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1 **Thermoacclimation and genome adaptation of the membrane lipidome in**
2 **marine *Synechococcus***

3

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18

19 **Running title:** Membrane thermoadaptation in marine *Synechococcus*

20

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27

28 **Originality and significance statement**

29 Our study constitutes the first comprehensive lipidomic work on marine *Synechococcus*, in
30 which we show that these ecologically relevant cyanobacteria have a specific membrane lipidome
31 structure and use particular thermoacclimation processes to maintain photosynthetic activity. Our
32 work strongly suggests that the structure and thermoregulation of cyanobacterial membranes is thus
33 actually much more diverse than was previously thought, to some extent rewriting the dogmas that
34 have been established by the numerous studies on freshwater cyanobacteria. In addition, using
35 sequences retrieved from 53 genomes, including many new genomes, we show that the enzymatic
36 machinery catalyzing the last reactions of the membrane lipid biosynthetic pathways differ between
37 *Synechococcus* temperature ecotypes and that a number of strains have acquired different lipid
38 desaturation capacities through horizontal gene transfer. This work thus includes major new progress
39 in our knowledge of the diversity and evolution of such important biosynthetic pathways. The
40 understanding of these physiological and evolutionary processes is critical to assess how these
41 organisms will respond to a warming planet, given their importance as key marine primary producers
42 at the global scale.

43

44 **Abstract**

45 The marine cyanobacteria of the genus *Synechococcus* are important primary producers,
46 displaying a wide latitudinal distribution that is underpinned by diversification into temperature
47 ecotypes. The physiological basis underlying these ecotypes is poorly known. In many organisms,
48 regulation of membrane fluidity is crucial for acclimating to variations in temperature. Here, we reveal
49 the detailed composition of the membrane lipidome of the model strain *Synechococcus* sp. WH7803
50 and its response to temperature variation. Unlike freshwater strains, membranes are almost devoid of
51 C18, mainly containing C14 and C16 chains with no more than two unsaturations. In response to cold,
52 we observed a rarely observed process of acyl chain shortening that likely induces membrane thinning,
53 along with specific desaturation activities. Both of these mechanisms likely regulate membrane
54 fluidity, facilitating the maintenance of efficient photosynthetic activity. A comprehensive examination
55 of 53 *Synechococcus* genomes revealed clade-specific gene sets regulating membrane lipids. In
56 particular, the genes encoding desaturase enzymes, which are key to the temperature stress response,
57 appeared to be temperature ecotype-specific, with some of them originating from lateral transfers.
58 Our study suggests that regulation of membrane fluidity has been among the important adaptation
59 processes for the colonization of different thermal niches by marine *Synechococcus*.

60

61 Introduction

62 Picocyanobacteria are a major component of phytoplankton communities across wide
63 expanses of the world Ocean and responsible for up to 25% of global net marine primary production
64 (Flombaum *et al.*, 2013). Contained within this group is the genus *Synechococcus* which occurs all the
65 way from the equator to the poles (Zwirgmaier *et al.*, 2008; Huang *et al.*, 2012), suggesting that this
66 widespread picocyanobacterium has developed efficient adaptive strategies to cope with temperature
67 variations (Mackey *et al.*, 2013; Pittera *et al.*, 2014).

68 Marine *Synechococcus* exhibit a large genetic diversity, with 15 clades and 28 subclades
69 delineated within the main radiation (subcluster 5.1; Herdman *et al.*, 2015) based on the *petB* gene
70 marker encoding cytochrome *b₆* (Mazard *et al.*, 2012). Phylogeography studies have shown that the
71 major marine *Synechococcus* lineages occupy distinct ecological niches. Clades I and IV are confined to
72 nutrient-rich, cold or temperate waters, with clade I seemingly occurring at higher latitudes than clade
73 IV (Paulsen *et al.*, 2016), whereas clades II and III preferentially thrive in warm waters, with the former
74 being prevalent in (sub)tropical open ocean waters and the latter dominating more oligotrophic
75 systems, e.g. the eastern Mediterranean Sea (Sohm *et al.*, 2015; Farrant *et al.*, 2016).

76 Several studies have demonstrated that temperature is one of the main factors impacting the
77 genotypic composition of marine *Synechococcus* assemblages, although other factors such as
78 nutrients, light quantity and quality can also be important (Zwirgmaier *et al.*, 2008; Sohmi *et al.*, 2015;
79 Farrant *et al.*, 2016). Interestingly, members of *Synechococcus* clades I, II, III and IV have been shown
80 to exhibit thermal *preferenda* consistent with the seawater temperature at their isolation site (Pittera
81 *et al.*, 2014; Varkey *et al.*, 2016). These genetically defined lineages, physiologically adapted to specific
82 thermal niches, therefore correspond to different temperature ecotypes, a concept previously defined
83 for the very abundant marine cyanobacterium *Prochlorococcus* (Johnson *et al.*, 2006; Zinser *et al.*,
84 2007; Iskandar *et al.*, 2013).

85 The adaptive physiological processes conferring competitiveness to the different
86 *Synechococcus* temperature ecotypes in their respective thermal niche remain poorly known.
87 Membranes are among the cell components that are the most sensitive to temperature, a factor that
88 may drastically change their fluidity and therefore the activity of membrane-embedded proteins
89 (Mikami and Murata, 2003). Therefore, the ability to modulate membrane fluidity can be critical for
90 the fitness of an organism in a specific thermal niche. In most freshwater and halotolerant
91 cyanobacteria studied so far, the membrane lipid matrix comprises four main glycerolipids, including
92 mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively), sulfoquinovosyldiacylglycerol
93 (SQDG) and phosphatidylglycerol (PG), on which two acyl chains of usually 18 and 16 carbons long, are
94 esterified at the *sn*-1 and *sn*-2 positions of the glycerol backbone, respectively (Fork *et al.*, 1979; Los
95 and Mironov, 2015). Membrane fluidity can notably be adjusted by modifying the average length of

96 the fatty acid moiety of these glycerolipids, a thinner membrane being more fluid than a thicker one
97 (Marsh, 2010). Another well-known mechanism, essential to tuning membrane fluidity, is the
98 regulation of the level of unsaturation of the acyl chains, *i.e.* the number of carbon-carbon double
99 bonds. Highly unsaturated membranes indeed exhibit increased fluidity levels and are commonly
100 observed in cold-adapted organisms (Chintalapati *et al.*, 2007; Iskandar *et al.*, 2013).

101 Unsaturations are inserted into acyl chains by regiospecific enzymes called acyl desaturases.
102 The catalytic site of these enzymes comprises histidine-rich boxes, including a non-heme iron center
103 whose activity requires electrons and oxygen (Los and Murata, 1998; Behrouzian and Buist, 2003).
104 Cyanobacterial acyl desaturases have been extensively studied mostly in freshwater model organisms
105 (Sato and Wada, 2009; Los and Mironov, 2015). For example, *Synechocystis* sp. PCC 6803 possesses
106 four genes encoding acyl-desaturases, *desA*, *B*, *C* and *D*, which catalyze the insertion of double bonds
107 at positions $\Delta 12$, 15, 9, and 6 of glycerolipid acyl chains (Murata and Wada, 1995). These enzymes have
108 been shown to be major players in the temperature stress response since, by adjusting thylakoid
109 fluidity, they notably prevent temperature-induced photoinhibition (Ludwig *et al.*, 2012; Mizusawa and
110 Wada, 2012).

111 It has been reported that marine picocyanobacteria display unusual desaturase gene contents
112 with regard to their freshwater counterparts (Chi *et al.*, 2008). A recent study by Varkey *et al.* (2016)
113 further showed that marine *Synechococcus* strains isolated from cold waters exhibited a higher total
114 unsaturation level than strains from warmer environments that might be related to their different sets
115 of lipid desaturase genes. This suggests that marine *Synechococcus* use specific ways of membrane
116 lipid regulation, which may vary depending on the thermal niches of strains. However, the lack of
117 knowledge about the composition of membrane lipids has so far prevented a clear understanding of
118 acclimation and adaptation mechanisms by which marine *Synechococcus* adjust their lipidomes in
119 response to temperature changes.

120 Here, we analysed in detail the structure and composition of the membrane lipidome of the
121 model marine *Synechococcus* strain WH7803 and its response to long- and short-term temperature
122 variations. We also performed an extensive comparative genomic study of membrane lipid
123 biosynthetic pathways in different marine *Synechococcus* ecotypes. These analyses revealed specific
124 adaptations, notably with regard to their lipid desaturase content, which are likely critical for the
125 ubiquity of *Synechococcus* in the marine environment.

126

127 **Results**

128 *Response of Synechococcus sp. WH7803 growth rate and photosynthesis to temperature*

129 Under our defined laboratory culture conditions *Synechococcus* sp. WH7803 grew between
130 16°C and 30°C. A plot of temperature vs. growth rate showed a typical shouldered shape with an abrupt
131 drop after the optimal growth temperature was reached, which was close to 28°C (Fig. 1A). The
132 quantum yield of the photosystem II reaction center (F_V/F_M) increased from 16°C to 25°C, reaching a
133 maximum value of 0.65 at 25°C, before decreasing down to 0.53 at 30°C (Fig. 1B). Spectrofluorometric
134 measurements showed that the phycoerythrin:phycocyanin fluorescence emission ratio was stable
135 within the range 18-28°C and high at the thermal limits of growth, especially in the cold (Fig. 1C). In
136 contrast, the fluorescence emission ratio of phycocyanin to the phycobilisome terminal acceptor
137 remained constant at all growth temperatures. The phycourobilin:phycoerythrobilin fluorescence
138 excitation ratio was also stable at 0.4 (data not shown). In addition, acclimation to the coldest
139 temperature was accompanied by changes in the photosynthetic pigment ratios in the thylakoid
140 membranes (Fig. 1D). The zeaxanthin:chl a mass ratio decreased from 0.52 ± 0.06 at 16°C to $0.36 \pm$
141 0.05 fg cell⁻¹ whilst the β -carotene:chl a mass ratio remained stable at 0.12 ± 0.10 . These variations in
142 pigment ratios suggest a decrease of the chl a and β -carotene cell content relative to zeaxanthin (Kana
143 *et al.*, 1988; Moore *et al.* 1995; Six *et al.* 2004).

144

145 Composition of the membrane lipidome in *Synechococcus* sp. WH7803

146 Separation of membrane lipid classes by 2-dimensional thin layer chromatography showed
147 four major spots that corresponded to the four lipid classes typical of cyanobacteria, MGDG, DGDG,
148 SQDG and PG (Fig. S1). We did not detect the galactolipid precursor monoglucosyldiacylglycerol
149 (MGLcDG) with the lipid quantities we loaded on the plates. LC-MS/MS analyses showed that the
150 proportions of each glycerolipid remained stable at all growth temperatures, with MGDG being the
151 dominant lipid (45.4 ± 4.1 %) followed by DGDG (23.0 ± 3.6 %) and SQDG (24.1 ± 3.9 %). PG was a minor
152 lipid in these membranes (7.5 ± 2.3 %). In contrast to most freshwater strains, the fatty acid moiety of
153 *Synechococcus* sp. WH7803 lipids mostly comprised C14 and C16 chains, with only traces of C18 chains
154 (Meritt *et al.*, 1991) and no more than two saturations per chain were detected. Although our
155 analyses do not provide this information, it is likely that the unsaturated fatty acid species were
156 C14:1 ^{Δ^9} , C16:1 ^{Δ^9} , C18:1 ^{Δ^9} and C16:2 ^{$\Delta^9,12$} , as in all other cyanobacteria described so far (Los and Mironov,
157 2015). The *sn*-1 position most often bind a C16 chain, whereas C14 chains were more frequent at the
158 *sn*-2 position.

159

160 Molecular thermoacclimation of the glycerolipids in *Synechococcus* sp. WH7803

161 The global membrane lipidome showed clear responses to long- and short-term temperature
162 variations. Whereas the proportion of the four glycerolipids did not vary, the fatty acid moieties of the

163 three glycolipids were modified in response to temperature. With decreasing growth temperature, the
164 average acyl chain length of the galactolipids decreased and the global proportion of unsaturated
165 chains in the membranes strongly increased from 33.4 ± 0.9 at 30°C to 54.5 ± 0.4 % at 16°C (Table S1).
166 We hereafter present the molecular changes specific to each glycerolipid, which explain these
167 observed global changes.

168 **Monogalactosyldiacylglycerol** – The acyl chain esterified at the *sn*-2 position of MGDG was invariably
169 a C14 chain, whereas the *sn*-1 position was most often a C16 chain (Fig. 2, Tables S2, S3). The average
170 length of the MGDG *sn*-1 acyl chain slightly decreased with decreasing temperature, from about 15.8
171 carbon atoms at $28\text{-}30^\circ\text{C}$ to 15.3 carbon atoms at 16°C (Table S3), due to an increase in the
172 monounsaturated C14:1 at the expense of the C16 chains. This C14:1 synthesis did not seemingly
173 originate in a dynamically induced desaturation of C14:0 chains, since the latter chains were not
174 abundant and did not decrease proportionally (Fig. 2, Table S2). C14:1 synthesis was not observed
175 when cells acclimated to 22°C were suddenly shifted to 18°C and 13°C for 4 days (Fig. 3A, B). However,
176 when cells were shifted to 30°C , the reverse reactions were completed after 24 h (Fig. 4A, B).

177 Only two weak desaturation activities were detected on the MGDG. At the *sn*-2 position, some
178 C14:0 chains were desaturated into C14:1, the latter being undetectable at temperatures higher than
179 25°C (Fig.2, Table S2). Similarly, a small fraction of the C16:1 chains at the *sn*-1 position were
180 desaturated into C16:2 (Fig. 2, Table S2). These two reactions were also apparent during the cold-shift
181 experiments. When cells acclimated to 22°C were shifted to 13°C , the *sn*-1 C16:2 was synthesized up
182 to 10% of the MGDG *sn*-1 bound chains, while the *sn*-1 16:1 chain decreased from a similar proportion
183 (Fig. 3B). This desaturation was hardly detectable when cells were shifted to 18°C , with less than 0.5%
184 16:2 at the end of the experiment. Both cold shifts induced the synthesis of C14:1 at the *sn*-2 position,
185 but this was stronger at 13°C (Fig. 3C).

186 **Digalactosyldiacylglycerol** – The acyl chains esterified to DGDG were similar to those of the MGDG, as
187 the glycerol *sn*-2 position was exclusively occupied by a C14 chain, almost systematically saturated,
188 whereas the *sn*-1 position was dominated by C16 chains (Fig. 2, Tables S2, S3). As for MGDG, a
189 shortening of the *sn*-1 position by synthesis of C14:1 was observable. This was also induced during the
190 cold-shift experiments (Fig. 3E) and the reverse reactions were completed one day after cells were
191 shifted from 22°C to 30°C (Fig. 4E).

192 Similar to MGDG, we observed at the lowest acclimation temperatures a slight induction of
193 C14:1 at both *sn*-2 and *sn*-1 positions and C16:2 at the *sn*-1 position (Fig. 2, Table S2). These weak
194 desaturation activities were also detected during the two cold-shift experiments (Fig. 3E, F). In contrast
195 to MGDG, the C16:0 chain of the DGDG *sn*-1 position was the site of a strong desaturation activity.
196 Cells acclimated to 30°C showed 75% C16:0 and 11 % C16:1 at this position whereas at 16°C , C16:0

197 decreased to 18% while C16:1 increased to 51% (Fig. 2). This monodesaturation was also strongly
198 induced in both cold shift experiments and the reverse reaction was rapidly induced when cultures
199 were shifted from 22°C to 30°C (Fig. 3D, 4D, E).

200 **Sulfoquinovosyldiacylglycerol** – In contrast to the galactolipids, SQDG *sn*-2 acyl chains included both
201 C14 and C16 chains, with roughly 50% of each. However, at 16°C there was more C14, indicating a
202 shortening of the average chain length at this position (Fig. 2, Tables S2, S3). Comparable variations
203 were observed during the temperature shift experiments. The acyl chains bound at the *sn*-1 position
204 were predominantly C16 chains (with only 4-7% C14), independent of the growth temperature (Fig. 2,
205 Table S2).

206 With decreasing growth temperature, the *sn*-2 position was enriched in C16:2 whereas the *sn*-
207 1 position bound more C14:1 and much more C16:1, as the result of the desaturation of the C14:0 and
208 C16:0 chains (Fig. 2, Table S2). Similar variations were observed during both cold shift experiments,
209 with desaturation activities more marked when the cells were shifted from 22°C to 13°C (Fig. 3G, H).
210 The reverse reaction 16:1 → 16:0 was induced at high efficiency when cells were shifted from 22°C to
211 30°C (Fig. 4G, H).

212 **Phosphatidylglycerol** – PG appeared to be totally different from the three glycolipids as no C14 was
213 detected and the PG molecules contained almost only C16 chains. Some C18:1 chains were
214 occasionally detected at the *sn*-1 position (Fig. 2, Table S2). Overall, the composition in acyl chains was
215 dominated by C16:1 chains, and poorly influenced by temperature (Fig. 2, 3J, K, L, and Table S2). Some
216 slight variations were however observed during shifts from 22°C to 30°C, mostly comprising a decrease
217 in the 16:1:16:0 ratio at the *sn*-2 position (Fig. 4J, K, L).

218 Membrane lipid biosynthetic pathways in marine *Synechococcus* and *Cyanobium*

219 The bacterial fatty acid synthase (FAS II) has been extensively studied in *Synechocystis* sp. PCC
220 6803 (see *e.g.* Liu *et al.*, 2011; Hu *et al.*, 2013). However, the dearth of knowledge of these enzymes in
221 marine *Synechococcus* spurred us to search the 53 complete *Synechococcus* and *Cyanobium* genomes
222 for homologs of known FAS II genes. Expectedly, most of the genes involved in the FAS II pathway are
223 present as a unique copy in all the searched genomes, including the four enzymes (AccA-D) comprising
224 the initiation module and the following steps catalyzed by the β-ketoacyl-ACP synthase III (KAS III), the
225 β-ketoacyl reductase (KR), the β-hydroxyacyl-ACP dehydratase (DH) and the enoyl-ACP reductase
226 (ENR). In cyanobacteria, the KAS II enzyme is thought to be responsible for the entire fatty acid
227 elongation, condensing the growing acyl-ACP with malonyl-ACP to extend the chain by adding two
228 carbons at each cycle (White *et al.*, 2005). Search for KAS II in *Synechococcus* genomes revealed that
229 all of them possess at least one *fabF* gene copy but, interestingly, 20 out of 53 strains, mostly belonging
230 to clades II, III, IV and WPC1, actually possess a second copy that we called *fabF2* (Table S4). Both

231 copies are significantly more related to *E. coli fabF* (KAS II; e.g. 54 and 38 % aa identity of WH8102 FabF
232 and FabF2 to *E. coli* K12 FabF, respectively) than they are to *fabB* (KAS I; 38 and 29 % aa identity of
233 WH8102 FabF and FabF2 to *E. coli* K12 FabB, respectively).

234 The incorporation into the membranes starts with the acylation of G3P catalyzed by the PlsX-
235 GPAT system, then the membrane-associated protein PlsX catalyzes the formation of an acyl-
236 phosphate (Acyl-P; Cross, 2016) and the G3P acyltransferase (GPAT) acylates the 1-position of G3P
237 forming lysophosphatidic acid (LPA). Finally, the LPA acyltransferase (LPAAT) acylates the *sn*-2 position
238 of LPA to form phosphatidic acid (PA), the central intermediate of membrane glycerolipids. All these
239 enzymes are encoded by single core genes in the *Synechococcus* and *Cyanobium* genomes.

240 The biosynthetic pathways of membrane lipids in cyanobacteria then divide into two branches,
241 leading to the synthesis of the glycolipids or to PG (Petroutsos *et al.*, 2014). For the galactolipid
242 pathway, an ortholog of the PA phosphatase (Nakamura *et al.*, 2007) is present in four halotolerant
243 strains (CB0101, CB0205, WH5701 and PCC 6307) but not in the ‘truly’ marine *Synechococcus* strains,
244 suggesting that another enzyme is involved in this process in the latter strains. A possible candidate is
245 a membrane protein possessing a PA phosphatase-like domain (Cyanorak cluster CK_0000099). The
246 diacylglycerol produced is then used as a substrate for the synthesis of MGLcDG, which in
247 cyanobacteria, is further epimerized into MGDG (Awai, 2016). In many freshwater cyanobacterial
248 strains, the MGLcDG epimerase is encoded by the *mgdE* gene (Awai *et al.*, 2014; Sato, 2015), which
249 includes a C-terminus Rossmann fold domain and a fatty acid hydroxylase at the N-terminus, the
250 function of which remains unclear (Awai, 2016). In marine *Synechococcus*, the best hit to *mgdE* is a
251 gene that includes only the C-terminal Rossmann-fold domain of the *Synechocystis* gene (Table S5).
252 DGDG is synthesized from MGDG by the *dgdA* gene product (Sakurai *et al.* 2007), and SQDG by the
253 UDP-sulfoquinovose synthase (SqdB) and the SQDG synthase (SqdX; Sanda *et al.*, 2001). PG is
254 synthesized by the phosphatidyl-glycerophosphate synthase (PgsA). All these proteins are encoded by
255 single core genes in marine *Synechococcus* spp. (Table S5). Additional information is available in the
256 supplementary material.

257

258 Lipid desaturases in marine *Synechococcus* and *Cyanobium*

259 The acyl desaturases of marine cyanobacteria have so far been poorly studied, even though
260 they have already been reported to differ from those of their freshwater counterparts (Chi *et al.*, 2008;
261 Varkey *et al.*, 2016). We identified 11 gene clusters encoding putative lipid desaturase enzymes (Table
262 1), with one to six desaturases per strain. These genes encode proteins ranging from 259 to 428 amino
263 acids, as compared to 318 to 359 amino acids in *Synechocystis* sp. PCC 6803 (Murata and Wada, 1995).

264 Phylogenetic analysis of the 11 marine acyl desaturases together with freshwater
265 cyanobacteria desaturases (Fig. 5, datasets 1-2) and comparison of their 3 conserved histidine-rich

266 motifs with those previously determined based on 37 cyanobacterial genomes (Chi *et al.*, 2008; Fig.
267 S2-4), allowed us to identify six major marine *Synechococcus* lipid desaturases, including three putative
268 $\Delta 9$ desaturases (DesC3, C4, C6; Fig. S2) and three putative $\Delta 12$ desaturases (DesA2, A3, A4; Fig. S3). In
269 addition, five other proteins, present only in one or two *Synechococcus* strains (Table 1), also displayed
270 two to three histidine-rich motifs but could not be assigned with confidence to a specific desaturase
271 type (Fig. S4). Each of the six major desaturases indeed form well-supported monophyletic groups
272 within the DesC or DesA/B clusters, and their histidine-rich motifs were typical of $\Delta 9$ or $\Delta 12$
273 desaturases, respectively (more details in the supplemental material). Noteworthy, while DesC3 and
274 DesC4 display a quite high degree of similarity between strains, and especially within sub-cluster 5.1
275 (Average % identity: 87.3 % and 85.5 %, respectively), DesA2 and particularly DesA3 sequences proved
276 to be much more variable with 79.4 % and 58.5% identity on average within sub-cluster 5.1,
277 respectively.

278 In order to decipher the origin and evolution of this gene family in marine *Synechococcus*, the
279 phyletic profiles (Table 1), the genomic context of each gene (Fig. 6), their potential occurrence in
280 genomic islands (Fig. 6), the local nucleotide composition (Fig. S7) and their phylogenetic relatedness
281 (Fig. S5-6 and S8-9) were examined for the 4 main acyl-desaturases. While *desC3* is a core gene, *i.e.*
282 present in all strains, the *desC4* gene is specifically absent from clades II, III, WPC1, XX and UC-A (Table
283 1). Although there is little doubt given their close phylogenetic relatedness that the accessory *desC4*
284 gene arose from a duplication event of the core *desC3* gene ancestor (Fig. 5), it is difficult to conclude
285 with certainty whether the absence of *desC4* in clades II, III, WPC1, XX and UC-A is due to a specific loss
286 in these lineages after their diversification, or to a loss (in their common ancestor with clade IV),
287 followed by a secondary reacquisition of this gene in clade IV strains by lateral transfer, potentially
288 from clade I (Fig. S6; more details in the supplementary material).

289 As for the *desA2* gene, although the genomic context is very well conserved among most
290 strains of sub-cluster 5.1, it is very different in strain BIOS-U3-1 (clade CRD1), and in both sub-cluster
291 5.2 strains CB0101 and CB0205, suggesting that this gene has been laterally transferred in these
292 lineages (Fig. 6). This is supported for BIOS-U3-1 both by Alien Hunter genomic island prediction (Fig.
293 6) and comparative phylogenetic analyses (Fig. S8), and for CB0101 and CB0205 by the absence of
294 *desA2* in all other 5.2 strains (Table 1). As concerns *desA3*, both its highly variable genomic context
295 between strains (data not shown) and comparative phylogenetic analyses (Fig. S9), which group
296 together strains distantly related based on ribosomal protein phylogeny (belonging to clades II, IV, XX
297 and 2 out of the 3 clades VII strains), strongly suggest the occurrence of multiple lateral transfers for
298 this gene. This hypothesis is further strengthened by the detection of this gene in a genomic island for
299 12 out of 53 *Synechococcus* strains (Fig. 6).

300

301 Discussion

302 Growth and photosynthesis response to temperature

303 *Synechococcus* sp. WH7803 that was isolated in the Sargasso Sea in summer, *i.e.* in rather
304 warm waters (25.8°C; Pittera *et al.*, 2014), displays a thermal *preferendum* corresponding to warm
305 temperate ecotypes. In this study, under continuous low light irradiance (20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), we
306 were able to grow this strain from 16°C to 30°C, with an optimal growth rate at ca. 28°C. Curiously,
307 growing this strain under the same conditions, but at a light intensity of 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$,
308 extended the thermal growth range up to 34°C with optimal growth at 33°C (Pittera *et al.*, 2014). This
309 shows that *Synechococcus* growth capacity depends on the interaction between light and temperature,
310 as seen for other cyanobacteria (Miśkiewicz *et al.*, 2000) and phytoplankton (Edwards *et al.* 2016), the
311 latter study showing that light-limitation can reduce the optimal growth temperature of phytoplankton
312 by $\sim 5^\circ\text{C}$.

313 *Synechococcus* sp. WH7803 cells were able to maintain high photosynthetic efficiency
314 throughout the thermal growth range as shown by the photosystem II quantum yield which was
315 generally higher than 0.5. The maximal yield was close to 25°C, *i.e.* at a temperature slightly lower than
316 the maximal growth temperature, indicating that optimal photosynthesis is not necessarily coupled to
317 optimal growth over a temperature range. Photosystem II quantum yield was lower at the thermal
318 growth limits, especially at 16°C, probably due to chronic photoinhibition and/or non photochemical
319 quenching of fluorescence. It is worth noting that the phycoerythrin fluorescence increase at 16°C (see
320 below) also contributes to the low F_v/F_m at this temperature since, in cyanobacteria, phycobiliprotein
321 fluorescence contributes much to the F_0 fluorescence level (Ogawa *et al.*, 2017).

322 The functioning of the photosynthetic antenna, the phycobilisome, was also disturbed at the
323 thermal growth limits. Indeed, *in vivo* fluorescence emission spectra showed an increase of
324 phycoerythrin fluorescence relative to phycocyanin, indicating a chronic decrease of the energy
325 transfer rate, *i.e.* an energy leak, between these two phycobiliproteins. This impairment, mostly visible
326 at 16°C, is likely related to a temperature induced change of conformation of the phycobiliproteins.
327 Indeed, Pittera *et al.* (2016) recently showed that the stability of marine *Synechococcus* phycobilisomes
328 varies according to the average sea surface temperature at the strain isolation site. The phycoerythrin
329 fluorescence increase can also be interpreted as a way to dissipate excess light at a temperature at
330 which it cannot be fully utilized by the photosystem reaction centers. In addition, our pigment analyses
331 show a response to temperature that mimics high light acclimation (Kana *et al.*, 1988; Moore *et al.*,
332 1995; Six *et al.* 2004). This has been described in numerous photosynthetic organisms and notably
333 implies the down regulation of the photosystem cell content, in order to adjust light utilization
334 capacities at low temperature. In *Synechococcus* sp. WH7803, these processes are observable only at

335 the cold growth limits, suggesting that other mechanisms stabilize the photosynthetic apparatus
336 throughout the rest of the thermal growth range.

337

338 Cold-induced changes in membrane composition and thickness

339 Thylakoid membranes are by far the predominant membranes in cyanobacteria and thus their
340 lipid composition is close to that of the total cellular membranes (Sakurai *et al.*, 2006). Membrane
341 fluidity adjustments are critical for the biological reactions occurring in membranes, particularly in
342 thylakoids. Membrane fluidity is in large part determined by the ambient temperature, the degree of
343 unsaturation and the length of the constituent fatty acids (*i.e.* membrane thickness). We did not
344 observe any significant changes in the proportions of the four main membrane lipids, indicating that
345 *Synechococcus* sp. WH7803 does not acclimate to temperature variations by modifying the polar lipid
346 head groups in the membranes. MGDG was always the dominant lipid, followed by DGDG, SQDG and
347 PG, as commonly described in freshwater cyanobacteria and eukaryotic chloroplasts (see *e.g.* Murata
348 *et al.*, 1992; Somerville *et al.*, 2000; Wada and Mizusawa 2009; Dormann and Holzl, 2009; Shimojima
349 *et al.*, 2009a; Awai, 2016). Our data are thus somewhat at odds with studies by Van Mooy *et al.* (2006,
350 2009) that describe marine *Synechococcus*, *Prochlorococcus* and the model freshwater
351 cyanobacterium *Synechocystis* sp. PCC 6803 as containing relatively less MGDG, a surprising result in
352 view of the numerous reports of the membrane composition of the latter, intensively studied strain
353 (Awai *et al.*, 2014; Sato and Wada, 2009). Indeed, a recent study reported MGDG as the dominant
354 membrane lipid in several strains of the marine picocyanobacterium *Prochlorococcus* (Biller *et al.*,
355 2014).

356 In many freshwater cyanobacteria, MGlcDG, the precursor of MGDG, is often detected in
357 lipidomic analyses. In *Synechocystis* sp. PCC 6803, the MGlcDG synthase MgdA is activated by high
358 temperature whereas MgdE is inhibited, leading to the accumulation of MGlcDG (Shimojima *et al.*,
359 2009b; Awai *et al.*, 2014). In marine *Synechococcus*, the best hit to *mgdE*, encoding the MGlcDG
360 epimerase, is a gene that includes only the C-terminal Rossmann-fold domain of the *Synechocystis*
361 gene (Table S5), suggesting that the fatty acid hydroxylase domain is probably not essential to the
362 epimerase activity. However, we did not notice such MGlcDG accumulation in high temperature
363 acclimated cells nor in warm shift experiments.

364 *Synechococcus* sp. WH7803 membranes seem to contain only traces of C18 chains and are C14-
365 rich, whereas most freshwater strains bind C18 fatty acids at the *sn*-1 position of the glycerol backbone
366 on all lipids, and a shorter C16 chain at the *sn*-2 position (Sato and Wada, 2009; Los and Mironov,
367 2015). The glycolipids of *Synechococcus* sp. WH7803 have nevertheless a similar global structure to the
368 freshwater strain lipids, as the glycerol *sn*-1 position most often binds a C16 chain, and the *sn*-2 position
369 a shorter C14 chain. Consequently, *Synechococcus* sp. WH7803 membranes are on average thinner

370 than most freshwater strains. This feature might be related to the constraint of the picoplanktonic size
371 of this type of organism and/or an adaptation trait to high salt environment, as membrane fluidization
372 is a response mechanism to salt stress in many microorganisms (Los and Murata, 2004; Rodriguez-
373 Vargas *et al.*, 2007). The nature of the fatty acid bound to the *sn*-2 position is regulated by the acyl-
374 ACP pools and the lysophosphatidic acid acyltransferases (*plcC*), which may have different affinities for
375 specific fatty acid lengths. The two distinct enzymes present in the genome of *Synechococcus* sp.
376 WH7803, like in all other marine *Synechococcus* (Table S5), may be responsible for this possibility to
377 bind either a C14 or a C16 chain at the *sn*-2 position, as shown in *Synechocystis* sp. PCC 6803 for C16
378 and C18 chains (Okazaki *et al.*, 2006).

379 Our results suggest that variations in the acyl chain length are involved in the response to
380 temperature in *Synechococcus* sp. WH7803. At the *sn*-1 position of both galactolipids, a significant
381 proportion of the C16 chains were replaced by C14:1 in response to cold. Since there was no apparent
382 concomitant desaturation of the myristic acid chains (C14:0), it is likely that C14:1 were synthesized *de*
383 *novo* to replace C16 chains. This induced both a shortening and an increase of the unsaturation level
384 of the galactolipids at the glycerol *sn*-1 position, likely leading to an increase in membrane fluidity in
385 response to cold temperature. The *de novo* synthesis is also supported by the fact that this mechanism
386 was observed only in long-term thermoacclimated cells and warm shift experiments, as *de novo*
387 synthesis requires time and metabolically active cells. SQDG was not subjected to such a process.

388 Determination of the length of the acyl chains is thought to rely on a complex enzymatic
389 regulation system, based on competition among elongation synthases, the supply of malonyl-ACP and
390 the utilization of acyl-ACPs by the acyltransferase (Heath *et al.*, 1994; Heath and Rock, 1995). It has
391 also been shown that the β -ketoacyl synthase II is essential for the regulation of fatty acid composition
392 in response to temperature fluctuations (Garwin *et al.*, 1980, Heath *et al.*, 2002). The regulatory role
393 of KAS enzymes in the length of fatty acid chain synthesis has notably been evidenced by the
394 characterization of KAS IV enzymes, which display strong preferences for the elongation of short chain
395 acyl-ACPs (Schutt *et al.*, 2002). Interestingly, searches for KAS II in the *Synechococcus* and *Cyanobium*
396 genomes revealed they all possess at least one *fabF* gene copy, but 20 out of 53 strains, mostly
397 belonging to clades II, III, IV and WPC1, actually possess an additional gene copy, *fabF2* (Table S4). The
398 function of this second KAS II remains unclear but one might hypothesize that it is related to an ecotype-
399 specific ability to incorporate different lengths of acyl chains into the membranes, in order to modulate
400 the fluidity in response to temperature changes.

401

402 Cold-induced desaturations of acyl chains in *Synechococcus* sp. WH7803

403 The extent of unsaturation of the fatty acids in *Synechococcus* sp. WH7803 is rather low, as
404 only mono- and dienoic acyl chains were detected. The fatty acid desaturation activities were more

405 pronounced when the cells were transferred to 13°C than at 18°C, clearly showing the temperature
406 sensitivity of the acclimation system. The results of the warm shift experiment (30°C) also support this
407 and illustrate well the dynamic plasticity of the membrane lipidome of *Synechococcus* sp. WH7803.

408 At the *sn*-2 position of both galactolipids, a weak cold-induced desaturation activity induced
409 the conversion of C14 chains into C14:1 chains, as seen in the fully acclimated cells and the cold shift
410 experiments. Similarly, low amounts of C16:2 were synthesized in response to cold only in the
411 galactolipids. The major cold-induced desaturation activities occurred on the C16 chains at the *sn*-1
412 position of the SQDG and the DGDG, leading to efficient conversion of palmitic acid into palmitoleic
413 acyl chain. These two reactions were immediately induced upon a rapid temperature decrease,
414 reaching a plateau corresponding to the long-term acclimated state in about 24 h (Fig. 3D, E, G, H).
415 SQDG was the only lipid that could be desaturated at the *sn*-2 position (C16:0 → C16:1) in response to
416 cold temperature, although to a low extent.

417 When all these processes are summed, the total cell content of unsaturated acyl chains
418 significantly increases from about 35% at 30°C to 55% at 16°C. These are values comparable to the
419 study of Varkey *et al.* (2016), who measured the percentage of unsaturated fatty acids in three
420 *Synechococcus* strains acclimated to two different temperatures. These mechanisms differ from those
421 known in freshwater cyanobacteria since so far there has been no positive evidence for a desaturation
422 activity on DGDG in the latter organisms (Sato and Wada, 2009). However, studies on *Synechocystis*
423 mutants devoid of DGDG showed that this galactolipid is involved in thermotolerance by influencing
424 the sensitivity to photoinhibition at different temperatures (Mizusawa *et al.*, 2009a, 2009b). By
425 contrast, in the marine *Synechococcus* sp. WH7803, we show here that DGDG is a major target for cold-
426 induced acyl desaturation.

427 In *Synechococcus* sp. WH7803, PG is a minor lipid that appears quite different from the three
428 main glycolipids. At both glycerol positions, the dominant acyl chain was palmitoleic acid whilst C18:1
429 chains were occasionally detected at the *sn*-1 position. In contrast to most freshwater strains in which
430 the C18 chain bound to the *sn*-1 position can be desaturated (Sato and Wada, 2009), the fatty acid
431 content of PG did not show any clear desaturation response to temperature acclimation. In
432 *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, a number of mutant studies have
433 demonstrated that PG is physiologically essential and is notably involved in the activity of both
434 photosystems, influencing the dimerization and reactivation of core complexes (Sakurai *et al.*, 2003;
435 Yamamoto, 2016; Bogos *et al.*, 2010). X-ray crystallographic analysis of photosystem II at 1.9 Å
436 resolution has identified 5 PG molecules bound to photosystem II, directly connected to the D1 protein
437 and plastoquinone Q_B (Itoh *et al.*, 2012; Mizusawa and Wada, 2012). It is thus probable that low
438 amounts of PG in *Synechococcus* sp. WH7803 are associated with PSII, playing an important structural
439 role that requires a fixed composition in fatty acids.

440 Evolution of the desaturase family in marine *Synechococcus*

441 The desaturation of fatty acids involves O₂-dependent dehydrogenation reactions catalyzed
442 by non-heme di-iron desaturase enzymes *via* an electron donor molecule (Los and Murata, 1998;
443 Behrouzian and Buist, 2003). These reactions are highly stereoselective and regioselective, a
444 desaturase being able to insert a double bond at a specific position of the fatty acid, located at a given
445 *sn*- position on the glycerol backbone. These enzymes, which prevent membranes from undergoing
446 transition to the gel phase, a state which is lethal for cells, are encoded by cold-inducible genes that
447 have been well characterized in *Synechocystis* sp. PCC 6803 (see *e.g.* Sato and Wada, 2009). We
448 screened 53 genomes of marine and halotolerant *Synechococcus/Cyanobium* for lipid desaturase
449 genes. Whereas freshwater (Los and Mironov, 2015) and halotolerant (this study) strains often contain
450 a high number of desaturase genes, most of the truly marine *Synechococcus*, belonging to subcluster
451 5.1, usually contain three or four genes encoding DesC3, DesC4, DesA3 and DesA4 proteins. This
452 number is even lower for clade II, for which most of the representative strains have only two
453 desaturase genes. This suggests that these warm-adapted cyanobacteria have a globally low capacity
454 to modulate membrane fluidity, consistent with their thermal niche. The variability in lipid-desaturase
455 gene number among *Synechococcus* strains can also be seen as an adaptation to low seasonal
456 variability in warm, tropical waters compared to strong seasonality at higher latitudes. More generally,
457 the globally low number of desaturase genes in marine *Synechococcus* is in agreement with the low
458 level of unsaturation that we observed in the model strain WH7803, which contains four desaturase
459 genes, and therefore expected to be able to undertake only two types of Δ 9-desaturations and two
460 types of Δ 12-desaturations.

461 In freshwater cyanobacteria that contain both *desC1* and *desC2* genes, the products of these
462 genes insert an unsaturation at the ninth carbon from the carboxyl end of the C18 acyl chain bound at
463 the *sn*-1 position and the C16 chain bound at the *sn*-2 position, respectively. Although this requires
464 experimental evidence, it is thus possible that the marine DesC3 and DesC4 enzymes carry out the Δ 9-
465 desaturation activities on the palmitic (C16:0) and myristic (C14:0) chains which, similar to freshwater
466 strains, are most often bound to the *sn*-1 and *sn*-2 glycerol positions of the membrane lipids in
467 *Synechococcus* sp. WH7803, respectively.

468 The phylogenetic relatedness of *desC3* and *desC4* as well as their immediate vicinity in
469 genomes suggests that these genes originate from a duplication event. In freshwater cyanobacteria,
470 DesC1 is usually a constitutive enzyme whose activity is not necessarily temperature induced, while
471 DesC2 desaturates the acyl chain at the *sn*-2 position in response to cold stress (Chintalapati *et al.*,
472 2006, 2007). By analogy, DesC3, a Δ 9 desaturase present in all marine *Synechococcus* genomes, could
473 be a constitutive enzyme that likely desaturates the acyl chains bound to the *sn*-1 position. As for

474 DesC4, among the phylogenetic clades whose thermal niche has been studied (Pittera *et al.*, 2014;
475 Farrant *et al.*, 2016), it is only absent in clades adapted to warm environments (clades II and III). Thus,
476 similarly to DesC2 in freshwater cyanobacteria, DesC4 could well provide additional $\Delta 9$ desaturation
477 capacity, which would be induced under cold conditions (Varkey *et al.* 2016). The fact that DesC4 is
478 present in most *Synechococcus* strains (except clades II and III), including halotolerant members of sub-
479 cluster 5.2, tends to support the hypothesis that this gene has been lost in the warm-adapted clades II
480 and III, rather than gained in all other clades.

481 The two marine enzymes DesA3 and DesA4 are expected to be responsible for the few double
482 unsaturations (C16:2) that we detected in *Synechococcus* sp. WH7803, located exclusively on the *sn*-1
483 of the two galactolipids. These double unsaturations appear to be scarce and only induced when the
484 cells were acclimated to a temperature lower than the optimal growth temperature. In this context,
485 the specificities of the two enzymes DesA3 and DesA4 in *Synechococcus* sp. WH7803 remain unclear,
486 but one may hypothesize that each of them acts on a specific galactolipid. DesA3 is present in almost
487 all marine *Synechococcus* and *Cyanobium*, but is absent from most strains of the tropical clade II.
488 Interestingly, the only clade II strains possessing DesA3, PROS-U-1 and WH8109, were isolated from
489 an upwelling area located off the Moroccan coast and in northern water of the Sargasso Sea, where
490 the temperature is rather low compared to tropical waters (Pittera *et al.*, 2014). Thus, in addition to
491 the absence of the DesC4 enzyme, clade II ecotypes, adapted to the warmest waters of the world
492 Ocean, also exhibit decreased capacities to synthesize dienoic acyl chains, compared to most marine
493 *Synechococcus*. Although the phyletic profile of *desA2* is a bit less clear cut, it seems that DesA2 is
494 counter selected in cold environments (clades I and IV habitats) and mostly found in strains isolated in
495 rather warm waters (clades II, III, V and VI; Pittera *et al.*, 2014). Still, characterization of its function is
496 necessary to better understand its potential significance for thermal niche adaptation.

497

498 **Conclusion**

499 Several studies suggest that thylakoids require a particularly high level of fluidity regulation for
500 the proper functioning of the embedded proteins, which occupy about 70% of the membranes
501 (Kirchhoff *et al.*, 2008; Dormann and Holzl, 2009; Yamamoto, 2016). As these membranes provide the
502 matrix for the photosynthetic machinery, the fluidity regulation processes we highlight in this study
503 are likely essential mechanisms for survival and competitiveness of a cyanobacterial strain at different
504 temperatures. Our results show that the marine picocyanobacterium *Synechococcus* sp. WH7803
505 maintains optimal photosynthetic rates over most of its growth temperature range. To do so, this
506 cyanobacterium undergoes a remodeling of the composition of the acyl moiety of the membrane lipids
507 in order to adjust membrane fluidity. The membrane lipidome regulation mechanisms used by this

508 marine strain notably rely on specific desaturation processes of *sn*-1 bound acyl chains of the three
509 glycolipids, as well as a shortening of the *sn*-1 position of the major membrane constituents, the
510 galactolipids. The latter temperature-induced process has so far only been rarely reported (Shivaji and
511 Prakash, 2010). In organisms that use a limited set of desaturase enzymes, such a mechanism may
512 constitute an important additional component of the response to thermal changes in order to
513 adequately adjust membrane fluidity and successfully acclimate to temperature variations. These
514 processes constitute significant differences with the mechanisms described so far in freshwater
515 cyanobacteria.

516 Our study shows that, during the diversification of the marine *Synechococcus* radiation into
517 different temperature ecotypes, membrane lipid metabolism pathways have been globally well
518 conserved. However, the enzymatic machinery catalyzing the last reactions of the biosynthetic
519 pathways seem to be less evolutionary constrained and distinct *Synechococcus* ecotypes have acquired
520 different lipid desaturation capacities, notably through horizontal gene transfer events. These
521 mechanisms appear to be directly linked to temperature adaptation and niche partitioning, since
522 *Synechococcus* ecotypes adapted to the warmest environments generally show lower fatty acid
523 desaturation capacities than those adapted to temperate and subpolar waters. This observation
524 highlights the importance of the capacity of *Synechococcus* cells to regulate their membrane
525 composition for colonizing distinct thermal niches, likely a key factor for the ecological success of these
526 picocyanobacteria in the world Ocean. Future studies should aim at characterizing the biochemical
527 function of thermotype-specific lyases, e.g. through gene inactivation and heterologous expression
528 approaches.

529

530 **Experimental procedures**

531 Culture conditions and experimental design

532 The axenic strain *Synechococcus* sp. WH7803 was retrieved from the Roscoff culture collection
533 (<http://roscoff-culture-collection.org/>) and grown in PCR-S11 culture medium (Rippka *et al.*, 2000)
534 supplemented with 1 mM sodium nitrate. Continuous light was provided by multicolor LED systems
535 (Alpheus, France) at 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance. The axenic nature of the cultures was regularly
536 checked by flow cytometry using SYBR-Green staining.

537 To study the temperature induced differences in the homeostatic composition of the
538 membranes, cultures were acclimated for several weeks to a range of temperatures, from 16 to 30°C,
539 within temperature-controlled chambers, and sampled during the exponential growth phase. To study
540 the dynamics of the temperature-induced remodeling of the membranes, we carried out temperature
541 shift experiments. Ten liters of early exponentially growing cultures maintained at 22°C were split and

542 transferred to 13, 18 or 30°C, under identical light conditions. Then, the subcultures were sampled
543 during four days. All experiments were repeated at least three times.

544

545 Flow Cytometry, in vivo fluorometry and pigment analyses

546 Aliquots of cultures were preserved using 0.25% (v/v) glutaraldehyde (grade II, Sigma Aldrich,
547 St Louis, MO, USA) and stored at -80°C until analysis. Cell concentrations were determined using a flow
548 cytometer (FACS Canto II, Becton Dickinson, San Jose, CA, USA), as described previously (Marie *et al.*,
549 1999). Growth rates were computed as the slope of a Ln(Nt) vs. time plot, where Nt is the cell
550 concentration at time t.

551 The photosystem II quantum yield (F_V/F_M) was measured using a Pulse Amplitude Modulation
552 fluorometer (PhytoPAM, Walz, Effeltrich, Germany) in the presence of 100 mM of the PSII blocker 3-
553 (3,4-dichlorophenyl)-1,1-dimethylurea, following a previously described procedure (Pittera *et al.*,
554 2014). The quantum yield was calculated as:

$$555 \quad F_V/F_M = (F_M - F_0)/F_M$$

556 where F_0 is the basal fluorescence level, F_M the maximal fluorescence level and F_V is the variable
557 fluorescence (Campbell *et al.*, 1998; Ogawa *et al.*, 2017).

558 Furthermore, in order to study phycobiliprotein coupling in the phycobilisome, fluorescence
559 emission spectra were recorded with a LS-50B spectrofluorometer (Perkin-Elmer, Waltham, MA, USA),
560 as described elsewhere (Pittera *et al.*, 2016). Fluorescence excitation spectra were recorded to
561 determine the phycourobilin to phycoerythrobilin ratio (Six *et al.*, 2007).

562 For pigment analyses, 50 mL volumes of culture were harvested by centrifugation in the
563 presence of 0.01% (v/v) pluronic acid final concentration (Sigma Aldrich, St Louis, MO, USA). After
564 extraction in methanol, pigment extracts were supplemented with distilled water. Pigments were then
565 measured by high pressure liquid chromatography using an HPLC 1100 Series System (Hewlett Packard,
566 St Palo Alto, CA, USA), as described previously (Pittera *et al.*, 2014).

567

568 Membrane lipidome analyses

569 **Lipid extraction** - Cells were harvested by centrifugation and stored at -80°C until analysis. Membrane
570 lipids were extracted in glass hardware following a modified version of the Bligh and Dyer (1959)
571 procedure, using methanol/dichloromethane/water at ratios of 1.1/1/1.4, then evaporated under
572 nitrogen and stored at -20°C until analysis.

573 **Fatty acid regiolocalization** - We first identified the positional distribution of the fatty acids esterified
574 to the four main glycerolipids of *Synechococcus* sp. WH7803. To do so, 400 mL culture grown at 16, 22
575 and 30°C was harvested and the lipids extracted as described above. The glyceroplipid classes were
576 separated by 2-dimensional thin layer chromatography on 20 x 20 cm silica plates (Merck, Darmstadt,

577 Germany), using chloroform/methanol/water and chloroform/acetone/methanol/acetic acid/water at
578 ratios of 65/25/4 and 50/20/10/10/5 v/v, respectively, (Simionato *et al.*, 2013). Glycerolipid spots were
579 revealed under UV light in the presence of 8-anilino-1-naphthalene sulfonic acid (0.2 % in pure
580 methanol) and scraped off the plates. Each separated lipid class was recovered from the silica powder
581 after addition of 1.35 mL chloroform:methanol 1:2 v/v, thorough mixing and addition of 0.45 mL
582 chloroform and 0.8 mL H₂O and collection of the chloroform phase. Lipids were then dried under argon
583 and analyzed by mass spectrometry (MS). Purified lipid classes were dissolved in 10 mM ammonium
584 acetate in pure methanol. The glycerolipids were introduced by direct infusion (ESI-MS) into a trap
585 type mass spectrometer (LTQ-XL, Thermo Scientific), and their identity was confirmed by MS/MS
586 analysis as described in Abida *et al.* (2016). Under these conditions, the produced ions were mainly
587 present as H⁻, H⁺, NH₄⁺ or Na⁺ adducts. The position of the fatty acid molecular species esterified to
588 the glycerol backbone of the purified glycerolipids was determined by MS/MS analyses. Depending on
589 the glycerolipid species and the ionic adduct, the substituents at *sn*-1 and *sn*-2 positions were
590 differently cleaved upon low energy collision-induced dissociation. This was reflected in MS/MS
591 analyses by the preferential loss of one of the two fatty acids, leading to a dissymmetrical abundance
592 of the collision fragments, and following dissociation patterns of MS² fragments described in previous
593 studies (Abida *et al.*, 2016).

594 **Lipid quantification** - The lipid extracts corresponding to about 25 nmol of total fatty acids were
595 dissolved in 100 μ L chloroform/methanol [2/1, (v/v)] containing 125 pmol of each internal standard.
596 Internal standards were obtained from Avanti Polar Lipids Inc. for PG 18:0-18:0 or synthesized by D.
597 Lafont (Amara *et al.*, 2009, 2010) for MGDG 18:0-18:0 and DGDG 16:0-16:0 or extracted from spinach
598 thylakoid (Demé *et al.*, 2014) and hydrogenated as previously described for SQDG 16:0-18:0 (Buseman
599 *et al.*, 2006). Lipids were then separated by HPLC and quantified by MS/MS.

600 The HPLC separation method was adapted from Rainteau *et al.* (2012). Lipid classes were
601 separated using an Agilent 1200 HPLC system using a 150 mm \times 3 mm \times 5 μ m diol column (Macherey-
602 Nagel), at 40°C. The mobile phases consisted of hexane/isopropanol/water/ammonium acetate 1M,
603 pH5.3 [625/350/24/1, (v/v/v/v)] (A) and isopropanol/water/ammonium acetate 1M, pH5.3
604 [850/149/1, (v/v/v)] (B). The injection volume was 20 μ L. After 5 min, the percentage of B was
605 increased linearly from 0% to 100% in 30 min and kept at 100% for 15 min at a flow rate of 200 μ L min⁻¹.
606 The distinct glycerolipid classes eluted successively depending on the polar head group.

607 Mass spectrometric analysis was done on an Agilent 6460 triple quadrupole mass
608 spectrometer equipped with a jet stream electrospray ion source under following settings: Drying gas
609 heater: 260°C, Drying gas flow 13 L min⁻¹, Sheath gas heater: 300°C, Sheath gas flow: 11 L min⁻¹,
610 Nebulizer pressure: 25 psi, Capillary voltage: \pm 5000 V, Nozzle voltage \pm 1000. Nitrogen was used as
611 the collision gas. The quadrupoles Q1 and Q3 were operated at widest and unit resolution,

612 respectively. SQDG analysis was carried out in negative ion mode by scanning for precursors of m/z -
613 225 at a CE of -56eV. PG, MGDG and DGDG measurements were performed in positive ion mode by
614 scanning for neutral losses of 189 Da, 179 Da and 341 Da at CEs of 16 eV, 8 eV and 8 eV, respectively.
615 Quantification was done by multiple reaction monitoring (MRM) of all the molecules detected in the
616 TLC-MS experiment with 100 ms dwell time. Mass spectra were processed with the Agilent
617 MassHunter Workstation software for lipid identification and quantification. Lipid amounts were
618 corrected for response differences between internal standards and endogenous lipids.

619

620 Comparative genomics and detection of lateral gene transfers

621 Among the 53 *Synechococcus* and *Cyanobium* genomes used for comparative analyses in the
622 present study, which encompass marine and halotolerant strains, 22 complete or high quality genome
623 sequences were retrieved mostly from NCBI and 31 are still unpublished. The latter strains were cloned
624 and purified by three transfers onto agarose plates and their DNA extracted, as previously described
625 (Humily *et al.*, 2013). Whole genomes were sequenced by Genoscope (Evry, France) or the NERC
626 Biomolecular Analysis Facility (NBAF) located at the Centre for Genomic Research (University of
627 Liverpool, UK). The genomic sequences were assembled using the CLC Assembly Cell software (CLC Bio,
628 Aarhus, Denmark) and scaffolded using WiseScaffolder (Farrant *et al.*, 2016). After an automatic
629 structural and functional annotation performed by the Institute of Genome Sciences (Maryland, USA)
630 using the Manatee annotation pipeline (<http://manatee.sourceforge.net/igs/index.shtml>), individual
631 sequences were grouped into clusters of orthologous genes using OrthoMCL (Li *et al.*, 2003), then
632 uploaded into the custom-designed information system Cyanorak v2 (www.cyanorak.sb-roscoff.fr) for
633 further manual curation. All genes involved in the biosynthesis of fatty acids, membrane lipids and
634 acyl-desaturases, as well as 52 ribosomal protein coding genes, were manually curated and their
635 sequences deposited in Genbank (datasets 1-3). The potential occurrence of each acyl-desaturase gene
636 in genomic islands was analyzed using Alien Hunter (Vernikos and Parkhill, 2006).

637

638 Phylogenetic analyses

639 Amino acid sequences of the six major acyl-desaturase proteins and 52 ribosomal proteins that
640 were used to make a refined analysis of the phylogeny of marine *Synechococcus* were aligned using
641 MAFFT v7.164b with FFT-NS-2 parameters (Kato and Standley, 2014). Individual ribosomal protein
642 alignments were then concatenated in one super-alignment of 7,072 amino acid sites and trimmed to
643 remove ambiguously aligned regions using Geneious® 8.1.5 (Kearse *et al.*, 2012). Maximum likelihood
644 trees were inferred using PHYML v3.0 – 20120412 (Guindon and Gascuel, 2003), with the LG
645 substitution model for acyl-desaturase proteins and JTT for the ribosomal proteins, and with the
646 estimation of the distribution of the gamma distribution shape parameter and of the proportion of

647 invariables sites for both trees. Confidence of branch points was determined by performing bootstrap
648 analyses including 1000 replicate datasets. Phylogenetic trees were edited using the Archaeopteryx
649 v0.9901 beta program (Han and Zmasek, 2009). The single acyl-desaturase tree was drawn using iTOL
650 (<http://itol.embl.de>; (Letunic and Bork, 2007) and tree comparison was made using the Dendextend R
651 package (Galili, 2015).

652

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663

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906 **Table and figure legends**

907 **Table 1:** Genome screening for putative lipid desaturase genes in 53 marine *Synechococcus* and
908 *Cyanobium* genomes, ordered by sub-clusters and phylogenetic clades. Cells filled with grey indicate
909 the presence of one gene copy in the genome. Absence of color indicates that no orthologous gene
910 was found in the genome.

911 **Figure 1:** Variations of growth rate (A), photosystem II quantum yield (F_V/F_M ; B), phycobiliprotein
912 fluorescence emission ratio (C) and membrane pigments (D) in *Synechococcus* sp. WH7803 acclimated
913 from 16°C to 30°C. **PE:** Phycoerythrin; **PC:** Phycocyanin; **TA:** Terminal acceptor of the phycobilisome;
914 **Zea:** Zeaxanthin; **β -car:** β -carotene. The measurements were repeated four times.

915 **Figure 2:** Variations in the acyl chains esterified at the two glycerol positions of the four membrane
916 glycerolipids, monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG),
917 sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) of *Synechococcus* sp. WH7803

918 acclimated to a range of temperatures (see also Table S2). The left bar chart refers to the fatty acid
919 species bound to the *sn*-1 position, the right one to the fatty acid species bound *sn*-2 position and the
920 *sn*-3 position binds the polar head.

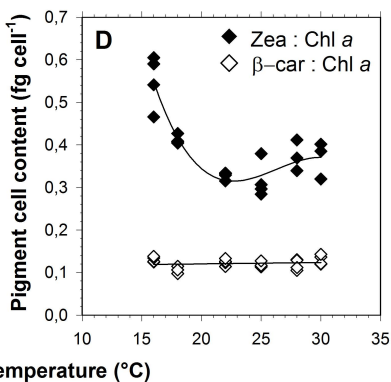
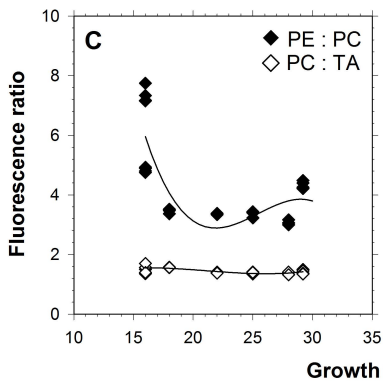
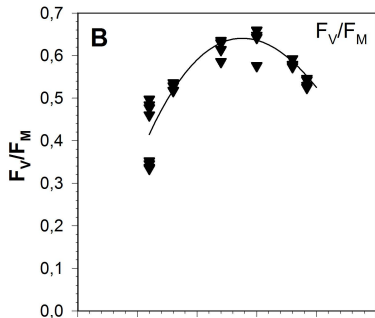
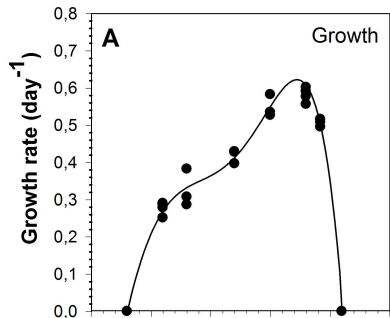
921 **Figure 3:** Variations of the acyl chains esterified at the two glycerol positions *sn*-1 (left panels) and *sn*-
922 2 (right panel) of monogalactosyldiacylglycerol (MGDG; A-C), digalactosyldiacylglycerol (DGDG; D-F),
923 sulfoquinovosyldiacylglycerol (SQDG; G-I) and phosphatidylglycerol (PG; J-L), as induced in response to
924 a shift from 22°C to either 13°C (circles) or 18°C (triangles) in *Synechococcus* sp. WH7803. The results
925 are expressed in percentages of total acyl chain esterified at the stereospecific position of the
926 glycerolipid. The experiments were repeated three times.

927 **Figure 4:** Variations of the acyl chains esterified at the two glycerol positions *sn*-1 (left panels) and *sn*-
928 2 (right panel) of monogalactosyldiacylglycerol (MGDG; A-C), digalactosyldiacylglycerol (DGDG; D-F),
929 sulfoquinovosyldiacylglycerol (SQDG; G-I) and phosphatidylglycerol (PG; J-L), as induced in response to
930 a shift from 22°C to 30°C, in *Synechococcus* sp. WH7803. The results are expressed in percentages of
931 total acyl chain esterified at the stereospecific position of the glycerolipid. The experiments were
932 repeated three times.

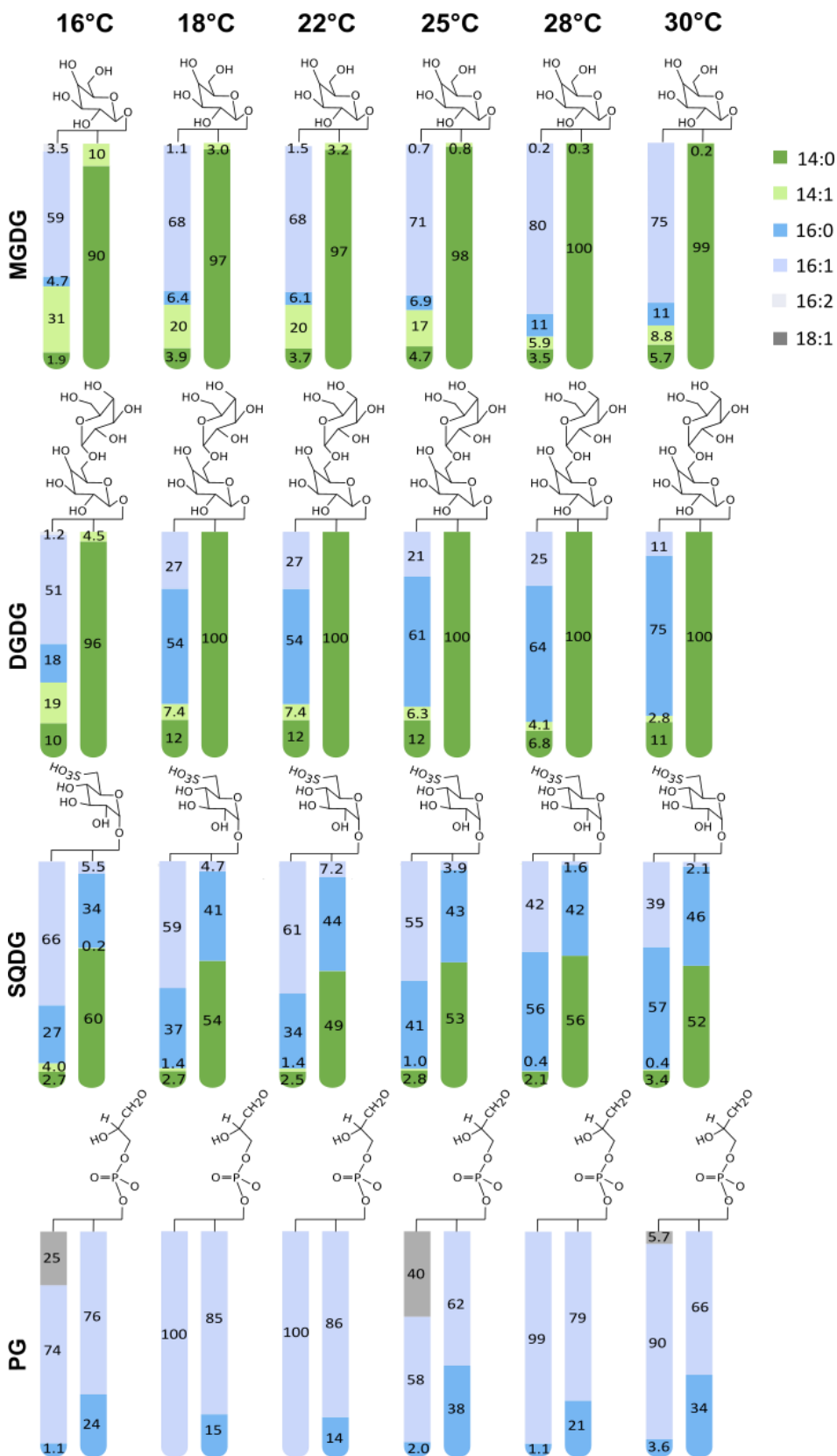
933 **Figure 5:** Maximum likelihood analysis of cyanobacterial lipid desaturase enzymes, including marine
934 *Synechococcus*, *Cyanobium* and a selection of freshwater cyanobacteria (see Supplementary datasets
935 1-2). Clusters including marine cyanobacteria are shown in green and blue colors while those including
936 exclusively freshwater cyanobacteria are in grey. Circles at nodes indicate bootstrap support over 70%.
937 The scale bar represents the number of substitutions per amino acid position.

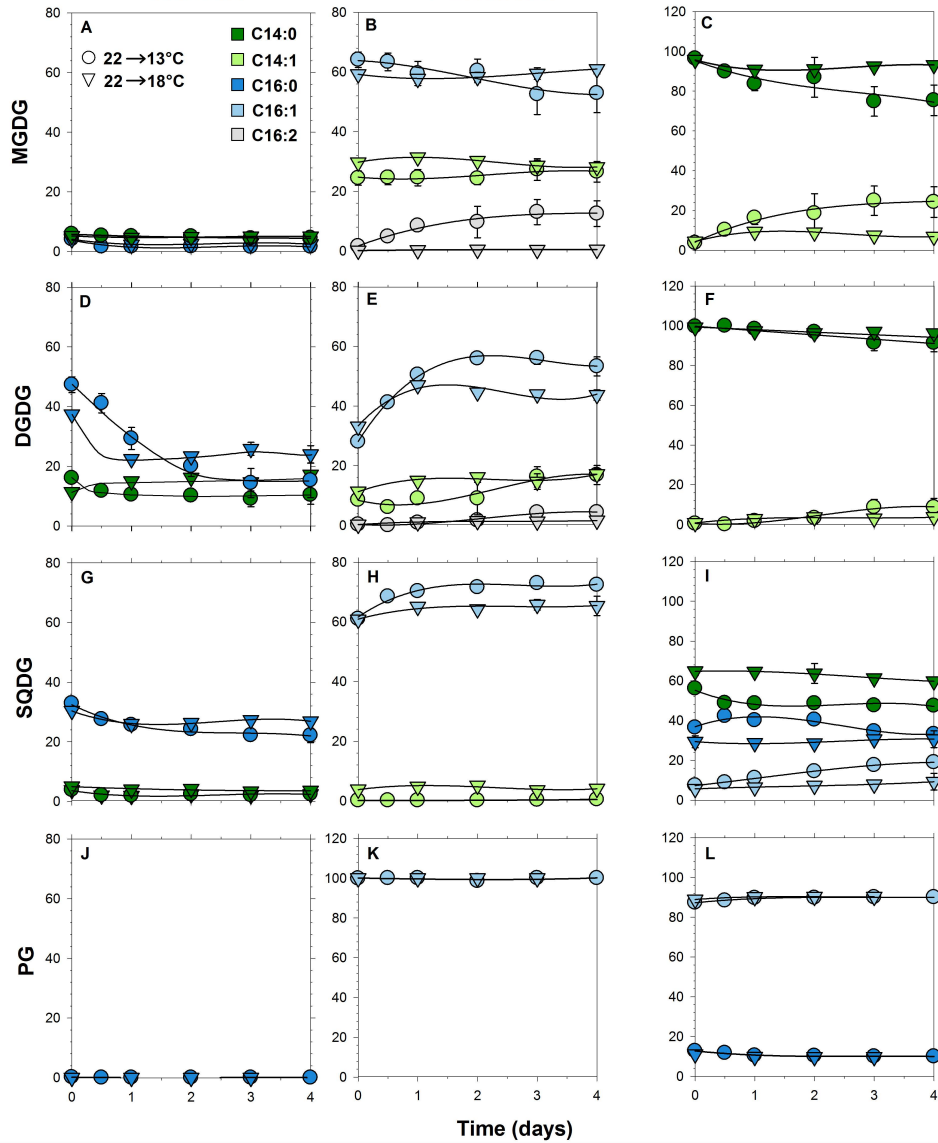
938 **Figure 6:** Clade- or strain-specific variability of the genomic context for *desc3*, *desC4* and *desA2* genes
939 among the 53 sequenced *Synechococcus* strains. Note that *desA3* is not shown as its genomic context
940 is too variable between strains even within clades. Gene names are indicated as a four letter code
941 except for conserved hypothetical protein genes indicated as “chp” followed by a number. The table
942 shows the acyl-desaturase genes predicted to be located in horizontally transferred genomic islands
943 by the Alien Hunter software, among the 53 *Synechococcus/Cyanobium* genomes
944 (<http://www.sanger.ac.uk/science/tools/alien-hunter>; Vernikos and Parkhill, 2006).

945



Pittera *et al.*, Figure 2



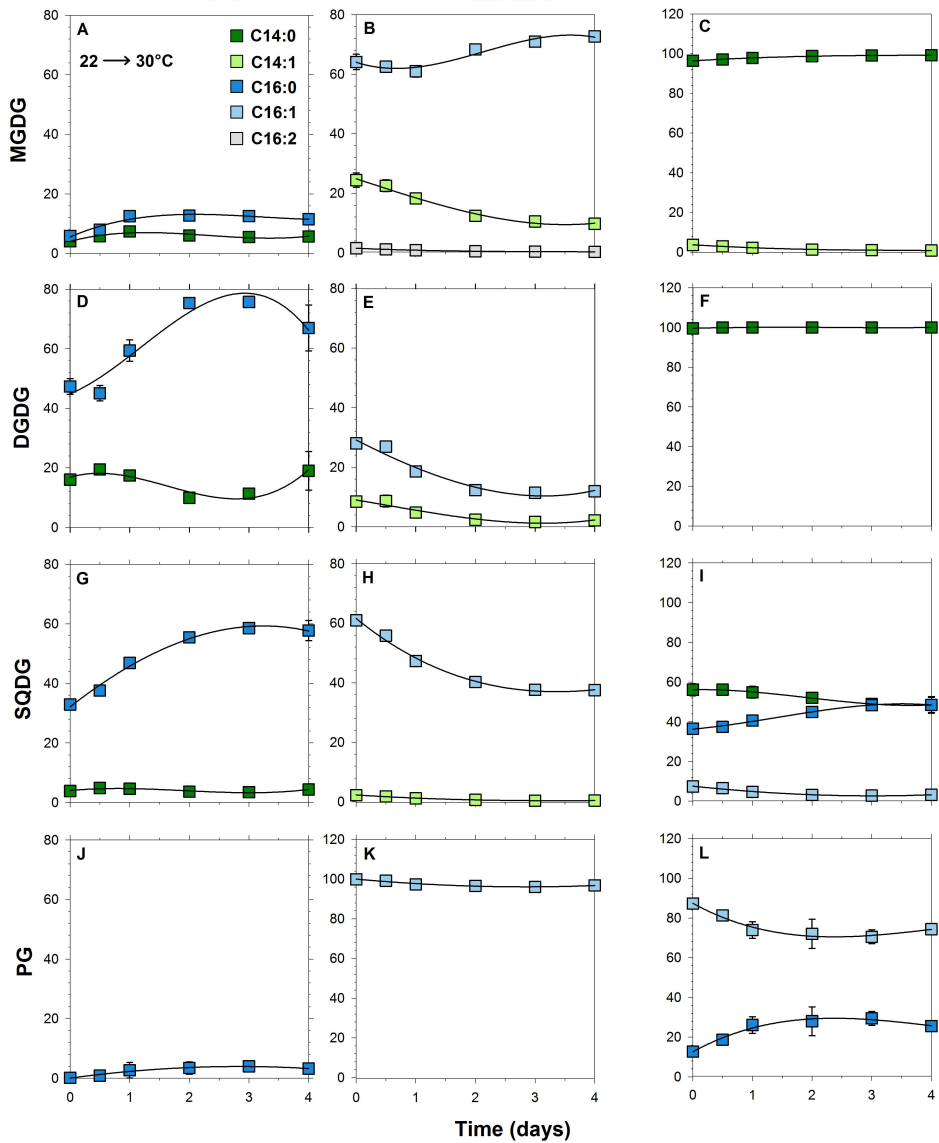
sn-1 position**sn-2 position****Saturated****Desaturated**

sn-1 position

sn-2 position

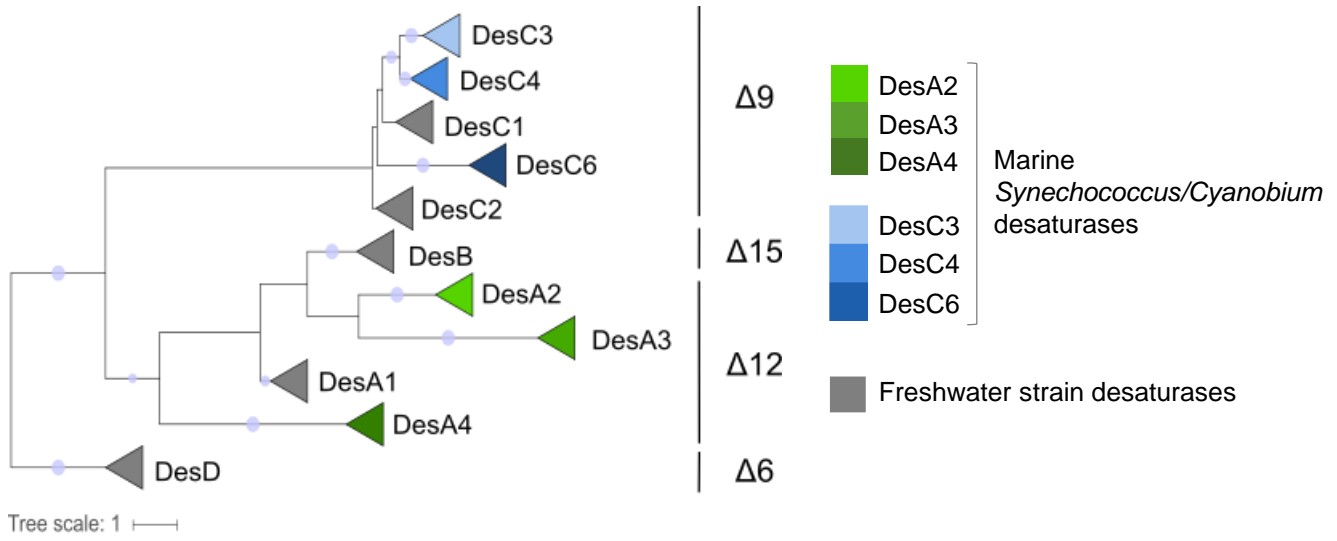
Saturated

Desaturated

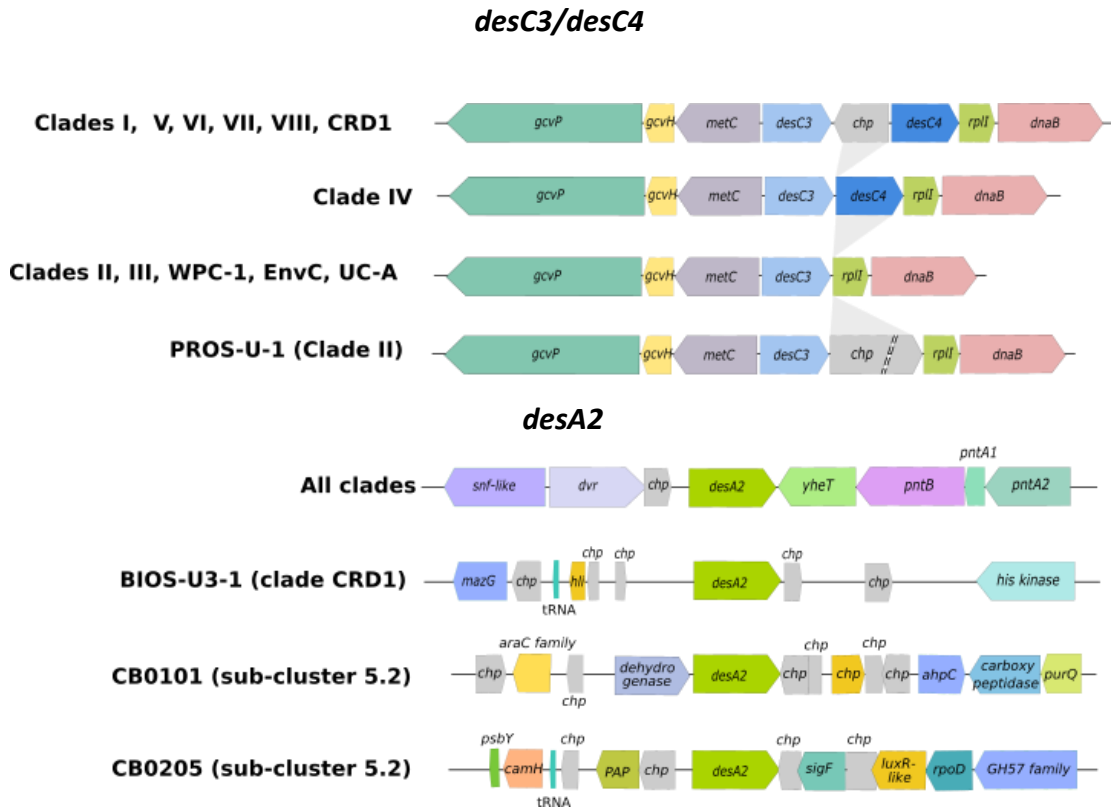


Time (days)

Pittera *et al.*, Figure 5



Pittera *et al.*, Figure 6



<i>des</i> gene	Prediction for gene inclusion in a genomic island
<i>desC3</i>	No strain
<i>desC4</i>	No strain
<i>desA2</i>	BIOS-U3-1
<i>desA3</i>	A15-24, A15-28, A15-60, A18-25, A18-46, BIOS-E4-1, BIOS-U3-1, BMK-MC-1, CC9616, PROS-U-1, WH8102, WH8109

Table 1

Sub-cluster ¹	Clade ²	Representative sequenced strains	Δ9 desaturases			Δ12 desaturases			Other desaturases					Number of <i>des</i> genes	
			<i>desC3</i>	<i>desC4</i>	<i>desC6</i>	<i>desA2</i>	<i>desA3</i>	<i>desA4</i>	<i>desC</i>	<i>desC</i>	<i>des</i>	<i>des</i>	<i>des</i>		
5.1	I	CC9311, MVIR-18-1, PROS-9-1, WH8016, ROS8604													3
		SYN20													4
	II	A15-62, CC9605, M16.1, RS9902, RS9907, TAK9802													2
		KORDI-52													1
		A15-44													3
		WH8109, PROS-U-1													3
	III	WH8102, WH8103, A15-24, A18-46.1, BOUM118, RS9915, A15-28, A18-40													3
	IV	BL107, CC9902													3
	V	WH7803, BMK-MC-1													4
	VI	WH7805, MEDNS5													4
		PROS-7-1													6
	VII	A15-60, A18-25c													3
		NOUM97013													4
	VIII	RS9909, RS9917													3
		WH8101													2
	IX	RS9916													2
	CRD1	MITS9220, BIOS-E4-1													3
BIOS-U3-1														4	
WPC1	A15-127, KORDI-49													3	
XX	CC9616													2	
UC-A	KORDI-100													1	
5.2		NS01, WH5701													6
		PCC6307													7
		CB0101													6
		CB0205													5
		PCC7001													4
5.3		RCC307, MINOS11												3	

¹ *sensu* Herdman et al. (2001); ² see Mazard et al. (2012) and Choi & Noh (2009).