A novel Δ^9 acyl-lipid desaturase, DesC2, from cyanobacteria acts on fatty acids esterified to the *sn*−2 position of glycerolipids

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Acyl-lipid desaturases are enzymes that convert a C–C single bond into a C=C double bond in fatty acids that are esterified to membrane-bound glycerolipids. Four types of acyl-lipid desaturase, namely DesA, DesB, DesC, and DesD, acting at the Δ^{12}, Δ^{15}, Δ^9, and Δ^6 positions of fatty acids respectively, have been characterized in cyanobacteria. These enzymes are specific for fatty acids bound to the *sn*−1 position of glycerolipids. In the present study, we have cloned two putative genes for a Δ^9 desaturase, designated *desC1* and *desC2*, from *Nostoc* species. The *desC1* gene is highly similar to the *desC* gene that encodes a Δ^9 desaturase that acts on C_{18} fatty acids at the *sn*−1 position. Homologues of *desC2* are found in genomes of cyanobacterial species in which Δ^9-desaturated fatty acids are esterified to the *sn*−2 position. Heterologous expression of the *desC2* gene in *Synechocystis* sp. PCC 6803, in which a saturated fatty acid is found at the *sn*−2 position, revealed that DesC2 could desaturate this fatty acid at the *sn*−2 position. These results suggest that the *desC2* gene is a novel gene for a Δ^9 acyl-lipid desaturase that acts on fatty acids esterified to the *sn*-2 position of glycerolipids.

Key words: cyanobacteria, desaturase, DesC2, fatty acids, glycerolipids, *Nostoc* sp.

INTRODUCTION

Cyanobacteria have been classified into four groups according to the positional distribution of fatty acids that are esterified to specific *sn*− positions in glycerolipids (for reviews, see [1–4]). Strains in Group 1 (*Prochlorothrix hollandica*, *Synechococcus* sp. PCC 6301, *Synechococcus* sp. PCC 7942, *Synechococcus elongatus*, *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus*) are characterized by the presence of mono-unsaturated fatty acids at both *sn*−1 and *sn*−2 positions. Strains in Group 2 (*Anabaena variabilis*, *Anabaena* sp. PCC 7120, *Synechococcus* sp. PCC 7002, *Nostoc punctiforme*, *Nostoc* sp. 36, *Trichodesmium erythraeum* and *Gloeobacter violaceus*) contain a tri-unsaturated C_{18} fatty acid (α-linolenic acid) at the *sn*−1 position and a mono-unsaturated C_{16} fatty acid, $C_{16:1(9)}$, at the *sn*−2 position. Strains in Group 3 (*Spirulina platensis* and *Prochlorococcus marinus*) have a tri-unsaturated C_{18} fatty acid (γ-linolenic acid) at the *sn*−1 position and a $C_{16:0}$ (saturated C_{16} acid), at the *sn*−2 position. Strains in Group 4 (*Synechocystis* sp. PCC 6803) are similar to strains in Group 3 except that they have, in addition, tri-unsaturated C_{18} (α-linolenic acid) and tetra-unsaturated C_{18} fatty acids ($C_{18:4}$) at the *sn*-1 position.

Fatty acid desaturases are enzymes that convert a C–C single bond into a C=C double bond in a fatty-acyl chain [5]. In particular, Des (acyl-lipid desaturases) proteins act on fatty acids that are esterified to the glycerol backbone of glycerolipids. Several acyl-lipid desaturases and their genes have been characterized in cyanobacteria [6] and plants [7–10]. In general, desaturases have strict specificity with respect to the position in the fatty acid at which a double bond is introduced, and to the *sn*-position of the glycerol moiety of glycerolipids to which fatty acids are esterified. Wada et al. [11] cloned the *desA* gene encoding DesA, which introduces a double bond at the Δ^{12} position of fatty acids at the *sn*-1 position, from *Synechocystis* sp. PCC 6803 (hereafter termed *Synechocystis* sp.). Subsequently, genes for various acyl-lipid desaturases were cloned from a variety of cyanobacteria, such as *Synechocystis* sp. [12–14], *Synechocystis* PCC 6714 [15], *Synechococcus* PCC 7002 [15,16], *Synechococcus vulcanus* [17], *Spirulina platensis* [18], *Anabaena variabilis* [14,15] and *Anacystis nidulans* (now reclassified as *Synechococcus* sp. PCC 6301; [19]).

Des proteins and their genes have been extensively studied in *Synechocystis* sp., which belongs to Group 4 cyanobacteria [3,20]. This cyanobacterium encodes four Des proteins, DesA, DesB, DesC and DesD, that introduce a double bond at the Δ^{12}, Δ^{15} (ω^3), Δ^9 and Δ^6 positions of the C_{18} fatty acid at the *sn*−1 position respectively [3,12–14,20–22]. In *Spirulina platensis*, a strain in Group 3, three genes for acyl-lipid desaturases named *desA*, *desC* and *desD* have been cloned [18]. In addition, three genes for acyl-lipid desaturases named *desA*, *desB* and *desC* have also been cloned from the cyanobacterial strains *Anabaena variabilis* [14,15] and *Synechococcus* sp. PCC7002, which are characterized in Group 2 [16,23]. The strict specificity of DesC from *Synechocystis* sp. with respect to the *sn*−1 position has been definitively established [14]. However, the specificity of DesC with respect to the *sn*−1 position is unclear in the case of acyl-lipid desaturases of cyanobacteria that belong to Groups 1 and 2. Cyanobacterial strains in Groups 1 and 2 introduce a double bond into fatty acids at both the *sn*−1 and the *sn*−2 position [4]. Therefore it is conceivable that DesC from these strains might be unspecific with respect to a particular *sn*− position or,

Abbreviations used: Des, acyl-lipid desaturase; DGDG, digalactosyl diacylglycerol; LB, Luria–Bertani; MGDG, monogalactosyl diacylglycerol; ORF, open reading frame; PG, phosphatidylglycerol; SQDG, sulphoquinovosyl diacylglycerol.

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alternatively, that two Δ^9 desaturases might exist, each of which catalyses Δ^9 desaturation at a specific position, either *sn*−1 or *sn*−2.

Earlier studies have clearly demonstrated that when mesophilic cyanobacteria that grow optimally at 35 °C are subjected to low-temperature stress, by a downward shift in growth temperature to 25 °C, they can overcome this stress by increasing the synthesis of polyunsaturated fatty acids. The synthesis of the polyunsaturated fatty acids in cyanobacteria is catalysed by fatty acyl-lipid desaturases, encoded for by the *des* genes (*desA*, *desB*, *desC* and *desD*) [3,22]. In *Synechocystis* sp. PCC 6803, cold stress induced the expression of *desA*, *desB* and *desD* [3,22]. Further, the importance of the *des* genes with respect to cold adaptation has been unequivocally demonstrated by the observations that cyanobacterial mutants defective in these genes are cold-sensitive and grow slower than the wild-type cells [3,22]. These studies clearly indicated that polyunsaturated fatty acids and fatty acyl-lipid desaturases are essential for the acclimation of cyanobacteria to low temperatures [3,22]. However, in contrast with mesophilic cyanobacteria, psychrotolerant cyanobacteria grow optimally at 25 °C and are also capable of growing at 10 °C, a temperature at which the mesophilic cyanobacteria barely grow. Therefore it would be of interest to identify and characterize the *des* genes to ascertain the expression patterns of these genes, which will establish whether they are crucial for low-temperature survival in psychrotolerant cyanobacteria that are already adapted to low-temperature survival and growth. As part of this long-term project, the present study was undertaken on *Nostoc* sp. strain SO-36, a psychrotolerant strain isolated from a lake in Antarctica.

In the present study, we cloned two *desC* homologous genes from *Nostoc* sp. strain SO-36 (hereafter termed *Nostoc* sp.), which belongs to Group 2, and demonstrated that one of these genes encodes a Δ^9 desaturase that introduces double bonds in fatty acids that are bound to the *sn*−2 position of the glycerol moiety of membrane glycerolipids. We designated this gene *desC2* and the other gene *desC1*.

EXPERIMENTAL

Bacterial strains and growth conditions

Nostoc sp. strain SO-36 was isolated from a water sample from an Antarctic lake and identified as *Nostoc* sp. on the basis of its filamentous morphology, binary fission and characteristic trichomes, which are neither branched nor tapered and are made up of cells of equal size. The strain grows at temperatures between 10 and 30 °C. The partial sequence of the gene for 16 S rRNA from this micro-organism was highly similar to that of *Nostoc punctiforme* and *Nostoc commune*, indicating that all three micro-organisms are closely related to one another (results not shown). *Synechocystis* sp. PCC 6803 was obtained originally from Dr J. G. K. Williams (DuPont de Nemours and Co., Wilmington, DE, U.S.A.). *Nostoc* sp. and *Synechocystis* sp. were grown at 25 °C in BG-11 medium [24] supplemented with 10 mM Hepes buffer, pH 8.0, in light from a tungsten lamp at 350 and 3500 lux respectively, with a constant supply of 1 % CO_2 in air.

Escherichia coli DH10B cells, which served as the host for cloning, were grown at 37 °C in LB (Luria–Bertani) medium that contained 1 % (w/v) tryptone, 0.5 % (w/v) yeast extract and 1 % (w/v) sodium chloride. The final pH of the medium was 7.2.

Analysis of fatty acid composition and the positional distribution of fatty acids in MGDG (monogalactosyl diacylglycerol)

Total cell lipids were extracted as described by Bligh and Dyer [25] and fatty acids were analysed essentially as described by Sato and Murata [26]. Lipase from *Rhizopus delemar* (Seikagaku Kogyo, Tokyo, Japan), which specifically dissociates fatty acids at the *sn*−1 position of polar glycerolipids, was used to identify fatty acids esterified to the *sn*−1 and *sn*−2 positions of the glycerol moieties of glycerolipids [27]. In this study, the major lipid fraction MGDG was purified by TLC and then treated with lipase from *R. delemar* to liberate the fatty acids esterified to the *sn*−1 position of polar glycerolipids and to simultaneously generate the 2-monoacyl product. The fatty acid at the *sn*−2 position was determined by analysing the fatty acids in the lysolipid. In addition, the fatty acid at the *sn*−1 position was determined by comparing the fatty acid composition of undigested MGDG with the fatty acid composition of the 2-monoacyl product.

Cloning of the *desC* genes

The genome of *Anabaena* sp. PCC 7120 includes two putative *desC* homologous genes. The nucleotide sequence of one is more similar than the other to that of the *desC* gene from *Synechocystis* sp. The former gene is designated *desC1* and the latter is *desC2*. Referring to the sequences of these genes, we designed primers for PCR: desC1F (5'-ACTCAAAGGGACTGTTTCTGGTGG-3') and desC1R (5'-TGAGTGAGTTAGTTAGTGCCA-3') for amplification of *desC1*; and desC2F (5'-CGCCAGCATCATGCTCACACCGAAG-3') and desC2R (5'-ATGGTTGTCTTGCTAAATCAGGCGC-3') for amplification of *desC2*. We extracted and purified the genomic DNA from *Nostoc* sp. as described by Williams [28] and used this for PCR amplification using the above primers, which produced 350 bp and 160 bp fragments with sequences that were identical with the partial sequences of the *desC1* and *desC2* genes from *Anabaena* sp. PCC 7120 respectively. These PCR products were used in the following screening step.

Two partial genomic libraries of *Nostoc* sp. were constructed. One was constructed by digestion of the genomic DNA of *Nostoc* sp. with EcoRI and HindIII for cloning of the *desC1* gene. Electrophoresis on a 0.8 % agarose gel yielded fragments of approx. 6 kb that were recovered from the agarose gel using a silica-gel-membrane column (Qiagen GmbH, Hilden, Germany). The other library was constructed by digestion with DraI for cloning of the *desC2* gene, and electrophoresis on a 0.8 % agarose gel yielded fragments of approx. 1.3 kb. The fragments were ligated to pBluescript II KS(+) (Stratagene, La Jolla, CA, U.S.A.) that had been digested with EcoRI and HindIII or with DraI (for cloning of the *desC1* or the *desC2* genes respectively) and dephosphorylated with shrimp alkaline phosphatase (Boehringer-Mannheim GmbH, Mannheim, Germany). The ligation mixture was then used to transform *E. coli* DH10B cells by electroporation. Transformed cells were selected on agar-solidified LB medium with 150 μg/ml ampicillin. Screening of the two libraries was performed using the products of PCR as probes, using the method described by Sambrook et al. [29], which under high-stringency conditions yielded a single positive clone (pC36C) with an ORF (open reading frame) of 822 bp that corresponded to *desC1*, and a single clone (pC36C2) with an ORF of 855 bp that corresponded to *desC2*. Sequencing confirmed that the ORFs corresponded to the full-length *desC1* and *desC2* genes.

Conjugal transfer of the *desC2* gene into *Synechocystis* sp.

Plasmid pC36C2 was digested with ClaI and SmaI to release the *desC2* gene from the vector. The *desC2* gene was then cloned into the shuttle vector pVZ321 [30], which carries kanamycin- and chloramphenicol-resistance genes (the nucleotide sequence of the plasmid is available in GenBank® under accession number AF100175), to generate pVZC2. *E. coli* DH5α cells were then

Table 1 Fatty acid composition of total lipids, in MGDG and at *sn*−1 and *sn*−2 positions of MGDG of *Nostoc* sp. (strain 36)

Results are means ± S.D. of three experiments and are expressed as mol % of total fatty acids. T implies a trace amount that was less than 0.5 mol %. ND, not detected.

		Fatty acid (mol % of total)		
Lipid	Total lipids	MGDG	*sn*−1 of MGDG	*sn*−2 of MGDGTT
$C_{16:0}$	12.0 ± 0.3	6.0 ± 0.6	T	5.0 ± 0.3
$C_{16:1(9)}$	30.0 ± 0.4	41.0 ± 0.7	T	41.0 ± 0.5
$C_{18:0}$	4.0 ± 0.1	1.2 ± 0.2	T	T
$C_{18:1(9)}$	17.0 ± 0.3	18.0 ± 0.4	15.0 ± 0.3	3.0 ± 0.2
$C_{18:1(11)}$	1.0 ± 0.3	T	T	T
$C_{18:2(9,12)}$	29.0 ± 0.5	27.0 ± 0.5	27.0 ± 0.5	T
$C_{18:3(9,12,15)}$	7.0 ± 0.3	7.0 ± 0.3	7.0 ± 0.2	ND

transformed with pVZC2 by heat shock at 42 °C for 90 s as described previously [22]. *E. coli* DH5α cells were also transformed with a control vector pVZ321. The resultant transformants of *E. coli* DH5α were isolated on plates of agar-solidified LB medium with 20 μg/ml chloramphenicol. The presence of the *desC2* gene was confirmed by PCR, using the plasmid isolated from transformants as the template. The plasmid was then transferred from *E. coli* DH5α to *Synechocystis* sp. by tri-parental mating, using the method described previously [30]. *E. coli* DH5α cells transformed with pVZ321 or pVZC2 were used as donor strains to generate control and transformed strains of *Synechocystis* sp. respectively, with *E. coli* R591 cells as the helper strain. Donor and helper cells were grown to stationary phase, while the recipient cells (*Synechocystis* sp.) were grown to a turbidity (D_{730}) of 0.5. Suspensions of donor, helper and recipient cells were mixed in the following proportions, 1:1:10 (by vol.; total volume 1.2 ml). The cells were collected by centrifugation at 1000 ***g*** for 1 min in a microcentrifuge tube, then rinsed with BG-11 medium. After re-suspension in 40 μl of BG-11 medium, the cells were transferred on to a sterile Durapore™ membrane filter (13 mm diameter, 22 μm pore size; Millipore, Billerica, MA, U.S.A.). Membranes were placed on plates of agar-solidified BG-11 and 5 % (v/v) LB medium and incubated overnight at 34 °C in light at 900 lux. Each membrane was then placed in a micro-centrifuge tube, to which 200 μl of BG-11 medium was added. Mixing on a vortex mixer separated the *Synechocystis* and *E. coli* cells from the membrane. The washed membrane was removed and the released cells were collected by centrifugation at 1000 *g* for 5 min. The supernatant was discarded, the cells were washed with fresh BG-11 medium and then pelleted by centrifugation as described above. The cells were then spread on agar-solidified BG-11 medium that contained 50 μg/ml trimethoprim, 20 μg/ml chloramphenicol and 25 μg/ml kanamycin for isolation of pVZ321/PCC 6803 transformants and 50 μg/ml trimethoprim and 20 μg/ml chloramphenicol for isolation of pVZC2/PCC 6803 transformants. The plates were incubated in light at 350 lux at 34 °C. Resultant colonies were lifted on to fresh plates.

RESULTS AND DISCUSSION

The fatty acid composition and positional distribution of fatty acids in *Nostoc* sp. strain SO-36 indicate that it belongs to Group 2 cyanobacteria

Cyanobacteria contain four classes of lipids [6,27,31,32], which are MGDG, PG (phosphatidylglycerol), SQDG (sulphoquinovosyl diacylglycerol) and DGDG (digalactosyl diacylglycerol). MGDG accounts for more than 50 % of the total lipids. In the present study, lipid analysis revealed that the major lipid classes in *Nostoc* sp. are MGDG, DGDG, PG, and SQDG and that MGDG accounts for 55 % of the total lipids (results not shown).

Fatty acid analysis indicated that the predominant fatty acids in total lipids were $C_{16:0}$, $C_{16:1(9)}$, $C_{18:0}$, $C_{18:1(9)}$, $C_{18:2(9,12)}$ and $C_{18:3(9,12,15)}$, with their composition in MGDG shown in Table 1. The distribution of fatty acids at the *sn*-positions of the glycerol backbone of MGDG revealed that the C_{18} fatty acids were present predominantly at the *sn*−1 position, whereas the C_{16} fatty acids were present exclusively at the *sn*−2 position of the glycerol moiety (Table 1). Moreover, the C_{16} fatty acid bound to *sn*−2 was strongly desaturated. The presence of $C_{18:3(9,12,15)}$ and $C_{16:1(9)}$ at the *sn*−1 and the *sn*−2 positions respectively indicated that the *Nostoc* sp. belongs to Group 2 of cyanobacteria with respect to the distribution of fatty acids at specific *sn*-positions.

Characteristics of DesC1 and DesC2 of *Nostoc* sp.

To examine the details of the desaturation of fatty acids in *Nostoc* sp., we cloned two *desC* homologous genes from this organism and determined their nucleotide sequences (accession numbers AJ621244 for *desC1* and AJ621247 for *desC2* in the NCBI database). These two genes were found at separate loci of the *Nostoc* sp. genome. The *desC1* gene encodes an ORF of 822 bp and its predicted product contains 274 amino acids. The *desC2* gene encodes an ORF of 855 bp and its predicted product contains 285 amino acids. The predicted amino acid sequence of DesC1 is more similar than DesC2 to the predicted amino acid sequence of DesC of *Synechocystis* sp., with 77 % and 65 % similarity respectively. Since DesC from *Synechocystis* sp. catalyses desaturation exclusively at the *sn*−1 position [3,14,32], we predicted that DesC1 would desaturate fatty acids at the *sn*-1 position. Thus we postulated that DesC2 of *Nostoc* sp. would catalyse the desaturation of C_{16} saturated fatty acids at the *sn*-2 position.

Table 2 Distribution of fatty acids at *sn*−1 and *sn*−2 positions of MGDG from *Synechocystis* sp. cells that had been transformed with either pVZ321 (control) or pVZC2 carrying the *desC2* gene from *Nostoc* sp. (strain 36)

Results are means ± S.D. from three independent experiments, and are expressed as mol % of total fatty acids. T implies a trace amount that corresponded to less than 0.5 mol %. ND, not detected.

		Fatty acid (mol %)							
Vector	Position	$C_{16:0}$	$C_{16:1(9)}$	$C_{18:0}$	$C_{18:0(9)}$	$C_{18:2(9,12)}$	$C_{18:3(6,9,12)}$	$C_{18:3(9,12,15)}$	$C_{18:4(6,9,12,15)}$
pVZ321	*sn*−1	3 ± 0.2	2 ± 0.3	T	T	5 ± 0.3	29 ± 0.4	0.7 ± 0.2	9 ± 0.3
	sn−2	49 ± 0.4	0.5 ± 0.2	T	T	ND	T	ND	ND
pVZC2	*sn*−1	T	4.0 ± 0.3	T	1.5 ± 0.3	5.6 ± 0.3	28.5 ± 0.3	1.0 ± 0.1	9.0 ± 0.3
	sn−2	11.8 ± 0.3	36.1 ± 0.4	T	0.9 ± 0.2	T	ND	ND	ND

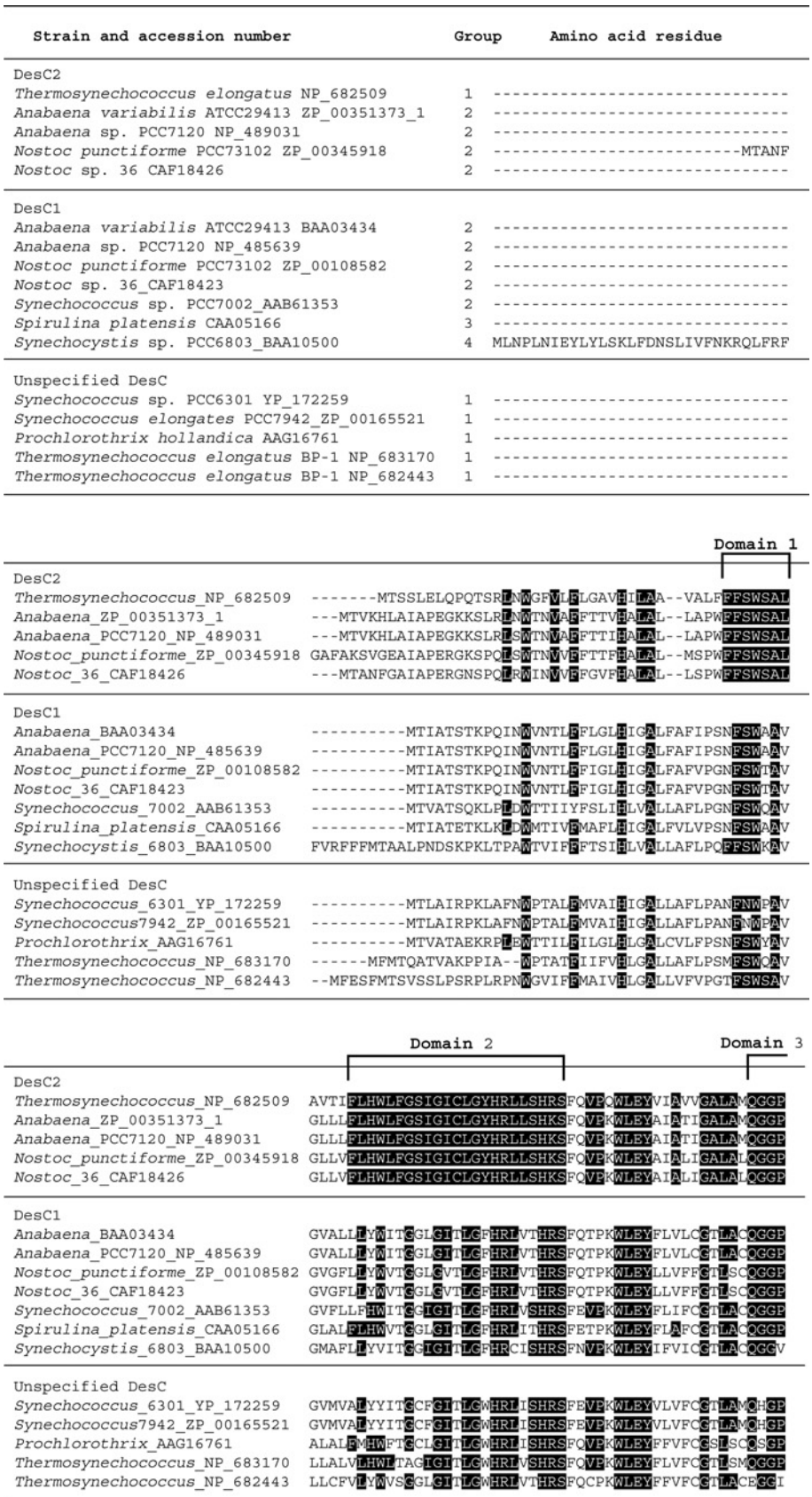

Figure 1 **For legend see page no. 212**

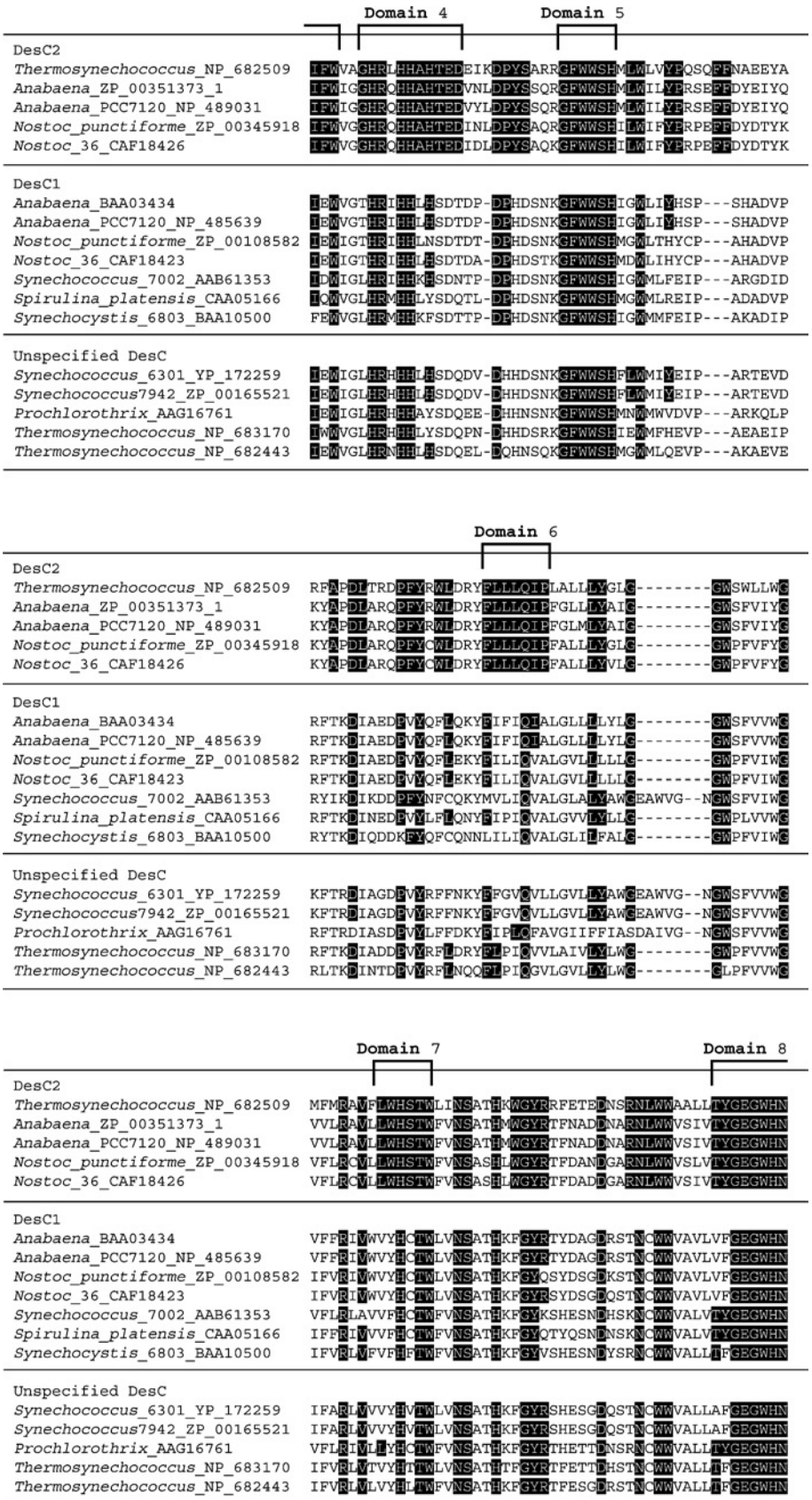

Figure 1 **For legend see page no. 212**

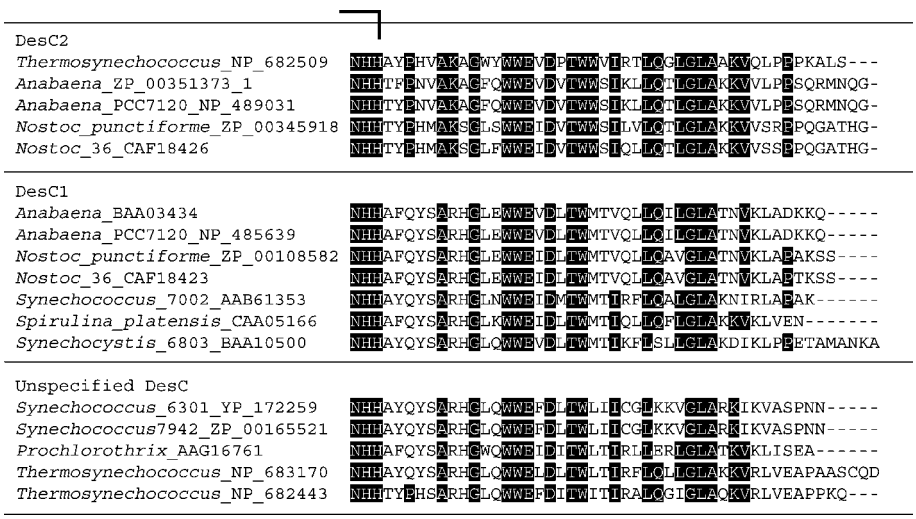

Figure 1 Alignment of the complete deduced amino acid sequences of DesC1 and DesC2 of cyanobacteria, grouped on the basis of the distribution of fatty acids at *sn*− positions

Amino acid residues that are conserved in DesC2 are highlighted in black boxes.The eight domains that are conserved in DesC2 are indicated by square brackets. The deduced amino acid sequences were obtained from databases (GenBank®, EMBL and DDBJ) and the sequences were aligned using CLUSTAL W version 1.83 software. The accession number of each DesC sequence and the name of corresponding cyanobacterium are indicated on the left.

Functional expression of DesC2 in *Synechocystis* sp. introduces Δ^9 desaturation at the *sn*−2 position

To examine whether DesC2 acts on fatty acids at the *sn*−2 position, we transformed *Synechocystis* sp., which cannot desaturate fatty acids at the *sn*−2 position, with the *desC2* gene from *Nostoc* sp. by tri-parental mating, as described in the Experimental section. The distribution of fatty acids at *sn*−1 and *sn*−2 positions of MGDG indicated that in *Synechocystis* sp. cells that had been transformed with the *desC2* gene (pVZC2), there was a prominent increase in the level of $C_{16:1}$ at the *sn*−2 position (Table 2). These results suggest, that when DesC2 is expressed in *Synechocystis* sp., it catalyses the desaturation of fatty acids at the *sn*−2 position. We also observed that the level of $C_{16:0}$ at the *sn*−1 position decreased, even though it is normally only a minor constituent at this *sn*− position. This observation suggests that DesC2 might also act on fatty acids at the *sn*−1 position.

Homologues of DesC1 and DesC2 in cyanobacteria

The complete nucleotide sequences of the genomes of several strains of cyanobacteria (http://www.kazusa.or.jp/cyano/) indicate that each strain has one or two putative *desC* genes for Δ^9 acyl-lipid desaturases that are homologous with the *desC* gene of *Synechocystis* sp. Genes homologous with the *desC2* gene are found in genomes of cyanobateria that belong to Group 2, namely *Anabaena* sp. PCC 7120, *Anabaena variabilis*, and *Nostoc punctiforme*, and also in *Thermosynechococcus elongatus*, which belongs to Group 1. By contrast, there is apparently no *desC2* gene in *Spirulina platensis* and *Synechocystis* sp., which belong to Group 3 and Group 4, respectively (Figure 1). Previous studies have demonstrated that the *sn*−2 position of the glycerol moiety of glycerolipids is associated with saturated and unsaturated fatty acids in strains in Groups 1 and 2, but is associated only with $C_{16:0}$ in strains in Groups 3 and 4 [16,22]. The *desC2* gene is present in all strains in Group 2 that have been examined and in one strain in Group 1, but it is not present in any strains that have been examined in Groups 3 and 4. These findings are consistent with the mode of desaturation of fatty acids at the *sn*−2 position. By contrast, the *desC1* gene has been found in all strains examined in Groups 1–4 (Figure 1). Therefore, it is likely to be generally true that DesC2, encoded by the *desC2* gene, acts on fatty acids at the *sn*−2 position, whereas DesC1, encoded by the *desC1* gene, is specific to the *sn*−1 position.

Three strains in Group 1, namely *Synechococcus* sp. PCC 6301, *Synechococcus* sp. PCC 7942, and *Prochlorothrix hollandica*, each contain a single gene homologous to *desC*. The MGDG in these cyanobacterial strains is esterified with $C_{16:1}$ at the *sn*−2 position [3,4]. It seems likely that the DesC in these strains might be non-specific with respect to *sn*− position. We demonstrated that overexpression of the *desC* gene from *Synechococcus* sp. PCC 6301 in tobacco plants raised the level of $C_{16:1(9)}$ in MGDG at the expense of $C_{16:0}$ at the *sn*−2 position [19]. Therefore it is possible that most cyanobacterial strains in Group 1 contain only one type of DesC, which acts on fatty acids at both the *sn*−1 and the *sn*−2 position.

Conserved domains in DesC2

All acyl-lipid desaturases contain three histidine clusters, whose structures are unique to individual classes of acyl-lipid desaturases and are related to the specificity of individual desaturases to the position of carbon atoms in fatty acids at which unsaturated bonds are introduced [4,20,33]. In a previous study, we demonstrated that the structures of the three histidine clusters in DesC can be represented as H-X_4-H, W-X_3-H-X_2-H-H and H-X_2-H-H (where X represents any amino acid) [14,15,33]. The deduced amino acid sequence of DesC2 includes histidine clusters with the same sequences (Figure 1).

Figure 1 shows an alignment of the deduced amino acid sequences of the DesC proteins from cyanobacteria for which the genome sequences are known and/or the distributions of fatty acids at *sn*− positions have been determined or predicted. There are eight domains with strongly conserved amino acid sequences in all the DesC2 proteins examined to date: domain 1 is partly conserved in DesC1; domain 2 is a large conserved domain and includes the first histidine cluster, this sequence is also partly conserved in DesC1; domain 3 is relatively strongly conserved in

DesC1; domain 4 (includes one amino acid that is either glutamine or leucine) contains the second histidine cluster. This domain is poorly conserved in DesC1; domain 5 is fully conserved in DesC1. Only a single glutamine residue in domain 6 of DesC2 is conserved in DesC1; domain 7 is only partly conserved in DesC1, and; domain 8 (11 amino acids) contains the third histidine cluster and is well conserved in DesC1, with only one or two amino acids differences in the examined DesC1 proteins. Many amino acids that are not included in these eight domains are also conserved between both DesC1 and DesC2 proteins. It is likely that domains that are well conserved in both DesC1 and DesC2 proteins are responsible for the specificity of each enzyme with respect to the position of the double bond that is introduced into a fatty acid, and that domains that are conserved within the DesC1 and DesC2 proteins, but not between them, are responsible for the specificity with respect to the *sn*− position of the glycerol moiety of glycerolipids.

Phylogenetic relationships between DesC1 and DesC2 proteins

We constructed a phylogenetic tree using the deduced amino acid sequences of the DesC proteins available in databases. The tree indicates that DesC2 proteins from *Nostoc* sp., *Nostoc punctiforme, Anabaena* sp. PCC 7120, and *Anabaena variabilis* form a robust clade with bootstrap values of 100 % (Figure 2). This clade is closely related to another formed by DesC2 proteins of *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus*. The tree indicates that two desaturases of *G. violaceus* are related to the DesC2 clade. However, *G. violaceus* seems to belong to Group 3 or 4 of cyanobacteria, even though its lipid and fatty acid composition is unusual [34,35]. DesC1 proteins from *Anabaena variabilis*, *Anabaena* sp. PCC 7120, *Nostoc punctiforme*, *Nostoc* sp. strain SO-36, *Trichodesmium erythraeum*, *Spirulina platensis*, *Synechocystis* sp. PCC 6803, and *Synechococcus* sp. PCC 7002 constitute a separate clade (Figure 2). DesC proteins from *Prochlorothrix hollandica*, *Synechococcus* sp. PCC 6301, and *Synechococcus* sp. PCC 7942 and two DesC proteins from *Thermosynechococcus elongatus* are related to the DesC1 clade. The genomic data also indicate the presence of the third robust clade. However, no fatty acid composition of any of the cyanobacteria whose DesC homologues are included in this clade has yet been determined. The analysis of fatty acids in the cyanobacteria in this clade should provide useful information to establish whether these enzymes are of the DesC1 or DesC2 type.

The results in Figures 1 and 2 suggest that strains in Group 2 might have both a d*esC1* and a *desC2* gene, whereas strains in Groups 3 and 4 might only have a *desC1* gene. Such a distribution of *desC* homologous genes would be consistent with the characterization of the fatty acids esterified to the *sn*-positions of glycerol moieties of glycerolipids, that is, strains in Groups 3 and 4 have only $C_{16:0}$ at the *sn*−2 position whereas strains in Group 2 have $C_{16:1(9)}$ in addition to $C_{16:0}$ at the *sn*−2 position. The *desC* homologous genes of strains in Group 1 are widely distributed on the phylogenetic tree (Figure 2). *Thermosynechococcus elongatus* has three *desC* homologous genes, which consist of one *desC2* and two unspecified *desC* genes. By contrast, *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 6301 and *P. hollandica* have only one *desC* homologous gene, while *Thermosynechococcus vulcanus* has potentially only one *desC* homologous gene [17], which is included in the *desC2* clade. The relationship between phylogenetic groupings and the specificity of DesC1 and DesC2 with respect to *sn*− positions in strains in Group 1 remains an open question.

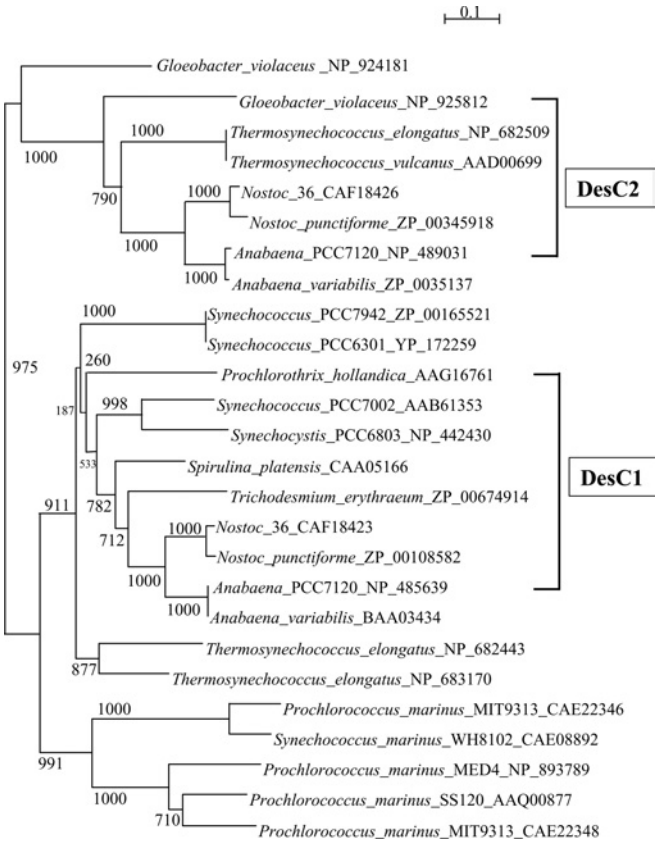

Figure 2 Phylogenetic tree determined on the basis of deduced amino acid sequences of DesC homologues in cyanobacteria

The amino acids sequences corresponding to the *desC* genes in cyanobacteria were obtained from databases (GenBank, EMBL and DDBJ) and the sequences were aligned with CLUSTAL W version 1.83 (as shown in Figure 1). The phylogenetic tree was drawn with NJplot (http://pbil.univ-lyon1.fr/software/njplot.html) using PHYLIP (phylogeny inference package) version 3.5c. The accession number of each *desC* homologue is indicated beside the name of the corresponding cyanobacterium. Bootstrap values (expressed relative to 1000 replications; [3]) are given at the respective nodes.

This work was supported by a grant from the India–Japan Cooperative Science Programme of the Department of Science and Technology, Government of India, and the Japanese Society for the Promotion of Science to I. S., N. M. and S. S. and by the Programme for Cooperative Research on Stress Tolerance of Plants of the National Institute for Basic Biology, Japan. S. C. thanks the University Grants Commission, New Delhi, Government of India, for a Junior and a Senior Research Fellowship.

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Received 5 January 2006/19 April 2006; accepted 11 May 2006
Published as BJ Immediate Publication 11 May 2006, doi:10.1042/BJ20060039