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1 **Higher sensitivity to hydrogen peroxide and light stress conditions of the microcystin producer**
2 ***Microcystis aeruginosa* sp PCC7806 compared to non-producer strains.**

3

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5

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15

16 **Abstract**

17 The increasing incidence of cyanobacterial blooms with their associated production of
18 cyanotoxins lead managers of aquatics systems to control their biomass to limit the health risk.
19 Among the variety of existing treatment approaches, hydrogen peroxide (H₂O₂) shows increasing use
20 but the effects of environmental parameters on its effectiveness are still not completely known. With
21 the aim to assess the efficiency of H₂O₂ treatments in the control of cyanobacterial blooms and
22 decrease toxic risk, we tested three *Microcystis* strains according to their ability to produce
23 cyanotoxins (a microcystin-producing, non-microcystin-producing and *mcyB*-knockout mutant).
24 Photochemical efficiency, percentage of living cells and microcystin cell content were compared
25 under various hydrogen peroxide concentrations coupled with stress conditions encountered during
26 the life cycle of cyanobacteria as darkness and high light. The microcystin-producing strain appeared
27 the more sensitive to hydrogen peroxide treatment and to light condition, probably due to a lower
28 rate of repair of Photo System II (PSII). We also highlighted various responses of PSII activity
29 according to *Microcystis* strains which could partly explain the shift of dominant genotypes often
30 occurring during a bloom event. Our results confirm the link between light and microcystin content
31 and variations of microcystin contents appear as a consequence of photosynthetic activity. These
32 findings could be of particular interest regarding water quality management, especially the use of
33 H₂O₂ as a potential algaecide which seems to be more effective to use during periods of high light.

34

35 **Key words:**

36 *Microcystis*, hydrogen peroxide, light and oxidative stress, photochemical efficiency, *mcyB*-knockout
37 mutant, microcystin.

38

39 **Highlights:**

40 Higher sensitivity of microcystin producer to hydrogen peroxide and light stress.

41 Efficiency to repair photodamage is genotype-dependant.

42 Variations of microcystin quotas appear as a consequence of photosynthetic activity.

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45

46 Introduction

47 *Microcystis* is one of the most widespread cyanobacteria. Massive proliferation of this genus
48 affects the balance of aquatic trophic networks and induces an increasing threat to the use and
49 management of freshwater ecosystems (Huisman et al., 2018; Paerl and Paul, 2012) . However, the
50 main risk associated with *Microcystis* proliferations relates to the ability to synthesise hepatotoxins,
51 called microcystins, which are hazardous to animal and human health (Merel et al., 2013). The
52 toxicity of *Microcystis* blooms is not only due to microcystin cellular production but is also linked to
53 the variable proportion of microcystin-producing cells within the whole *Microcystis* population
54 (Briand et al., 2009; Kardinaal et al., 2007; Kurmayer et al., 2003; Sabart et al., 2010). In some cases, a
55 positive correlation between cell concentration and microcystin cellular quotas have been shown,
56 suggesting an association between microcystin production and cell density (Wood et al., 2021).
57 Nevertheless, even if in some cases correlation between cyanobacterial biomass and microcystin
58 concentrations is evident, most of the time, this relationship is weak and site specific (Sinang et al.,
59 2013). Indeed, many environmental parameters are suspected to impact the regulation of the toxic
60 potential such as light intensity, temperature, nutrient concentrations (mostly nitrogen and
61 phosphorus), trace metals, pH, oxidative stress and predators, which can both affect the production
62 of microcystins and the proportion of MC-producing strains (Bittencourt-Oliveira et al., 2011;
63 Kardinaal et al., 2007; Kurmayer et al., 2003; Natumi and Janssen, 2020; Neilan et al., 2013; Rinta-
64 Kanto et al., 2009). Although the ecological role of microcystins remains unknown, several studies
65 suggest that the ability to produce these molecules could confer a competitive advantage. In some
66 cases, these molecules seem to give a particular advantage under suboptimal conditions for growth,
67 as the highest cellular concentrations of microcystins were often found at the onset or at the end of
68 a blooming event (Briand et al., 2009; Kardinaal et al., 2007; Sabart et al., 2010). Some results also
69 suggest an intracellular function of microcystins related to photosynthetic activity and oxidative
70 stress (Phelan and Downing, 2011; Wang et al., 2018; Zilliges et al., 2011).

71 In parallel, in order to reduce cyanobacterial blooms, among the multiple methods tested,
72 hydrogen peroxide (H₂O₂) is emerging as a potential algaecide, because it is environmentally friendly
73 and easy to apply in natural waters (Matthijs et al., 2012). Most studies suggest that H₂O₂ can
74 selectively suppress cyanobacteria in natural environments. Nevertheless, some factors can modify
75 the expected results such as nutrient limitation and light intensity (Matthijs et al., 2012; Moradinejad
76 et al., 2020; Piel et al., 2020; Sandrini et al., 2020; Wang et al., 2018). Indeed, at low light levels and
77 limited nutrient conditions, *Microcystis* were less sensitive to H₂O₂ (Sandrini et al., 2020). Conversely,
78 under high light conditions, cyanobacteria seem to be more sensitive to H₂O₂ due to a decrease of
79 photosynthetic activity (Piel et al., 2020; Wang et al., 2018). Indeed, results suggested that H₂O₂

80 causes major damage to photosystem II (PSII) and interferes with PSII repair, which makes cells more
81 sensitive to photoinhibition (Piel et al., 2020). Considering toxin production, Schuurmans et al. (2018)
82 showed that a microcystin-producing strain did not degrade H₂O₂ and did not recover, so it appeared
83 to be less prepared for high levels of oxidative stress than a non-toxic mutant and a naturally non-
84 toxic *Microcystis* strain. Moreover a global effect of H₂O₂ on MC production has been shown with a
85 decrease of transcriptional levels of microcystin-synthesis genes (*mcyA* and *mcyD*) but an increase in
86 microcystin-release gene (*mcyH*) (Wang et al., 2018). Nevertheless, no direct relationship has been
87 established between microcystin concentration and the use of H₂O₂.

88 It is therefore timely to investigate how oxidative stress induced by H₂O₂ can act on toxic
89 cyanobacterial potential: whether by regulating the production of microcystin toxins, or more
90 globally by interfering on the relative selection of MC-producing and non-MC-producing strains. To
91 answer this question, we compared three different strains of the cyanobacteria *Microcystis*: a
92 microcystin-producing strain, a non-microcystin-producing strain and a *mcyB*- knockout mutant with
93 impaired microcystin biosynthesis on *mcyB* gene. Moreover, as light conditions appear to be a key
94 factor in both microcystin production and sensitivity to H₂O₂, we tested the effects of several light
95 conditions (from dark to high light) on the photochemical efficiency, the microcystin cellular quotas
96 and the percentage of living cells of each *Microcystis* strain. The main aim of this study was thus to
97 assess both the stress response of H₂O₂ and light conditions on different *Microcystis* strains,
98 according to their ability to produce microcystin toxins.

99

100 **Material & methods**

101 ***Experimental settings and sampling***

102 Four experiments were conducted to compare the physiological response of three
103 *Microcystis aeruginosa* strains exposed to various concentrations of H₂O₂. In addition, to better
104 understand the response of cyanobacteria in natural environments, we decided to test H₂O₂
105 treatments under different light stress conditions as light appears to be an important factor in the
106 sensitivity to H₂O₂. Moreover, as for water management, concentration of cyanotoxins is the most
107 important factor to control, for each experiment, three *Microcystis* strains were used according to
108 their ability to produce microcystin: a microcystin-producing strain, *mcy+* (PCC 7806), a non-
109 microcystin-producing strain, *mcy-* (PCC 7005) and a mutant strain, *mcy^{mut}* (PCC 7806) with
110 impaired microcystin biosynthesis on *mcyB* gene (provided by the team of Dittman, Department of
111 Microbiology, University of Potsdam, Germany). Each strain was grown in BG11 medium (Sigma-
112 Aldrich). For each experiment, 1.5 litres of culture of each strain were grown at 25°C and irradiance
113 of 10 μmol.photon.m⁻²s⁻¹ until cells reached the mid-exponential growth phase. On the first day of

114 the experiment, cultures were gently mixed and a sample of each strain was taken to determine
115 cellular abundance. All cultures were composed of individual cells which were never seen grouped
116 during experiments. If necessary, cellular abundance was adjusted between the three *Microcystis*
117 cultures by diluting the most concentrated culture with BG11 medium and new counts performed to
118 confirm the cellular concentration. The cultures were then distributed into 250 mL glass conical
119 flasks, in triplicate for each strain and experimental condition.

120 *Dark stress*

121 As the life cycle of cyanobacteria include both light and dark phases (i.e. during
122 overwintering), the three *Microcystis* strains were cultured either under continuous light conditions
123 ($10 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$, 24:0) or complete darkness. Samples were collected every 2-3 days during
124 18 days of incubation (table 1).

125 *Oxidative stress*

126 Cyanobacteria cultures were incubated for 24h under continuous light conditions
127 ($10 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$, 24:0) with three different H_2O_2 concentrations: 0, 2 and 10 mg.L^{-1} (table 1).
128 These concentrations were chosen in agreement with the H_2O_2 concentration required to suppress
129 bloom-forming cyanobacteria which is in the range of 1–10 mg/L (Matthijs et al., 2012; Piel et al.,
130 2020). Each experimental condition was sampled at 0-2h-4h-6h-8h and 24h of the experiment.

131 *High light stress*

132 To test the effects of high light stress, the three *Microcystis* strains were placed under two
133 light conditions of 25 and $45 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$ during the first 2 hours of the experiment then
134 incubated under low light of $10 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$ to follow the repair of the PSII for a further 22
135 hours (table1). In order to inhibit the synthesis of new proteins potentially implicated in the repair of
136 PSII, an addition of inhibitors of protein synthesis was performed at the onset of the experiment
137 (Nishiyama et al. 2005). Thus, under light condition of $25 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$, for each *Microcystis*
138 strain, one third of flasks received an addition of 50 mg.mL^{-1} of chloramphenicol, one third was
139 incubated with 50 mg.mL^{-1} of kanamycin and the other third served as control. Each experimental
140 condition was sampled after 0-2h-4h-7h and 24h of the experiment.

141 *Oxidative stress coupled with high light stress*

142 For this last experiment, we combined the effect of high light and oxidative stress on the
143 same three *Microcystis* strains to better reflect the effect of H_2O_2 during a summer bloom. Cultures
144 in triplicate were incubated with or without 2 mg.L^{-1} of H_2O_2 and then placed under the two same
145 light conditions as the third experiment (25 and $45 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$ during the first 2 hours of the

146 experiment then incubated under low light of $10 \mu\text{mol.photon.m}^{-2}\text{s}^{-1}$ (table 1). Each experimental
147 condition was sampled after 0-2h-4h-7h and 24h of the experiment.

148 ***Cellular abundance***

149 Cell counts were determined on a 500 μL sample fixed with glutaraldehyde (1% final and kept
150 at 4°C) using a Thoma counting chamber under a Nikon microscope (Model Eclipse E200, Nikon
151 Instruments Inc., Melville, NY, USA). The cell growth rate (μ) was calculated as:

$$152 \quad \mu = \frac{X_{t_f} - X_{t_0}}{(t_f - t_0) X_{t_0}}$$

153

154 where X_{t_0} is the cellular density of *Microcystis* population at the beginning of a time interval, X_{t_f} is the
155 cellular density at the end of the time interval and $t_f - t_0$ is the length of the time interval in days.

156 ***Photosynthetic efficiency - F_v/F_m ratio***

157 The maximum photochemical efficiency of PSII fluorescence, i.e. the ratio of variable
158 fluorescence to maximum fluorescence (F_v/F_m ratio) was determined using a fluorescence induction
159 and relaxation (FIRe) fluorometer (Satlantic Inc, Halifax, NS, Canada). Cells were dark-acclimated for
160 15 min then the F_v/F_m ratio was determined as $(F_m - F_0)/F_m$ where F_0 is the minimal fluorescence when
161 all the reaction centres are open and F_m , the maximal fluorescence measured when all of the
162 reaction centres are closed. The fluorescence rise was analyzed using the FIREWORX script for
163 MATLAB software (MathWorks, Natick, MA, USA).

164 ***Living cells***

165 Cell death was quantified using SYTOX-green (Invitrogen), a nucleic acid-specific mortal stain
166 that only passes across the plasma membrane of dead cells, staining the DNA green under blue light
167 illumination. SYTOX-green concentration and incubation times were adapted from (Bouchard and
168 Purdie 2011). SYTOX-green working stocks were prepared in Milli Q water to a final concentration of
169 50 μM . Two μl of the SYTOX-green working stock was added to 500 μL of each sample (final
170 concentration of 0.05 μM) and incubated in the dark, at room temperature for 30 min. The ratio
171 between SYTOX-positive cells (green colour) and SYTOX-negative cells (red colour due to chlorophyll
172 fluorescence) were measured using a Zeiss epifluorescence microscope (Carl Zeiss Ltd, Hertfordshire,
173 UK) equipped with a FITC filter set (Ex: 465-495 nm; Em: 515-555 nm). Results are expressed in terms
174 of percentage of living cells considering that SYTOX-positive cells + SYTOX-negative cells give the total
175 cellular abundance.

176

177 ***Microcystin extraction and analysis***

178 The intracellular microcystins of PCC 7806 were extracted twice by sonication with 75%
179 methanol (MeOH). The lysates were centrifuged to remove cell debris, and then the supernatants
180 placed in Eppendorf tubes, and evaporated using a concentrator (Concentrator 5301; Eppendorf,
181 Hamburg Germany). The extracts obtained were dissolved in 200 µl 20% MeOH and stored at -20°C
182 prior to analysis.

183 Microcystin concentrations were determined by high-pressure-liquid chromatography (HPLC,
184 Agilent Technologies Waghäusel-Wiesental, Germany). A mobile-phase system consisting of water
185 (A) and acetonitrile (B) (each containing 0.1% formic acid) was used and separation was performed
186 with an RP-18 column (100 by 2.1 mm, 3.5-µm particle size; Agilent Zorbax Eclipse Plus) and the
187 following solvent gradient: 0 to 2 min, 98% A; 2 to 12 min, 98% to 2% A (linear); and 12 to 13 min, 2%
188 to 98% A. Data sets were acquired in positive electrospray (ESI) mode in a scan range from 50 to
189 1,500 m/z at a sampling rate of 2 Hz. Sodium formate solution was used for the calibration and
190 injected at the beginning of each chromatographic run by infusion (Harvard Apparatus; flow rate, 220
191 µl/h). Quality control samples (MC-LR) and blank runs were interspersed between the samples under
192 investigation. The microcystin concentrations in the samples were quantified on the basis of
193 standard curves.

194 ***Chemicals***

195 All reagents were of HPLC-MS grade (99.9%) or analytical grade. Methanol was purchased
196 from Sigma-Aldrich (Saint Quentin Fallavier, France), and acetonitrile and water were obtained from
197 Rhiedel de Hahn (Chromasolv LC-MS grade; Sigma-Aldrich, Saint Quentin Fallavier, France).
198 Microcystin standard MC-LR was purchased from Alexis Biochemicals (Enzo Life Sciences, Lausanne,
199 Switzerland).

200 ***Data analysis***

201 All statistical analyses were performed with XLStat (Addinsoft, 2021, Paris, France).
202 Spearman's correlation tests and analyses of variance (ANOVA) were performed. One-way ANOVA,
203 coupled with the post hoc Tukey's test with a 5% confidence interval, was performed in order to
204 determine differences between strains and tested conditions (light/dark, light intensity, H₂O₂
205 concentrations). The level of significance was set at 5%. The normality of the data was checked with
206 the Shapiro–Wilk normality test.

207

208

209 **Results**

210 ***Darks stress***

211 For all the parameters measured and each *Microcystis* strain, results showed a significant
212 difference between light and dark conditions. For each strain, a positive growth rate was found
213 under light conditions while a negative one was observed under dark conditions (figure 1A). The
214 *mcy+* strain grew faster ($0.52 \pm 0.004 \text{ d}^{-1}$) than the two other strains *mcy-* and *mcy mut* ($0.47 \pm 0.01 \text{ d}^{-1}$
215 for both) ($P < 0.001$). Thus, *mcy+* reached a mean of $14.5 \times 10^3 \text{ cells} \cdot \mu\text{l}^{-1}$ after 18 days of culture while
216 both *mcy-* and *mcy mut* attained lower mean values of 7.1 and $7.2 \times 10^3 \text{ cells} \cdot \mu\text{l}^{-1}$ respectively (figure
217 1A).

218 The initial F_v/F_m values for all three strains were around 0.2 (figure 1B). The F_v/F_m ratio of
219 cultures kept in the light showed a slight increase during the experiment (more noticeable for the
220 *mcy+* strain), whereas F_v/F_m remained quite stable (*mcy-*) or decreased (*mcy mut* and *mcy+*) for
221 strains placed in darkness. The comparison of the F_v/F_m ratio between dark and light conditions
222 revealed for each strain a significant difference ($P < 0.0001$). The *mcy+* strain showed a stronger and
223 more rapid decrease in F_v/F_m ratio in dark incubated cultures, and was close to zero from day 10. The
224 *mcy mut* revealed a lesser drop in F_v/F_m remaining around 0.1 for the last two sampling times while
225 the *mcy-* strain decreased less remarkably (apart from an apparent anomalous increase on day 10) to
226 a value of 0.15 after 18 days in the dark (figure 1B).

227 The percentage of living cells also showed a significant difference between the strains and
228 the light or dark incubation conditions ($P < 0.0001$). No significant difference was observed over time
229 between the three strains incubated in the light, with living cells making up to 90% of total cells
230 counted. Differences in percentage of living cells were detected under dark conditions (figure 1C)
231 with the *mcy+* strain showing the stronger and more rapid decrease in percentage of living cells, from
232 day 10 reaching only around 5% at the end of the experiment. The *mcy mut* culture also showed a
233 decrease of percentage of living cells but was only significant for the last two sampling days
234 ($P < 0.0001$) with around 50% of living cells detected on day 18. Conversely, the *mcy-* strain kept under
235 darkness showed no significant change in percentage of living cells over the entire 18 days of
236 incubation.

237 The microcystin cellular content measured on *mcy+* cells showed constant values around
238 $0.13 \pm 0.01 \text{ pg Mc-LR} \cdot \text{cell}^{-1}$ when maintained under light conditions (figure 1D) whereas, the culture
239 kept in darkness, showed an initial increase from 0.13 at day zero to $0.19 \pm 0.01 \text{ pg Mc-LR} \cdot \text{cell}^{-1}$ after 8
240 days then decreased steadily to $0.02 \text{ pg Mc-LR} \cdot \text{cell}^{-1}$ after 18 days. An increase in microcystin per cell
241 was observed during the first 8 days, reaching $0.2 \pm 0.01 \text{ pg Mc-LR} \cdot \text{cell}^{-1}$, then the microcystin cellular

242 content decreased slowly with a significant drop until day 15 and, at the end of the experiment,
243 values were close to zero.

244 ***Oxidative stress***

245 Under various concentrations of H₂O₂ tested, the dynamics of the photosynthetic yield
246 showed significant differences between the different strains (figure 2A). The *mcy mut* and the *mcy-*
247 strains showed a similar trend of photosynthetic yield while the *mcy+* strain was significantly
248 different (P<0.003) and appeared more sensitive to the addition of H₂O₂. Indeed, at the lower
249 addition of 2mg.L⁻¹ of H₂O₂, the photosynthetic yield of the *mcy+* strain showed a slight decrease to
250 63% after 24h of incubation whereas the other strains remained around 100%. When a
251 concentration of 10mg.L⁻¹ of H₂O₂ was added, only the photosynthetic yield of the *mcy+* strain
252 decreased initially to 70%, after only two hours of incubation (P<0.038) and then reaching zero after
253 24 hours.

254 In parallel, the microcystin-LR cellular content revealed variations according to the addition
255 of different concentrations of H₂O₂ (figure 2B). In the control culture (no added H₂O₂) and the culture
256 with 2 mg.L⁻¹ of H₂O₂ added, the microcystin-LR cellular quotas remained quite stable over 24h
257 (around 0.12 pg Mc-LR.cell⁻¹). However, when 10 mg.L⁻¹ of H₂O₂ was added to the *mcy+* culture, the
258 microcystin cell content decreased markedly to less than 0.01 pg Mc-LR.cell⁻¹.

259 ***High light stress***

260 The three *Microcystis* strains showed different patterns of response under light stress
261 conditions (figure 3A). The photosynthetic efficiency of *mcy-* strain was not affected by light stress
262 and remained quite stable over time. A similar trend was observed with the *mcy mut* strain but only
263 under the lower light stress condition. Conversely, with higher light stress of 45 μmol.photon.m⁻²s⁻¹,
264 the F_v/F_m ratio of *mcy mut* cells showed a decrease during the first 2 hours of increased light and
265 then, when placed back under low light conditions remained stable at a low value of 0.1 and
266 recovered its initial value of around 0.2 only after 24 hours of incubation. The *mcy+* strain showed a
267 similar response for both light stress conditions tested, but with a higher decrease in F_v/F_m ratio
268 under the higher light stress of 45 μmol.photon.m⁻²s⁻¹. Moreover, the *mcy+* cells showed a more
269 rapid recovery back to the initial F_v/F_m value than *mcy- cells*, after only 5 hours back in the low light
270 condition (figure 3A). The microcystin cellular contents remained quite stable in the *mcy+ strain* with
271 values around 0.15±0.015 pg Mc-LR.cell⁻¹, regardless of the light intensity tested (figure 4A).

272 To examine photodamage independently of repair, photochemical efficiency was measured
273 in the presence of two different inhibitors of protein synthesis (Chloramphenicol and Kanamycin).
274 The addition of these inhibitors had different effects on the dynamics of the F_v/F_m ratio detected for

275 each strain and revealed differences compared to those previously described without any added
276 inhibitors (figure 3 A&C). Concerning the *mcy+* strain, the addition of chloramphenicol produced a
277 similar pattern of F_v/F_m ratio changes to those cells incubated without the inhibitor present, with a
278 decrease after the light stress, followed by return to initial value after 24 hours. The addition of
279 Kanamycin induced a strong decrease of F_v/F_m ratio close to zero immediately after the light stress.
280 Then, after only 2h placed back in low light conditions, the F_v/F_m ratio returned to its initial value of
281 around 0.2. In parallel, microcystin cellular content showed major changes over time with differences
282 between the two inhibitor treatments. A large increase in microcystin cellular quota up to 0.4 ± 0.025
283 pg Mc-LR.cell⁻¹ was observed after 2 hours of incubation with the addition of chloramphenicol while
284 no change appeared under kanamycin condition (figure 4B). In the case of the *mcy mut* strain, the
285 F_v/F_m ratios remained high and quite stable after the light stress with the addition of
286 chloramphenicol. Similarly to *mcy+* cells, Kanamycin addition induced a quite different result with a
287 decrease of the F_v/F_m ratio of *mcy mut* cells but only after 2 hours placed in low light ($P < 0.039$)
288 before recovering to a higher value after 24 hours of the experiment (figure 3C). In contrast, the
289 addition of chloramphenicol revealed a stronger effect on the F_v/F_m ratio of *mcy-* with a rapid
290 decrease to zero value after 2 hours of light stress and remained at zero until the end of the
291 experiment. Under kanamycin conditions, the F_v/F_m ratio changed little after 2 hours in high light
292 condition then decreased close to zero over the next 22 hours (figure 3C).

293 ***Oxidative stress coupled with high light stress***

294 The combination of H₂O₂ addition and light stress had various effects on the F_v/F_m ratios of
295 the three *Microcystis* strains (figure 3E). The more sensitive strain was the *mcy+*, which showed a
296 large and rapid decrease of the F_v/F_m ratio to zero after 2 hours of light stress in both H₂O₂
297 concentrations. In parallel, microcystin cellular content, which remained stable after two hours of
298 light stress, decreased markedly to under 0.4 pg Mc-LR.cell⁻¹ after 24 hours of the experiment (figure
299 4C). The *mcy mut* strain also showed a decrease in the F_v/F_m ratio but more slowly than in the case of
300 *mcy+* strain and reached zero after 4 or 7 hours under respectively 25 or 45 $\mu\text{mol.photon.m}^{-2}\text{s}^{-1}$. The
301 *mcy-* strain appeared less sensitive and was only affected under the higher light stress which induced
302 a slow decrease of the F_v/F_m ratio over time while under low light stress no change of the
303 photosynthetic efficiency was observed (figure 3E).

304

305 **Discussion**

306 This study was conducted to compare the response of three different strains of *Microcystis*,
307 one (*mcy+*) that is able to produce microcystins, *mcy-* that does not produce microcystins and a

308 mutant strain (*mcy mut*), to the addition of H₂O₂ under several light conditions. The aim was to
309 improve our understanding of the dynamics of toxic and non-toxic strains and microcystin
310 concentrations in response to the use of H₂O₂ as an algaecide in natural environments. Interestingly,
311 under all tested stress conditions, the *mcy+* strain revealed a quicker and stronger decrease of the
312 F_v/F_m ratio than the two other strains that do not produce microcystin, as (Schuurmans et al., 2018)
313 showed. Typically, a decrease of photochemical efficiency is interpreted as an indicator of stress of
314 the photosynthetic apparatus (Allakhverdiev et al., 2008; Nishiyama et al., 2005). Photosystem II
315 (PSII) is very sensitive to changes in the environment and under unfavourable environmental
316 conditions, the activity of PSII declines more rapidly than other physiological activities (Campbell et
317 al., 1998) providing a good indicator of global cellular stress. The toxic strain appeared more
318 sensitive to stress conditions than the other two strains, as (Schuurmans et al., 2018) has shown
319 with H₂O₂ addition, and as Kardinaal et al. (2007) observed for light competition. Our results,
320 combining light and H₂O₂ stress, suggest that various responses from one strain to another could be
321 due to different rates of photodamage and/or rate of repair of photodamage between strains. The
322 activity of PSII is defined by the balance between the rate of damage to PSII and the rate of repair of
323 damaged PSII. In living cells, damage of PSII is repaired simultaneously and it is difficult to distinguish
324 these two processes (Allakhverdiev and Murata, 2004). To examine photodamage independently of
325 repair, we measured the photochemical efficiency in the presence of inhibitors of protein synthesis
326 which thus inhibit the repair of PSII by blocking the synthesis of new proteins. It appears that the
327 non-toxic strain underwent the strongest photodamage with a rapid reduction of the F_v/F_m ratio,
328 which reached zero in less than 24h. This means that inactivation of PSII of the non-toxic strain
329 occurred with inhibitors, as was previously shown with a *Synechocystis* strain, due to a lack of repair
330 of photodamage (Allakhverdiev and Murata, 2004; Nishiyama, 2001). As a consequence, the highest
331 and more stable values of F_v/F_m ratio of the *mcy-* strain observed without any protein inhibitors was
332 probably the result of a faster and more efficient rate of repair of photodamage of this strain
333 compared to the toxic and mutant ones, suggesting that the rate of repair of PSII could be genotype-
334 dependant. Nevertheless, the comparison of three different strains does not allow to easily give a
335 general conclusion. Indeed, natural populations contain a mixture of genotypes that vary in
336 morphology, colony size, gas vesicle concentration, types of thylakoids and amounts of various
337 compounds such as carboxysomes, polyphosphate granules and pigments which can influence
338 microcystin production (Jacinavicius et al., 2019). The contradictory results reported in the literature
339 clearly show the difficulty to identify general traits explaining the regulation of microcystin
340 production. Our results suggest that the higher capacity to repair PSII seems to give an advantage, in
341 our case, to the non-toxic strain, as its photochemical efficiency always appeared less affected by
342 environmental stress than the toxic or mutant strains. The different rates of damage and repair of

343 PSII could thus partly explain the shift, and then, the dominance of few genotypes among the
344 population in case of light stress conditions during a bloom event (Briand et al., 2009; Sabart et al.,
345 2010). Nevertheless, there is no evidence from our results that the ability to produce microcystins
346 would be involved in this selective process.

347 When the ability to synthesize new proteins was blocked for the toxic strain, the dynamics of
348 F_v/F_m ratio revealed unexpected results and we surprisingly observed that after a decrease of PSII
349 activity, a repair of PSII can still occur during hours following the light stress. This suggests that the
350 toxic strain was able to repair damage to PSII without any need to synthesize new molecules. This
351 could partly explain the similar dynamics of F_v/F_m ratio observed with and without protein inhibitors
352 for this *Microcystis* strain. Nevertheless, microcystins seem not to be involved in this process as a
353 noticeable stability of microcystin cellular quotas was observed in parallel with the repair of PSII
354 under different light stress conditions. Moreover, the similar trend of F_v/F_m ratio between the mutant
355 and the toxic strain under the highest light stress reinforce the idea of a non-specific need of
356 microcystins to repair the PSII and/or protect against photooxidation, as previously suggested by
357 several authors (Phelan and Downing, 2011; Zilliges et al., 2011). In contrast to the expected use and
358 decrease of microcystin cellular quotas, a substantial rise was noticed with the addition of
359 chloramphenicol. This cannot correspond to a new synthesis of these molecules, as chloramphenicol
360 prevents protein chain elongation by inhibiting the peptidyltransferase activity (Wolfe and Hahn,
361 1965) while Kanamycin exhibits a lesser effect by inducing mistranslation. Moreover, our
362 experimental period might have been too short to stimulate microcystin production: short-term
363 exposures to high light intensities can induce transcript increases within 2 hours (Kaebernick et al.,
364 2000) but furthermore, increase in toxicity was shown to take longer than 5 days (Utkilen and
365 Gjørlme, 1995) and thus may not be observed in our toxin data. So, as new synthesis of microcystins
366 within 2 hours seems unlikely under chloramphenicol conditions, the measured rise could then
367 correspond to a lack of use and/or exportation of this molecule, which could usually occur when cell
368 functions are complete. In addition, results obtained under dark conditions, where metabolic
369 functions are also reduced (Latour et al., 2007; Verspagen et al., 2004), indicated likewise a rise of
370 microcystins compared to light condition, strengthening the idea that microcystins can be stored in
371 cells under unfavourable growth conditions. This ability to store microcystins could partly explain the
372 higher microcystin contents often measured in the field in benthic *Microcystis* cells during the dark
373 overwinter phase (Ihle et al., 2005; Latour et al., 2007; Misson et al., 2011) and more generally under
374 unfavourable growth conditions (Briand et al., 2009; Sabart et al., 2010).

375 Light can damage PSII directly while other forms of environmental stress act primarily by
376 inhibiting repair of PSII (Nishiyama et al., 2005). Oxidative stress seems to be mostly implicated in the

377 inhibition of repair of PSII. Indeed, H₂O₂ is now known to inhibit the synthesis of D1 protein, that is
378 required for the repair of PSII (Frangeul et al., 2008; Kojima et al., 2007; Nishiyama et al., 2006).
379 More generally, hydrogen peroxide seems also to suppress the synthesis of other proteins in
380 thylakoid membranes (Allakhverdiev and Murata, 2004; Nishiyama, 2001). Studies have thus shown
381 that H₂O₂ treatment down-regulates *mcyA* and *mcyD* transcription (Qian et al., 2010; Wang et al.,
382 2018). This result is consistent with the decrease of microcystin content observed after the addition
383 of H₂O₂ in our experiments and previously found by several authors (Barrington et al., 2013; Matthijs
384 et al., 2012). This decrease could also correspond to a stronger binding of microcystins to proteins, as
385 was shown by Zilliges et al. (2011) under high light and oxidative conditions. These authors then
386 proposed that microcystins can play a role related to the protection against oxidative stress.
387 Nevertheless, even if binding protein could occur in our study, this seems not to constitute an
388 advantage against photooxidation as the photochemical activity of the mutant strain exhibited
389 always higher values under H₂O₂ and light stress than the toxic strain. In parallel, our results indicated
390 also that the decrease of microcystin cellular content occurred each time after the fall of
391 photochemical activity of the toxic strain (noticeable in cases of the addition of H₂O₂, H₂O₂ coupled
392 with light stress and in dark conditions). This repeated pattern under different stress conditions
393 suggests that the decrease of microcystin intracellular concentrations would be a consequence of the
394 fall of PSII activity. The decrease of PSII activity is accompanied by a phase of repair, during which
395 numerous molecular changes occur in the thylakoid membrane of cyanobacteria (Komenda et al.,
396 2012). So, due to its localisation on thylakoid membranes (Young et al., 2005), microcystin contents
397 could be consequently affected after damage of PSII. This sustains the idea that photosynthetic
398 reactions modulate microcystin contents at a cellular level (Deblois and Juneau, 2010).

399

400 In conclusion, it appeared that the toxic *Microcystis* strain exhibited the lowest resistance
401 against stress conditions compared to the two other non-toxic strains. Even if we are aware that a
402 greater number of tested *Microcystis* strains would be now required to improve our data set, it
403 seems that PSII activity and in particular, various rates of repair of photodamage from one strain to
404 another, could explain the differences in sensitivity of *Microcystis* strains under stress conditions. In
405 this way, variations of F_v/F_m ratio according to the strains, could then promptly promote few
406 genotypes and thus partly account for the shift of dominant genotypes of *Microcystis*, as Hodoki et
407 al. (2011) suggested concerning the shift of dominance from *Microcystis* to *Aphanizomenon*.
408 However, the ability to produce microcystin seems not to provide a specific advantage in
409 environmental stress conditions or against photooxidation. Both stable microcystin cell contents,
410 while repair of PSII occurred, and higher mutant's photochemical efficiency under stress conditions,
411 reinforce the idea of a non-specific need of microcystins to protect or repair PSII. The link between

412 light and microcystin contents suggested by several authors is though still confirmed. More precisely,
413 in our study, variations of microcystin content appear as a consequence of photosynthetic activity as,
414 systematically, a decrease of photochemical efficiency was followed by a fall in microcystin content.
415 So, regarding water quality management, it seems more efficient to use H₂O₂ treatment in
416 combination with high light, i.e. at midday in summer, as cumulative oxidative stress could reduce
417 both the fitness of toxic genotypes and the microcystin contents. Nevertheless, these results were
418 obtained only with the highest H₂O₂ concentration which can also have an effect on exposing non-
419 target organisms, especially zooplankton, but currently only a few studies have examined this aspect
420 (Escobar-Lux et al., 2019). Therefore, before using H₂O₂ treatment in natural environments as a
421 method to selectively suppress cyanobacterial blooms, it seems necessary to improve our
422 understanding of its overall impact on aquatic organisms and the food chain.

423

424 **Author contributions**

425 DL: conceptualization - methodology, investigation - original draft, writing – review & editing; FP:
426 methodology, investigation; DP: conceptualization - original draft, writing - review & editing.

427 **Declaration of competing interest**

428 The authors declare that they have no known competing financial interests or personal relationships
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432

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578

Table 1: Experimental culture conditions used and parameters measured in the four experiments.

Stress condition	Experimental conditions				Parameters measured			
	Light ($\mu\text{mol.photon.m}^{-2}\text{s}^{-1}$)	H ₂ O ₂ (mg.L ⁻¹)	Chloramphenicol or kanamycin (mg.mL ⁻¹)	Duration	Cells count	F _v /F _m	Living cells	Mc content
	Darkness	10 or darkness; 24h:0	None	None	18 d	×	×	×
Oxidative	10; 24h:0	2, 10	None	24 h	×	×		×
High light	25 or 45 for 2h then 10	None	50	24 h	×	×		×
Oxidative + high light	25 or 45 for 2h then 10	2	None	24 h	×	×		×