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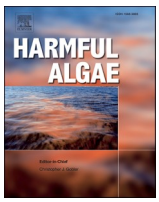

The success of the bloom-forming cyanobacteria *Planktothrix*: Genotypes variability supports variable responses to light and temperature stress

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ABSTRACT

Cyanobacterial blooms can modify the dynamic of aquatic ecosystems and have harmful consequences for human activities. Moreover, cyanobacteria can produce a variety of cyanotoxins, including microcystins, but little is known about the role of environmental factors on the prevalence of microcystin producers in the cyanobacterial bloom dynamics. This study aimed to better understand the success of *Planktothrix* in various environments by unveiling the variety of strategies governing cell responses to sudden changes in light intensity and temperature. The cellular responses (photosynthesis, photoprotection, heat shock response and metabolites synthesis) of four *Planktothrix* strains to high-light or high-temperature were studied, focusing on how distinct ecotypes (red- or green-pigmented) and microcystin production capability affect cyanobacteria's ability to cope with such abiotic stimuli. Our results showed that high-light and high-temperature impact different cellular processes and that *Planktothrix* responses are heterogeneous, specific to each strain and thus, to genotype. The ability of cyanobacteria to cope with sudden increase in light intensity and temperature was not related to red- or green-pigmented ecotype or microcystin production capability. According to our results, microcystin producers do not cope better to high-light or high-temperature and microcystin content does not increase in response to such stresses.

1. Introduction

Aquatic ecosystems are complex and dynamic adaptive systems driven by multiple biotic and abiotic factors. In these environments, cyanobacteria (photosynthetic prokaryotes) play critical roles at the base of the food chain and contribute significantly to primary production. However, over the past three decades, the increasing anthropic pressures on the natural aquatic environment have caused massive cyanobacterial developments, including those containing toxin-producers (*i.e.* cyanobacteria, Harmful Algal Blooms; cHAB) (Huisman et al., 2018). The increasing occurrence of these blooms is a source of concern since they can modify the dynamic of ecosystems and have deleterious consequences for the production of drinking water or recreational aquatic activities (Cheung et al., 2013; Chorus et al., 2021; Chorus and Welker, 2021; Massey et al., 2020; Otten and Paerl, 2015). Studies have been dedicated to environmental drivers affecting cyanobacterial growth and blooms (Huisman et al., 2018; Manning and Nobles, 2017; Paerl and Paul, 2012).

Planktothrix is one of the main bloom-forming cyanobacteria identified in temperate areas (Anagnostidis and Komárek, 1988; Gaget et al., 2015; Suda et al., 2002) and is globally distributed in lakes and reservoirs (Kurmayer et al., 2011). Recent genomic analyses revealed that all planktic *Planktothrix* belongs to the same species (Pancrace et al., 2017). Within this species, two central ecotypes exist a red-pigmented ecotype

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(*Planktothrix rubescens*) and a green-pigmented ecotype (*Planktothrix agardhii*) containing respectively phycoerythrin (PE) or phycocyanin (PC) as the principal constituent of phycobilisomes (*i.e.* the light-harvesting antennae of cyanobacteria). To dominate microbial ecosystems (bloom conditions), cyanobacteria need to be able to adapt to sudden changes of abiotic environmental factors (*i.e.* light, temperature, nutrients). Aquatic organisms are often exposed to rapid fluctuations in light intensity due the position of the sun and clouds or the movements of waves. In aquatic environments, the light intensity can increase by up to five times, reaching values as high as 9000 µmol photons $m^{-2} s^{-1}$ (Allahverdiyeva et al., 2015). In shallow lakes, water temperature responds rapidly to changes in air temperature, sometimes with episodic changes in water temperature of >5 °C; in the littoral zone the water temperature may even vary daily by 5–10 °C (Zhang et al., 2016).

Light is essential to drive photosynthesis and hence provide the fuel needed for cell functioning. However, excessive light can damage photosystems leading to photoinhibition (Murata et al., 2007). To cope with sudden increase in light intensity, cyanobacteria have evolved a variety of defensive mechanisms. One of these pathways involves the orange carotenoid protein and is related to phycobilisomes (OCP) (Kirilovsky, 2007). Flavodiiron and high-light-induced proteins (HLIPs) also play an essential role in photoprotection (Allahverdiyeva et al., 2013; Komenda and Sobotka, 2016). Light is not the sole abiotic factor damaging cell components when in excess. High-temperature (HT) also provokes adverse effects at the cellular level. An increase in temperature leads to denaturation of proteins, disruption of cell activity and eventually cell death. Heat shock proteins (HSPs) are produced in response to various stresses, including HT (Wang et al., 2004). They modulate protein homeostasis and can be classified into five families according to their size: HSPs100, HSPs90, HSPs70/ HSPs40/ HSPs25, HSPs60/ HSPs10 and small HSPs. The regulation and the multiple roles of HSPs in cyanobacteria are well documented (Rajaram et al., 2014). Their actions notably involve the protection of the photosynthetic apparatus from stress-induced damage (Chaurasia and Apte, 2009; Hihara et al., 2001; Huang et al., 2002; Nakamoto et al., 2000; Sato et al., 2010; Thurotte et al., 2020; Tran et al., 2013).

In parallel, numerous studies have been conducted to understand better the factors modulating the prevalence of toxin-producing strains in blooms and their toxin content. Under field and laboratory conditions, the effects of environmental elements such as light, temperature, nitrogen, phosphorous, and trace metals on microcystin (MC; the most prevalent toxins generated by cyanobacteria) production have been explored (Neilan et al., 2013; Omidi et al., 2018; Pearson et al., 2016). Interestingly, it has been previously shown that high-light (HL) increases transcription of genes belonging to the *mcy* gene cluster (Kaebernick et al., 2000; Sevilla et al., 2010; Tonk et al., 2005; Tran et al., 2013). The hypothesis that MC may play an intracellular role and modulates protein stability and function was then fueled by discovering a noticeable binding capability between MC and cysteins of various proteins, notably under HL (Zilliges et al., 2011). Altogether, these results strongly suggest a biological role of MC related to the limitation of cell damages induced by HL, especially at the level of the photosynthetic apparatus. However, figuring out the precise biologic roles of MC remains still under investigation (Omidi et al., 2018).

This study aimed at better understanding the reason for the success of *Planktothrix* in various changing environments by unveiling the variety of strategies governing cell responses to a sudden change in light intensity or temperature. We investigated the response of four *Planktothrix* genotypes to HL or HT and how ecotype (red- or green-pigmented) and MC production capability affect the ability of cyanobacteria to cope with such stresses. We hypothesized that: (i) red and green *Planktothrix* respond differently since the two ecotypes bloom in contrasting environments (deep and shallow lakes, respectively), (ii) *Planktothrix* strains producing MC will be more tolerant to HL than non-MC producers as MC is suspected of allowing the cells to limit damages induced by such stress on the photosynthetic apparatus, and (iii) responses to HL and HT will be contrasted as the two stresses impact different cellular mechanisms. *Planktothrix* responses were assessed at the gene expression, metabolomics, physiological, and ultrastructural levels using high throughput qPCR, untargeted metabolomics, *in vivo* fluorescence measurements, Transmission Electron Microscopy (TEM) techniques in order to better understand the mechanisms that enable these cyanobacteria to cope with fluctuating light intensities or temperatures. We mainly focused on cellular functions related to photosynthesis, photoprotection, heat shock response and metabolites synthesis (including cyanotoxins).

2. Material and methods

2.1. Cyanobacterial cultures

Four *Planktothrix* strains isolated from European blooms were selected upon their MC production: two producing strains, *P. agardhii* PCC 10110 (France) and *P. rubescens* PCC 7821 (Norway), a wild-type non-producing strain *P. agardhii* PCC 7805 (the Netherland) and an impaired MC production by mutagenesis of *P. agardhii* NIVA CYA 126/8 *ΔmcyD* (Finland; Gaget et al., 2015). The PCC strains further named 10110GreenMC+, 7821RedMC+, and 7805GreenMC- were axenic, whereas the mutant strain 126GreenMC- was living with bacteria (details related to the mutant and the ecotypes are given in supplementary Appendix A). The strains were maintained in 40 mL of BG11 (Rippka et al., 1979) and supplemented with 1 µg mL^{-1} chloramphenicol (Sigma) for the mutant strain. Each of the four cultures was separated into three independent Erlenmeyer diluted at OD_{750nm} 0.01 to 0.04. The cultures were synchronized under a 13:11 h light/dark cycle at 6 µmol photons $m^{-2} s^{-1}$ at 20 °C until they reached an OD_{750nm} to 0.5–0.7. They were progressively transferred in larger volumes until 1 L culture into a 3 L flask continuously agitated by magnetic stirring bars. Each 1-L culture was transmitted and divided into three subcultures of 1 L in 2 L bottles under the same conditions as above to obtain the expected biomasses for the HL stress, HT stress and control experiments. After four weeks of growth, the cultures reached an OD_{750nm} about 0.15–0.32 and were adjusted to 0.2–0.3. The 24-hour investigation was conducted the day after.

2.2. Experimental conditions

The 24-hour experiment was composed of a 4-hour stress exposure followed by a recovery phase. The first sampling point (T0) was made 1h30 after the light switch on in control conditions (Ctrl: 20 °C, 6 µmol photons $m^{-2} s^{-1}$) (Fig. 1). The stresses were applied after 2 h of light on to HL (150 µmol photons $m^{-2} s^{-1}$, 20 °C) or to HT (33 °C, by transferring the flask in 33 °C baths) for 4 h. Each experiment was tested in triplicate. For the RNA analysis, samplings were performed after 20 min (T0.3), 1 hour (T1), and 4 h (T4) of stress, as well as in control flasks. Further, samplings were performed at the end of the stress phase named recovery, after 3 h (T7), 5h30 (T10) and 20 h (T24) in all flasks. A volume of 50 mL of culture was filtered through a polycarbonate membrane (diam. 47 mm, pore size 3.0 µm Whatman Nucleopore). The filter was transferred into a 2 mL Eppendorf tube containing 1.5 mL of RNAlater (Sigma), stored at 4 °C overnight and then moved at −80 °C until RNA extraction. For *in vivo* fluorescence measurements, 10 mL of culture were sampled and immediately analyzed at the exact sampling times. For chlorophyll a (Chl-*a*) quantification, 10 mL of culture were sampled at T0, T1, T4, T7, T10 and T24, filtered through a GF/C filter (diam. 47 mm, pore size 1.2 µm Whatman) and stored at −20 °C until Chl-*a* extraction. 10 mL of culture were sampled for metabolomic analyses at T0, T4, T10 and T24. After centrifugation (3220 g; 10 min; 4 °C), the supernatant was discarded, and the pellet was conserved at −20 °C until extraction of metabolites. For TEM analyses, 10 mL of culture were sampled at T0 and T4.

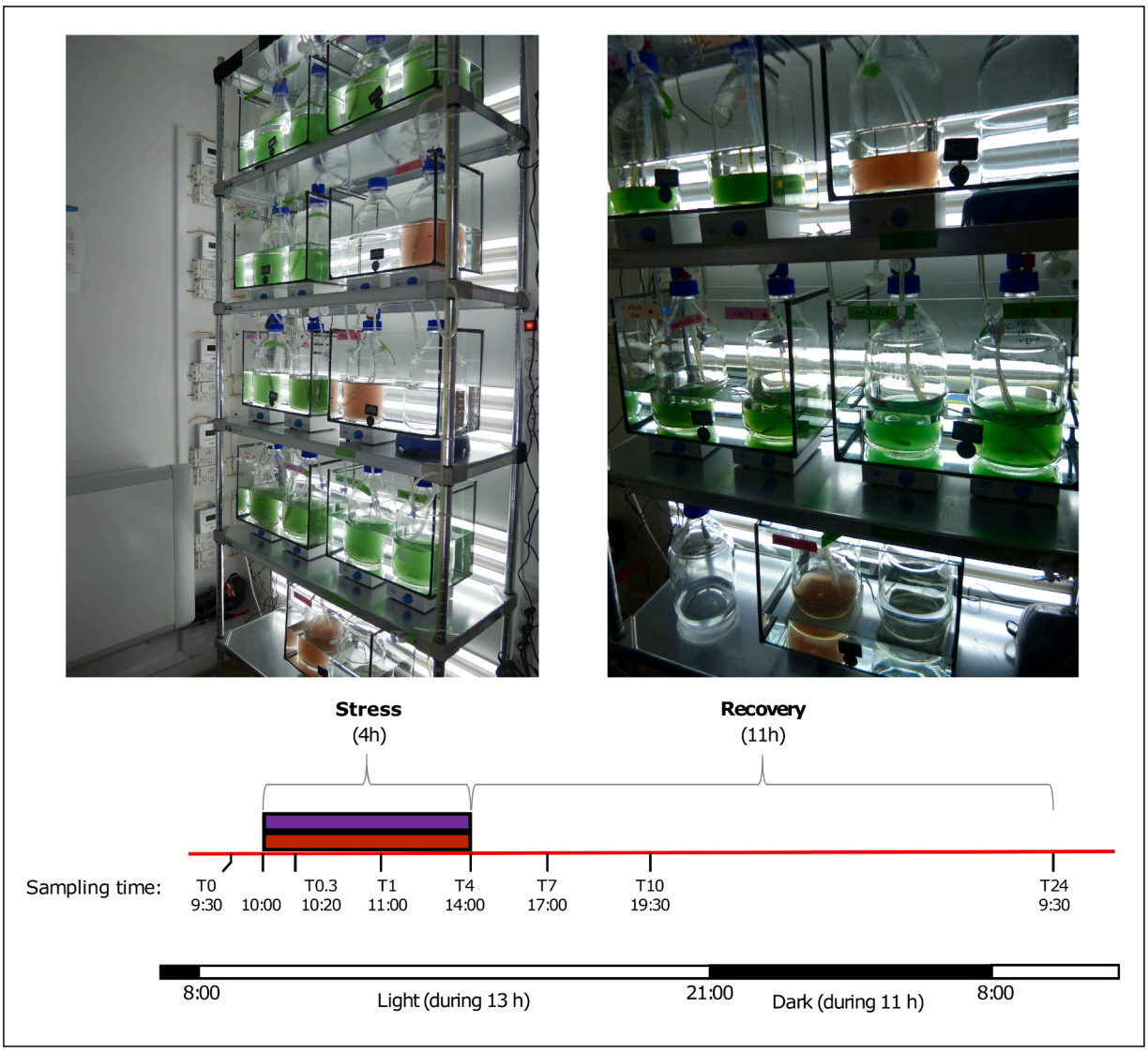

Fig. 1. Short-time stress experiment considering several *Planktothrix* genotypes belonging to two ecotypes and with different MC production capabilities. The 24-hour experiment was composed of a 4-hour stress exposure to HL or HT, followed by a recovery phase with a night shift of 11 h. All samplings were performed in triplicate during light phase.

2.3. Genome and gene selection

The complete genomes of the strains 7805GreenMC-, 7821RedMC+ are publicly available (LO018304–6 and CZCZ01000001–13, respectively), as well as the draft version of 126GreenMC- (NZ_ASAK00000000.1). The culture of 10110GreenMC+ (40 mL) was centrifuged rinsed twice with sterile water. The pellet of this culture was freeze-dried and lyophilized before DNA extraction. Genomic DNA was extracted with the NucleoBond Genomic DNA purification kit (Macherey-Nagel) as previously used in various pure cyanobacteria (Shih et al., 2013). The draft genome of the strain 10110GreenMC+ was obtained by Illumina sequencing resulting in a 5.42 Mb with a GC content of 39.55% in 148 scaffolds and deposited under the accession number GCA_903,969,095. The genome was deposited in the MicroScope platform for genomic analysis and compared with the other genomes (Vallenet et al., 2019).

2.4. Reverse transcription qPCR (RT-qPCR)

The RNA extraction followed the method based on high-intensity ultra-sonication recently developed for *Planktothrix* (Kim Tiam et al., 2019a) briefly described in supplementary Appendix B. The extracted RNA samples were reverse transcribed into cDNA and analyzed by qPCR at the High Throughput qPCR Core Facility of the ENS. Multiplex quantitative PCR were performed using the BioMark™ HD System with 48.48 integrated fluidic circuits (Fluidigm), following the process described in supplementary Appendix B.

Impacts of stresses were assessed on the relative expression of genes involved in photosynthesis (*psaA, psbA, apcA, cpcA, fnr*), photoprotection (*frp, ocp, flv1, flv3, hliA*), temperature tolerance (*dnaK, groEL, groES, hslO, hspA, hspG*), oxidative stress regulation (*sodB, gor*), microcystin synthesis (*mcyA, mcyB, mcyE*) and the synthesis of other secondary metabolites (*mdnB, aerA, aerB, aerF, ociB, ociC, ociD, aptA, aptB, aptC, micA, micC, micD*). The many phases in the reliable transcript quantification procedure (including primer design, validation, and correct normalization with the relevant reference genes, supplementary Table A and supplementary Appendix B) were carried out following MIQE recommendations (Bustin et al., 2009). Relative quantification of each gene expression level was normalized according to gene expression of *rpoC, gyrB* and *rpsL*, stable throughout the experiments. It was generated using Pfaffl's method, which considers the efficiencies of the primers (Pfaffl, 2001).

2.5. Sample analyses

Photoinhibition (inhibition of Photosystem II activity) monitoring, Chl-*a* quantification, metabolomic analyses and TEM analyses were performed as described earlier (Djediat et al., 2020; Kim Tiam et al., 2019b; Yéprémian et al., 2016) and are explained in detail in supplementary Appendix C. Results concerning the effects of HL on ultrastructure (obtained with TEM) are published elsewhere (Djediat et al., 2020) and will not be presented in this paper.

2.6. Statistical analyses

Normality and homogeneity of variance were checked before data analysis. The effects of light and temperature stresses on genetic expression analysis ($n = 3$) and Chl-*a* concentration ($n = 3$) were tested by analysis of variance (ANOVA), and multiple comparisons were conducted with Tukey-HSD tests using MetaboAnalyst 4 tool (Chong et al., 2018). Metabolomic data were treated using MetaboAnalyst 4 tool for Pareto's normalization and Partial Least Squares-Discriminant Analysis (PLS-DA). The effects of light and temperature on metabolomic composition were tested by Permutational Analysis of Variance (PERMANOVA) using R software's ade4 and vegan packages (R Core Team, 2019). Principal Component Analysis (PCA) were made using R software.

3. Results

Overall, the high throughput qPCR, *in vivo* fluorescence measurements and TEM techniques showed that the four *Planktothrix* strains exhibit clearly distinguishable responses when exposed to HL or HT, while untargeted metabolomics showed no significant or more moderate impact of these stresses (supplementary Figures A, B, C and D). The PCA (Fig. 2) representing the mean ($n = 3$) relative quantity of transcripts of the four strains in control condition or exposed to stresses revealed that *Planktothrix* strains had specific gene expression responses regarding the nature of stress as the first axis (31.8% of the variance explained by axis 1), clearly separated samples exposed to HL (orange) from the ones exposed to HT (purple). The HL treatment had the most substantial effects on the expression of genes involved in photosynthesis (*i.e. apcA* and *cpcA* expression decreased of x2.2 for both in HL), photoprotection (*i.e. frp, ocp, flv1, flv3* and *hliA* expression increased of x3.6, x1.7, x6.0, x5.1 and x61.4 in HL, respectively) and heat shock genes used as a marker of the general stress response (*i.e. groEL, groES, hspA* and *hspG* expression increased of x3.8, x3.1, x10.1 and x4.2 in HL, respectively) (Table 1). The HT treatment had the most substantial effects on genes involved in heat shock response (*i.e. groEL, groES, hspA* and *hspG* expression increased of x17.4, x13.4, x182.5 and x10.0 in HT, respectively).

3.1. Specific responses of the strains to HL

First, the expression patterns of the genes involved in photosynthesis, photoprotection and general stress response were globally similar for the four strains (Fig. 3a). These patterns showed a down-regulation of the expression of genes involved in photosynthesis (*psaA, psbA, apcA, cpcA* and *fnr*) and up-regulations of the expression of genes involved in photoprotection (*frp, flv1, flv3* and *hliA*) or general stress response (*groEL, groES, hspA* and *hspG*). While these gene expression patterns were similar for the four strains, their answers differed noticeably ($p<0.05$, Tukey-HSD) from one strain to another for some specific genes. Notably, the up-regulation of *frp, flv1, flv3* and *hliA* in the 126GreenMC- stress occurs early with the vigorous intensity of the response (value of expression factor of 9.4 ± 0.5, 4.5 ± 0.1, 16.0 ± 2.2, 10.0 ± 0.4 and 155.6 ± 41.9, respectively; Fig. 3b). Regarding the recovery phase, the expression patterns of these genes were also globally similar for the four strains with still a down-regulation of the expression of genes involved in photosynthesis, and down-regulations of the expression of genes involved in photoprotection or general stress response. Nevertheless, the strong response in the genes involved in photoprotection in the 126GreenMC- was still visible during the recovery phase with the up-regulation of *frp* and *hliA* only observed for this strain (value of expression factor 3.1 ± 0.6 and 35.7 ± 6.8, respectively; Fig. 3b).

The mean of photosynthetic capability was used to characterize physiological responses (short-term responses, in seconds). The findings revealed that HL had a significant impact on photosynthetic capacity (Fig. 4). Under the HL stress (from T0 to T4), variable fluorescence (F_v)

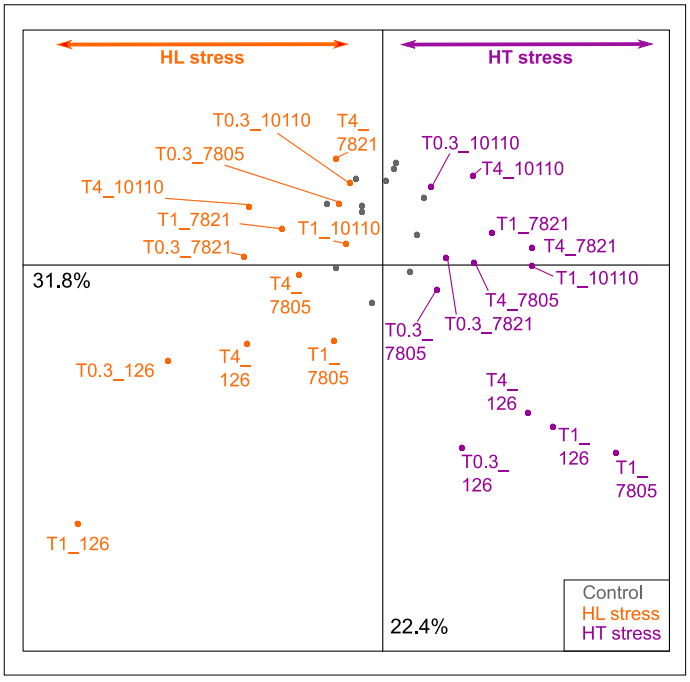

Fig. 2. Principal component analysis of the mean transcript abundance of 17 genes from the four *Planktothrix* strains (7805GreenMC-, 7821RedMC+, 10110GreenMC+, and 126GreenMC-) under control condition (gray), HL (orange) or HT (purple) during stress phase (T0.3, T1 and T4).

Table 1
Expression factors (mean of all strains) for *Planktothrix* exposed to high light or high temperature during stress and recovery phase compared to the control. NRPS: non-ribosomal peptide synthetase; PKS: polyketide synthase; MC: microcystin.

Mechanism	Gene	Product	High Light		High Temperature	
			Stress	Recovery	Stress	Recovery
Photosynthesis	*psaA*	photosystem I P700 chlorophyll a apoprotein A1	−1.5	−1.8	1.7	−1.5
	psbA	photosystem Q(B) protein 1	−1.1	−1.1	1.9	−1.4
	apcA	allophycocyanin alpha chain	−2.2	−2.6	1.2	−1.5
	cpcA	C-phycocyanin alpha chain	−2.2	−2.7	1.5	−1.2
	fnr	flavoprotein ferredoxin-NADP reductase	−1.1	−1.6	1.3	−1.2
Photoprotection and oxidative stress	*frp*	fluorescence recovery protein	3.6	1.3	−4.0	1.2
	ocp	orange carotenoid protein	1.7	−1.3	−2.1	−1.1
	flv1	flavodiiron proteins 1	6.0	−1.3	1.9	1.1
	flv3	flavodiiron protein 3	5.1	−1.3	2.9	−1.1
	hliA	high-light-induced protein A	**61.4**	**9.5**	1.2	1.2
	sodB	superoxyde dismutase	1.2	−1.0	1.2	−1.2
	gor	glutathione reductase	−1.2	−1.3	−1.2	1.0
General stress responses	*dnaK*	70 kDa heat shock protein dnaK	1.0	1.1	1.5	1.5
	groEL	large subunit chaperonin GroESL	3.8	1.6	**17.4**	−2.8
	groES	small subunit chaperonin GroESL	3.1	1.4	**13.4**	−4.3
	hslO	33 kDa heat shock protein HSLO	1.1	−1.3	−1.4	1.0
	hspA	small heat shock protein HSPA	**10.1**	2.0	**182.5**	1.3
	hspG	90 kDa heat shock protein HSPG	4.2	−1.4	**10.0**	−2.0
Microcystin synthesis	*mcyA*	NRPS, involved in MC biosynthesis	1.2	−1.1	−1.3	1.3
	mcyB	NRPS, involved in MC biosynthesis	1.0	−1.3	−2.2	1.1
	mcyE	NRPS/PKS hybrid, involved in MC biosynthesis	2.7	−1.0	2.1	1.2
Other secondary metabolites	Mean expression factors of genes involved in aeruginosin, anabaenopeptin, microviridin, microginin synthesis in the range [−2;2].					

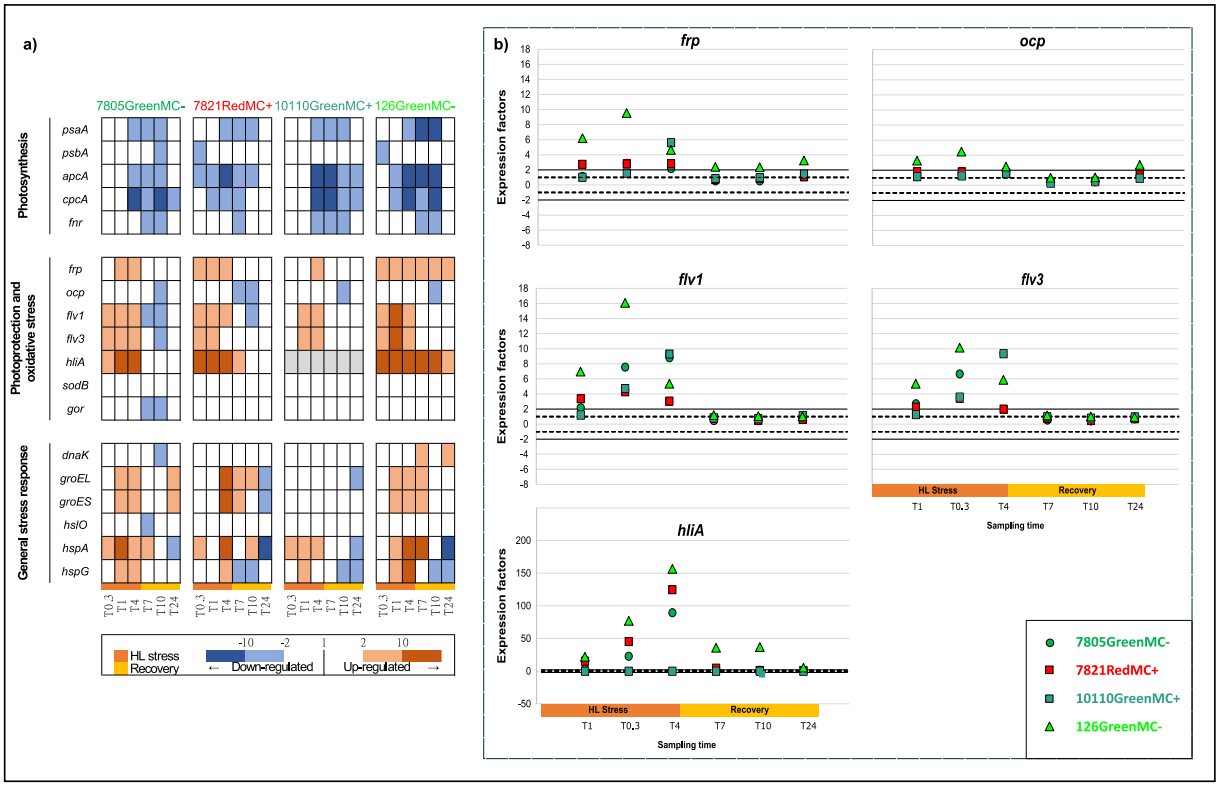

Fig. 3. a) Expression of genes involved in photosynthesis, photoprotection and oxidative stress and general stress response under a 4-hour HL stress for the four *Planktothrix* strains (7805GreenMC-, 7821RedMC+, 10110GreenMC+, and 126GreenMC-). b) Mean expression factors ($n = 3$) of genes involved in photoprotection (*frp, ocp, hliA, flv1* and *flv3*) for 7805GreenMC-, 7821RedMC+, 10110GreenMC+, and 126GreenMC- exposed to HL compared to the controls for the different sampling times. Dashed and solid lines delimit the expression factors outside of the intervals [−1; 1] and [−2; 2] respectively. *frp*, fluorescence recovery protein; *ocp*, orange carotenoid protein; *flv1*, flavodiiron proteins 1; *flv3*, flavodiiron proteins 3; *hliA*, the high-light-induced-protein A.

decreased to reach a maximal inhibition of 81 ± 10%, while during the recovery phase under low light conditions (from T4 to T10), F_v increased. These results indicate that photosystem II (PSII) activity decreased during the HL stress and then increased during the recovery. The loss of PSII activity appears to vary from one strain to another. Notably, 7805GreenMC- PSII activity was more resistant to HL than others. After 4 h of HL, only 60% of the initial activity was lost in this strain, while others lost 90% of initial F_v. The recovery of the PSII activity under low light conditions (from T4 to T10) was also faster in 7805GreenMC- and completed by T7.

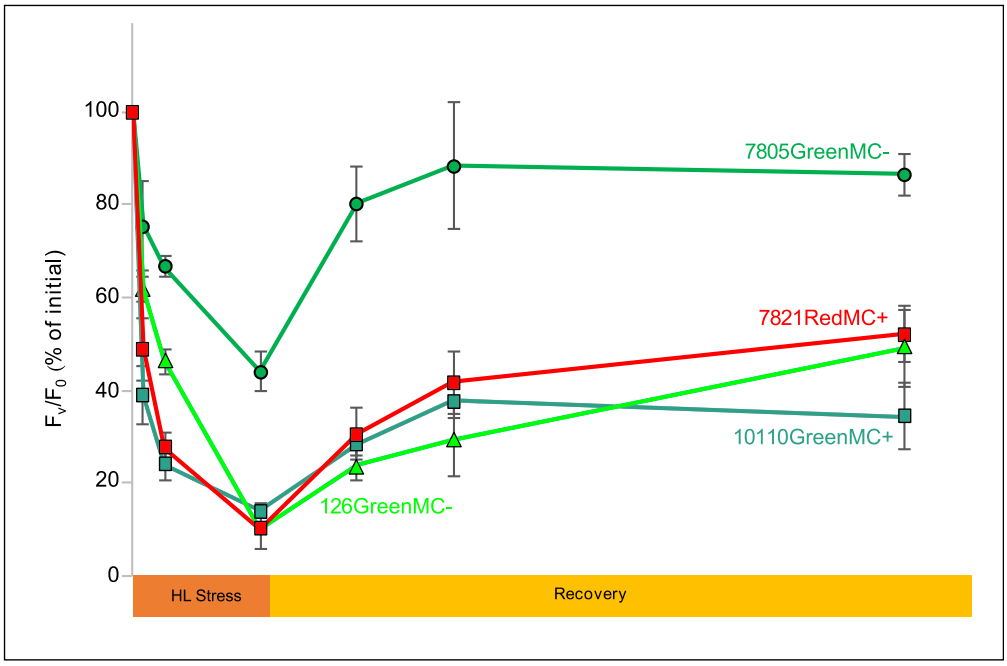

Fig. 4. PSII activity (F_v/F_0) under a 4-hour HL stress for the four *Planktothrix* strains (7805GreenMC-, 7821RedMC+, 10110GreenMC+, and 126GreenMC-).

Metabolome composition appears to be affected by HL only for the 7805GreenMC- strain (PERMANOVA, $p<0.01$; supplementary Figure D). The global metabolomic composition was shifted after 4 h of HL and is still observable after 24 h (Fig. 5). The Partial Least Squares-Discriminant Analysis (PLS-DA) and the associated scores of the Variables Importance in the Prediction (VIP) showed that the concentration of the majority of metabolites decreased when exposed to HL (supplementary Figure E). Among the 33 metabolites detected in 7805GreenMC-, 13 belong to secondary metabolites such as prenylagaramide, aeruginosin, oscillapeptin, and cyanopeptolin.

HL also had substantial effects on *Planktothrix* ultrastructure. As previously mentioned, the results concerning the impact of HL on ultrastructure showed that after 4 h of HL, thylakoids became less compact and interspaced by large clear electron vesicles (Djediat et al., 2020).

3.2. Strains also had specific responses under HT

In the experimental conditions tested, HT only impacted the expression of genes related to general stress responses (*groEL, groES, hspA* and *hspG*), with an evident up-regulation observed during the stress and a down-regulation phase during the recovery (Table 1 and supplementary Figure Fa). Maximal induction factors reached values of 60.0 ± 14.3, 51.6 ± 7.3 and 34.7 ± 23.9 for *groEL, groES* and *hspG,* respectively,

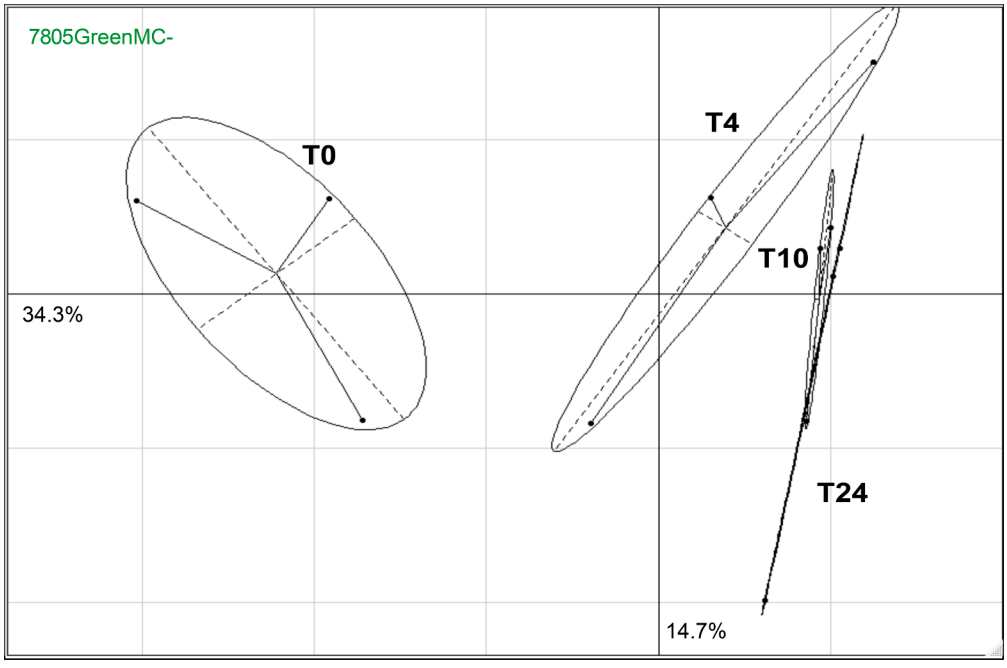

Fig. 5. Principal component analysis representing the metabolites production for 7805GreenMC- at T0, T4, T10 and T24 under a 4-hour HL stress.

while *hspA* got an induction factor of relative superior to 100 for the four strains (supplementary Figure Fb).

HT did not significantly affect the chlorophyll content of the different strains (supplementary Figure G). Still, critical ultrastructural modifications on thylakoids (swollen and less compact) were observed for all strains after 4 h of HT (supplementary Figure C). In addition, large inter-thylakoidal spaces and a detachment of the cellular membranes resulting in the apparition of large intermembrane rooms were observed. Despite the striking effects of HT stress on the ultrastructure of the four strains, this stress only affected the PSII activity of 10110GreenMC+ with the most substantial impact after 4 h where F_v decreased by 29 ± 4% (Fig. 6). The impact on the PSII activity of 10110GreenMC+ was still visible at the first sampling time of the recovery phase (T7) where 16% of the initial activity was still lost in this strain. Nevertheless, we can note that PSII activity of 10110GreenMC+ returned to initial values at T10 and T24 highlighting a complete recovery of PSII activity within a day after the end of HT stress (Fig. 6).

4. Discussion

This study focused on cellular processes by using high throughput qPCR, untargeted metabolomics, *in vivo* fluorescence measurements, and TEM techniques to describe responses to HL and HT stresses in four *Planktothrix* strains. Red- and green-pigmented *Planktothrix*, representative of two planktic ecotypes, belonged to the same species as revealed by recent genomic analyses (Pancrace et al., 2017). The authors compared the genetic relationships between different *Planktothrix* strains using the fifteen genomes available in 2017 and applying a Maximum Likelihood method on 586 concatenated proteins and the ANIm values between all genomes. Both methods showed that *P. agardhii* and *P. rubescens* belong to the same species. The four selected *Planktothrix* strains exhibited global gene content similarities with a core-genome and strain-specific genomes (*i.e.* flexible genes) represent 41.8% and 10.9–14.6% of the pan-genome respectively (Kim Tiam et al., 2019b). The clustering of the four strains revealed a close relationship between the two strains, 7805GreenMC- and 126GreenMC-, while the 7821RedMC+ and the 10110GreenMC+ were more distantly related (Kim Tiam et al., 2019b). Secondary metabolites gene clusters represented 1%–2% of the whole genome size; the strains possessed different secondary metabolites gene clusters resulting in a remarkable chemo diversity including MC and other secondary metabolites (*e.g.* prenylagaramide, aeruginosin, oscillapeptin, cyanopeptolin) (Kim Tiam et al., 2019b). We then investigated the cellular responses to HL and HT stresses on these four strains regarding the red- and green-pigmented ecotypes, and the capacity to produce MC and secondary metabolites, and of different cellular mechanisms. Cyanobacteria responses were evaluated during stress and recovery periods in order to compare their tolerance to HL or HT but also their capacity to recover from such stress as strains with both a higher tolerance to stress and better recovery will be advantaged in the environment. The main results of this study showed that HL and HT stress-induced contrasted responses, whatever the biological level considered (genes expression, photosynthetic capacities, metabolites production, and ultrastructure modification) and that individual strains had specific responses visible not only during the stress phase but also during the recovery phase that interestingly appears not related to MC production nor their respective ecotypes.

4.1. Strain-specific responses: The need to consider genetic diversity

The results highlighted specificities at the genotype level besides the contrasting responses to HL or HT of the four studied *Planktothrix* strains. In other words, each genotype has individual capacities to respond to abiotic stresses. Among these variations, the photosynthetic capacities appeared less impacted for the 7805GreenMC- strain in HL conditions considering both the stress phase and the recovery phase. This result follows the recent findings on photosynthesis and photoprotection mechanisms of three *Planktothrix* strains. Djediat et al. (2020) found that 7805GreenMC- was characterized by more efficient recovery machinery as the better resistance to HL was related to a better replacement of the damaged D1 protein. Up-regulations of *psbA* is observed during the first minutes (Kulkarni et al., 1992) or hours (Huang et al., 2002) of HL stress. The D1 protein (*i.e.* encoded by the *psbA* gene) has a fast turnover since it is the main target of damages caused by oxidative stress due to light and is sacrificed to avoid the destruction of PSII.

Moreover, the lower impact of HL on the photosynthetic capacities of 7805GreenMC- was associated with a modification of its intracellular metabolome characterized by a decrease of concentration of the

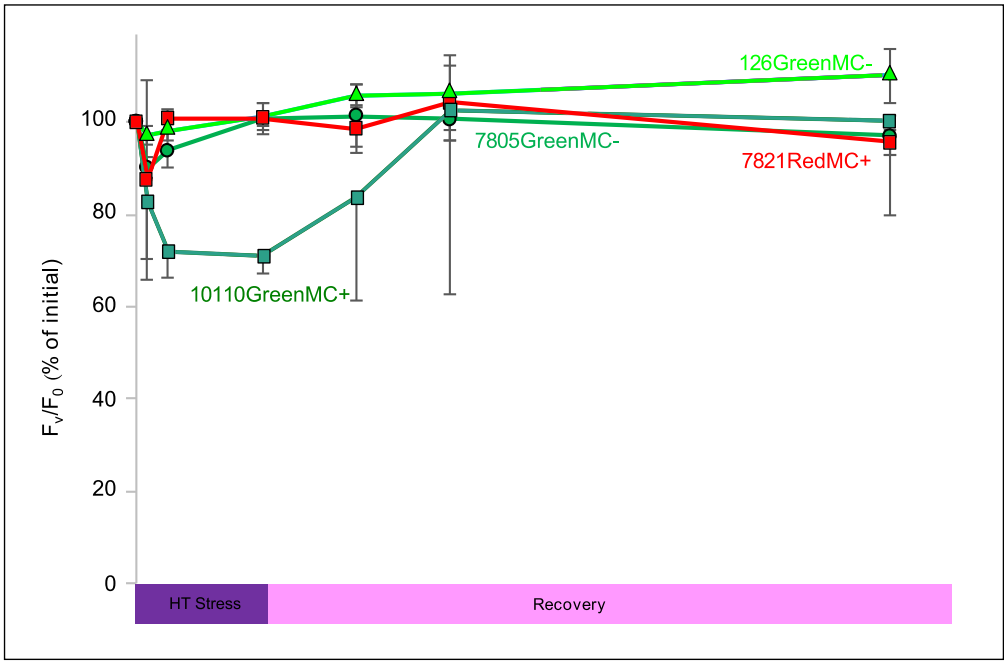

Fig. 6. PSII activity (F_v/F_0) under a 4-hour HT stress for the four *Planktothrix* strains (7805GreenMC-, 7821RedMC+, 10110GreenMC+, and 126GreenMC-).

majority of metabolites including secondary metabolites such as prenylagaramide, aeruginosin, oscillapeptin and cyanopeptolin. Producing secondary metabolites has a high energetically cost because their production involves giant enzymes (Christiansen et al., 2003). We hypothesized that in *Planktothrix* 7805GreenMC- cells reallocated energy dedicated to secondary metabolites synthesis to reducing damages caused by HL (*e.g.* production of molecules involved in photoprotection, replacement of proteins damages by HL). Differences in sensitivity between strains also appeared facing HT as photosynthetic capacities were much more impacted for 10110GreenMC+.

The response of 126GreenMC- exposed to HL differed from the other strains regarding the higher impact of genes involved in photoprotection (Fig. 3b). Actually, it is interesting to note that the stronger up-regulation of these genes in the 126GreenMC- was not concomitant with a higher impact on photosynthetic capability (Fig. 4). To explain this result, we could hypothesize that the strong up-regulation observed in 126GreenMC- was particularly efficient in order to limit the damages caused by HL. Indeed, it seems that the very strong up-regulation of *frp, flv1, flv3* and *hliA* allows 126GreenMC- to maintain its photosynthetic capability at the same level than 7805RedMC+ and 10110GreenMC+. The sensitivity of this strain to similar HT was previously demonstrated with a notable incapacity to survive at 30 °C (Gaget et al., 2015).

The four studied *Planktothrix* strains had similar global responses to either HL or HT (*i.e.* HL mainly impacted photosynthesis-related processes as shown previously in a transcriptomic study in the cyanobacterial model *Synechocystis* sp. PCC 6803 (Huang et al., 2002) while HT mainly impacted HSPs expression), but also that the responses to these abiotic stresses are strain-specific (*i.e.* 7805GreenMC- is the least impacted by HL while 10110GreenMC+ is the most impacted by HT). This study increased knowledge about relative sensitivity of *Planktothrix* strains to HL and HT. Our results showed that 7805GreenMC- was more tolerant to HL than 126GreenMC-, 7821RedMC+ and 10110GreenMC+ (Fig. 4) while 7805GreenMC-, 126GreenMC- and 7821RedMC+ were more tolerant to HT than 10110GreenMC+ (Fig. 6). In the environment, both stresses usually occur at the same time. Considering these results about the relative sensitivity of the four strains to HL or HT, we could hypothesize that 7805GreenMC- will be the most tolerant to a combined stress HL+HT while 10110GreenMC+ will be the least tolerant. These strain-specificities allow us to understand better how *Planktothrix* blooms may be able to last over a considerable period (up to several years) and develop in various environments (Briand et al., 2005). Although our study using high throughput qPCR, *in vivo* fluorescence measurements, untargeted metabolomics, and TEM enabled us to evaluate the effect of HL and HT on several *Planktothrix* genotypes, it certainly does not consider the whole genetic diversity of the natural population encountered in the aquatic environments. Field studies performed during the two last decades gave precious information about blooms dynamics and their genetic diversity (*e.g.* Briand et al., 2008, Sabart et al., 2010). They showed that cyanobacterial blooms are often dominated by several genotypes of the same species from which the relative proportions vary over time in response to environmental factor fluctuations. For instance, variations in the ratio of the different genotypes were recorded in a perennial bloom of *P. agardhii* over a 2-year survey (Briand et al., 2008). Such genotypes successions were also described in blooms dominated by others cyanobacteria such as *Microcystis* (Sabart et al., 2010).

4.2. MC producers do not cope better to HL or HT and MC content does not increase in response to such stresses

The negative impacts caused by the development of cyanobacterial blooms involve the production of cyanotoxins, including MC. In this context, numerous studies (mainly focused on *Microcystis*) have been carried out to understand better the link between MC production and environmental factors (recently reviewed by Chorus et al., 2021; Massey et al., 2020; Omidi et al., 2018). These studies can be classified into three groups: how environmental factors affect i/ intracellular MC concentrations, ii/ regulation of the MC gene cluster, and iii/ MC producers and non-MC producers. Laboratory studies have shown that MC content increased with light intensity until optimal light growth and decreased under light-saturated development after seven days (Utkilen and Gjølme, 1992; Wiedner et al., 2003). We could wonder if the decrease of MC content under light-saturated conditions observed in these studies traduced a reallocation of energy from MC synthesis to mechanisms linked with stress coping. Actually, producing MC has a high energetically cost because MC is synthesized nonribosomally *via* a giant enzyme complex comprising peptide synthetases, polyketide synthases (PKSs), and additional modifying enzymes. The genes needed for the biosynthesis of MC are included in a 55-kb cluster (Christiansen et al., 2003). Other studies did not report significant trends in MC content from cells grown under different light intensities (Kaebernick et al., 2000; Song et al., 1998) despite a change in the ratio of MC variants (Tonk et al., 2005). Similar results were obtained with temperature. It was found that increasing temperature up to optimal growth values enhanced MC content, while it decreased when temperatures exceeded its optimal level (Mowe et al., 2015; van der Westhuizen and Eloff, 1985). Whereas studies evaluating the MC content from cells grown under different light intensity or temperatures (like those cited above) are abundant, studies assessing the impact of fluctuating light intensity or temperature remain scarce. To our knowledge, this study is the first to assess the impact of a sudden increase in light intensity or temperature on MC and other metabolites content. Moreover, it revealed that these short-term stresses did not increase MC content.

Other works aiming to unravel the link between environmental factors and MC production have focused on regulating the MC biosynthesis gene cluster (Kaebernick et al., 2000; Sevilla et al., 2010; Tonk et al., 2005). These studies reported that HL increased the expression of the cluster. Our results follow the studies cited above since an up-regulation of the *mcyE* was observed during HL stress in *Planktothrix*. This up-regulation was not followed by a detectable increase of MC intracellular content during the 20 following hours. This last point underlines the need to link better the *mcy* gene cluster regulation and the MC production that is not strictly linear.

Finally, the third group of studies has focused on understanding how environmental factors could regulate cyanobacterial community composition, particularly looking at MC producers and non-MC producers. Several studies found that HL could favor MC producers (LeBlanc Renaud et al., 2011; Tran et al., 2013) and that HT promoted the frequency of MC producers over non-MC producers (Davis et al., 2009; Lehman et al., 2013; Yu et al., 2014). Our study did not support these findings since the tolerance to HL or HT is not related to the presence or absence of the MC gene cluster.

5. Conclusion

Altogether, these data demonstrate that HL and HT impact different cellular processes on *Planktothrix* and strain-specific *Planktothrix* responses. While some strains struggle to maintain photosynthesis in stressful conditions, others cope with them. This genotype diversity contributes to explaining the success of *Planktothrix* blooms in lakes presenting different environmental characteristics (*i.e.* deep alpine lakes or shallow urban lakes) and over a perennial period. Investigating the cellular mechanisms considering different cellular levels (transcriptional, metabolomic, physiological and ultrastructural) underlie stress response regulation in *Planktothrix* is heterogeneous. This heterogeneity should exist within the cyanobacterial population and is one way for cyanobacteria to adapt to large spectra of environments and/or quick-time adaption to abiotic factors within one local environment. According to our results, MC producers do not cope better to HL or HT and MC content does not increase in response to such stresses. As changes related to global warming would extend the severity of harmful cyanobacterial blooms (Chorus et al., 2021; Manning and Nobles, 2017; Massey et al.,

2020; Omidi et al., 2018), studies on the genetic diversity within monospecific population and genotypes-stress responses are a way to understanding better physiological traits that may support bloom dynamics.

CRediT authorship contribution statement

All authors performed sampling. CY and SH performed chlorophyll analyses. DK, KF and PS performed photoinhibition monitoring. SK and KC performed qPCR related analyses. MD and BD helped analyze qPCR data. BM, SK and SLM performed metabolomics analyses. C Djediat performed TEM analyses. SKT, MG, KC, BM and CB wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2022.102285.

References

Allahverdiyeva, Y., Mustila, H., Ermakova, M., Bersanini, L., Richaud, P., Ajlani, G., Battchikova, N., Cournac, L., Aro, E.-.M., 2013. Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. Proc. Natl. Acad. Sci. 110, 4111. https://doi.org/10.1073/pnas.1221194110.

Allahverdiyeva, Y., Suorsa, M., Tikkanen, M., Aro, E.-.M., 2015. Photoprotection of photosystems in fluctuating light intensities. J. Exp. Bot. 66, 2427–2436.

Anagnostidis, K., Komárek, J., 1988. Modern approach to the classification system of cyanophytes. 3-Oscillatoriales. Algol. Stud. Hydrobiol. 50–53, 327–472. Suppl.

Briand, E., Gugger, M., François, J.-.C., Bernard, C., Humbert, J.-.F., Quiblier, C., 2008. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (cyanobacterium) population. Appl Env. Microbiol. 74, 3839–3848.

Briand, J.-.F., Jacquet, S., Flinois, C., Avois-Jacquet, C., Maisonnette, C., Leberre, B., Humbert, J.-.F., 2005. Variations in the microcystin production of *Planktothrix rubescens* (cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. Microb. Ecol. 50, 418–428. https://doi.org/10.1007/s00248-005-0186-z.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622.

Chaurasia, A.K., Apte, S.K., 2009. Overexpression of the groESL operon enhances the heat and salinity stress tolerance of the nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC7120. Appl. Environ. Microbiol. 75, 6008–6012.

Cheung, M.Y., Liang, S., Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: a review of the problems, impact on drinking water safety, and efforts for protecting public health. J. Microbiol. 51, 1–10.

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D.S., Xia, J., 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res 46, W486–W494.

Chorus, I., Fastner, J., Welker, M., 2021. Cyanobacteria and cyanotoxins in a changing environment: concepts, controversies, challenges. Water (Basel) 13, 2463.

Chorus, I., Welker, M., 2021. Toxic Cyanobacteria in water: a Guide to Their Public Health consequences, Monitoring and Management. Taylor & Francis.

Christiansen, G., Fastner, J., Erhard, M., Börner, T., Dittmann, E., 2003. Microcystin biosynthesis in *Planktothrix*: genes, evolution, and manipulation. J. Bacteriol. 185 https://doi.org/10.1128/JB.185.2.564-572.2003.

Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8, 715–725. https://doi.org/10.1016/j.hal.2009.02.004.

Djediat, C., Feilke, K., Brochard, A., Caramelle, L., Kim Tiam, S., Sétif, P., Gauvrit, T., Yéprémian, C., Wilson, A., Talbot, L., et al., 2020. Light stress in green and red *Planktothrix* strains: the orange carotenoid protein and its related photoprotective mechanism. Biochim. Biophys. Acta Bioenerg. 1861, 148037.

Gaget, V., Welker, M., Rippka, R., de Marsac, N.T., 2015. A polyphasic approach leading to the revision of the genus *Planktothrix* (Cyanobacteria) and its type species, P. agardhii, and proposal for integrating the emended valid botanical taxa, as well as three new species, *Planktothrix paucivesiculata* sp. nov.ICNP, *Planktothrix tepida* sp. nov.ICNP, and *Planktothrix serta* sp. nov.ICNP, as genus and species names with nomenclatural standing under the ICNP. Syst. Appl. Microbiol. 38, 141–158. https://doi.org/10.1016/j.syapm.2015.02.004.

Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., Ikeuchi, M., 2001. DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell 13, 793–806.

Huang, L., McCluskey, M.P., Ni, H., LaRossa, R.A., 2002. Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. J. Bacteriol. 184, 6845–6858.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471–483. https://doi.org/10.1038/s41579-018-0040-1.

Kaebernick, M., Neilan, B.A., Börner, T., Dittmann, E., 2000. Light and the transcriptional response of the microcystin biosynthesis gene cluster. Appl. Environ. Microbiol. 66, 3387–3392.

Kim Tiam, S., Comte, K., Dalle, C., Duval, C., Pancrace, C., Gugger, M., Marie, B., Yéprémian, C., Bernard, C., 2019a. Development of a new extraction method based on high-intensity ultra-sonication to study RNA regulation of the filamentous cyanobacteria *Planktothrix*. PLoS ONE 14, e0222029. https://doi.org/10.1371/journal.pone.0222029.

Kim Tiam, S., Gugger, M., Demay, J., Le Manach, S., Duval, C., Bernard, C., Marie, B., 2019b. Insights into the diversity of secondary metabolites of *Planktothrix* using a biphasic approach combining global genomics and metabolomics. Toxins Basel 11, 498.

Kirilovsky, D., 2007. Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. Photosynth. Res. 93 (7) https://doi.org/10.1007/s11120-007-9168-y.

Komenda, J., Sobotka, R., 2016. Cyanobacterial high-light-inducible proteins — Protectors of chlorophyll–protein synthesis and assembly. Organ. Dyn. Bioenerg. Syst. Bact. 1857, 288–295. https://doi.org/10.1016/j.bbabio.2015.08.011.

Kulkarni, R.D., Schaefer, M.R., Golden, S.S., 1992. Transcriptional and posttranscriptional components of psbA response to high light intensity in *Synechococcus* sp. strain PCC 7942. J. Bacteriol. 174, 3775. https://doi.org/10.1128/jb.174.11.3775-3781.1992.

Kurmayer, R., Schober, E., Tonk, L., Visser, P.M., Christiansen, G., 2011. Spatial divergence in the proportions of genes encoding toxic peptide synthesis among populations of the cyanobacterium *Planktothrix* in European lakes. FEMS Microbiol. Lett. 317, 127–137.

LeBlanc Renaud, S., Pick, F.R., Fortin, N., 2011. Effect of light intensity on the relative dominance of toxigenic and nontoxigenic strains of *Microcystis aeruginosa*. Appl. Environ. Microbiol. 77, 7016. https://doi.org/10.1128/AEM.05246-11.

Lehman, P.W., Marr, K., Boyer, G.L., Acuna, S., Teh, S.J., 2013. Long-term trends and causal factors associated with *Microcystis* abundance and toxicity in San Francisco Estuary and implications for climate change impacts. Hydrobiologia 718, 141–158. https://doi.org/10.1007/s10750-013-1612-8.

Manning, S.R., Nobles, D.R., 2017. Impact of global warming on water toxicity: cyanotoxins. Food Toxicol. Food Saf. 18, 14–20. https://doi.org/10.1016/j.cofs.2017.09.013.

Massey, I.Y., Al Osman, M., Yang, F., 2020. An overview on cyanobacterial blooms and toxins production: their occurrence and influencing factors. Toxin Rev 1–21.

Mowe, M.A.D., Porojan, C., Abbas, F., Mitrovic, S.M., Lim, R.P., Furey, A., Yeo, D.C.J., 2015. Rising temperatures may increase growth rates and microcystin production in tropical *Microcystis* species. Harmful Algae 50, 88–98. https://doi.org/10.1016/j.hal.2015.10.011.

Murata, N., Takahashi, S., Nishiyama, Y., Allakhverdiev, S.I., 2007. Photoinhibition of photosystem II under environmental stress. Struct. Funct. Photosyst. 1767, 414–421. https://doi.org/10.1016/j.bbabio.2006.11.019.

Nakamoto, H., Suzuki, N., Roy, S.K., 2000. Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria. FEBS Lett 483, 169–174.

Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E., 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ. Microbiol. 15, 1239–1253.

Omidi, A., Esterhuizen-Londt, M., Pflugmacher, S., 2018. Still challenging: the ecological function of the cyanobacterial toxin microcystin–What we know so far. Toxin Rev 37, 87–105.

Otten, T.G., Paerl, H.W., 2015. Health effects of toxic cyanobacteria in U.S. drinking and recreational waters: our current understanding and proposed direction. Curr. Environ. Health Rep. 2, 75–84. https://doi.org/10.1007/s40572-014-0041-9.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res 46, 1349–1363.

Pancrace, C., Barny, M.-.A., Ueoka, R., Calteau, A., Scalvenzi, T., Pédron, J., Barbe, V., Piel, J., Humbert, J.-.F., Gugger, M., 2017. Insights into the *Planktothrix* genus: genomic and metabolic comparison of benthic and planktic strains. Sci. Rep. 7, 41181. https://doi.org/10.1038/srep41181.

Pearson, L.A., Dittmann, E., Mazmouz, R., Ongley, S.E., D'Agostino, P.M., Neilan, B.A., 2016. The genetics, biosynthesis and regulation of toxic specialized metabolites of cyanobacteria. Harmful Algae 54, 98–111.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29. https://doi.org/10.1093/nar/29.9.e45 doi: 10.1093/nar/29.9.e45.

R Core Team, 2019. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/. Vienna Austria. ISBN 3-900051-07-0.

Rajaram, H., Chaurasia, A.K., Apte, S.K., 2014. Cyanobacterial heat-shock response: role and regulation of molecular chaperones. Microbiology 160, 647–658.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111, 1–61.

Sabart, M., Pobel, D., Briand, E., Combourieu, B., Salençon, M.J., Humbert, J.F., Latour, D., 2010. Spatiotemporal variations in microcystin concentrations and in the proportions of microcystin-producing cells in several *Microcystis aeruginosa* populations. Appl. Environ. Microbiol. 76, 4750. https://doi.org/10.1128/AEM.02531-09.

Sato, T., Minagawa, S., Kojima, E., Okamoto, N., Nakamoto, H., 2010. HtpG, the prokaryotic homologue of Hsp90, stabilizes a phycobilisome protein in the cyanobacterium Synechococcus elongatus PCC 7942. Mol. Microbiol. 76, 576–589.

Sevilla, E., Martin-Luna, B., Vela, L., Bes, M.T., Peleato, M.L., Fillat, M.F., 2010. Microcystin-LR synthesis as response to nitrogen: transcriptional analysis of the *mcyD* gene in Microcystis aeruginosa PCC7806. Ecotoxicol. 19, 1167–1173.

Shih, P.M., Wu, D., Latifi, A., Axen, S.D., Fewer, D.P., Talla, E., Calteau, A., Cai, F., De Marsac, N.T., Rippka, R., et al., 2013. Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proc. Natl. Acad. Sci. 110, 1053–1058.

Song, L., Sano, T., Li, R., Watanabe, M.M., Liu, Y., Kaya, K., 1998. Microcystin production of *Microcystis viridis* (cyanobacteria) under different culture conditions. Phycol. Res. 46, 19–23. https://doi.org/10.1046/j.1440-1835.1998.00120.x.

Suda, S., Watanabe, M.M., Otsuka, S., Mahakahant, A., Yongmanitchai, W., Nopartnaraporn, N., Liu, Y., Day, J.G., 2002. Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. Int. J. Syst. Evol. Microbiol. 52, 1577–1595.

Thurotte, A., Seidel, T., Jilly, R., Kahmann, U., Schneider, D., 2020. DnaK3 is involved in biogenesis and/or maintenance of thylakoid membrane protein complexes in the cyanobacterium *Synechocystis* sp. PCC 6803. Life 10, 55.

Tonk, L., Visser, P.M., Christiansen, G., Dittmann, E., Snelder, E.O.F.M., Wiedner, C., Mur, L.R., Huisman, J., 2005. The microcystin composition of the cyanobacterium *Planktothrix agardhii* changes toward a more toxic variant with increasing light intensity. Appl. Environ. Microbiol. 71, 5177–5181. https://doi.org/10.1128/AEM.71.9.5177-5181.2005.

Tran, T.D.C., Bernard, C., Ammar, M., Chaouch, S., Comte, K., 2013. Heat shock transcriptional responses in an MC-producing cyanobacterium (*Planktothrix agardhii*) and its MC-deficient mutant under high light conditions. PLoS ONE 8, e73198.

Utkilen, H., Gjølme, N., 1992. Toxin production by *Microcystis aeruginosa* as a function of light in continuous cultures and its ecological significance. Appl. Environ. Microbiol. 58, 1321.

Vallenet, D., Calteau, A., Dubois, M., Amours, P., Bazin, A., Beuvin, M., Burlot, L., Bussell, X., Fouteau, S., Gautreau, G., Lajus, A., Langlois, J., Planel, R., Roche, D., Rollin, J., Rouy, Z., Sabatet, V., Médigue, C., 2019. MicroScope: an integrated platform for the annotation and exploration of microbial gene functions through genomic, pangenomic and metabolic comparative analysis. Nucleic Acids Res 48, D579–D589. https://doi.org/10.1093/nar/gkz926.

van der Westhuizen, A.J., Eloff, J.N., 1985. Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). Planta 163, 55–59. https://doi.org/10.1007/BF00395897.

Wang, W., Vinocur, B., Shoseyov, O., Altman, A., 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9, 244–252.

Wiedner, C., Visser, P.M., Fastner, J., Metcalf, J.S., Codd, G.A., Mur, L.R., 2003. Effects of light on the microcystin content of *Microcystis* strain PCC 7806. Appl. Environ. Microbiol. 69, 1475. https://doi.org/10.1128/AEM.69.3.1475-1481.2003.

Yéprémian, C., Catherine, A., Bernard, C., Congestri, R., Elersek, T., Pilkaityte, R., 2016. Chlorophyll a extraction and determination. Handb. Cyanobacterial Monit. Cyanotoxin Anal. 331–334.

Yu, L., Kong, F., Zhang, M., Yang, Z., Shi, X., Du, M., 2014. The dynamics of *Microcystis* genotypes and microcystin production and associations with environmental factors during blooms in Lake Chaohu. China. Toxins 6, 3238–3257. https://doi.org/10.3390/toxins6123238.

Zhang, M., Qin, B., Yu, Y., Yang, Z., Shi, X., Kong, F., 2016. Effects of temperature fluctuation on the development of cyanobacterial dominance in spring: implication of future climate change. Hydrobiologia 763, 135–146.

Zilliges, Y., Kehr, J.-.C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Börner, T., Dittmann, E., 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. PLoS ONE 6, e17615. https://doi.org/10.1371/journal.pone.0017615.