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Enzymatic and physiological characterization of fatty acid activation in *Synechocystis* sp. PCC6803

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Free fatty acids are typically activated by thioesterification processes and catalyzed by the fatty acyl-CoA synthetase or fatty acyl-ACP synthetase. However, the routes for fatty acid activation in cyanobacteria are not well understood. In this investigation, the *slr1609* gene, which encodes the fatty acid activation enzyme, was cloned from *Synechocystis* sp. PCC6803. This gene was identified by heterologous expression and *in vitro* enzymatic activity analyses. Different from previous reports stating that free fatty acids are only activated through the fatty acyl-ACP synthetases encoded by these genes in cyanobacteria, this gene was also proven to possess a fatty acyl-CoA synthetase activity, by *in vitro* enzymatic activity analyses and *in vivo* complementation experiments. The protein Slr1609 is located in both the cell membrane and the cytosol of *Synechocystis* sp. PCC6803. The differences in the transcriptional profiles between the wild type and the *slr1609* deletion mutant strain were evaluated using microarray analyses. These analyses indicated that 299 differentially expressed genes are involved in fatty acid metabolism, photosynthesis, carbon fixation, stress tolerance and other metabolic processes. Our experiments demonstrate the observed compositional changes in the unsaturated fatty acids from the membrane lipids of the *slr1609* deletion mutant when shifted from 30 to 24 °C.

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Introduction

Fatty acid metabolism is ubiquitous and plays a pivotal role in nearly all organisms. Fatty acids must be activated by the formation of fatty acyl-thioesters, through the catalysis of the fatty acyl-CoA synthetase (ACS, EC 6.2.1.3) or fatty acyl-ACP synthetase (AAS, EC 6.2.1.20), before participating in their physiological roles. Currently, our understanding of the roles played by ACS and AAS in fatty acid metabolism has been gained mainly through studies in *Escherichia coli* and *Arabidopsis thaliana*. An inner membrane-associated ACS, encoded by the *fadD* gene, has an important function in the coupled import and activation of fatty acids in *E. coli* [1]. An AAS also exists in *E. coli*. Free fatty acids can be activated in the form of fatty acyl-ACPs and further transformed into lipids and lipid derivatives [2]. Enzymes encoded by the nine identified ACS genes in *Arabidopsis* participate in cutin synthesis, lipid mobilization, and seedling development [3]. *Arabidopsis* possesses an AAS for the direct activation and elongation of exogenous medium-chain fatty acids to enable remodeling of the plastid membrane [4].

Based on the results of a sequence identity analysis, cyanobacterial strains such as *Synechocystis* sp. PCC6803 and *Synechococcus elongatus* PCC7942 have been found to code for only a single gene for fatty acid activation, which is annotated as ACS and is designated as *slr1609*

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and *aas*PCC 7942, respectively. A membrane protein fraction containing heterologously expressed AasPCC 7942 in *E. coli* was found to be an AAS [5]. Cyanobacterial mutants containing an *aas* deletion were observed to be incapable of utilizing exogenous fatty acids and secreted these substances from the membrane into the medium. These data suggest that a remarkable turnover of lipids occurs and that AAS activity plays a role in recycling the released fatty acids [5].

In the study described below, we found that the protein Slr1609 possessed activities towards the fatty acyl-CoA synthetase. Slr1609 is located in the cytosol of *Synechocystis* sp. PCC6803 and in its membranes. We also analyzed the physiological role of *slr1609* in *Synechocystis* sp. PCC6803 using DNA microarrays. The differences in the contents and in the compositions of fatty acids were then compared between the wild type and the *slr1609* deletion strain.

Materials and methods

Strains and general cell growth conditions

E. coli was grown in Luria-Bertani (LB) medium. When necessary, the following antibiotics were added: ampicillin (100 μg/ml), kanamycin (50 μg/ml), spectinomycin (50 μg/ml), and chloramphenicol (17 μg/ml). Liquid cultures of *Synechocystis* sp. PCC6803 were grown photoautotrophically in flasks containing BG 11 medium [6] at 30 °C under constant illumination at a photoflux density of approximately 30 μmol photons $m^{-2}s^{-1}$ and with aeration by sterile air or in a shaker. Growth was monitored by measuring the optical density (OD) at 730 nm.

Plasmids and mutant strains

The plasmid pGQ7 [7], which was based on the protein expression vector pET21b, was constructed previously for overexpressing *slr1609* in *E. coli*. GQ3 [7] is the *slr1609* overexpression mutant strain of *Synechocystis* sp. PCC6803, in which another copy of the *slr1609* gene is driven by the promoter of Rubisco. The *slr1609* deletion mutant GQ8 [7] was constructed previously by replacing the open reading frame (ORF) of *slr1609* with the chloramphenicol and erythromycin resistance gene cassettes (see Supporting Information Tables S1 and S2).

In vivo complementation assay

The plasmids pGQ7 and pET21b were transformed into *E. coli* XL100 [9], which is a deletion mutant for *fadD* in *E. coli* BL21 (DE3), and a single colony was inoculated into liquid LB medium supplemented with ampicillin (100 μg/ml) and shaken at 37 °C overnight. After being washed three times with M9 minimal medium (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 18.5 mM NH_4Cl, 8.5 mM NaCl, 2 mM $MgSO_4$, and 0.1 mM $CaCl_2$) supplemented with 11 mM glucose, the cells were suspended, diluted to an OD_{600} of 0.05 in M9 medium supplemented with 7 mM oleate and cultured at 37 °C with shaking at 200 rpm. The cultures were sampled each hour by measuring the OD_{600}. For comparison and as controls, *E. coli* BL21 (DE3) harboring pET21b and *E. coli* XL100 harboring pET21b were grown under the same conditions and at the same time.

Protein expression and purification

Protein expression was induced by adding 0.5 mM isopropylthiogalactoside (IPTG) at $OD_{600} = 0.6$. The cultures were then incubated at 16 °C with shaking at 180 rpm for another 12 h. The cells were harvested by centrifugation, and the cell pellet was resuspended in 20 ml binding buffer (20 mM phosphate-buffered saline, 0.5 M NaCl, pH 6.9) and lysed by sonication on ice. The target proteins were purified with Ni-NTA resin (Novagen, USA). The eluted fractions were immediately dialyzed against 20 mM Tris-HCl (pH 7.4), and the protein concentration was determined using Bradford's method [10].

Western blot analysis

The proteins were separated on 12% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were probed with an anti-6 × His-tag antibody (Tiangen, China), visualized by using an Alkaline Phosphatase Color Development kit (Amresco, USA) according to the manufacturer's instructions.

In vitro enzymatic assay for the putative ACS

In the spectrophotometric assay, the rate of AMP formation was determined by coupling the reaction of acyl-CoA synthetase with those of adenylate kinase, pyruvate kinase and lactate dehydrogenase, and by monitoring the absorbance change resulting from the oxidation of NADH at 340 nm with a UV spectrophotometer (Varian, USA) [11]. The standard reaction mixture for the assay contained 100 μmol Tris-HCl buffer (pH 7.4), 5 μmol dithiothreitol, 1.6 μmol Triton X-100, 7.5 μmol ATP, 10 μmol $MgC1_2$, 0.25 μmol potassium oleate, 1 μmol CoA, 0.2 μmol potassium phosphoenol pyruvate, 0.15 μmol NADH, 11 U adenylate kinase, 9 U pyruvate kinase, 9 U lactate dehydrogenase and ~1.8 μmol ACS-PCC6803 in a total volume of 1 ml. A negative control contained the mixture described above without the addition of the enzyme or the oleate substrate.

Isolation of soluble and membrane proteins

Liquid cultures of *Synechocystis* were grown photoautotrophically in BG 11 medium at 30 °C under continuous illumination at 30 μmol photons $m^{-2}s^{-1}$ until the stationary phase was reached. The cultures (1.6 l) were harvested by centrifugation. The cell pellet was resuspended in 13 ml extraction buffer (50 mM Tris-HCl, pH 8.0) and lysed by sonication. Cell debris was removed by centrifugation at 5000 × g (4 °C) for 25 min. After ultracentrifugation at 105,000 × g at 4 °C for 1 h, the supernatant was collected as soluble protein fraction, while the membrane pellet was washed three times with extraction buffer and resuspended in 13 ml extraction buffer. Equivalent amounts of the soluble and membrane proteins were loaded on an SDS-PAGE gel, and Western blot analyses were performed following the procedure described above.

RNA isolation

The wild type and the *slr1609* mutant of *Synechocystis* were grown under normal conditions with aeration by sterile air. Cells were collected by centrifugation and frozen in liquid nitrogen. The frozen pellet was ground in liquid nitrogen, and approximately 8 ml of TRIzol reagent (Invitrogen, USA) was used for the isolation of total RNA according to the manufacturer's instructions. The RNA was then treated with DNase (Takara, Japan) to eliminate DNA contamination.

DNA microarray analysis

DNA microarray analyses were performed with the help of a commercial *Synechocystis* cDNA microarray (CyanoCHIP 2.0; TaKaRa, Japan), covering 3079 of the 3264 ORFs of the *Synechocystis* genome. CyeDye-labeled cDNA from 30 μg total RNA was synthesized using the MMLV RNA fluorescent labeling kit (TaKaRa, Japan) for hybridizations, which was allowed to proceed at 65 °C overnight. The microarrays were then rinsed with 2× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate) at room temperature, washed twice with 2× SSC and 0.2% SDS at 55 °C for 5 min, rinsed with 2× SSC and 0.2% SDS at 65 °C for 5 min and 0.05× SSC for 5 min. Before analysis with the array scanner (GMS428; Affymetrix, USA), the samples were dried under a flowing stream of air. Each signal was quantified using ImaGene (ver. 4.2 Program; BioDiscovery, USA). The signal from each gene on the microarray was normalized by Global Lowess and by using a housekeeping gene. On the array, duplicate spots allowed for signal evaluation and the exclusion of errors. A change in gene expression of more than 2-fold was defined as differential expression [12].

Real-time PCR analysis

Primers were designed to amplify 150–200 bp of the internal coding region of each gene (Supporting Information Table S3). Quantitative real-time PCR was performed using SYBR Premix Dimer-Eraser (Takara, Japan) on a Light Cycler (Roche, USA), and the data were analyzed as previously described [13]. The analysis was performed in triplicate.

Total lipid extraction and analysis

Total lipids were extracted using the method of Bligh and Dyer [14] with some modifications. The culture (10 ml) was completely mixed with 2 ml acetic acid. Heptadecanoic acid (20 μg) was added to the mixture for quantification before 20 ml $CHCl_3/CH_3OH$ 1:1 (v/v) was added. The bottom organic phase was separated by centrifugation, transferred into an esterification tube and dried under a stream of nitrogen at 55 °C [15]. The dried residues were resuspended in 2 ml 0.4 M KOH in CH_3OH and incubated at 60 °C for 1 h. The esterification process was then ended by the addition of 4 ml HCl/CH_3OH 1:9 (v/v). Finally, the samples were extracted with 2 ml hexane. Aliquots of the upper phase were analyzed using an Agilent 7890A-5975C system (GC-MS) equipped with a HP-INNOWax column (30 m × 250 μm × 0.25 μm). The temperature of the injector was 250 °C, and the following temperature program was applied: 100 °C for 1 min, an increase of 5 °C min^{-1} to 200 °C and then an increase of 25 °C min^{-1} to 240 °C for 15 min.

Results

Heterologous expression, purification and enzymatic characterization of the proteins encoded by the *slr1609* gene from the cytoplasmic fraction

To identify the enzymatic activities of the protein encoded by *slr1609*, the ORF from the genomic DNA of *Synechocystis* sp. PCC6803 was cloned into pET21b with a C-terminal His-tag and heterologously expressed in *E. coli* BL21 (DE3). Proteins from the soluble cytoplasmic fraction were collected and purified to apparent homogeneity in His-tagged form by using Ni-NTA affinity chromatography. The protein fractions from each preparation were analyzed by SDS-PAGE (Supporting Information Fig. S1). The molecular weight of the Synechocystis protein was estimated to be 66 kDa. The target band was verified by Western blot analysis (Supporting Information Fig. S1). Finally, 5 mg of the purified Synechocystis protein was obtained from 1 l of E. coli cell culture.

The enzymatic activity of the purified protein was determined by using a coupled enzymatic reaction assay (see Materials and methods). Slr1609 was found to function as an ACS and to utilize oleic acid and coenzyme A as substrates to generate oleoyl-CoA. The K_m and k_{cat} values of Slr1609 are 1.10 ± 0.06 mM and 3.0 ± 0.3 min^{-1}, respectively.

Partial recovery of the fatty acid-activating function of the *fadD*-deleted *E. coli* mutant strain by *slr1609*

To confirm that the protein encoded by *slr1609* physiologically functions as an ACS, an *E. coli fadD* deletion mutant (XL100) [9] that could not activate free fatty acids was used in *in vivo* complementation experiments. Whereas the *E. coli* XL100 strain containing pET21b did not grow under these conditions owing to the lack of a fatty acid-activating function, an *E. coli* BL21 (DE3) strain harboring the empty plasmid pET21b as a positive control was found to grow normally (Fig. 1). In contrast, the *E. coli* XL100 strain harboring the pGQ7 plasmid grew well under these conditions. This observation indicates that the fatty acid-activating function, which enables conversion of fatty acids into CoA thioesters in a process that is catalyzed by the ACS (encoded by *fadD* in *E. coli*), can be promoted by the presence of the protein encoded by *slr1609 in vivo* (Fig. 1). Thus, the protein encoded by *slr1609* also has the capability of catalyzing the fatty acyl-CoA synthetase reaction.

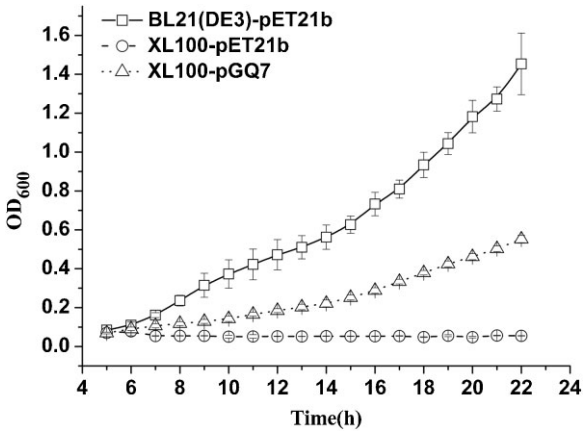

Figure 1. Cell growth curves for the four *E. coli* strains used in the *in vivo* complementation experiments in M9 medium with oleate as the sole carbon source: BL21 (DE3)/pET21b (open squares), XL100/pET21b (open circles), XL00/pGQ7 (open triangles). The positive control strain (BL21 (DE3)/pET21b) grew well in the medium with oleate as the sole carbon source, while XL100/pET21b, which is a fatty acyl-CoA synthetase mutant strain, could not grow under these conditons. By contrast, XL100/pGQ7, which has an introduced copy of the *slr1609* gene, gained the ability to grow in M9 medium with oleate.

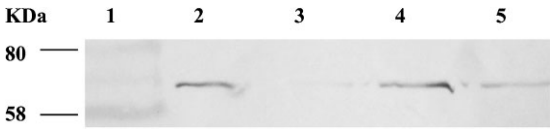

Figure 2. Western blot analysis of homogeneously overexpressed proteins encoded by the *slr1609* gene with a C-terminal 6× histidine tag in the *Synechocystis* sp. PCC6803 mutant strain GQ3. Lane 1: protein molecular weight marker; lane 2: 40 μg of whole proteins of BL21 DE3)/pGQ7 as positive control; lane 3: the blank control (no loading); lane 4: 96 μg of GQ3 soluble proteins; lane 5: 96 μg of GQ3 membrane protein fraction.

Subcellular localization of the Slr1609 protein in *Synechocystis* sp. PCC6803

Synechocystis mutant strains homogenously overexpressing *slr1609* were constructed by performing transformations with the pGQ9 and pGQ11 plasmids. The mutant cells were collected, lysed and separated into soluble and membrane fractions by ultracentrifugation (see Materials and methods). Western blot analyses were performed to detect expression of the proteins and to identify the subcellular localization of Slr1609. The results indicate that the protein is present in both the cytosolic and membrane fractions (Fig. 2).

Microarray analysis of the genome-wide response to *slr1609* deletion

The products of the reaction catalyzed by fatty acyl-CoA synthetase (fatty acyl-CoA or fatty acyl-ACP) are important intermediates in lipid and fatty acid metabolism and thus affect several cellular systems and functions. To investigate the potential role of *slr1609* in cell metabolism and signaling, DNA microarray analyses were performed by comparing the *slr1609* deletion mutant with wild-type *Synechocystis* sp. PCC6803. This analysis indicated that 299 of the 3725 genes involved in photosynthesis and respiration, translation, regulatory functions, fatty acid metabolism, etc. were differentially expressed (Table 1). The validation of these results was performed by microarray analysis. The transcriptional changes of 10 selected genes were evaluated by performing a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis. As shown in Table 2, the data obtained from the qRT-PCR are consistent with those obtained from the microarray data.

Effects of *slr1609* deletion on the transcription of genes involved in fatty acid metabolism

Based on the data obtained from both the gene chip (Supporting Information Table S4) and qRT-PCR analyses (Table 2), six genes related to fatty acid metabolism were found to be down-regulated 2–3-fold in the mutant. The

Table 1. Differentially regulated genes in response to *slr1609* gene deletion, according to functional categories and as defined in CyanoBase.

General pathway	No. of genes	Differentially expressed genes[a)]
Amino acid biosynthesis	97	2
Biosynthesis of cofactors, prosthetic groups, and carriers	125	11
Cell envelope	67	9
Cellular processes	80	7
Central intermediary metabolism	31	3
Energy metabolism	93	5
Fatty acid, phospholipid and sterol metabolism	39	6
DNA replication, restriction, recombination, and repair	75	1
Hypothetical	1277	93
Other categories	369	21
Photosynthesis and respiration	143	33
Purines, pyrimidines, nucleosides, and nucleotides	43	0
Regulatory functions	156	9
Transcription	30	3
Translation	168	35
Transport and binding proteins	200	5
Unknown	679	56
RNA	53	0
Total	3725	299

[a)]Genes were considered differentially regulated when the change was more than 2-fold.

largest change, approximately 3.7-fold down-regulation, occurred with the *fabG* (*sll0330*) gene, which codes for the 3-ketoacyl-acyl carrier protein reductase, catalyzes the fourth step in the fatty acid synthesis pathway in prokaryotes and is strongly induced by osmotic stress [16]. The transcriptional levels of some other proteins involved in this pathway were also decreased 2–3-fold. These proteins included the acyl carrier protein (encoded by *ssl2084*), beta-ketoacyl-acyl carrier protein synthase (encoded by *slr1332*), beta-ketoacyl-acyl carrier protein synthase II (encoded by *sll1069*) and enoyl-[acyl carrier protein] reductase (encoded by *slr1051*). The gene *sll0262* codes for a fatty acid desaturase (delta-6 desaturase), which introduces double bonds directly into the fatty acid chains of membrane lipids at the Δ6 site [17]. This protein was down-regulated by up to 2-fold, indicating that the deletion of *slr1609* leads to the repression of fatty acid biosynthesis and the desaturation of membrane lipids.

Effects of *slr1609* deletion on the transcription of genes involved in other functions

Some genes coding for the subunits of photosynthetic protein complexes, including photosystem I (*psaF*, *psaA*, *psaB*, *psaJ*, and *psaC*), photosystem II (*psbA3*, *psaA2*, and *psbI*), the cytochrome b_6/f complex (*petC1* and *petA*), ATP synthase (*atpI*, *atpG*, *atpF*, *atpB*, and *atpH*), and phycobilisome (*apcE*, *apcF*, and *apcB*) were down-regulated by 2–5-fold in the mutant compared with the wild-type strain of *Synechocystis* sp. PCC6803. The *chlL* (*slr0749*), *chlN* (*slr0750*), and *chlG* (*slr0056*) genes, which are responsible for the synthesis of chlorophyll, were down-regulated by the deletion of *slr1609*. The protein Slr1609 was previously identified in purified photosystem I monomers, indicating its important role in photosystem I [18]. The transcriptional level of *ctpA*, encoding a carboxyl-terminal processing

Table 2. Transcriptional changes of selected genes in the *slr1609* deletion mutant GQ8 of *Synechocystis* sp. PCC6803.

ORF[a)]	Product	qRT-PCR (fold change)[b)]	Microarray (fold change)[b)]	Remarks
sll0330	3-Ketoacyl-acyl carrier protein reductasesepiapterin reductase	0.45 ± 0.16	0.27 ± 0.07	Fatty acid biosynthesispathway
sll0262	Delta-6 desaturase	0.43 ± 0.17	0.42 ± 0.08	Desaturation of membrane lipids
ssl2084	Acyl carrier protein	0.29 ± 0.04	0.42 ± 0.12	Fatty acid biosynthesispathway
sll1069	Beta-ketoacyl-acyl carrier protein synthase	0.34 ± 0.04	0.46 ± 0.07	Fatty acid biosynthesispathway
slr1051	Enoyl-[acyl carrier protein] reductase	0.37 ± 0.18	0.51 ± 0.11	Fatty acid biosynthesispathway
slr1332	Beta-ketoacyl-acyl carrier protein synthase	0.54 ± 0.02	0.55 ± 0.1	Fatty acid biosynthesispathway
sml0008	Photosystem I subunit IX PsaJ	0.41 ± 0.17	0.20 ± 0.05	Photosynthesis and respiration
sml0001	Photosystem II reaction center PsbI protein	0.40 ± 0.21	0.46 ± 0.18	Photosynthesis and respiration
ssl2615	ATP synthase subunit c	0.22 ± 0.11	0.34 ± 0.13	Photosynthesis and respiration
sll1732	NADH dehydrogenase subunit 5	2.43 ± 0.42	2.16 ± 0.9	Photosynthesis and respiration
sll1030	Carbon dioxide concentrating mechanism protein Ccml	0.17 ± 0.05	0.28 ± 0.09	Photosynthesis and respiration, carbon fixation

[a)]Gene identification numbers are based on CyanoBase.
[b)]The data represent the mean ± SD of measurements from three independent assays.

protease for the maturation of the D1 protein of the photosystem II reaction center complex [19], was also up-regulated 2-fold. Our findings indicate that Slr1609 functions in photosynthesis not only through a protein-protein interaction with photosystem I but also in transcriptional regulation and that it represses the transcription of photosynthetically relevant genes.

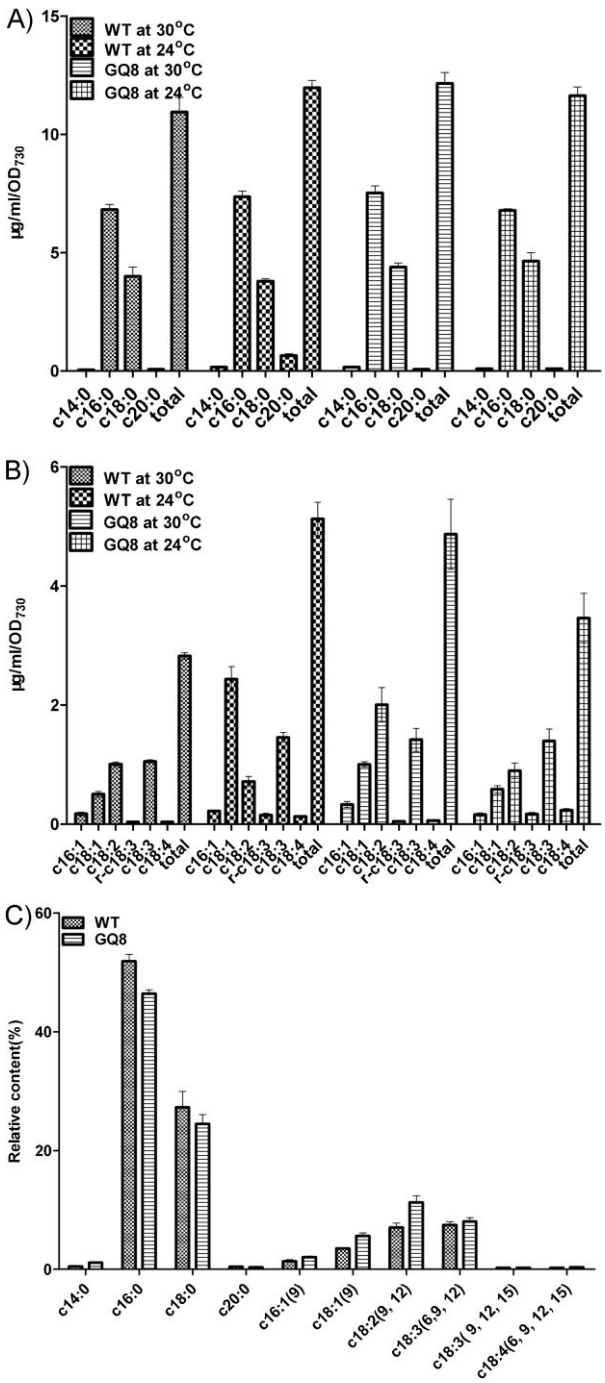

Figure 3. Fatty acid composition. (A) Saturated fatty acid contents of the total lipids of the wild type (WT) and the *slr1609* deletion mutant (GQ8) at 30 and 24 °C, (B) unsaturated fatty acid contents of the total lipids of the WT and GQ8 at 30 and 24 °C, (C) total lipid compositions from the WT and GQ8 at 30 °C.

Effects of *slr1609* deletion on the temperature response of the cell membrane

The deletion of *slr1609* apparently changes the content and composition of fatty acids in the mutant. Whenever cultured at 30 or 24 °C, the content of saturated fatty acids in the *slr1609* mutant is almost the same as that of the wild type (Fig. 3A). The content of unsaturated fatty acids in the *slr1609* mutant is 72.2% higher than the content found in the wild type when cultured at 30 °C but is 32.5% lower when cultured at 24 °C (Fig. 3B). There was also an 81.2% increase in the content of unsaturated fatty acids in the wild-type strain, as previously reported [20]. However, there was a 28.9% decrease in the mutant when the cultures were shifted from 30 to 24 °C. The relative composition of saturated fatty acids (%) dropped from 80.1% in the wild type to 72.3% in the *slr1609* mutant, while the content of unsaturated fatty acids increased from 19.8 to 27.6% (Fig. 3C). These results indicate that the deletion of *slr1609* not only increased the content of total fatty acids but also altered the responding patterns of total fatty acids in the cells under cold stress conditions.

Discussion

Kaczmarzyk *et al*. [5] studied the enzymatic function of the protein encoded by s*lr1609* and reported earlier that a protein arising from the membrane fraction of *E. coli* heterologously overexpressing the protein can catalyze the conversion of free fatty acids into fatty acyl-ACPs and that this protein only functions as an AAS. In this work, the *slr1609* gene was transformed into an *fadD* deletion mutant strain (XL100) and the complementation assay showed that the gene complemented the function of *fadD*. To find the difference between the present and previous work, the protein was purified from the cell plasma and the enzyme activity of Slr1609 was assayed, demonstrating its ACS activity. Consequently, it was important to determine if the native protein encoded by *slr1609* in *Synechocystis* is also located in the cytosol of *Synechocystis* cells. Proteomics studies of *Synechocystis* revealed that Slr1609 is present in the cellular membrane and, more specifically, in the plasma [18] and in thylakoid membranes [21, 22]. However, our results from the Western blot analysis revealed that the protein Slr1609 is present in both the cytosolic and membrane fractions of *Synechocystis* sp. PCC6803. Taken together,

these results suggest that the protein encoded by *slr1609* in *Synechocystis* might be a bifunctional fatty acyl-thioester synthetase with activities that are dependent upon its subcellular localization.

Although five genes involved in the fatty acid biosynthesis pathway are down-regulated in the *slr1609* deletion mutant, its content of total lipids does not decrease as expected. However, this result is consistent with a previous report describing this same mutant [5]. The *slr1609* protein has been previously reported as involved in the activation of free fatty acids released from membrane lipids, and the deletion of *slr1609* also leads to an increase of either intercellular or total free fatty acids [5].

As discussed before, *desD*, a gene regulating the extent of unsaturation of membrane lipids in response to temperature [17], was found to be down-regulated 2.5-fold in the *slr1609* deletion mutant, by microarray analysis (Supporting Information Table S4). Normally, fatty acyl chains are first desaturated by constitutively expressing DesC (Δ9) to form monounsaturated fatty acids and then sequentially desaturated by cold-induced DesA (Δ12), DesD (Δ6), and DesB (Δ15) in *Synechocystis* sp. PCC6803 [23]. It has been reported that the complete disruption of *desD* leads to the accumulation of mono- (C16:1 (9) and C18:1 (9)) and biunsaturated fatty acids (C18:2 (9, 12)) [24]. The composition of mono- and biunsaturated fatty acids increased from 11.9 to 18.9% in the mutant while the composition of the polyunsaturated fatty acids (including C18:3 (6, 9, 12), C18:3 (9, 12, 15)) slightly increased from 7.7 to 8.3%. Therefore, the deletion of *slr1609* leads to the down-regulation of *desD* and thus alters the composition of unsaturated fatty acids.

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Conflict of interest

We declare that no financial or commercial conflict of interest exists.

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