1 for white light), and the MIB cell quota (c) and fluroscense intensities of Chl *a* and PE (d) during the logarithmic phase (day 15 - day 30)

The MIB concentrations generally followed the same pattern as the cell growth for red and white light cultures. Maximum MIB concentrations ((2436 ± 23) μg L^{-1}) along with the maximum cell density were obtained from the samples under red light on day 30; the samples under white light showed a relatively earlier increase of MIB concentrations, with the highest concentrations observed on day 19 (578 μg L^{-1}). MIB concentrations remained low for blue light samples due to low cell densities. Nevertheless, the samples under green light showed slightly lower MIB concentrations (69% ± 13.4%) than red light during the logarithmic phases (Fig. 2b), in spite of the much lower cell density (Fig. 2a); hence the maximum MIB cell quota (median: 147 fg $cell^{-1}$, LQR: 112 fg $cell^{-1}$) was obtained from green light cultures in comparison with red ones (median: 71 fg $cell^{-1}$, LQR: 31 fg $cell^{-1}$) and white ones (median: 63 fg $cell^{-1}$, LQR: 17 fg $cell^{-1}$) with significant difference between red and green ($p < 0.001$, wilcox test, Fig. 2c).

The absorption near 570 nm was different for the cultures exposed to red and green light, representing a great distinction in PE production (Fig. 2d and Fig. S2). The *Pseudanabaena* produced little PE under red light, resulting in a very low ratio of Chl *a* to PE under red light (14.2 ± 1.2) in comparison with those under green light (1.2 ± 0.3) and white light (1.9 ± 0.4) ($p < 0.005$, wilcox test, Fig. 2d). The fluorescence emission intensity of Chl *a* per cell under green light was 1.69 times and 2.51 times higher than those under red and white light (Fig. 2d), which followed similar pattern with MIB yield (Fig. 2c).

3.3. The relationship between MIB production and photosynthetic pigments

The MIB cell quotas of all cultured samples are positively correlated with Chl *a* abundance regardless of light color. The correlation can be well described with a log-log linear model as illustrated in Fig. 3a ($R^2 = 0.68$, $p < 0.001$). Meanwhile, the relative abundance of celluar PE showed positive correlation with cellular Chl *a* for green lighting cultures during logarithmic phase ($R^2 = 0.57$, $p = 0.019$, Fig. 3b). Nevertheless, no correlation was observed for the cultures in red light ($p > 0.05$, Fig. 3b).

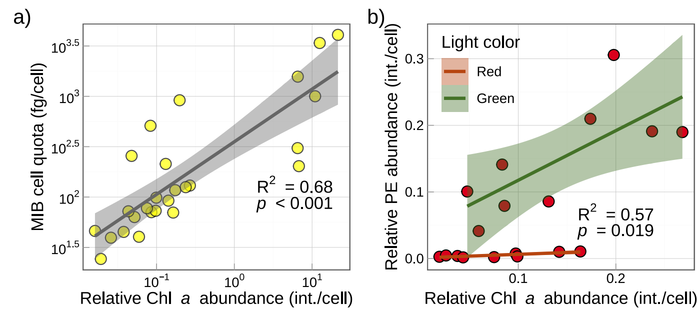

Fig. 3Correlation analysis of MIB and photosynthetic pigments; (a) MIB cell quota and Chl *a* abundance (b) PE abundance and and Chl *a* abundance under red and green light

4. Discussions

4.1. Biosynthesis pathway of MIB and photosynthetic pigments in cyanobacteria

Chl *a* is the primary pigment responsible for photosynthesis for cyanobacteria (Kirk, 2011), this molecule contributes around 1.5% dry weight for cyanobacteria cells (Jeon et al., 2014; Zavřel et al., 2017), suggesting that the biosynthesis of Chl *a* is of great importance for cyanobacteria. Fig. 4a illustrated a brief pathway map of Chl *a* associated with other pigments. The biosynthesis of Chl *a* can be broken down into 2 parts, including 1) the complex ring structure that begins with 5-aminolevulinic acid (5-ALA), and 2) the attachment of the phytol tail that synthesizes from isopentenyl pyrophosphate (IPP) etc. (Taiz and Zeiger, 2010). Along with this pathway, carotenoids can be synthesized from IPP, and phycobilins including PE, PC and APC can be synthesized from 5-ALA. MIB is a terpenoid produced as a secondary metabolite by some cyanobacteria and actinomycetes (Watson, 2004), it uses GPP as the monoterpenes' precursor (Giglio et al., 2011) (Fig. 4a). GPP undergoes a SAM-dependent methylation driven by GPP methyltransferase (GPPMT), resulting in the generation of an intermediate 2-methyl-GPP; this 2-methyl-GPP then undergoes direct cyclization to produce MIB driven by MIB synthase (MIBS) (Giglio et al., 2011).

Positive correlation between MIB yield and Chl *a* abundance was observed regardless of the light color for *Pseudanabaena* sp. (Fig. 3a), verifying that the MIB is synthesized along with the side branch of pigment biosynthesis pathway as occurs in actinomycetes (Bentley and Meganathan, 1981) and other cyanobacteria (Zimba et al., 1999); our evidence also supports

the consistent allocation of carbon into MIB during the biosynthesis of pigments including Chl *a* and carotenoids under different culture conditions, which is coincident with the evidences using biosynthectic inhibition treatments on phytogene to phytofluene (Zimba et al., 1999). Comparable studies are not found for MIB, but the other common earthy-musty odorant in drinking water - geosmin, has been well studied. As illustrated in Fig. 4a, this irregular sesquiterpene C15 compound is synthesized along with another side branch from Farnesyl diphosphate (FPP) in both actinomycetes (Bentley and Meganathan, 1981; Seto et al., 1998; Cane et al., 2006) and cyanobacteria (Naes et al., 1985, 1988, 1989; Jüttner and Watson, 2007). Some strains can simultaneously produce MIB and geosmin, such as *Oscillatoria f. granulata* (Tsuchiya and Matsumoto, 1999) and *Pseudanabaena catenata* (Jüttner and Watson, 2007), further studies are required to understand the proportions of carbon fluxes along with the two side branches for MIB and geosmin biosynthesis.

4.2. The correlation between MIB biosynthesis and chromatic acclimation of Pseudanabaena

Photosynthetic organisms capture energy from light via antenna pigments and transfer to photoreaction center for following metabolic processes (Watanabe and Ikeuchi, 2013). The light-harvesting antennas show large variation in structure and pigment composition, which enables them to adapt to the light conditions in their natural habitat (Pandit et al., 2017). Cyanobacteria use phycobilins as antenna pigments (Bryant, 1994). Phycobilins are covalently linked via a thioether bond to phycobiliproteins to form water-soluble pigment-protein complexes that can aggregate to form phycobilisomes. These proteins are organized into disks that are themselves stacked into rods, with disks containing shorter wavelength pigments on one end, and longer wavelength pigments at the other end next to a central core. Thus, the shorter wavelength absorbers: phycoerythrins (PE), 570 nm; are on the outside, phycocyanins (PC), 630 nm; within them, allophycocyanins (APC), 650 nm; in the core, followed by carotenoids, Chl *a* inside the photosynthetic membrane (Hayashi et al., 2003).

It is clear that cyanobacterial photosynthetic pigment components affect growth in a variety of light colors. Cyanobacteria were less efficiently than most eukaryotic in using blue light for

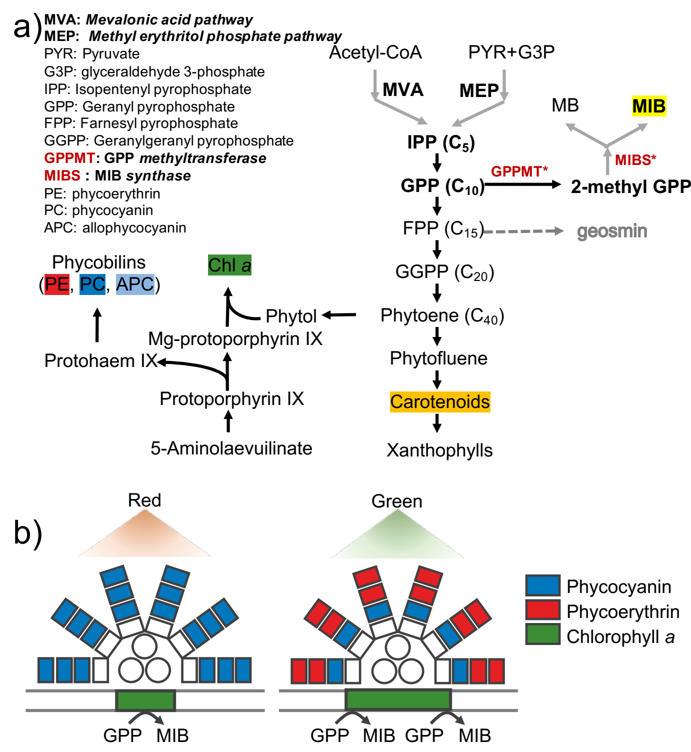

Fig. 4Chromatic acclimation regulates MIB production in *Pseudanabaena*. Schematic representation of the biosynthetic pathway of MIB and photosynthetic pigments, adapted from Zimba et al. (1999) and Giglio et al. (2011) (a), and the changes of photosynthetic pigments and MIB production under red and green light culture conditions (b)

photosynthesis, owing to the imbalance between PSI and PSII imparted by blue light (Luimstra et al., 2018). Mishra et al. (2012) observed that a *Pseudanabaena* strain can reproduce normally under solely blue light only when the intensity is very low (85 lux, approx. 1 µmol $m^{-2}s^{-1}$), and Lima et al. (2018) showed blue light combined with red light could favor both biomass and pigment productivity. A culture experiment of 8 cyanobacteria strains showed much lower growth rates under blue light than white, red and green light, with identical photon flux densities of 12 µmol $m^{-2}s^{-1}$ (Wyman et al., 1986); a plausible hypothesis is that blue light results in limited energy transfer to PSII, because cyanobacteria invest most Chl *a* in PSI, whereas their phycobilisomes including PE are mostly associated with PSII but do not absorb blue photons (Luimstra et al., 2019).

No adverse effects of cultivation in red/green light were observed for *Pseudanabaena*, indicating that this spectral range serves photosynthetic demands of growth in this cyanobacterium. The better growth rate in red than in green light was probably due to higher photosynthetic efficiency and quantum yield in the red light, which is coincident with red alga *Porphyra umbilicalis* (Figueroa et al., 1995). PE efficiently captures green, but not red, light. This strain increased their PE content in green light to optimize the light-harvesting efficiency through CA (Fig. 2d), as observed and revealed in many photosynthetic organisms (Bryant, 1994; Kehoe and Gutu, 2006). Among the six CA forms, our evidence supports that this *Pseudanabaena* performs CA3, by altering both the PE and PC in the outer rod regions (Fig. 4b) (Sanfilippo et al., 2019). In green light, PE makes up the outer rods, whereas in red light, PC is instead present, maximizing light-harvesting effectiveness, as observed in another freshwater cyanobacteria *Fremyella diplosiphon* (Haney and Kehoe, 2019; Wiltbank and Kehoe, 2019). RceA was identified as the major CA3 regulating gene in *Fremyella diplosiphon* (Terauchi et al., 2004), future studies on the gene expression of *Pseudanabaena* CA3 process are required.

In this study, the cellular MIB yield under green light is significantly higher than that under red light (Fig. 2b). Nevertheless, the MIB synthesis is irrelevant with PE according to the pathway map of MIB and typical pigments (Fig. 4a) and the evidences from the coincident relations

between MIB and Chl *a* abundance under different light color (Fig. 3). Since Chl *a* can not use green light, the photosynthesis reaction center can only use the light energy transferred along with PE⟶PC⇢Chl *a* in solely green light, while Chl *a* can use the red light photon directly and use the light energy transferred from PC simultaneously in red light (Wehrmeyer, 2003). In addition, We have evaluated the differences of pigment abundances under different light conditions. The relative PE abundance was significantly increased from 0 under red light to 0.20 under green light, relative Chl *a* abundance was increased from 0.14 under red light to 0.23 under green light, and the MIB cell quota is increased from 0.07 pg under red light to 0.12 pg under green light. Therefore, we speculate that the photosynthetic efficiency of PE⟶PC⇢Chl *a* light-harvesting system under solely green light is lower than the PC⇢Chl *a* system under red light, more Chl *a* was synthesized as the inevitable point of energy transport under green light. The mechanism responsible for the higher MIB yield under green light is probably the side effect of CA3 process.

5. Conclusion

By investigating the biosynthesis of MIB and photosynthesis pigments of a MIB-producing *Pseudanabaena*, we revealed that *Pseudanabaena* performed CA3, which alters the abundance of photosynthetic pigments including Chl *a*), PE and PC under various light color conditions. MIB yield is positively correlated with Chl *a* abundance under various light conditions, therefore it results in higher MIB yield in green light than red light. Our findings reveal the causal correlation between biosynthesis of MIB and photosynthetic pigments.

Acknowledgement

We would like to express our appreciation to the anonymous re- viewers for their great comments and suggestions. This work was fi- nancially supported by the National Natural Science Foundation of China (51878649, 52030002), the National Key R&D Program of China (2018YFE0204101), Shanghai Chengtou Raw Water Co. Ltd. and Chinese Academy of Sciences (KFJ-SW-YW036) and Youth Inno- vation Promotion Association CAS.

CRediT authorship contribution statement

M.S designed research. Z.J carried out experiment. Y.L, Y.Z and B.W prepared samples M.S and Z.J analyzed data, wrote the manuscript. J.F, J.L, J.Y and M.Y revised the manuscript with all authors contributing comments.

References

BEDDARD, G.S., PORTER, G., TREDWELL, C.J., BARBER, J., 1975. Fluorescence lifetimes in the photosynthetic unit. Nature 258, 166–168. URL: https://doi.org/10.1038/258166a0, doi:10.1038/258166a0.

Bentley, R., Meganathan, R., 1981. Geosmin and methylisoborneol biosynthesis in streptomycetes. evidence for an isoprenoid pathway and its absence in non-differentiating isolates. FEBS Letters 125, 220.

Bertone, E., Burford, M.A., Hamilton, D.P., 2018. Fluorescence probes for real-time remote cyanobacteria monitoring: A review of challenges and opportunities. Water Research 141, 152–162. doi:10/gdzhmn.

Bryant, D.A., 1982. Phycoerythrocyanin and phycoerythrin: Properties and occurrence in cyanobacteria. Microbiology 128, 835–844. URL: https://www.microbiologyresearch.org/content/journal/micro/10.1099/00221287-128-4-835, doi:10.1099/00221287-128-4-835.

Bryant, D.A. (Ed.), 1994. The Molecular Biology of Cyanobacteria. Springer Netherlands. URL: https://doi.org/10.1007/978-94-011-0227-8, doi:10.1007/978-94-011-0227-8.

Cane, D.E., He, X., Kobayashi, S., ömura, S., Ideda, H., 2006. Geosmin biosynthesis in *Streptomyces Avermitilis*. molecular cloning, expression, and mechanistic study of the germacradienol/geosmin synthase. The Journal of Antibiotics 59, 471–479. URL: https://www.nature.com/articles/ja200666.pdf, doi:10.1038/ja.2006.66.

Chang, D.W., Hobson, P., Burch, M., Lin, T.F., 2012. Measurement of cyanobacteria using in-vivo fluoroscopy - effect of cyanobacterial species, pigments, and colonies. Water Research 46, 5037–5048. doi:10.1016/j.watres.2012.06.050.

Ficner, R., Huber, R., 1993. Refined crystal structure of phycoerythrin from Porphyridium cruentum at 0.23-nm resolution and localization of the γ subunit. European Journal of Biochemistry 218, 103–106. URL: https://febs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1432-1033.1993.tb18356.x, doi:10.1111/j.1432-1033.1993.tb18356.x.

Figueroa, F.L., Aguilera, J., Niell, F.X., 1995. Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, Rhodophyta). European Journal of Phycology 30, 11–18. URL: https://doi.org/10.1080/09670269500650761, doi:10.1080/09670269500650761.

Gan, F., Zhang, S., Rockwell, N.C., Martin, S.S., Lagarias, J.C., Bryant, D.A., 2014. Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. Science 345, 1312–1317. URL: https://doi.org/10.1126/science.1256963, doi:10.1126/science.1256963.

Giglio, S., Chou, W.K.W., Ikeda, H., Cane, D.E., Monis, P.T., 2011. Biosynthesis of 2-methylisoborneol

in cyanobacteria. Environmental Science & Technology 45, 992–998. doi:10.1021/es102992p, arXiv:http://pubs.acs.org/doi/pdf/10.1021/es102992p.

Gillbro, T., Cogdell, R.J., 1989. Carotenoid fluorescence. Chemical Physics Letters 158, 312–316. URL: https://doi.org/10.1016/0009-2614%2889%2987342-7, doi:10.1016/0009-2614(89)87342-7.

Glazer, A., Stryer, L., 1983. Fluorescent tandem phycobiliprotein conjugates. emission wavelength shifting by energy transfer. Biophysical Journal 43, 383–386. URL: https://doi.org/10.1016/s0006-3495%2883%2984361-6, doi:10.1016/s0006-3495(83)84361-6.

Grébert, T., Doré, H., Partensky, F., Farrant, G.K., Boss, E.S., Picheral, M., Guidi, L., Pesant, S., Scanlan, D.J., Wincker, P., Acinas, S.G., Kehoe, D.M., Garczarek, L., 2018. Light color acclimation is a key process in the global ocean distribution of *Synechococcus cyanobacteria*. Proceedings of the National Academy of Sciences 115. URL: https://doi.org/10.1073/pnas.1717069115, doi:10.1073/pnas.1717069115.

Gutu, A., Kehoe, D.M., 2012. Emerging perspectives on the mechanisms, regulation, and distribution of light color acclimation in cyanobacteria. Molecular Plant 5, 1–13. URL: https://doi.org/10.1093/mp/ssr054, doi:10.1093/mp/ssr054.

Halstvedt, C.B., Rohrlack, T., Andersen, T., Skulberg, O., Edvardsen, B., 2007. Seasonal dynamics and depth distribution of *Planktothrix* spp. in Lake Steinsfjorden (Norway) related to environmental factors. Journal of Plankton Research 29, 471–482. URL: http://plankt.oxfordjournals.org/content/29/5/471.abstract, doi:10.1093/plankt/fbm036, arXiv:http://plankt.oxfordjournals.org/content/29/5/471.full.pdf+html.

Haney, A.M., Kehoe, D.M., 2019. Fremyella diplosiphon. Trends in Microbiology 27, 562–563. URL: https://doi.org/10.1016/j.tim.2019.01.011, doi:10.1016/j.tim.2019.01.011.

Hayashi, M., Chang, Y., Wang, T., Lin, S., Knee, J.L., 2003. Ultrafast spectroscopy and its applications, in: Encyclopedia of Physical Science and Technology. Elsevier, pp. 217–226. URL: https://doi.org/10.1016/b0-12-227410-5/00797-3, doi:10.1016/b0-12-227410-5/00797-3.

Ho, M.Y., Soulier, N.T., Canniffe, D.P., Shen, G., Bryant, D.A., 2017. Light regulation of pigment and photosystem biosynthesis in cyanobacteria. Current Opinion in Plant Biology 37, 24–33. URL: https://doi.org/10.1016/j.pbi.2017.03.006, doi:10.1016/j.pbi.2017.03.006.

Holtrop, T., Huisman, J., Stomp, M., Biersteker, L., Aerts, J., Grébert, T., Partensky, F., Garczarek, L., Woerd, H.J.v.d., 2021. Vibrational modes of water predict spectral niches for photosynthesis in lakes and oceans. Nature Ecology & Evolution 5, 55–66. URL: https://doi.org/10.1038/s41559-020-01330-x, doi:10.1038/s41559-020-01330-x.

Holzwarth, A.R., 1986. FLUORESCENCE LIFETIMES IN PHOTOSYNTHETIC SYSTEMS. Photochemistry and Photobiology 43, 707–725. URL: https://doi.org/10.1111/j.1751-1097.1986.tb05650.x, doi:10.1111/j.1751-1097.1986.tb05650.x.

Ikeuchi, M., Ishizuka, T., 2008. Cyanobacteriochromes: a new superfamily of tetrapyrrole-binding photoreceptors in cyanobacteria. Photochemical & Photobiological Sciences 7, 1159. URL: https://doi.org/10.1039/b802660m, doi:10.1039/b802660m.

Izaguirre, G., Taylor, W.D., 1998. A *Pseudanabaena* species from Castaic Lake, California, that produces 2-methylisoborneol. Water Research 32, 1673–1677. URL: http://www.sciencedirect.com/science/article/pii/s0043135497003795, doi:10.1016/s0043-1354(97)00379-5.

Izaguirre, G., Taylor, W.D., Pasek, J., 1999. off-flavor Problems in Two Reservoirs, Associated With Planktonic *Pseudanabaena* Species. Water Science and Technology 40, 85–90. URL: https://doi.org/10.2166/wst.1999.0268, doi:10.2166/wst.1999.0268.

Jeon, H.J., Choi, Y.K., Eom, H.S., Kang, G.H., Kim, K.J., Yang, Y.H., Lee, S.H., Kim, K.H., Kim, S.J., Kim, H.J., 2014. Comparison among dry cell weight, chlorophyll a concentration, and amperometric signal during a batch cultivation of spirulina maxima. Sensors and Actuators B: Chemical 205, 9–11. URL: https://doi.org/10.1016/j.snb.2014.08.047, doi:10.1016/j.snb.2014.08.047.

Jüttner, F., Watson, S.B., 2007. Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Applied and Environmental Microbiology 73, 4395–4406. URL: http://dx.doi.org/10.1128/AEM.02250-06, doi:10.1128/aem.02250-06.

Kehoe, D.M., Gutu, A., 2006. Responding to color: The regulation of complementary chromatic adaptation. Annual Review of Plant Biology 57, 127–150. URL: https://doi.org/10.1146/annurev.arplant.57.032905.105215, doi:10.1146/annurev.arplant.57.032905.105215.

Kirk, J.T.O., 2011. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press.

Leech, D.M., Pollard, A.I., Labou, S.G., Hampton, S.E., 2018. Fewer blue lakes and more murky lakes across the continental u.s.: Implications for planktonic food webs. Limnology and Oceanography 63, 2661–2680. URL: https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.1002/lno.10967, doi:10.1002/lno.10967, arXiv:https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.1002/lno.10967.

Li, Z., Hobson, P., An, W., Burch, M.D., House, J., Yang, M., 2012. Earthy odor compounds production and loss in three cyanobacterial cultures. Water Research 46, 5165–5173. URL: http://www.sciencedirect.com/science/article/pii/s0043135412004046, doi:10.1016/j.watres.2012.06.008.

Lima, G.M., Teixeira, P.C.N., Teixeira, C.M.L.L., Filócomo, D., Lage, C.L.S., 2018. Influence of spectral light quality on the pigment concentrations and biomass productivity of Arthrospira platensis. Algal Research 31, 157–166. doi:10.1016/j.algal.2018.02.012.

Lorenzen, C.J., 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep Sea Research and Oceanographic Abstracts 13, 223–227. doi:10/dm5vbm.

Luimstra, V.M., Schuurmans, J.M., de Carvalho, C.F.M., Matthijs, H.C.P., Hellingwerf, K.J., Huisman, J., 2019. Exploring the low photosynthetic efficiency of cyanobacteria in blue light using a mutant lacking phycobilisomes. Photosynthesis Research 141, 291–301. URL: https://doi.org/10.1007/s11120-019-00630-z, doi:10.1007/s11120-019-00630-z.

Luimstra, V.M., Schuurmans, J.M., Verschoor, A.M., Hellingwerf, K.J., Huisman, J., Matthijs, H.C.P., 2018. Blue light reduces photosynthetic efficiency of cyanobacteria through an imbalance between photosystems I and II. Photosynthesis Research 138, 177–189. URL: https://doi.org/10.1007/s11120-018-0561-5, doi:10.1007/s11120-018-

0561-5.

Luimstra, V.M., Verspagen, J.M.H., Xu, T., Schuurmans, J.M., Huisman, J., 2020. Changes in water color shift competition between phytoplankton species with contrasting light-harvesting strategies. Ecology 101, e02951. URL: https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1002/ecy.2951, doi:10.1002/ecy.2951, arXiv:https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1002/ecy.2951.

de Marsac, N.T., 1977. Occurrence and nature of chromatic adaptation in cyanobacteria. Journal of Bacteriology 130, 82–91. URL: https://doi.org/10.1128/jb.130.1.82-91.1977, doi:10.1128/jb.130.1.82-91.1977.

Mishra, S.K., Shrivastav, A., Maurya, R.R., Patidar, S.K., Haldar, S., Mishra, S., 2012. Effect of light quality on the c-phycoerythrin production in marine cyanobacteria *Pseudanabaena* sp. isolated from Gujarat coast, India. Protein Expression and Purification 81, 5–10. URL: https://doi.org/10.1016/j.pep.2011.08.011, doi:10.1016/j.pep.2011.08.011.

Mishra, S.K., Shrivastav, A., Mishra, S., 2011. Preparation of highly purified c-phycoerythrin from marine cyanobacterium *Pseudanabaena* sp. Protein Expression and Purification 80, 234–238. URL: https://www.sciencedirect.com/science/article/pii/S104659281100163X, doi:10.1016/j.pep.2011.06.016.

Naes, H., Aarnes, H., Utkilen, H.C., Nilsen, S., Skulberg, O.M., 1985. Effect of photon fluence rate and specific growth rate on geosmin production of the cyanobacterium *oscillatoria brevis* (kütz.) gom. Applied and Environmental Microbiology 49, 1538–1540. URL: https://doi.org/10.1128/aem.49.6.1538-1540.1985, doi:10.1128/aem.49.6.1538-1540.1985.

Naes, H., Utkilen, H., Post, A., 1989. Geosmin production in the cyanobacterium *Oscillatoria brevis*. Archives of Microbiology 151, 407–410. URL: https://link.springer.com/article/10.1007/bf00416598, doi:10.1007/bf00416598.

Naes, H., Utkilen, H.C., Post, A.F., 1988. Factors influencing geosmin production by the cyanobacterium *Oscillatoria Brevis*. Water Science and Technology 20, 125. URL: http://dx.doi.org/10.2166/wst.1988.0233, doi:10.2166/wst.1988.0233.

Nichols, H., 1973. Growth media–freshwater, in: Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, pp. 8–24.

Oberhaus, L., Briand, J.F., Leboulanger, C., Jacquet, S., Humbert, J.F., 2007. Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. Journal of Phycology 43, 1191–1199. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1529-8817.2007.00414.x, doi:10.1111/j.1529-8817.2007.00414.x.

O'Carra, P., Murphy, R.F., Killilea, S.D., 1980. The native forms of the phycobilin chromophores of algal biliproteins. a clarification. Biochemical Journal 187, 303–309. URL: https://doi.org/10.1042/bj1870303, doi:10.1042/bj1870303.

Pandit, A., van Stokkum, I.H.M., van Amerongen, H., Croce, R., 2017. Introduction: light harvesting for photosynthesis. Photosynthesis Research 135, 1–2. URL: https://doi.org/10.1007/s11120-017-0457-9, doi:10.1007/s11120-017-0457-9.

Sanfilippo, J.E., Garczarek, L., Partensky, F., Kehoe, D.M., 2019. Chromatic acclimation in cyanobacteria: A diverse and

widespread process for optimizing photosynthesis. Annual Review of Microbiology 73, 407–433. URL: https://doi.org/10.1146/annurev-micro-020518-115738, doi:10.1146/annurev-micro-020518-115738.

Seto, H., Orihara, N., Furihata, K., 1998. Studies on the biosynthesis of terpenoids produced by actinomycetes. part 4. formation of be-40644 by the mevalonate and nonmevalonate pathways. Tetrahedron Letters 39, 9497–9500. URL: http://www.sciencedirect.com/science/article/pii/s0040403998021546, doi:10.1016/s0040-4039(98)02154-6.

Solomon, C.T., Jones, S.E., Weidel, B.C., Buffam, I., Fork, M.L., Karlsson, J., Larsen, S., Lennon, J.T., Read, J.S., Sadro, S., Saros, J.E., 2015. Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: Current knowledge and future challenges. Ecosystems 18, 376–389. URL: https://doi.org/10.1007/s10021-015-9848-y, doi:10.1007/s10021-015-9848-y.

Stadnichuk, I.N., Krasilnikov, P.M., Zlenko, D.V., 2015. Cyanobacterial phycobilisomes and phycobiliproteins. Microbiology 84, 101–111. URL: https://doi.org/10.1134/s0026261715020150, doi:10.1134/s0026261715020150.

Stomp, M., Huisman, J., Vörös, L., Pick, F.R., Laamanen, M., Haverkamp, T., Stal, L.J., 2007. Colourful coexistence of red and green picocyanobacteria in lakes and seas. Ecology Letters 10, 290–298. doi:10.1111/j.1461-0248.2007.01026.x.

Su, M., An, W., Yu, J., Pan, S., Yang, M., 2014. Importance of underwater light field in selecting phytoplankton morphology in a eutrophic reservoir. Hydrobiologia 724, 203–216. URL: https://doi.org/10.1007/s10750-013-1734-z, doi:10.1007/s10750-013-1734-z.

Su, M., Andersen, T., Burch, M., Jia, Z., An, W., Yu, J., Yang, M., 2019. Succession and interaction of surface and subsurface cyanobacterial blooms in oligotrophic/mesotrophic reservoirs: A case study in Miyun Reservoir. Science of the Total Environment 649, 1553–1562. URL: http://www.sciencedirect.com/science/article/pii/s0048969718332789, doi:J.scitotenv.2018.08.307.

Su, M., Yu, J., Zhang, J., Chen, H., An, W., Vogt, R.D., Andersen, T., Jia, D., Wang, J., Yang, M., 2015. MIB-producing cyanobacteria (*Planktothrix* sp.) in a drinking water reservoir: Distribution and odor producing potential. Water Research 68, 444–453. URL: http://www.sciencedirect.com/science/article/pii/s004313541400668x, doi:10.1016/j.watres.2014.09.038.

Taiz, L., Zeiger, E., 2010. Plant Physiology. Sinauer Associates Inc.

Tan, X., Zhang, D., Duan, Z., Parajuli, K., Hu, J., 2020. Effects of light color on interspecific competition between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* in batch experiment. Environmental science and pollution research international 27, 344—352. URL: https://doi.org/10.1007/s11356-019-06650-5, doi:10.1007/s11356-019-06650-5.

Teale, F.W.J., Dale, R.E., 1970. Isolation and spectral characterization of phycobiliproteins. Biochemical Journal 116, 161–169. doi:10/gf6dmz.

Team, R.C., 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Terauchi, K., Montgomery, B.L., Grossman, A.R., Lagarias, J.C., Kehoe, D.M., 2004. RcaE is a complementary chromatic adaptation photoreceptor required for green and red light responsiveness. Molecular Microbiology 51, 567–577.

URL: https://doi.org/10.1046/j.1365-2958.2003.03853.x, doi:10.1046/j.1365-2958.2003.03853.x.

Tsuchiya, Y., Matsumoto, A., 1999. Characterization of oscillatoria f. granulata producing 2-methylisoborneol and geosmin. Water Science and Technology 40, 245–250. doi:10.1016/s0273-1223(99)00564-8.

Watanabe, M., Ikeuchi, M., 2013. Phycobilisome: architecture of a light-harvesting supercomplex. Photosynthesis Research 116, 265–276. URL: https://doi.org/10.1007/s11120-013-9905-3, doi:10.1007/s11120-013-9905-3.

Watson, S.B., 2004. Aquatic taste and odor: A primary signal of drinking-water integrity. Journal of Toxicology and Environmental Health, Part A 67, 1779–1795. URL: https://doi.org/10.1080/15287390490492377, doi:10.1080/15287390490492377, arXiv:https://doi.org/10.1080/15287390490492377. pMID: 15371216.

Wehrmeyer, W., 2003. Light-Harvesting Systems in the Photosynthetic Apparatus of Cyanobacteria, Red Algae and Cryptophytes. Springer Berlin Heidelberg, Berlin, Heidelberg. pp. 87–111. URL: https://doi.org/10.1007/978-3-642-56096-5_6, doi:10.1007/978-3-642-56096-5_6.

van der Weij-De Wit, C.D., Doust, A.B., van Stokkum, I.H.M., Dekker, J.P., Wilk, K.E., Curmi, P.M.G., Scholes, G.D., van Grondelle, R., 2006. How energy funnels from the phycoerythrin antenna complex to photosystem i and photosystem ii in cryptophyte rhodomonas cs24 cells. The Journal of Physical Chemistry B 110, 25066–25073. URL: https://doi.org/10.1021/jp061546w, doi:10.1021/jp061546w.

Wiltbank, L.B., Kehoe, D.M., 2019. Diverse light responses of cyanobacteria mediated by phytochrome superfamily photoreceptors. Nature Reviews Microbiology 17, 37–50. URL: https://doi.org/10.1038/s41579-018-0110-4, doi:10.1038/s41579-018-0110-4.

Wyman, M., Fay, P., Fogg, G.E., 1986. Underwater light climate and the growth and pigmentation of planktonic blue-green algae (cyanobacteria) ii. the influence of light quality. Proceedings of the Royal Society of London. Series B. Biological Sciences 227, 381–393. URL: https://royalsocietypublishing.org/doi/abs/10.1098/rspb.1986.0028, doi:10.1098/rspb.1986.0028, arXiv:https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.1986.0028.

Zavřel, T., Očenášová, P., Červený, J., 2017. Phenotypic characterization of *Synechocystis* sp. PCC 6803 substrains reveals differences in sensitivity to abiotic stress. PLOS ONE 12, e0189130. URL: https://doi.org/10.1371/journal.pone.0189130, doi:10.1371/journal.pone.0189130.

Zhang, T., Zheng, L., Li, L., Song, L., 2016. 2-methylisoborneol production characteristics of *Pseudanabaena* sp. FACHB 1277 isolated from Xionghe Reservoir, China. Journal of Applied Phycology , 1–10URL: http://dx.doi.org/10.1007/s10811-016-0864-x, doi:10.1007/s10811-016-0864-x.

Zheng, L., Zheng, Z., Li, X., Wang, G., Zhang, K., Wei, P., Zhao, J., Gao, N., 2021. Structural insight into the mechanism of energy transfer in cyanobacterial phycobilisomes. Nature Communications 12. URL: https://doi.org/10.1038/s41467-021-25813-y, doi:10.1038/s41467-021-25813-y.

Zimba, P.V., Dionigi, C.P., Millie, D.F., 1999. Evaluating the relationship between photopigment synthesis and 2-methylisoborneol accumulation in cyanobacteria. Journal of Phycology 35, 1422–1429. URL: http://dx.doi.org/10.1046/j.1529-8817.1999.3561422.x, doi:10.1046/j.1529-8817.1999.3561422.x.