Title

Construction of a cyanobacterium synthesizing cyclopropane fatty acids

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Abstract

Microalgae have received much attention as a next-generation source of biomass energy. However, most of the fatty acids (FAs) from microalgae are multiply unsaturated; thus, the biofuels derived from them are fluid, but vulnerable to oxidation. In this study, we attempted to synthesize cyclopropane FAs in the cyanobacterium *Synechocystis* sp. PCC 6803 by expressing the *cfa* gene for cyclopropane FA synthase from *Escherichia coli* with the aim of producing FAs that are fluid and stable in response to oxidization. We successfully synthesized cyclopropane FAs in *Synechocystis* with a yield of ~30% of total FAs. Growth of the transformants was altered, particularly at low temperatures, but photosynthesis and respiration were not significantly affected. C16:$1^{\Delta 9}$ synthesis in the *desA*$^{-}$/*desD*$^{-}$ strain by expression of the desC2 gene for *sn*-2 specific Δ9 desaturase positively affected growth at low temperatures via promotion of various cellular processes, with the exceptions of photosynthesis and respiration. Estimation of the apparent activities of desaturases suggested that some acyl-lipid desaturases might recognize the lipid side chain.

Keywords

Biofuel; Microalgae; Cyclopropane fatty acid; Acyl lipid desaturase; *Synechocystis* sp. PCC 6803

1. Introduction

Contemporary society is dependent on large quantities of fossil fuels. Fossil fuels are used not only as resources for large-scale transportation and generation of electricity, but also as raw materials for the production of various chemicals. However, use of fossil fuels accelerates global warming and environmental pollution. Moreover, the demand for fossil fuels is increasing due to global industrial expansion, while the availability of these fuels is gradually diminishing. Therefore, development of alternative sources of liquid fuels is required to sustain society. In recent years, microalgae have attracted attention as next-generation sources of biomass energy because their production does not directly compete with the production of land crops, which is sources of food, and because their production efficiency is extremely high [5,16]. Some microalgae accumulate large quantities of oils (mainly triacylglycerols), especially under conditions of stress, such as nitrogen starvation [8]. Fatty acids (FAs) in triacylglycerols are hydrolyzed and methylated, and their derivatives are utilized as biofuels. Most FAs from microalgae are multiply unsaturated; thus, the biofuels derived from them are fluid, but vulnerable to oxidation. Thus, direct use of natural FAs from microalgae in industry may be problematic. Use of FAs could be expanded if they were stable in response to oxidation during long-term storage and if they were sufficiently fluid. Saturated FAs are stable in response to oxidation, but saturated FAs with C16 or C18 chains (which are

56 the major acyl groups of lipids in living organisms) solidify at physiological temperatures.
57 Thus, saturated FAs are not suitable for use in biofuel production.

58 Some bacteria, such as *Escherichia coli* and *Lactobacillus arabinosus*, synthesize
59 cyclopropane FAs, which contain a cyclopropane ring in the acyl group. For example, 30% of
60 FAs in the total membrane lipids of *E. coli* cells are cyclopropane FAs. A green sulfur
61 bacterium, *Chlorobaculum tepidum*, also produces cyclopropane FAs attached to glycolipids
62 [15]. This suggests that heterotrophic bacteria are not the only producers of cyclopropane FAs.
63 However, oxygenic photosynthetic organisms do not synthesize cyclopropane FAs. Instead,
64 they produce unsaturated FAs to maintain membrane fluidity, suggesting that cyclopropane
65 FAs might negatively affect oxygenic photosynthesis. Cyclopropane FA synthase in *E. coli*
66 catalyzes the modification of acyl chains to their cyclopropane derivatives through
67 methylation of an unsaturated bond. In this reaction, *S*-adenosyl-L-methionine (SAM) is used
68 as a methyl donor. The enzyme acts on the double bond at the Δ9 or Δ11 in FAs attached to
69 lipids in the membrane [23]. Unsaturated FAs exist in the membrane lipids of most organisms
70 and maintain membrane fluidity. Additionally, SAM exists in most organisms as a methyl
71 donor for methylation reactions. Thus, organisms harboring Δ9 or Δ11 unsaturated FAs in
72 their membrane lipids and in which the cyclopropane FA synthase from *E. coli* is expressed
73 heterologously may have the ability to synthesize cyclopropane FAs. However, this has not

74 been evaluated to date.

75 Cyanobacterial acyl-lipid desaturases introduce double bonds at specific positions in FAs
76 that are esterified to the glycerol backbone of the membrane lipid [14]. The *desA*, *desB*, *desC*,
77 and *desD* genes of *Synechocystis* sp. PCC 6803 encode the acyl-lipid desaturases that
78 introduce double bonds at the Δ12, Δ15, Δ9, and Δ6 positions, respectively, of C18 FAs
79 attached at the *sn*-1 position of the lipids. In *Synechocystis*, a saturated C18 FA, stearic acid
80 (C18:0), is synthesized and incorporated into the *sn*-1 position of membrane lipids, followed
81 by desaturation. DesC introduces a double bond at the Δ9 position in the saturated FA. Then,
82 DesA and DesD unsaturate at the Δ12 and Δ6 positions, respectively, of oleic acid ($C18:1^{\Delta 9}$),
83 and the Δ12 unsaturated FAs are utilized by DesB as substrates to introduce an unsaturated
84 bond at the Δ15 position. The *desA* and *desD* genes were inactivated in the $desA^{-}/desD^{-}$ strain,
85 and this strain accumulated more $C18:1^{\Delta 9}$ than did the wild-type strain [18]. In *Synechocystis*,
86 the endogenous desaturase specifically unsaturates C18 FAs attached at the *sn*-1 position of
87 lipids but cannot unsaturate C16 FAs attached at the *sn*-2 position. On the other hand, DesC2
88 from *Nostoc* sp. strain 36 can unsaturate the Δ9 position of C16 FAs attached at the *sn*-2
89 position [4].

90 In this study, we attempted to synthesize cyclopropane FAs in the cyanobacterium
91 *Synechocystis* sp. PCC 6803 by expressing the cyclopropane FA synthase from *E. coli* to

92 produce FAs that are fluid and stable in response to oxidization. To increase the yield of
93 cyclopropane FAs *in vivo*, we also expressed the *desC2* gene from *Nostoc* sp. and examined
94 the effects of mutations in *desA* and *desD* of *Synechocystis*. We analyzed the changes in FA
95 composition, cell growth, and respiration, and photosynthesis activities in the *Synechocystis*
96 transformants.
97
98 **2. Materials and Methods**
99 *2.1. Organisms and culture conditions*
100 The *Synechocystis* sp. PCC 6803 glucose-tolerant strain [24] was used as the wild type in
101 this study. *Synechocystis* cells were grown in BG11 [17] buffered with 20 mM
102 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–NaOH (pH 7.5) at 34°C or
103 26°C under continuous illumination by white fluorescent lamps at 70 μmole photons m^{-2} s^{-1}
104 and aerated with 1% (v/v) CO_2-enriched air [21]. All transformants were maintained in BG11
105 medium solidified with 1.5% (w/v) Bacto-agar (BD Biosciences Japan, Tokyo) in the
106 presence of 25 μg/mL kanamycin sulfate, 25 μg/mL spectinomycin dihydrochloride
107 pentahydrate, or 25 μg/mL chloramphenicol, depending on the selection markers used.
108 *E. coli* strain JM109 [25] was grown in 1.5 mL of LB medium [1] at 37°C with shaking at
109 200 rpm. All transformants were maintained in LB medium solidified with 1.5% (w/v)

Bacto-agar (BD Biosciences Japan) in the presence of 50 μg/mL sodium ampicillin or 50 μg/mL spectinomycin dihydrochloride pentahydrate, depending on the selection markers used. To supply exogenous FAs, C18:$1^{\Delta 9}$, linoleic acid (C18:$2^{\Delta 9,12}$), γ-linolenic acid (C18:$3^{\Delta 6,9.12}$), and α-linolenic acid (C18:$3^{\Delta 9,12,15}$), to the *E. coli* cells, we cultivated the cells in liquid LB medium [1] containing 1 mM sodium salt of C18:$1^{\Delta 9}$ (Tokyo chemical industry, Japan), C18:$2^{\Delta 9,12}$ (Funakoshi, Tokyo, Japan), C18:$3^{\Delta 6,9.12}$ (Sigma Aldrich, Missouri, USA), or C18:$3^{\Delta 9,12,15}$ (Funakoshi).

2.2. Plasmid construction and transformation

For heterologous expression of the *cfa* and *desC2* genes in *Synechocystis* and overexpression of the *cfa* gene in *E. coli*, we constructed four plasmids—pTHT-cfaSp, pTHT-Sp, pTHT-desC2Sp, and pTHT-cfadesC2Sp—which are derivatives of an expression vector, pTCHT2031V, for this cyanobacterium [9]. Fig. S1 shows the construction scheme for these plasmids, and Table S1 shows the primers used. The plasmid pTCHT2031V contains five DNA fragments in the following order: the sequence upstream of the *slr2031* gene (2031up), a chloramphenicol resistance gene cassette (Cm-r), the *trc* promoter sequence (trc), the sequence downstream of the *slr2031* gene (2031dn), and the plasmid backbone of the pUC vector [9]. The derived plasmids had fragments introduced between 2031up and 2031dn

into the *Synechocystis* chromosome through homologous recombination with the coding sequence of *slr2031*, a non-essential gene. At first, to replace the selection marker from the chloramphenicol resistance gene cassette with the spectinomycin resistance gene cassette, we constructed a plasmid lacking the chloramphenicol resistance gene cassette, pTHT2031V, from pTCHT2031V by PCR amplification and using an In-Fusion HD Cloning Kit (Takara Bio, Ohtsu, Japan). The *cfa* gene was amplified by PCR by using *E. coli* chromosomal DNA as the template. The amplified DNA fragment was subcloned into a T-vector pMD19 vector (Takara Bio) to obtain the pMD-cfa plasmid, which was confirmed by DNA sequencing. We next amplified a DNA fragment including the spectinomycin resistance gene (Sp-r) using pAM1146 [20] as a template. The Sp-r fragment, digested with *BamH*I and *Bgl*II, was inserted into pMD-cfa, and pTHT2031V was cleaved with *Bgl*II in the same orientation as transcription of the *cfa* gene and the *trc* promoter, respectively, to obtain pMD-cfaSp and pTHT-Sp. The fragments containing the *cfa* and Sp-r genes in pMD-cfaSp were excised using *Nde*I and *Bgl*II and inserted into pTHT2031V digested using the same restriction enzymes to obtain pTHT-cfaSp. The *desC2* gene from the *Nostoc* sp. strain 36 [4] was artificially synthesized (Life Technologies Japan, Tokyo) and optimized for the codon usage of *Synechocystis*. Finally, we inserted the *desC2* gene into pTHT-cfaSp and amplified the fragment with two primer sets using an In-Fusion HD Cloning Kit (Takara Bio) to obtain

146 pTHT-desC2Sp and pTHT-cfadesC2Sp.

147 pTHT-cfaSp and pTHT-Sp were introduced into *E. coli* strain JM109 to construct the *cfa*
148 overexpression and vector control strains, respectively. The FA compositions of these cells
149 were analyzed. pTHT-cfaSp, pTHT-desC2Sp, and pTHT-cfadesC2Sp were used to transform
150 cells of the wild-type and *desA*$^{-}$/*desD*$^{-}$ strains of *Synechocystis* by homologous recombination
151 [24]. After verifying complete segregation of the mutated chromosomes from those
152 possessing the native *slr2031* gene by PCR, FA compositions and photosynthesis and
153 respiration activities were analyzed.

154

155 *2.3. FA analysis*

156 Profiles of FAs in the cells were examined by the method of Kotajima *et al*. [11]. Cells
157 were precipitated by centrifugation and re-suspended in 2 mL of methanol. The suspensions
158 were transferred to glass test tubes. After complete drying using a concentrating centrifuge
159 (CC-105, Tomy Seiko, Tokyo, Japan), the pellet was re-suspended in 0.1 M hydrochloric acid
160 methanolic solution (Wako Pure Chemicals, Osaka, Japan). Then, the tubes were tightly
161 capped and incubated at 100˚C for 1 h to allow saponification of the acyl-groups in lipids and
162 conversion into FA methyl esters (FAMEs). The resultant FAMEs were recovered using
163 *n*-hexane. The hexane phases recovered were evaporated, and the residues containing FAMEs

were dissolved in 100 μL of *n*-hexane.

To identify and quantify FAMEs, we applied 1 μl of the hexane solution to a GC-2014 gas chromatograph equipped with a flame-ionization detector (Shimadzu, Kyoto, Japan). Helium was used as a carrier gas at a constant flow rate of 1.25 mL/min in split-less mode. A CP-Sil5 CB column (Agilent Technologies, Santa Clara, CA) was used at the following temperatures: 60°C for 1.5 min, then 130°C at 20°C/min, and a further increase to 230°C at 4°C/min. Most FAMEs were provisionally identified based on retention time and confirmed using commercial FAME standards (Nu-Chek Prep, Elysian, MN). To identify *cis*-9,10-methylenehexadecanoic acid (C17:1cyclo$^{\Delta 9}$) and *cis*-9,10-methyleneoctadecanoic acid (C19:1cyclo$^{\Delta 9}$), we used a gas chromatograph, GC-2010, equipped with a mass spectrometer, QP-2010 (Shimadzu). Conditions of GC were identical to those used for the FAME quantification, as described above. We confirmed the retention times and mass spectrums of C17:1cyclo$^{\Delta 9}$ (Santa Cruz Biotechnology, Texas USA) and C19:1cyclo$^{\Delta 9}$(Santa Cruz Biotechnology). *cis*-9,10-methylene-*cis*-12-octadecenoic acid (C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$) and *cis*-9,10-methylene-*cis*-12,15-octadecadienoic acid (C19:3$^{\Delta 12,15}$cyclo$^{\Delta 9}$) were estimated from the differences in the mass of the parent ion of C19:1cyclo$^{\Delta 9}$ and pattern of fragmentation seen in the GC-MS results.

In order to analyze FAs attached to each lipid, the harvested cells were stored at −80°C

and the lipids were extracted with CH_3Cl:methanol (2:1, v/v). The cell debris were sedimented by centrifugation. The resulting supernatant was transferred into new tubes and evaporated. Then, the samples was re-suspended in 300 µl CH_3Cl:methanol (2:1, v/v) and applied to a silica gel plate. The lipids were separated by thin-layer chromatography using chloroform/methanol/acetic acid (65:25:8, v/v/v). The lipids were detected by staining with primulin and then the silica gel corresponding to the lipid spots was scraped off and extracted as described above.

2.4. Photosynthesis and respiration activities

Photosynthesis and respiration activities were measured as evolution and absorption of oxygen, respectively, using an oxygen electrode (Oxytherm System, Hansatech, Norfolk, UK). *Synechocystis* cells were grown at 34°C and 26°C for 2 d, and assayed at the same temperatures. Photosynthesis activity in these samples was measured at a light intensity of 600 µmole photons m^{-2} s^{-1}, which represented saturated light conditions. Sodium hydrogen carbonate (2.5 mM) was added to the cell suspensions as a carbon source. Respiration activity was measured in the dark.

2.5. RNA extraction and quantification of mRNA

For the RNA extraction, wild-type and transformant cells were cultivated at 34°C under standard growth condition for 3 d, and then the cultures were transferred to 26°C. After 1 d of cultivation, the cells were inoculated into fresh BG11 medium at an OD_{730} of 0.1 and further cultivated at 26°C for 1 d. The total RNAs were isolated by TRIzol Max Bacterial RNA Isolation Kit (Invitrogen, Carlsbad, CA), and purified by RNeasy Minikit (QIAGEN, Hilden, Germany) as previously described by Kotajima *et al*. [11].

The total RNAs extracted from the *Synechocystis* cells were reverse transcribed by PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Bio) to obtain cDNAs. Real-time PCR was performed using a GoTaq qPCR Master Mix (Promega, Fitchburg, WI) on a PikoPeal 96 Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA). The 10 μL of PCR reaction mixture contained 5 ng of cDNAs, 0.2 μL of 10 μM forward primer, and 0.2 μL of 10 μM reverse primer. To determine the expression levels of the *desC*, *desA*, and *desD* genes, we used primer sets desC_qPCR_F and desC_qPCR_R, desA_qPCR_F and desA_qPCR_R, and desD_qPCR_F and desD_qPCR_R, respectively (Table S1). As a reference, we amplified the *rnpB* gene encoding the RNase P subunit B using primers rnpB_RT_F and rnpB_RT_R. As a negative control, we used total RNA samples that were not reverse transcribed. The threshold cycle (C_T) values were determined by PikoReal Software 2.2 (Thermo Fisher Scientific) following the manual. Relative quantitation was

218 performed using the comparative C_T method [12]. The results of the wild-type cells were used
219 for calibration.
220
221 **3. Results and Discussion**
222 *3.1. FA composition of* cfa-*overexpressing* E. coli
223 *E. coli* cells synthesize the unsaturated FAs palmitoleic acid (C16:1$^{\Delta 9}$) and vaccenic acid
224 (C18:1$^{\Delta 11}$) as components of their membrane lipids, but not C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, or
225 C18:3$^{\Delta 6,9.12}$, which are the major unsaturated FAs in *Synechocystis* cells grown at 34°C.
226 FA-modifying enzymes, including the acyl-lipid FA desaturases, are position-specific [3]. It
227 was unclear whether Cfa in *E. coli* introduces cyclopropane groups at the Δ9 and Δ11
228 positions of the unsaturated FAs or specifically at the ω7 position. To determine whether Cfa
229 could modify C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, and C18:3$^{\Delta 6,9.12}$ *in vivo*, we analyzed the FA compositions
230 of the wild-type and *cfa*-overexpressing *E. coli* strains cultivated in a liquid medium
231 containing C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 9,12,15}$, or C18:3$^{\Delta 6,9.12}$.
232 Addition of each FA to a culture of the wild-type strain of *E. coli* resulted in total FAs in
233 membrane lipids comprising 10-30% of C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 9,12,15}$, and C18:3$^{\Delta 6,9.12}$
234 (Table 1). Wild-type cells cultivated with C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, and C18:3$^{\Delta 9,12,15}$ exhibited 0.3
235 ± 0.1%, 0.3 ± 0.1%, and 1.7 ± 0.7% of C19:1cyclo$^{\Delta 9}$, C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$, and C19:3$^{\Delta 12,15}$cyclo$^{\Delta 9}$,

236 respectively, among the total membrane FAs. The total FAs in membrane lipids of the
237 *cfa*-overexpressing *E. coli* strain comprised 8-12% of C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 9,12,15}$, and
238 C18:3$^{\Delta 6,9.12}$. Moreover, the *cfa*-overexpressing strain cultivated with C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, and
239 C18:3$^{\Delta 9,12,15}$ exhibited 14.5 ± 2.9%, 5.4 ± 0.7%, and 2.2 ± 0.2% of C19:1cyclo$^{\Delta 9}$,
240 C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$, and C19:3$^{\Delta 12,15}$cyclo$^{\Delta 9}$, respectively, among total membrane FAs. When
241 both wild-type and *cfa*-overexpressing *E. coli* cells were cultivated with C18:3$^{\Delta 6,9,12}$,
242 approximately 10% of it of the total FAs was incorporated into the membrane lipids. Both
243 cells synthesized C17:1cyclo$^{\Delta 9}$ and lactobacillic acid (C19:1cyclo$^{\Delta 11}$), but did not synthesize
244 novel cyclopropane FAs, such as *cis*-9,10-methylene-*cis*-6,12-octadecadienoic acid
245 (C19:3$^{\Delta 6,12}$cyclo$^{\Delta 9}$). C16:1$^{\Delta 7}$, C16:2$^{\Delta 7,10}$, C16:3$^{\Delta 4,7,10}$, and C16:3$^{\Delta 7,10,13}$ were detected when the
246 cells were cultured in media including C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 6,9,12}$, and C18:3$^{\Delta 9,12,15}$,
247 respectively, as exogenous FAs. The purities of C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 6,9,12}$, and
248 C18:3$^{\Delta 9,12,15}$ were higher than 97%, 99%, 99%, and 99%, respectively. In addition, when we
249 incubated these FAs in LB medium with shaking, these FAs were not changed into C16 FAs
250 (data not shown). Thus, we concluded that C16:1$^{\Delta 7}$, C16:2$^{\Delta 7,10}$, C16:3$^{\Delta 4,7,10}$, and C16:3$^{\Delta 7,10,13}$
251 were produced from C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 6,9,12}$, and C18:3$^{\Delta 9,12,15}$, respectively, in the *E.*
252 *coli* cells during the culture, perhaps via β-oxidation.

253 These results suggest that Cfa can specifically modify the Δ9 position of the C18

254 unsaturated FAs C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, and C18:3$^{\Delta 9,12,15}$ as substrates for production of
255 cyclopropane FAs. However, if both the Δ6 and Δ9 positions of the C18 unsaturated FAs
256 were unsaturated, Cfa could not introduce a cyclopropane ring at the Δ9 position. These
257 results suggested that C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, and C18:3$^{\Delta 9,12,15}$ are candidate substrates for Cfa in
258 *cfa*-expressing *Synechocystis* cells.
259
260 *3.2. FA compositions of* Synechocystis *transformants*
261 *3.2.1. Heterologous expression of the* cfa *gene in wild-type* Synechocystis
262 According to the results from FA feeding experiments in *E. coli* expressing the *cfa* gene,
263 Cfa can convert C18:1$^{\Delta 9}$ and C18:2$^{\Delta 9,12}$ to C19:1cyclo$^{\Delta 9}$ and C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$, respectively.
264 We examined whether the unsaturated FAs endogenously synthesized and incorporated into
265 membrane lipids are converted to cyclopropane FAs *in vivo* by heterologously expressing *cfa*
266 in wild-type *Synechocystis* cells.
267 In wild-type *Synechocystis* cells, C18:1$^{\Delta 9}$ and C18:2$^{\Delta 9,12}$, which are thought to be
268 substrates for Cfa, comprised 18.3 ± 0.6% and 16.6 ± 0.6% of total FAs, respectively.
269 C18:3$^{\Delta 6,9,12}$, which may not be catalyzed by Cfa, comprised 9.2 ± 0.8% of total FAs. In the
270 strain harboring the *cfa* gene (*cfa*$^{+}$), C19:1cyclo$^{\Delta 9}$ comprised 18.8 ± 0.4% of total FAs.
271 Simultaneously, the C18:1$^{\Delta 9}$ content was reduced to 5.1 ± 0.4% (Table 2). Interestingly,

272 C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$ was not produced from C18:2$^{\Delta 9,12}$ in *Synechocystis* cells. The abundance of
273 C18:2$^{\Delta 9,12}$ in the *cfa*$^{+}$ strain was 10.7 ± 0.7%, which was slightly lower than that in wild-type
274 cells. These results indicated that Cfa was functional in the *Synechocystis* cells, but that only
275 C18:1$^{\Delta 9}$ was used as a substrate and that DesA, a Δ12 acyl-lipid desaturase in *Synechocystis*,
276 also uses C18:1$^{\Delta 9}$ but not C19:1cyclo$^{\Delta 9}$ as a substrate. Cfa and DesA compete for use of
277 C18:1$^{\Delta 9}$.

278 Although Cfa converted C18:2$^{\Delta 9,12}$ to C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$ in *E. coli* cells, this did not happen
279 in *Synechocystis* cells. The reason for this discrepancy is unclear, but the head groups of the
280 membrane lipids differ between these two organisms. The membrane lipids in *E. coli* are
281 phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol
282 [PG], and cardiolipin) [6], whereas those in *Synechocystis* are mainly glycolipids (e.g.,
283 monogalactosyl diacylglycerol [MGDG], digalactosyl diacylglycerol [DGDG], and
284 sulfoquinovosyl diacylglycerol [SQDG]) and only one phospholipid, PG [14]. We next
285 separated the total lipids into each membrane lipid (i.e., MGDG, DGDG, SQDG, and PG) by
286 thin-layer chromatography and determined the FA profiles. As shown in Table S2, in the *cfa*$^{+}$
287 strain, C19:1cyclo$^{\Delta 9}$ was incorporated into all four types of lipids, and the C19:1cyclo$^{\Delta 9}$
288 content in SQDG was slightly lower than those in the other three lipids. Because a greater
289 quantity of palmitic acid (C16:0) was attached to SQDG than to the other lipids in

290 *Synechocystis* cells, as reported previously [22], the C19:1cyclo$^{\Delta 9}$ level in the lipids was lower.
291 Thus, Cfa activities were unaffected by differences in the lipid head groups.

292 The difference in lipid types does not explain the lack of production of C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$ in
293 *Synechocystis.* According to the literature, Cfa acts on the nonpolar portion of phospholipids
294 dispersed in vesicles [19]. Recently, S. J. Biller *et al*. [2] showed that many microorganisms
295 develop and release small membrane vesicles from cell surfaces. However, the presence of
296 such membrane vesicles in the cytosol of cyanobacterial cells is yet to be demonstrated. At
297 this time, we cannot clearly identify the substrate of Cfa. In *Synechocystis* cells, there are
298 three cell membranes, i.e., the outer membrane, inner membrane, and thylakoid membrane. In
299 these membranes, the lipids or FAs do not exist unevenly [10]. Thus, we did not consider the
300 presence of a different compartment of C18:2$^{\Delta 9,12}$ in *Synechocystis*. However, in unidentified
301 vesicles and possible microdomains where Cfa might locate and function, we cannot ignore
302 the possibility. It is unclear why C19:2$^{\Delta 12}$cyclo$^{\Delta 9}$ was not produced in *Synechocystis*.

303

304 *3.2.2. Heterologous expression of* cfa *in* desA$^-$/desD$^-$ Synechocystis *cells*

305 Cfa catalyzed the synthesis of only C19:1cyclo$^{\Delta 9}$ from C18:1$^{\Delta 9}$ in *Synechocystis* cells
306 (Table 2). To increase the abundance of C19:1cyclo$^{\Delta 9}$ in *Synechocystis* cells, we attempted to
307 introduce the *cfa* gene into the *desA$^-$/desD$^-$* strain (*desA$^-$/desD$^-$/cfa$^+$*), which does not produce

C18 polyunsaturated FAs [18]. Total FAs of the *desA*$^{-}$/*desD*$^{-}$ strain comprised 49.8 ± 0.5% of C18:1$^{\Delta 9}$, which is 2.5-fold greater than that of wild-type cells (Table 2). Although we attempted to increase the abundance of C19:1cyclo$^{\Delta 9}$ in the *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ strain, it unexpectedly comprised 22.2 ± 0.6% of the total FAs (Table 2). First, we hypothesized that Cfa prefers unsaturated FAs attached to phospholipids. However, the FA compositions of each lipid class—MGDG, DGDG, SQDG, and PG—of *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ were not markedly altered, suggesting that Cfa does not discriminate among lipid head groups (Table S2). Excess C19:1cyclo$^{\Delta 9}$ in the membrane lipids might exert deleterious effects on *Synechocystis* cells.

3.2.3. Heterologous coexpression of cfa *and* desC2 *in* Synechocystis *cells*

In the *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ strain, the abundance of C19:1cyclo$^{\Delta 9}$ was not increased compared with that in the *cfa*$^{+}$ strain. To increase the abundance of cyclopropane FAs in *Synechocystis* cells, we attempted to synthesize a novel cyclopropane FA, C17:1cyclo$^{\Delta 9}$, from C16:1$^{\Delta 9}$ in *Synechocystis*. The endogenous desaturases of *Synechocystis* specifically unsaturate C18 FAs attached at the *sn*-1 position of the lipids, but cannot unsaturate C16 FAs attached at the *sn*-2 position [14]. In contrast, DesC2 from *Nostoc* sp. strain 36 specifically unsaturates C16 saturated FA attached at the *sn*-2 position [4]. We aimed to synthesize C17:1cyclo$^{\Delta 9}$ by coexpression of the *cfa* and *desC2* genes in the wild-type and *desA*$^{-}$/*desD*$^{-}$

326 strains of *Synechocystis* (*cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$). Furthermore, we also
327 constructed the *desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ strains by introducing the corresponding
328 genes into the wild-type and *desA*$^{-}$/*desD*$^{-}$ cells, respectively, as controls.

329 In the *desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ strains, C16:1$^{\Delta 9}$ comprised approximately 25% of
330 total FAs. Because C19:1cyclo$^{\Delta 9}$ comprised ~20% of total FAs in the *cfa*$^{+}$ and
331 *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ strains, we expected that the *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$
332 strains would synthesize ~20% C17:1cyclo$^{\Delta 9}$ in addition to 20% C19:1cyclo$^{\Delta 9}$. However, the
333 *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strains produced only 7.7 ± 0.5% and 5.2 ± 0.4%
334 C17:1cyclo$^{\Delta 9}$, respectively. Moreover, the C16:1$^{\Delta 9}$ abundance was reduced to 2.3 ± 0.1% and
335 1.8 ± 0.1% in the *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strains, respectively. Interestingly,
336 C19:1cyclo$^{\Delta 9}$ abundance was maintained after introduction of the *desC2* gene, comprising
337 more than 20% in the *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strains. The abundance of
338 C17:1cyclo$^{\Delta 9}$ in the *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strains was lower than our
339 estimation. C16:1$^{\Delta 9}$ abundance in the *cfa*$^{+}$/*desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strains was
340 markedly lower than that in the *desC2*$^{+}$ and *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ strains.

341

342 *3.3. Growth of the* Synechocystis *transformants*

343 In this study, we constructed *Synechocystis* strains that synthesized cyclopropane FAs

344 and unsaturated C16 FAs. These are novel FAs in *Synechocystis* cells; therefore, we examined
345 the effects of synthesis of cyclopropane FAs and unsaturated C16 FA on the growth of
346 *Synechocystis* cells.

347 Both wild-type and *cfa*$^{+}$ cells grew well at high and low temperatures (34°C and 26°C,
348 respectively) (Fig. 1). The growth rates were not altered by introduction of the *cfa* gene in
349 wild-type cells. The growth of *desA*$^{-}$/*desD*$^{-}$ cells at 34°C was reduced compared with that of
350 wild-type cells, whereas growth at 26°C was almost halted, as reported previously [18]. The
351 growth rate of *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ cells was lower than that of *desA*$^{-}$/*desD*$^{-}$ cells at both
352 temperatures, and these cells were unable to grow at 26°C. These results suggest that
353 C19:1cyclo$^{\Delta 9}$ synthesis in cells that do not synthesize polyunsaturated FAs negatively affected
354 growth, irrespective of temperature. The melting point of C18:1$^{\Delta 9}$; i.e., 16.3˚C
355 (http://www.sciencelab.com/msds.php?msdsId=9927682), is lower than that of C19:1cyclo$^{\Delta 9}$
356 (i.e., 27.8–28.8˚C) [7], suggesting that maintenance of membrane fluidity in cells with
357 membrane lipids possessing C19:1cyclo$^{\Delta 9}$ instead of C18:1$^{\Delta 9}$ might be difficult.

358 The growth rate at 34°C of wild-type cells harboring the *desC2* gene was slightly
359 decreased compared with that of wild-type cells, and growth at 26°C was significantly
360 repressed. These results suggest that synthesis of C16:1$^{\Delta 9}$ in the wild-type cells negatively
361 affected their growth, particularly at low temperatures. In a previous report [4], the

362 unsaturation of the C16 FAs attached at the *sn*-2 position was suggested to contribute to the
363 growth of a *Nostoc* strain living in an Antarctic lake at low temperatures, but this was not so
364 in the case of *Synechocystis*. Although the growth of the *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ strain at 34°C
365 was not changed, growth at 26°C was significantly increased. However, the growth rate of the
366 *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ strain decreased to almost the same extent as that of the *desA*$^{-}$/*desD*$^{-}$
367 strain. Although C16:1$^{\Delta 9}$ synthesis in wild-type cells resulted in decreased growth at 26°C,
368 growth of the *desA*$^{-}$/*desD*$^{-}$ strain was stimulated at this temperature. If the C18 FA at the *sn*-1
369 position is not multiply unsaturated, unsaturation of the C16 FA at the *sn*-2 position might
370 contribute to growth at low temperatures. C16:1$^{\Delta 9}$ production by the *desA*$^{-}$/*desD*$^{-}$ strain might
371 have maintained the membrane fluidity under low-temperature conditions. Co-introduction of
372 the *cfa* gene abrogated the positive effects of the *desC2* gene on growth at low temperature.
373 Therefore, the conversion of C16:1$^{\Delta 9}$ at the *sn*-2 position of membrane lipids into
374 C16:1cyclo$^{\Delta 9}$ might decrease membrane fluidity.
375
376 *3.4. Effect of cyclopropane FA synthesis on photosynthesis and respiration activities*
377 Modification of FA profiles by expression of *cfa* and *desC2* altered the growth rates of
378 *Synechocystis* transformants, especially at 26°C (Fig. 1). To investigate these phenomena, we
379 analyzed photosynthesis and respiration activities in *Synechocystis* cells grown at 34°C and

380 26°C. Respiration and photosynthesis activities were not significantly altered in cells grown at
381 34°C (Fig. 2). As reported previously [18], the growth rate of the *desA*$^{-}$/*desD*$^{-}$ strain was
382 severely retarded at low temperature, although photosynthesis and respiration activities were
383 not significantly different from those of wild-type cells. However, the respiration and
384 photosynthesis activities in the wild-type cells harboring the *cfa* or *desC2* gene were
385 decreased to some extent, particularly at 26°C. Although expression of the *desC2* gene
386 resulted in recovery of the growth rate of the *desA*$^{-}$/*desD*$^{-}$ strain at 26°C, photosynthesis and
387 respiration activities were lower than those of the *desA*$^{-}$/*desD*$^{-}$ strain. These results suggest
388 that *cfa* and *desC2* expression in wild-type cells slightly decreased the photosynthesis and
389 respiration activities, which may be related to suppression of the growth rate of the
390 transformants. However, C16:1$^{\Delta 9}$ synthesis in the *desA*$^{-}$/*desD*$^{-}$ strain led to recovery of the
391 growth rate at 26°C via promotion of various cellular processes other than photosynthesis and
392 respiration.

393 The cultures in Fig. 1 were grown at 70 μmole photons m^{-2} s^{-1} and the photosynthetic
394 activity shown in Fig. 2 was measured at 600 μmole photons m^{-2} s^{-1}, which is near the light
395 saturation point in cells, to observe the maximum rate of O_2 evolution. We thought that there
396 was a discrepancy between the two experiments due to a difference in the light conditions.
397 Therefore, we further investigated the photosynthetic activity at various light intensities,

398 including 75 μmol photons/m^2/s, which is the closest to the growth condition. As a result,
399 even at a low light intensity, we could not observe clear differences in the photosynthetic
400 activity in the strains (Fig. S2). Therefore, modification of the FA composition might have
401 altered the activities of endogenous desaturase in *Synechocystis* and indirectly affected the
402 growth of the transformants.
403
404 *3.5. Apparent desaturase and cyclopropane FA synthetase activities in* Synechocystis *strains*
405 To compare apparent desaturase and cyclopropane FA synthetase activities, we
406 determined the total abundance of FAs synthesized by each enzyme (Table 2, Table S3, and
407 Fig. 3). The total abundance of FAs unsaturated by DesC is shown in Fig. 3A. The *cfa*$^+$,
408 *desC2*$^+$, and *cfa*$^+$*/desC2*$^+$ strains synthesized lower levels of FAs unsaturated by DesC than
409 the wild-type cells did. In addition, the *desA*$^-$*/desD*$^-$*/cfa*$^+$, *desA*$^-$*/desD*$^-$*/desC2*$^+$, and
410 *desA*$^-$*/desD*$^-$*/cfa*$^+$*/desC2*$^+$ strains synthesized lower levels of FAs unsaturated by DesC than
411 did the *desA*$^-$*/desD*$^-$ strain. This might be due to a mechanism regulating membrane fluidity.
412 In accordance with previous reports [13], the *desC* gene in *Synechocystis* is constitutively
413 expressed irrespective of culture conditions. However, these findings suggest the existence of
414 a novel mechanism of regulating *desC* expression to prevent excess membrane fluidity. We
415 analyzed expression levels of the *desC* gene by qPCR using total RNA extracted from the

416 cells of wild-type, *cfa*$^{+}$, *desC2*$^{+}$, and *cfa*$^{+}$/*desC2*$^{+}$ *Synechocystis* strains cultivated at 26°C. We
417 found that the *desC* gene expression levels in the *cfa*$^{+}$, *desC2*$^{+}$, and *cfa*$^{+}$/*desC2*$^{+}$ strains were
418 not remarkably different from those in the wild-type cells (Table S4A). These results suggest
419 that there might be a novel mechanism for regulating DesC activity, perhaps via
420 post-translationally, to maintain the suitable membrane fluidity..

421 Fig. 3B, C, and D show the total abundance of FAs unsaturated by DesA, DesD, and
422 DesB, respectively. These results demonstrated that the apparent activities of DesA, DesD,
423 and DesB were decreased to some extent by introduction of the *desC2* gene, especially at
424 26°C. *desA*, *desD*, and *desB* expression is induced at low temperature to maintain membrane
425 fluidity in *Synechocystis* cells [22]. *desC2* expression may downregulate the expression levels
426 of *desA*, *desD*, and *desB* via the regulatory mechanism for maintaining membrane fluidity
427 [13]. We analyzed expression levels of the *desA* and *desD* genes by qPCR using total RNA
428 extracted from the cells of wild-type, *cfa*$^{+}$, *desC2*$^{+}$, and *cfa*$^{+}$/*desC2*$^{+}$ *Synechocystis* strains
429 cultivated at 26°C. We found that the *desA* and *desD* gene expression levels in the *cfa*$^{+}$,
430 *desC2*$^{+}$, and *cfa*$^{+}$/*desC2*$^{+}$ strains were not remarkably altered from those in the wild-type cells
431 (Table S4B, C). Alternatively, DesC2 may compete with DesA, DesD, and DesB, and these
432 endogenous desaturases may specifically unsaturate C18 FAs attached at the *sn*-1 position of
433 lipids containing C16-saturated FAs at the *sn*-2 position, but not those containing

C16-unsaturated FAs. Fig. 4 summarizes these results, and the data therein suggest that no pathway for unsaturation by DesA, DesD, and DesB after unsaturation by DesC2 exists.

Fig. 3E shows the total abundance of FAs unsaturated by DesC2. These results demonstrated that cells harboring both the *cfa* and *desC2* genes produced lower levels of FAs unsaturated by DesC2 than did cells expressing the *desC2* gene under both the high- and low-temperature conditions. The total abundance of saturated FAs are shown in Fig. 3G. These results demonstrated that *Synechocystis* cells harboring only *desC2* produced lower levels of saturated FAs than did wild-type cells, but that the cells containing both *cfa* and *desC2* accumulated large quantities of saturated FAs. Indeed, the levels were almost identical to those in wild-type *Synechocystis* cells. These results suggested that DesC2 might unsaturate C16:0 attached at the *sn*-2 position of lipids possessing C18 unsaturated FAs at the *sn*-1 position but not lipids with C18-saturated FAs or C19 cyclopropane FAs (Fig. 4). The cyclopropane FA yield would be increased if the *sn*-2-specific desaturase could unsaturate C16:0 attached at the *sn*-2 position of lipids containing C18-saturated FAs or C19 cyclopropane FAs at the *sn*-1 position.

4. Conclusions

We successfully synthesized cyclopropane FAs in *Synechocystis* at a level comprising

452 ~30% of total FAs. Growth of *Synechocystis* cells harboring the *cfa* and *desC2* genes was
453 altered, particularly under low-temperature conditions, but photosynthesis and respiration
454 activities were not significantly affected. C16:1$^{\Delta 9}$ synthesis in the *desA*$^{-}$*/desD*$^{-}$ strain positively
455 affected growth under low-temperature conditions by promoting various cellular processes
456 other than photosynthesis and respiration. Our data suggest that Cfa and desaturases may
457 recognize lipid side chains. The cyclopropane FA yield would be increased if the
458 *sn*-2-specific desaturase could unsaturate C16:0 attached at the *sn*-2 position of lipids
459 containing C18-saturated FAs or C19 cyclopropane FAs at the *sn*-1 position.
460
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466
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536

537 **Figure Captions**

538 **Fig. 1.** Growth of wild-type and mutant cells at 34°C and 26°C. Mean ± S.D. values of three
539 independent experiments. Closed red circle, Wild-type cells; open red circle, *cfa*$^{+}$ cells; closed
540 green circle, *desC2*$^{+}$ cells; open green circle, *cfa*$^{+}$/*desC2*$^{+}$ cells; closed purple circle,
541 *desA*$^{-}$/*desD*$^{-}$ cells; open purple circle, *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$ cells; closed light blue circle,
542 *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ cells; and open light blue circle, *desA*$^{-}$/*desD*$^{-}$/*cfa*$^{+}$/*desC2*$^{+}$ cells.

543

544 **Fig. 2.** O_2 evolution and absorption of wild-type and mutant cells at 34°C and 26°C. Cells
545 were cultured at 34°C and 26°C for 2 days, and the activities were measured at the same
546 temperature using an O_2 electrode. Mean ± S.D. values of three independent experiments.
547 Black and white bars show respiratory and photosynthetic activities, respectively.

548

549 **Fig. 3.** Relative amounts of fatty acids produced by each desaturase and cyclopropane FA
550 synthetase. Mean ± S.D. values of three independent experiments, expressed as mol % of total
551 fatty acids. Red and blue bars indicate cells cultivated at 34°C and 26°C, respectively. A, total
552 production of Δ9 unsaturated C18 FAs by DesC, the sum of C18:1$^{\Delta 9}$, C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 9,12,15}$,
553 C18:3$^{\Delta 6,9,12}$, C18:4$^{\Delta 6,9,12,15}$, and C19:1cyclo$^{\Delta 9}$; B, total production of Δ12 unsaturated C18 FAs
554 by DesA, the sum of C18:2$^{\Delta 9,12}$, C18:3$^{\Delta 9,12,15}$, C18:3$^{\Delta 6,9,12}$, and C18:4$^{\Delta 6,9,12,15}$; C, total

555 production of Δ6 unsaturated C18 FAs by DesD, the sum of C18:3$^{\Delta6,9,12}$ and C18:4$^{\Delta6,9,12,15}$; D,
556 total production of Δ15 unsaturated C18 FAs by DesB, the sum of C18:3$^{\Delta9,12,15}$ and
557 C18:4$^{\Delta6,9,12,15}$; E, total production of Δ9 unsaturated C16 FAs by DesC2, the sum of C16:1$^{\Delta9}$
558 and C17:1cyclo$^{\Delta9}$; F, total production of cyclopropane FAs by Cfa, the sum of C17:1cyclo$^{\Delta9}$
559 and C19:1cyclo$^{\Delta9}$; and G, total production of saturated FAs, the sum of C16:0 and C18:0.
560
561 **Fig. 4.** Putative pathways of the desaturases and cyclopropane FA synthetases. Arrows
562 indicate unsaturation by DesA, DesB, DesC, DesC2, and DesD and modification by Cfa.
563 Doublets indicate that a reaction did not occur.
564
565 **Fig. S1.** Construction of the plasmids used in this study. Red, blue, and black arrows indicate
566 PCR amplification using the indicated primers, conjugation, and cyclization of the fragment
567 and vector using the In-Fusion HD Cloning Kit (Takara Bio), and ligation after digestion by
568 the indicated restriction enzymes.
569
570 **Fig. S2.** Photosynthesis activities of wild-type and mutant cells. A: Cells were cultured at
571 34°C and 26°C for 2 d, and the activities were measured at the same temperature using an O_2
572 electrode. Mean ± S.D. values of three independent experiments. Red, wild-type cells; green,

573 *desC2*$^{+}$ cells; purple, *desA*$^{-}$/*desD*$^{-}$ cells; and light blue, *desA*$^{-}$/*desD*$^{-}$/*desC2*$^{+}$ cells. B:

574 Magnification of ranges from 0 to 100 μmole photons m^{-2} s^{-1} of the graphs in A. Black allows

575 indicate light intensity at 70 μmole photons m^{-2} s^{-1} for cultivation.

576

Table 1

Fatty acid composition of total lipids from wild-type and *cfa*-overexpressing *E. coli* strains

	Fatty acid (mol %)									
	Wild type					*cfa*-overexpressing strain				
	Exogenous FA					Exogenous FA				
FA	-	$18:1^{\Delta9}$	$18:2^{\Delta9,12}$	$18:3^{\Delta9,12,15}$	$18:3^{\Delta6,9,12}$	-	$18:1^{\Delta9}$	$18:2^{\Delta9,12}$	$18:3^{\Delta9,12,15}$	$18:3^{\Delta6,9,12}$
14:0	6.8 ± 0.2	2.8 ± 1.5	5.3 ± 1.3	5.8 ± 0.6	4.8 ± 1.6	5.5 ± 1.2	3.9 ± 1.2	5.2 ± 0.9	6.4 ± 0.5	4.5 ± 1.0
16:0	44.9 ± 0.5	44.6 ± 1.3	58.7 ± 2.4	49.4 ± 2.0	41.3 ± 2.6	45.0 ± 0.2	35.8 ± 2.2	53.6 ± 3.1	47.8 ± 1.1	40.5 ± 3.2
$16:1^{\Delta9}$	16.8 ± 4.4	3.0 ± 1.4	3.9 ± 0.9	4.7 ± 1.6	11.0 ± 3.6	3.7 ± 0.8	1.8 ± 0.1	2.4 ± 0.9	2.3 ± 1.0	4.5 ± 1.9
$16:1^{\Delta7}$	-	2.5 ± 1.2	-	-	-	-	3.8 ± 1.1	-	-	-
$16:2^{\Delta7,10}$	-	-	2.9 ± 1.0	-	-	-	-	2.1 ± 0.1	-	-
$16:3^{\Delta7,10,13}$	-	-	-	5.2 ± 0.4	-	-	-	-	4.5 ± 0.7	-
$16:3^{\Delta4,7,10}$	-	-	-	-	2.6 ± 1.3	-	-	-	-	2.5 ± 1.0
$17:1cyclo^{\Delta9}$	11.1 ± 5.0	2.1 ± 1.3	6.0 ± 3.8	4.9 ± 2.8	6.4 ± 3.9	22.8 ± 2.1	5.4 ± 1.8	9.5 ± 2.1	10.6 ± 2.5	19.3 ± 3.4
18:0	3.3 ± 0.7	2.5 ± 0.7	2.7 ± 0.4	3.0 ± 0.8	8.8 ± 5.6	6.8 ± 2.0	6.1 ± 2.2	3.9 ± 0.7	4.2 ± 1.5	5.3 ± 1.9
$18:1^{\Delta11}$	15.9 ± 1.4	12.3 ± 2.4	5.5 ± 0.1	5.9 ± 0.6	13.1 ± 1.1	5.5 ± 1.8	12.6 ± 5.7	4.2 ± 1.0	3.3 ± 0.6	7.4 ± 2.0
$18:1^{\Delta9}$	-	29.4 ± 1.5	-	-	-	-	12.3 ± 3.6	-	-	-
$18:2^{\Delta9,12}$	-	-	12.9 ± 3.7	-	-	-	-	7.8 ± 1.1	-	-
$18:3^{\Delta9,12,15}$	-	-	-	17.6 ± 0.4	-	-	-	-	12.3 ± 1.2	-
$18:3^{\Delta6,9,12}$	-	-	-	-	10.9 ± 2.3	-	-	-	-	8.2 ± 0.6
$19:1cyclo^{\Delta11}$	1.2 ± 1.0	0.4 ± 0.1	1.8 ± 1.3	1.7 ± 0.7	1.1 ± 0.9	10.6 ± 1.0	4.0 ± 1.0	5.9 ± 0.8	6.5 ± 0.5	7.7 ± 1.7
$19:1cyclo^{\Delta9}$	-	0.3 ± 0.1	-	-	-	-	14.5 ± 2.9	-	-	-
$^{*}19:2^{\Delta12}cyclo^{\Delta9}$	-	-	0.3 ± 0.1	-	-	-	-	5.4 ± 0.7	-	-
$^{*}19:3^{\Delta12,15}cyclo^{\Delta9}$	-	-	-	1.7 ± 0.7	-	-	-	-	2.2 ± 0.2	-
$^{*}19:3^{\Delta6,12}cyclo^{\Delta9}$	-	-	-	-	-	-	-	-	-	-

The cells were grown at 37°C for 18 h. Exogenous fatty acids (1 mM) were added to the culture. Mean ± S.D. values of three independent experiments, expressed as mol % of total fatty acids. "-", not detected. "*", estimated from comparison with $19:1cyclo^{\Delta9}$.

582 **Table 2**

583 Fatty acid compositions of total lipids from *Synechocystis* wild-type and transformants

34˚C	Fatty acid (mol %)									
Strain	16:0	16:1Δ9	17:1cycloΔ9	18:0	18:1Δ9	18:2Δ9,12	18:3Δ6,9,12	18:3Δ9,12,15	18:4Δ6,9,12,15	19:1cycloΔ9
Wild type	55.0 ± 1.6	-	-	0.8 ± 0.1	18.3 ± 0.6	16.6 ± 0.6	9.2 ± 0.8	-	-	-
cfa$^+$	55.5 ± 0.8	-	-	2.1 ± 0.1	5.1 ± 0.4	10.7 ± 0.7	7.8 ± 0.6	-	-	18.8 ± 0.4
desC2$^+$	31.3 ± 0.8	25.3 ± 0.7	-	2.6 ± 0.4	15.2 ± 0.7	19.3 ± 1.2	6.3 ± 0.8	-	-	-
cfa$^+$/*desC2*$^+$	44.9 ± 0.4	2.3 ± 0.1	7.7 ± 0.5	5.6 ± 1.5	1.9 ± 0.1	11.2 ± 0.3	6.2 ± 0.4	-	-	20.2 ± 0.7
desA$^-$/*desD*$^-$	49.0 ± 0.4	-	-	1.2 ± 0.2	49.8 ± 0.5	-	-	-	-	-
desA$^-$/*desD*$^-$/*cfa*$^+$	59.7 ± 0.4	-	-	3.5 ± 0.2	14.6 ± 0.8	-	-	-	-	22.2 ± 0.6
desA$^-$/*desD*$^-$/*desC2*$^+$	35.8 ± 0.4	26.2 ± 1.0	-	4.1 ± 1.6	33.9 ± 1.0	-	-	-	-	-
desA$^-$/*desD*$^-$/*cfa*$^+$/*desC2*$^+$	54.6 ± 0.6	1.8 ± 0.1	5.2 ± 0.4	6.4 ± 1.8	3.7 ± 0.7	-	-	-	-	28.2 ± 1.9
26˚C	Fatty acid (mol %)									
Strain	16:0	16:1Δ9	17:1cycloΔ9	18:0	18:1Δ9	18:2Δ9,12	18:3Δ6,9,12	18:3Δ9,12,15	18:4Δ6,9,12,15	19:1cycloΔ9
Wild type	52.1 ± 0.1	-	-	1.1 ± 0.3	4.8 ± 0.7	13.5 ± 0.6	21.4 ± 1.0	4.5 ± 0.6	2.6 ± 0.3	-
cfa$^+$	53.9 ± 0.9	-	-	2.1 ± 0.6	4.3 ± 0.9	10.6 ± 0.8	16.9 ± 2.3	3.1 ± 0.6	1.9 ± 0.4	7.2 ± 2.4
desC2$^+$	26.5 ± 0.3	27.5 ± 0.6	-	3.5 ± 0.3	11.4 ± 1.0	16.7 ± 0.9	12.0 ± 0.7	1.5 ± 0.2	0.9 ± 0.1	-
cfa$^+$/*desC2*$^+$	42.5 ± 0.4	4.0 ± 0.2	8.2 ± 0.5	4.1 ± 0.4	4.0 ± 0.3	13.1 ± 1.0	12.5 ± 0.6	1.3 ± 0.2	0.9 ± 0.2	9.4 ± 2.0
desA$^-$/*desD*$^-$	54.0 ± 0.7	-	-	1.3 ± 0.3	44.7 ± 0.8	-	-	-	-	-
desA$^-$/*desD*$^-$/*cfa*$^+$	54.7 ± 1.1	-	-	7.2 ± 3.7	18.8 ± 2.5	-	-	-	-	19.3 ± 1.0
desA$^-$/*desD*$^-$/*desC2*$^+$	28.7 ± 0.7	25.4 ± 1.2	-	2.9 ± 0.2	43.1 ± 0.3	-	-	-	-	-
desA$^-$/*desD*$^-$/*cfa*$^+$/*desC2*$^+$	51.5 ± 1.0	3.3 ± 0.5	2.8 ± 0.1	2.7 ± 0.2	16.3 ± 1.8	-	-	-	-	23.4 ± 1.1

584 The cells were grown at 34˚C and 26˚C for 3 d. Mean ± S.D. values of three independent experiments, expressed as mol % of total fatty

585 acids. "-", not detected.

586 **Table S1**

587 Primers used in this study

No.	Name	Sequence (5'-3')
1	pTCHT_Cm_remove_InF_F	TTTTTGCTTCATCGCTTAAGGCAGTTATTG
2	pTCHT_Cm_remove_InF_R	ACTGCCTTAAAAAAAGAAGCAAAAAGCCTA
3	cfa_Nde_F	CATATGATGAGTTCATCGTGTATAGA
4	cfa_Bgl_R	AGATCTTTAGCGAGCCACTCGAAGGC
5	Sp_BamHI_F	GGGGATCCATCAATTCCCCTGCTCGCGC
6	Sp_Bgl_R	GGAGATCTTCCCAATTTGTGTAGGGCTT
7	desC2_F	TCAAGTAGGAGATTAATTCA
8	desC2_R	TTAGCCATGAGTTGCACCTT
9	pTHT_cfaup_inf_R	TAATCTCCTACTTGACATATGATCCTTATCG
10	pTHT_cfadn_inf_R	TAATCTCCTACTTGAGGATCTTTAGCGAGCCACTC
11	pTHT_Sprup_inf_F	GCAACTCATGGCTAAATCAATTCCCCTGCTCGCGC
12	desC_qPCR_F	AAATGCCCCAAATAACGAAGG
13	desC_qPCR_R	AAAGCTGATATTCCCCGCTACA
14	desA_qPCR_F	TATCCCCGTTGGGTGGAA
15	desA_qPCR_R	AAGGTGCGCTCGTAAAGAAAAG
16	desD_qPCR_F	GGTTGATGTGGGGATTGGA
17	desD_qPCR_R	CCTACTGGGTTGTATGGTTTTGG
18	rnpB_RT_F	GTAAGAGCGCACCAGCAGTATC
19	rnpB_RT_R	TCAAGCGGTTCCACCAATC

588

589

590 **Table S2**

591 Fatty acid composition in each lipid classes from the wild-type and transformants of

592 *Synechocystis*

		Fatty acid (mol %)							
Strain	Lipid class	16:0	16:1$^{\Delta 9}$	17:1cyclo$^{\Delta 9}$	18:0	18:1$^{\Delta 9}$	18:2$^{\Delta 9,12}$	18:3$^{\Delta 6,9,12}$	19:1cyclo$^{\Delta 9}$
Wild type	MGDG	56.6± 7.5	-	-	5.9 ± 3.6	18.3 ± 13.2	15.6 ± 4.5	4.1 ± 1.5	-
	DGDG	64.1 ± 14.2	-	-	11.7 ± 10.8	11.2 ± 5.4	7.9 ± 3.6	2.8 ± 1.9	-
	SQDG	74.4 ± 5.2	-	-	1.3 ± 0.1	18.2 ± 5.0	7.1 ± 1.7	T	-
	PG	80.4 ± 4.6	-	-	4.7 ± 0.9	8.5 ± 3.6	6.4 ± 2.4	T	-
	total	58.0 ± 3.9	-	-	3.8 ± 2.5	19.3 ± 2.1	13.6 ± 1.7	5.2 ± 0.7	-
cfa^{+}	MGDG	55.2 ± 6.7	-	-	5.1 ± 1.8	1.8 ± 0.1	2.2 ± 2.5	2.4 ± 1.5	34.4 ± 7.9
	DGDG	57.7 ± 7.1	-	-	3.1 ± 0.4	1.3 ± 0.1	2.4 ±2.2	2.1 ± 1.8	33.9 ±10.6
	SQDG	67 ± 4.1	-	-	5.2 ± 0.6	5.1 ± 3.4	6.6 ± 0.3	1.1 ± 0.4	16 ± 5.0
	PG	59.7 ± 4.0	-	-	15.6 ± 1.6	T	T	T	24.6 ± 4.4
	total	59.5 ± 2.1	-	-	2.1 ± 0.4	5.1 ± 0.9	6.7 ± 1.4	3.8 ± 0.8	22.8 ± 6.4
desC2^{+}	MGDG	25.8 ± 0.3	23.1 ± 1.5	-	7.9 ± 2.3	15.1 ± 1.3	18.3 ± 0.6	10.6 ±1.4	-
	DGDG	30.9 ± 1.1	25.9 ± 0.8	-	6.2 ± 1.9	13.9 ± 1.2	15.0 ± 3.4	5.7 ± 3.1	-
	SQDG	56.6 ± 3.0	14.5 ± 2.3	-	2.7 ± 1.0	20.8 ± 3.2	2.0 ± 0.4	1.7 ± 1.1	-
	PG	53.3 ± 2.3	11.1 ± 1.3	-	13.2 ± 1.1	13.3 ± 3.0	6.5 ± 2.6	1.4 ± 1.2	-
	total	31.2 ± 2.9	23.5 ± 1.0	-	4.5 ± 0.8	15.6 ± 1.3	16.7 ± 2.1	8.1 ± 1.3	-
cfa^{+}/*desC2*$^{+}$	MGDG	38.5 ± 3.0	1.7 ± 0.8	16.4 ± 1.3	2.8 ±0.3	1.9 ±0.2	8.6 ± 1.5	4.1 ± 1.3	24.9 ± 2.7
	DGDG	45.0 ± 1.2	1.1 ± 0.6	11.8 ± 0.9	3.8 ± 0.3	1.6 ± 0.5	11.1 ± 0.7	T	25.7 ± 2.0
	SQDG	72.9 ± 2.0	T	4.6 ± 1.9	4.6 ± 0.5	2.8 ± 0.8	6.5 ± 1.4	T	8.6 ± 4.4
	PG	43.4 ± 4.5	10.7 ± 5.4	3.3 ± 1.3	15.8 ± 12.2	2.8 ± 1.0	T	T	23.9 ± 1.0
	total	44.5 ± 2.3	2.3 ± 1.9	7.7 ± 0.4	5.6 ± 1.5	1.9 ± 0.8	11.1 ± 1.0	6.2 ± 2.2	20.1 ± 2.8
desA^{-}/*desD*$^{-}$	MGDG	46.3 ± 5.1	-	-	4.4 ± 1.4	49.3 ± 5.4	-	-	-
	DGDG	44.0 ± 2.5	-	-	5.7 ± 1.6	50.7 ± 3.1	-	-	-
	SQDG	82.4 ± 5.0	-	-	2.4 ± 0.8	15.4 ± 5.8	-	-	-
	PG	54.8 ± 2.9	-	-	20.8 ± 5.3	24.3 ± 2.5	-	-	-
	total	48.3 ± 2.1	-	-	1.9 ± 1.2	49.2 ± 2.6	-	-	-
desA^{-}/*desD*$^{-}$	MGDG	51.9 ± 2.0	-	-	5.2 ± 0.8	11.8 ± 5.0	-	-	29.9 ± 7.6
/*cfa*$^{+}$	DGDG	57.2 ± 4.9	-	-	4.4 ± 0.8	4.9 ± 2.5	-	-	31.1 ± 6.8

	SQDG	68.9 ± 4.7	-	-	6.2 ± 3.2	15.1 ± 3.1	-	-	10.9 ± 3.2
	PG	53.6 ± 4.8	-	-	19.2 ± 7.4	T	-	-	27.9 ± 6.6
	total	53.7 ± 2.9	-	-	4.5 ± 1.1	13.6 ± 1.9	-	-	28.2 ± 2.8
desA$^-$/*desD*$^-$	MGDG	22.4 ± 8.2	29.2 ± 3.8	-	7.3 ± 3.3	38.4 ± 7.3	-	-	-
/*desC2*$^+$	DGDG	21.1 ± 0.2	32.3 ± 1.4	-	6.9 ± 3.5	36.9 ± 2.2	-	-	-
	SQDG	79.8 ± 2.8	6.2 ± 0.1	-	3.7 ± 1.7	9.7 ± 1.4	-	-	-
	PG	71.5 ± 4.2	11.1 ± 0.6	-	9.2 ± 4.7	10.1 ± 0.9	-	-	-
	total	31.1 ± 3.9	25.5 ± 1.2	-	4.7 ± 1.7	32.4 ± 1.8	-	-	-
desA$^-$/*desD*$^-$	MGDG	49.4 ± 1.1	1.6 ± 1.0	7.9 ± 1.5	2.8 ± 1.0	3.9 ± 0.1	-	-	34.2 ± 0.7
/*cfa*$^+$/*desC2*$^+$	DGDG	47.8 ± 1.4	T	6.9 ± 1.1	4.1 ± 0.9	5.1 ± 0.7	-	-	36.1 ± 0.8
	SQDG	78.5 ± 1.6	T	2.1 ± 0.3	5.1 ± 1.0	3.1 ± 1.6	-	-	14.8 ± 2.7
	PG	40.9 ± 4.5	6.7 ± 2.6	1.7 ± 0.1	6.2 ± 1.2	6.7 ± 2.4	-	-	40.7 ± 2.2
	total	51.0 ± 1.8	2.9 ± 1.1	5.7 ± 0.8	4.4 ± 0.7	3.7 ± 0.6	-	-	31.9 ± 1.1

593 The cells were grown at 34˚C for 3 d. Mean ± S.D. values of three independent experiments,

594 expressed as mol % of total fatty acids. “-”, not detected and “T”, trace amount.

595

596 **Table S3**

597 Relative amounts of fatty acids produced by each desaturase and cyclopropane FA synthetase

34˚C	Percentage of total fatty acids (mol %)						
Strain	DesC	DesA	DesD	DesB	DesC2	Cfa	saturated
Wild type	44.2 ± 1.5	25.9 ± 1.4	9.2 ± 0.8	-	-	-	55.8 ± 1.5
cfa$^+$	42.4 ± 0.8	18.5 ± 1.1	7.8 ± 0.6	-	-	18.8 ± 0.4	57.6 ± 0.8
desC2$^+$	40.8 ± 1.8	25.6 ± 2.3	6.3 ± 1.0	-	25.3 ± 0.8	-	33.8 ± 1.3
cfa$^+$/*desC2*$^+$	39.5 ± 1.3	17.5 ± 0.8	6.2 ± 0.4	-	10.0 ± 0.6	27.9 ± 1.2	50.5 ± 1.6
desA$^-$/*desD*$^-$	49.8 ± 0.5	-	-	-	-	-	50.2 ± 0.5
desA$^-$/*desD*$^-$/*cfa*$^+$	36.8 ± 0.2	-	-	-	-	22.2 ± 0.6	63.2 ± 0.2
desA$^-$/*desD*$^-$/*desC2*$^+$	33.9 ± 1.2	-	-	-	26.2 ± 1.1	-	39.9 ± 2.2
desA$^-$/*desD*$^-$/*cfa*$^+$/*desC2*$^+$	31.9 ± 2.7	-	-	-	7.1 ± 0.5	33.5 ± 2.3	61 ± 2.6

26˚C	Percentage of total fatty acids (mol %)						
Strain	DesC	DesA	DesD	DesB	DesC2	Cfa	saturated
Wild type	46.8 ± 0.2	42.0 ± 0.8	23.9 ± 0.8	7.1 ± 0.8	-	-	53.2 ± 0.2
cfa$^+$	44.0 ± 1.5	32.5 ± 3.1	18.8 ± 2.5	5.1 ± 1.0	-	7.2 ± 2.4	56.0 ± 1.5
desC2$^+$	42.5 ± 0.9	31.1 ± 0.7	13.0 ± 0.7	2.3 ± 0.3	27.5 ± 0.7	-	30.0 ± 0.5
cfa$^+$/*desC2*$^+$	41.3 ± 1.5	27.8 ± 1.3	13.4 ± 0.5	2.2 ± 0.4	12.2 ± 0.8	17.6 ± 1.9	46.5 ± 0.9
desA$^-$/*desD*$^-$	44.7 ± 0.8	-	-	-	-	-	55.3 ± 0.8
desA$^-$/*desD*$^-$/*cfa*$^+$	38.1 ± 2.6	-	-	-	-	19.3 ± 1.0	61.9 ± 2.6
desA$^-$/*desD*$^-$/*desC2*$^+$	43.1 ± 0.4	-	-	-	25.4 ± 1.4	-	31.6 ± 1.0
desA$^-$/*desD*$^-$/*cfa*$^+$/*desC2*$^+$	39.7 ± 1.1	-	-	-	6.1 ± 0.5	26.2 ± 1.3	54.2 ± 1.4

598 The cells were grown at 34˚C and 26˚C for 3 d. Mean ± S.D. values of three independent

599 experiments, expressed as mol % of total fatty acids. "-", not detected.

600

Table S4

Relative quantitation of *Synechocystis* wild-type and transformants

A

Strain	$C_{T\ target}$	$C_{T\ rnpB}$	ΔC_T	$\Delta\Delta C_T$	Relative amount
Wild type	20.26 ± 2.28	19.18 ± 1.47	1.09 ± 0.85	0	1.00
cfa+	20.27 ± 1.99	19.41 ± 1.19	0.86 ± 0.81	-0.22 ± 0.23	1.18 ± 0.15
desC2+	20.01 ± 2.42	19.27 ± 1.48	0.74 ± 1.00	-0.35 ± 0.16	1.28 ± 0.12
cfa+/*desC2+*	20.33 ± 2.50	19.56 ± 1.57	0.77 ± 1.36	-0.31 ± 0.60	1.32 ± 0.44

B

Strain	$C_{T\ target}$	$C_{T\ rnpB}$	ΔC_T	$\Delta\Delta C_T$	Relative amount
Wild type	21.37 ± 1.78	19.18 ± 1.47	2.20 ± 0.74	0	1.00
cfa+	21.32 ± 1.73	19.41 ± 1.19	1.92 ± 0.68	-0.28 ± 0.45	1.25 ± 0.34
desC2+	21.14 ± 1.59	19.27 ± 1.48	1.86 ± 0.72	-0.33 ± 0.13	1.26 ± 0.09
cfa+/*desC2+*	21.20 ± 1.79	19.56 ± 1.57	1.64 ± 0.35	-0.55 ± 0.57	1.54 ± 0.44

C

Strain	$C_{T\ target}$	$C_{T\ rnpB}$	ΔC_T	$\Delta\Delta C_T$	Relative amount
Wild type	22.27 ± 2.18	19.18 ± 1.47	3.10 ± 0.71	0	1.00
cfa+	22.24 ± 2.51	19.41 ± 1.19	2.84 ± 1.32	-0.26 ± 61	1.27 ± 0.43
desC2+	22.19 ± 2.28	19.27 ± 1.48	2.92 ± 0.81	-0.18 ± 0.17	1.14 ± 0.11
cfa+/*desC2+*	22.61 ± 2.47	19.56 ± 1.57	3.05 ± 0.98	-0.05 ± 0.31	1.05 ± 0.17

The cells were grown at 26˚C for 1 d. Mean ± S.D. values of three independent experiments. A, B, and C show treatment of data when the *desC*, *desA*, and *desD* genes were targeted, respectively. $C_{T\ target}$ and $C_{T\ rnpB}$ mean the C_T values of the target gene and the *rnpB* gene, respectively. $\Delta C_T = C_{T\ target} - C_{T\ rnpB}$. $\Delta\Delta C_T = \Delta C_T - \Delta C_{T\ wild\ type}$. Relative amounts were calculated from $2^{-\Delta\Delta CT}$ [12].

Fig. 1

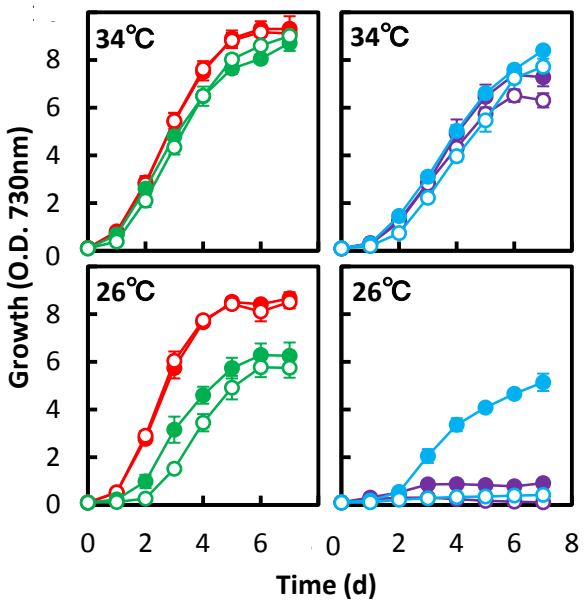


Fig. 2

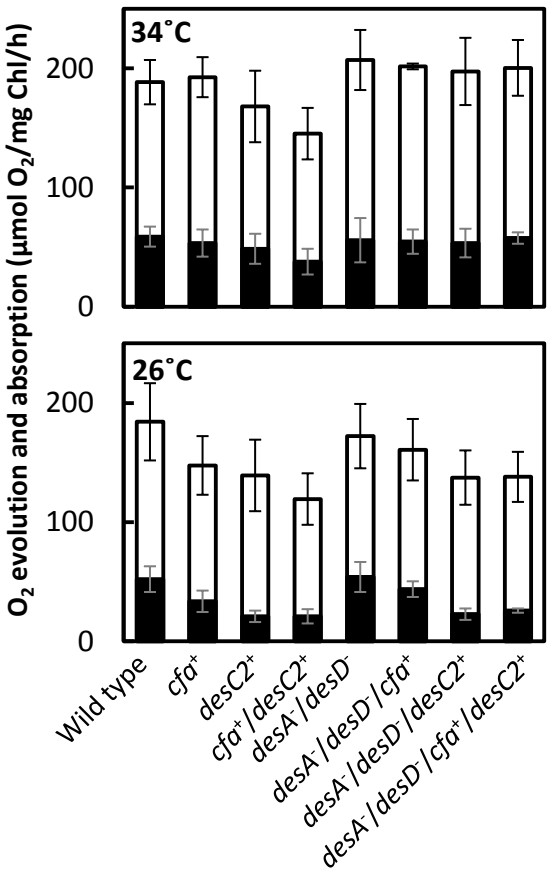

34˚C
26˚C
200
100
0
O2 evolution and absorption (µmol O2/mg Chl/h)
Wild type
cfa+
desC2+
cfa+/desC2+
desA-/desD-
desA-/desD-/cfa+
desA-/desD-/desC2+
desA-/desD-/cfa+/desC2+

Fig. 3

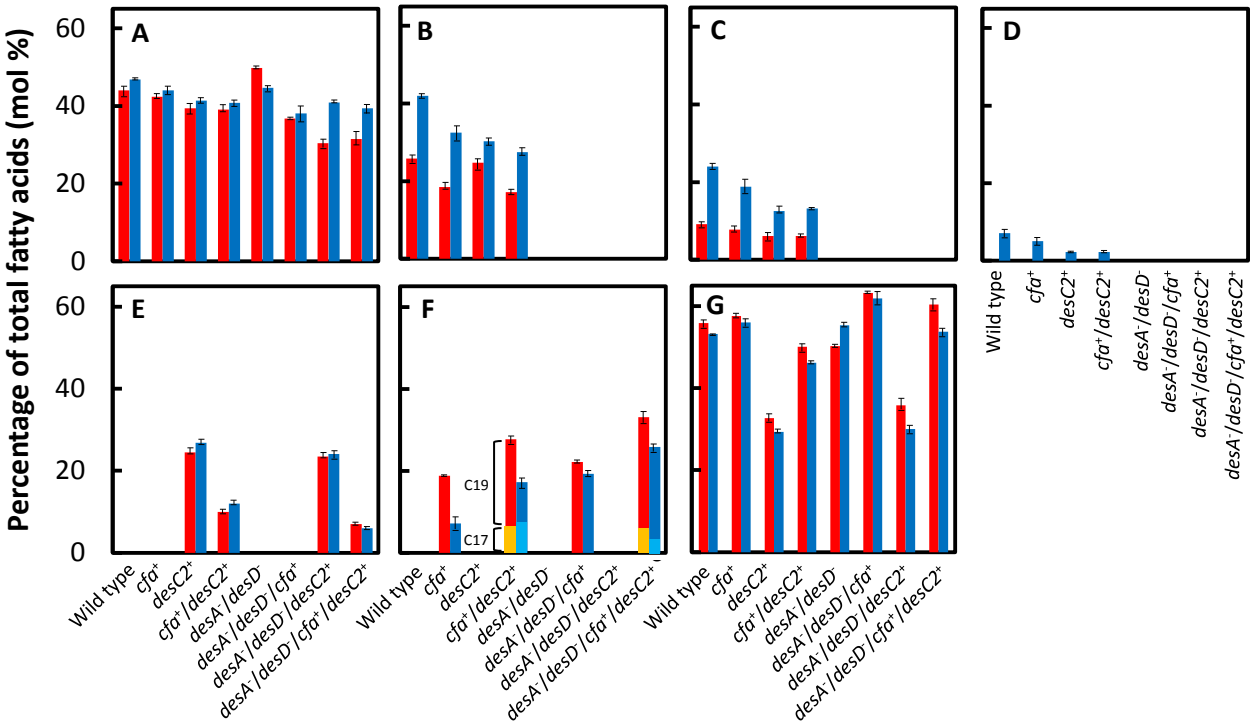
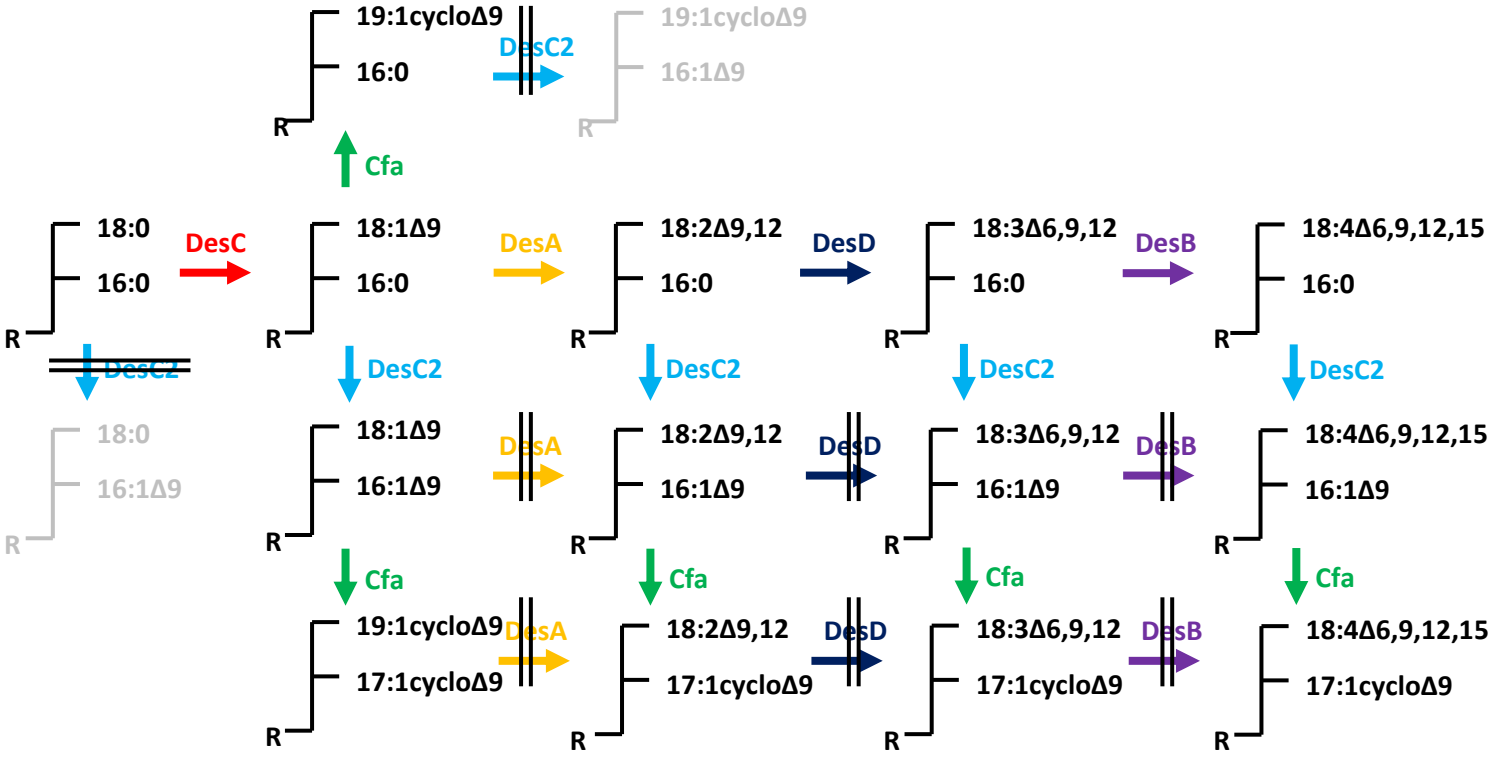

Percentage of total fatty acids (mol %)
A
B
C
D
E
F
G
C19
C17
Wild type
cfa+
desC2+
cfa+/desC2+
desA-/desD-
desA-/desD-/cfa+
desA-/desD-/desC2+
desA-/desD-/cfa+/desC2+

Fig. 4


19:1cycloΔ9
16:0
R
DesC2
19:1cycloΔ9
16:1Δ9
R
Cfa
18:0
16:0
R
DesC
18:1Δ9
16:0
R
DesA
18:2Δ9,12
16:0
R
DesD
18:3Δ6,9,12
16:0
R
DesB
18:4Δ6,9,12,15
16:0
R
DesC2
DesC2
DesC2
DesC2
DesC2
18:0
16:1Δ9
R
18:1Δ9
16:1Δ9
R
DesA
18:2Δ9,12
16:1Δ9
R
DesD
18:3Δ6,9,12
16:1Δ9
R
DesB
18:4Δ6,9,12,15
16:1Δ9
R
Cfa
Cfa
Cfa
Cfa
19:1cycloΔ9
17:1cycloΔ9
R
DesA
18:2Δ9,12
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R
DesD
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R
DesB
18:4Δ6,9,12,15
17:1cycloΔ9
R

Fig. S1

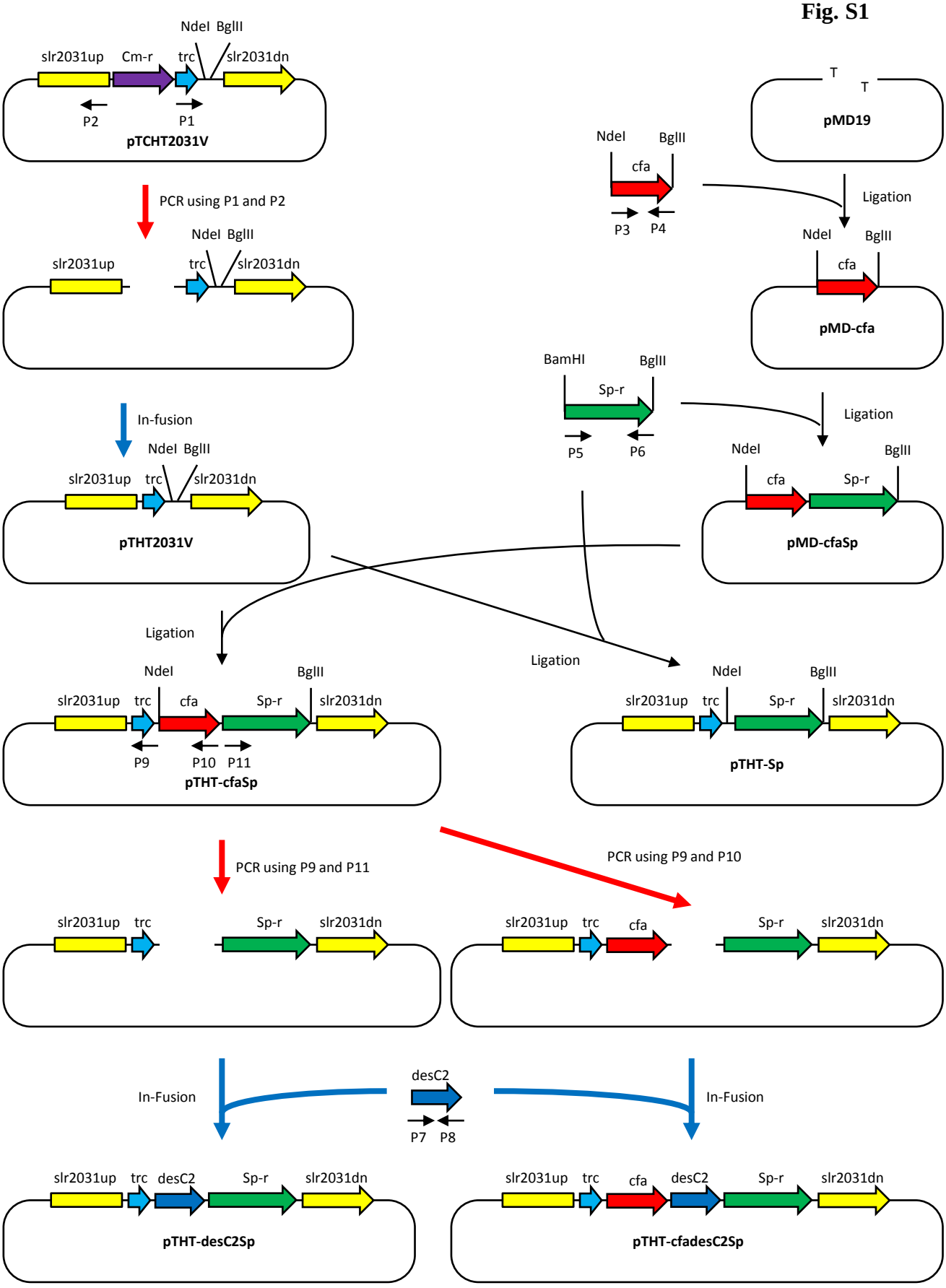
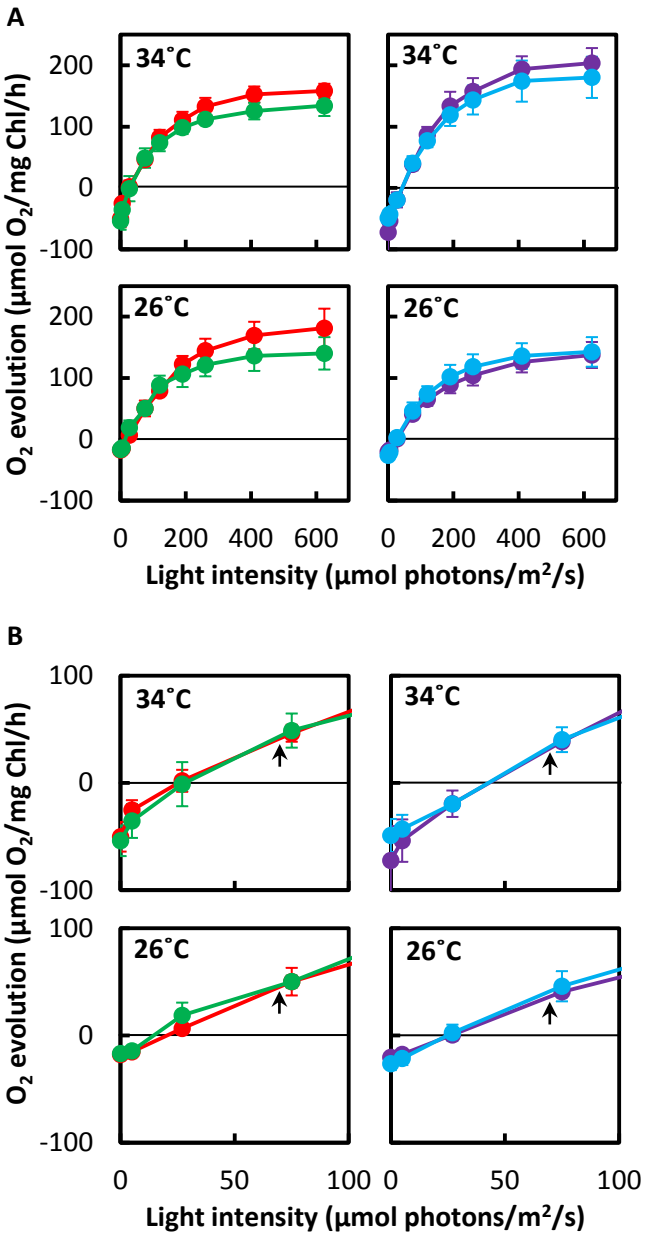

NdeI BglII
slr2031up Cm-r trc slr2031dn
P2 P1
pTCHT2031V
PCR using P1 and P2
NdeI BglII
slr2031up trc slr2031dn
In-fusion
NdeI BglII
slr2031up trc slr2031dn
pTHT2031V
T T
pMD19
NdeI BglII
cfa
P3 P4
Ligation
NdeI BglII
cfa
pMD-cfa
BamHI BglII
Sp-r
P5 P6
Ligation
NdeI BglII
cfa Sp-r
pMD-cfaSp
Ligation
Ligation
NdeI BglII
slr2031up trc cfa Sp-r slr2031dn
P9 P10 P11
pTHT-cfaSp
NdeI BglII
slr2031up trc Sp-r slr2031dn
pTHT-Sp
PCR using P9 and P11
slr2031up trc Sp-r slr2031dn
PCR using P9 and P10
slr2031up trc cfa Sp-r slr2031dn
In-Fusion
desC2
P7 P8
In-Fusion
slr2031up trc desC2 Sp-r slr2031dn
pTHT-desC2Sp
slr2031up trc cfa desC2 Sp-r slr2031dn
pTHT-cfadesC2Sp

Fig. S2


A
34˚C
34˚C
26˚C
26˚C
200
100
0
-100
0
200
400
600
O2 evolution (µmol O2/mg Chl/h)
Light intensity (µmol photons/m2/s)
B
34˚C
34˚C
26˚C
26˚C
100
0
-100
0
50
100
O2 evolution (µmol O2/mg Chl/h)
Light intensity (µmol photons/m2/s)